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Antagonistic properties of a probiotic preparation containing bacteria of the genera *Bacillus* and *Enterococcus*

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Complex probiotic preparations with spore-forming microorganisms of the genus *Bacillus* and other types of bacteria in their composition have a high biological activity and are of scientific and practical interest. In our laboratory experiments, we determined the level of antagonistic activity of the newly developed probiotic preparation containing strains of bacteria *B. subtilis*, *B. licheniformis*, *B. coagulans* in a complex with *Enterococcus faecium*, products of their own fermentation, other natural substances and a sorbent made of natural aluminosilicates. The antagonistic properties of the probiotic preparation were examined by diffusion methods of delayed antagonism and agar blocks. In order to identify the antagonistic action of the experimental probiotic preparation, we used Gram-negative and Gram-positive test cultures of bacteria from the Museum of Strains of Microorganism Cultures of the Research Bacteriology Department. The results of laboratory studies indicated the antagonistic effectiveness of the experimental probiotic preparation in its interaction with test microorganisms. This was confirmed by the indicators of the diameters of the growth inhibition zones of the tested bacteria, ranging 27–36 and more and corresponding to high and very high levels of antagonistic activity of the experimental probiotic. The use of the developed probiotic preparation in poultry farming would ensure the correct formation of microbiocenosis in gthe astrointestinal tract of poultry from hatching, increase local immunity, support a stable epizootic situation by preventing bacterial infections among poultry through biological means, and therefore contribute to production of ecologically high-quality and safe products of poultry farming.

Keywords: probiotic cultures; *Bacillus subtilis*; *Bacillus licheniformis*; *Bacillus coagulans*; *Enterococcus faecium*; symbiotic action.

Introduction

Preservation of the nation's health and prolonging life expectancy of the population is related to functional nutrition including high-quality agricultural products. The issue of obtaining clean ecological poultry products in Ukraine is extremely relevant (Dyshlyuk et al., 2017; Kucheruk et al., 2018; Chukhrai, 2019). The poultry industry of Ukraine has taken a course towards the development of organic poultry farming, the purpose of which is the production of organic products, that is, high-quality and safe raw materials and goods (Tochylyna et al., 2014; Romanovych, 2018; Hudzenko et al., 2019).

Modern poultry farming on an industrial basis is rapidly developing and is a promising industry in Ukraine. As known, the industrial system of poultry farming, especially under free-range conditions, limits the entry of normal microflora into the organism and colonization of the gastrointestinal tract, creating conditions for the development of pathogenic enterobacteria. Young poultry are especially sensitive to various bacterial diseases and rapid colonization by pathogenic microorganisms due to the unformed intestinal microbiocenosis, because they do not receive immunostimulating and nutritious compounds with mother's milk, as occurs in mammals (Sychevskyi et al., 2016; Stoianovskyi et al., 2018; Chukhrai, 2019; Tang et al., 2021).

At the current stage of development of the industry, the manifestation of pathological conditions in poultry should be considered as the influence of etiological factors. Due to increase in stocking density, more noise and less light, changes in the physico-chemical and microbiological content of the air, restriction of mobility, uniformity of nutrition, etc., the physiological ability of the poultry to resist the action of the main external stress factors is depleted. External stresses cause the formation of free radicals, overload cells with intracellular calcium, suppress energy production, protein synthesis and increase its degradation (Tochylyna et al., 2014; Haldar et al., 2016; Kucheruk et al., 2018).

A promising method of prevention, increasing indicators of nonspecific immunity, activity of immune cells, treatment and stimulation of productivity of animals and poultry is the use of probiotics, especially those with a complex content of probiotic strains of various types of bacteria (Haldar et al., 2016; Kim et al., 2017). Probiotics have high bioavailability, enhance the functions of regulation of redox processes, carbohydrate, protein and fat metabolism, support chemical balance and are powerful natural antioxidants. Probiotic preparations have antitoxic, hepatoprotective, antistress, adaptogenic, antiallergic, tonic, immunomodulatory, interferonogenic, anti-inflammatory, reparative, antibacterial and antiviral actions (Fadeenko et al., 2018). Mutual regulation and combined activities of the nervous, immune and antioxidant systems contribute to the maintenance of poultry homeostasis. When raising livestock, the key is to develop their protective mechanisms, immune and antioxidant abilities. These main links complement each other. The immune system supports the antigenic homeostasis of the organism and provides the normal functioning of all biochemical mechanisms. The antioxidant system ensures biochemical homeostasis and supports the normal and effective functioning of the organism and the immune system.

Intestinal microflora and the immune system of the digestive organs are a powerful peripheral complex of immune protection and directly or indirectly affect the immune function of the entire organism (Ashraf et al., 2014; Iegorov et al., 2022). The first barrier against the saprophytic microflora of the gastrointestinal tract which supports the immune homeostasis of poultry is the immune system of the mucous membrane with epithelial and dendritic cells. Probiotic strains of microorganisms in probiotics have high immunogenic properties due to their concentration of an important factor of local immunity – secretory Ig A. Secretory Ig A has the property of binding to pathogenic microorganisms, thereby preventing their adhesion to the intestinal epithelium (Hu et al., 2017; Chukhrai, 2019; Hubska et al., 2019).

Based on the principles of antagonism, the use of probiotic preparations ensures the replacement of pathogenic enterobacteria, their elimination from the organism, which contributes to the normalization of the balance of the microflora of the gastrointestinal tract of poultry, improves the metabolic processes, increases the viability of young poultry and ensures the production of environmentally safe products (Sychevskyi et al., 2016; Hedayati et al., 2018; Stoianovskyi et al., 2018).

Over the recent years, there have emerged reports on the scientific development of probiotic preparations based on spore-forming microorganisms, in particular bacteria of the *Bacillus* genus, focusing not only their antagonistic action against enterobacteria, but also confirming adhesive, enzymatic and synthesizing properties (Khariv et al., 2017; Hu et al., 2017; Hedayati et al., 2018). Bacteria of the *Bacillus* genus synthesize a number of enzymes of different classes that allow them to adapt to different types of substrates and survive in adverse environmental conditions. In particular, *B. subtilis*, a representative of this genus manifests its vital activity in a broad temperature ranges and acidic environments with pH = 3.0–4.0 (Fadeenko et al., 2018; Xiaopeng et al., 2021; Shkromada et al., 2022).

Probiotics based on *B. subtilis* and *B. licheniformis* strains have been confirmed to promote the normalization of hemoglobin and hematocrit in the organism and significantly increase the non-specific immunity of poultry (Fadeenko et al., 2018; Stoianovskyi et al., 2018; Shkromada et al., 2022).

Probiotics based on spore-forming bacteria of the genus *Bacillus* produce biologically active compounds that are used by the organism for the growth of other bacteria in the gastrointestinal tract of poultry, removal of harmful metabolic products, correction and maintenance of the microbial balance in the digestive tract (Kushnyr et al., 2015; Macelline et al., 2017; Mingmongkolchai et al., 2018).

Complex probiotic preparations with associated probiotic strains of various microorganisms are able to exhibit high biological activity, immunostimulating and antibacterial actions, thanks to the products of their own vital activity (Klaenhammer et al., 2012; Macelline et al., 2017; Kucheruk et al., 2018; Stoianovskyi et al., 2018; Hubska et al., 2019).

The objective of our study was the level of antagonistic activity of the newly developed probiotic preparation based on strains of *B. subtilis*, *B. licheniformis*, *B. coagulans* and *Enterococcus faecium* bacteria and products of their fermentation, other natural compounds and a sorbent made of natural aluminosilicates.

Materials and methods

The experiments were carried out in the laboratory for the diagnosis of diseases of bacterial etiology (LDDBE) of the State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE), Kyiv.

The composition of the newly developed probiotic preparation "Biomagn". Probiotic preparation "Biomagn" is a lyophilized preparation containing *B. subtilis* bacteria (strains Bs-5 and Bs-9) in the amount of 1.0×10^{12} CFU/g; *B. licheniformis* (strains Bfl-1 and Bfl-4) in the amount of 1.0×10^{10} CFU/g; *B. coagulans* (Bcg-5 strain) in the amount of 1.0×10^{10} CFU/g; *Enterococcus faecium* (strains Ef-3 and Ef-5) in the amount of 5.0×10^{10} CFU/g with their fermentation products, natural aluminosilicates, and other natural substances. To conduct the tests, a probiotic suspension was prepared: 1 g of the preparation was diluted in 9.0 cm^3 of sterile distilled water and thoroughly mixed. After the dilution, the content of the specified bacteria in the probiotic suspension was, respectively: *B. subtilis* – 1.0×10^{11} CFU/g; *B. licheniformis* and *B. coagulans* – 1.0×10^{9} CFU/g each; *E. faecium* – 5.0×10^{9} CFU/g.

To carry out the research, we used the Gram-negative test cultures *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 29630 and the Gram-positive test culture *Staphylococcus aureus* ATCC 6538, obtained from the Museum of Test Cultures of Microorganisms of the Laboratory of Diagnostics of Bacterial Etiology of the State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE). The test cultures of microorganisms were stored in the cryogenic state in a freezer at the temperature of –70 ± 10 ºС, in cryogenic test tubes with cryogranules (CRYO-Billes). To restore the metabolic processes of the test microorganisms, a cryogenic pellet was taken from the cryogenic test tubes, transferred to a test tube with meat-peptone broth and cultivated in a thermostat at the temperature of 37 ± 1 ºС for 24 hours. After the end of the incubation period, the corresponding test cultures were transplanted onto tryptone-soy agar, cultivated in the thermostat at the temperature of 37 ± 1 ºС for 24 hours. After verifying the compliance with the main typical properties, the restored test cultures of microorganisms *P. aeruginosa*, *E. coli*, *S. typhimurium* and *S. aureus* were approved for the experiments.

Study of antagonistic activity of the probiotic preparation "Biomagn" by the method of delayed antagonism. For the further study using the method of delayed antagonism to obtained individual macrocolonies of associated probiotic cultures in the content of the “Biomagn” preparation, subtitration of the probiotic suspension was carried out according to the generally accepted method of serial dilutions and its concentration was set at the level of 10^2 CFU/cm^3 (Ivchenko, 2004). The level of antagonistic activity of the probiotic preparation "Biomagn" was determined using the method of delayed antagonism on Petri dishes with 2.0% meat-peptone agar. The suspension of the probiotic preparation in a titrated concentration was inoculated on Petri dishes with meat-peptone agar, incubated at the temperature of 37 ± 1 ºС for 48 hours.

After the incubation, chloroform in the amount of up to 3 cm^3 was added to the Petri dishes with grown individual macrocolonies of the associated probiotic cultures. Chloroform covered the entire agar surface of the cup for 5 min. After the exposure, chloroform residues were drained and the surface of meat-peptone agar with macrocolonies was dried for 30 min in aseptic conditions (Lutgendorff et al., 2009).

At the same time, the test cultures of *P. aeruginosa*, *E. coli*, *S. typhimurium*, and *S. aureus* were cultivated on meat-peptone broth in the thermostat at the temperature of 37 ± 1 ºС for 24 hours. The obtained daily test cultures had been transplanted into test tubes with fresh meat-peptone broth and grown in the thermostat at the temperature of 37 ± 1 ºС for 6 hours. In the amount of 0.1 cm^3, the cultivated broth test cultures were placed in test tubes with 5.0 cm^3 of semi-liquid nutrient agar, melted and cooled to the temperature of 45 ± 1 ºС 0.7%. The mixture was quickly and thoroughly mixed, poured onto the agar surface of Petri dishes with macrocolonies of probiotic microorganisms, distributing it evenly over the surface. After complete solidification of the semi-liquid agar mixture with the corresponding test cultures, the test cups were incubated in the thermostat at the temperature of 37 ± 1 ºС for 24 hours.

Along with the main experiment, the control of the growth of test cultures of bacteria was carried out in the same way, but without introduction of a suspension of the experimental probiotic into semi-liquid agar.

To obtain agar blocks, the probiotic suspension was added to meat-peptone agar, melted and cooled to the temperature of 45 ± 1 ºС in a ratio of 1:10 (1st part of diluted suspension and 9 parts of meat-peptone agar), thoroughly mixed and poured into sterile 15.0 cm^3 Petri dishes. The dishes were left until the meat-peptone agar solidified with the probiotic completely. Then they were cultivated in the thermostat at the temperature of 37 ± 1 ºС for 24 hours. After the cultivating from meat-peptone agar with grown daily associated probiotic microorganisms, the agar blocks were cut under aseptic conditions using a sterile 9 mm-diameter punch.

The test cultures of microorganisms *P. aeruginosa*, *E. coli*, *S. typhimurium* and *S. aureus* were inoculated on meat-peptone agar, cultivated in the thermostat at the temperature of 37 ± 1 ºС for 24 hours. After the cultivation, the corresponding test cultures were rinsed with sterile physiological solution under aseptic conditions and a daily bacterial suspension in 0.5 OO concentration was prepared according to the McFarland optical turbidity standard. The resulting bacterial suspensions were inoculated on appropriate Petri dishes with meat-peptone agar, thoroughly rubbed over the entire surface of the medium to obtain a high-quality lawn. For diffusion into the agar of the corresponding test microorganisms, the dishes with the cultures were left at the room temperature for 15 minutes. Later, 3 agar blocks with associated probiotic cultures were placed on the surface of the dishes with seeds of the corresponding test cultures at the same distance from each other and cultivated at the temperature of 37 ± 1 ºC for 24 hours.

Based on the results, 6 colonies of associated cultures of each of the tested microorganisms were studied on a dish. The studies were carried out in three repetitions. The results were determined according to the size of the diameters of the growth inhibition zones of tested bacteria: around grown macrocolonies of associated probiotic microorganisms using the method of delayed antagonism and around agar blocks with associated probiotic microorganisms using the agar block method. The level of antagonistic activity of the probiotic preparation "Biomagn" was considered conditionally low, if the diameter of a growth inhibition zone ranged 7 to 14 mm; average – within 14–26 mm; high level – within 27–36 mm and a very high level – more than 36 mm with intensive growth of the corresponding tested bacteria microorganisms in the controls.

All the data were analyzed using Statistica 8.0 (StatSoft Inc., USA). The results in the tables are shown as x ± SE (mean ± standard error). The differences between values were considered significant at $P < 0.05$.

Results

The study of the influence of the probiotic preparation "Biomagn" on the test culture of *E. coli* revealed that the experimental probiotic inhibited the vital activity of the test strain. This was confirmed by the production of growth inhibition zone of the test culture with the average diameter of 40.3 ± 0.7 mm (Table 1). The obtained value was in the range of the indicators with a very high level of antagonistic activity. At the same time, intensive growth on the entire surface of the agar was observed in the growth controls of the *E. coli* test culture.

Evaluation of the effectiveness of the studied probiotic against the test culture of *S. typhimurium* showed a very high level of antagonistic activity. The average diameter of the growth inhibition zone of *S. typhimurium* was 38.7 ± 0.3 mm and significantly exceeded the determined threshold of the range of very high level of antagonism. Control of the growth of the *Salmonella* test culture was intensive on the entire surface of the agar.

Table 1
Antagonistic activity of the probiotic preparation "Biomagn" against the test cultures by the delayed antagonism method (x ± SE, n = 12)

The test culture	Average diameters of growth inhibition zones, mm
Escherichia coli ATCC 25922	40.3 ± 0.7
Pseudomonas aeruginosa ATCC 15442	32.5 ± 0.5
Salmonella typhimurium ATCC 29630	38.7 ± 0.3
Staphylococcus aureus ATCC 6538	40.1 ± 0.3

The experimental probiotic exerted effective antimicrobial action against the test culture of *S. aureus* microorganisms. During their interaction, the detected sizes of the average diameter of the growth inhibition zone was 40.1 ± 0.3 mm and was in the range of a very high level of antagonistic activity. At the same time, the growth control exhibited the intensive growth of *S. aureus* on the entire surface of the agar (Fig. 1).

According to the results of the study, the effective antibacterial action of the probiotic preparation "Biomagn" was found against the test-strain of *P. aeruginosa*. The average diameter of the growth inhibition of *P. aeruginosa* microorganisms was in the range of values of high level of antagonistic activity, measuring 32.5 ± 0.5 mm. The growth control of the test culture met the requirements and had vigorous growth of the culture over the entire surface of the agar.

According to the analysis of the results obtained by delayed antagonism method, of the "Biomagn" probiotic demonstrated effective antibacterial action against the Gram-negative and Gram-positive test bacteria. The experimental probiotic showed very high level of antagonistic activity and successfully inhibited the growth of the test microorganisms of *E. coli*, *S. typhimurium*, *S. aureus*, with the diameter of the growth inhibition zones equaling 40.3 ± 0.7, 38.7 ± 0.3 and 40.1 ± 0.3 mm, respectively, and high level of antagonistic activity against the test bacteria of *P. aeruginosa* with the diameter of the growth inhibition zone of 32.5 ± 0.5 mm, compared with intensive growth of the test cultures in the respective controls.

The complex probiotic cultures of the preparation "Biomagn" had negative impact on the viability of the test bacteria of *E. coli* and *S. typhimurium*, as determined using the agar block method (Fig. 2).

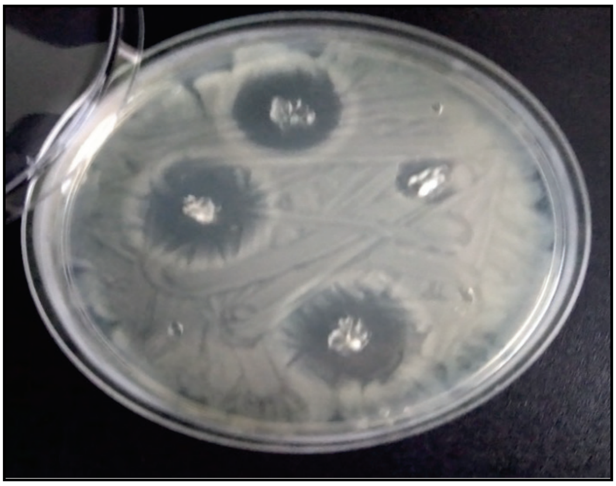

Fig. 1. Growth inhibition zones of test microorganisms *S. aureus* after interaction with associated probiotic cultures "Biomagn"

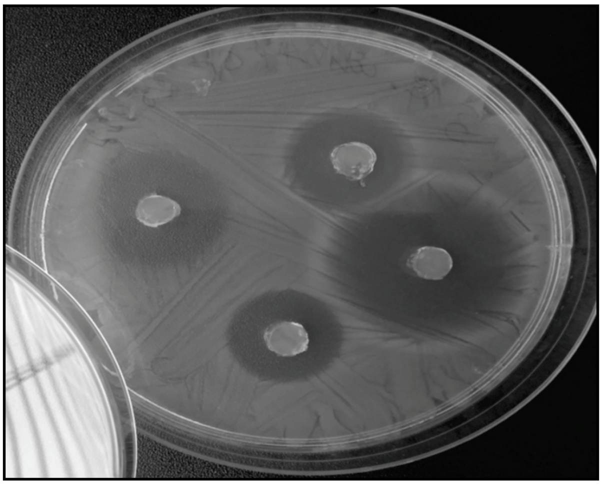

Fig. 2. Growth inhibition zones of the test microorganisms *E. coli* after interaction with associated probiotic cultures "Biomagn" in agar blocks

This is confirmed by the size of the diameters of the growth inhibition zones of test cultures of *Escherichia* and *Salmonella* – each 39.7 ± 0.3 mm. The data we obtained are in the range of values of very high level of antagonistic activity. The experimental probiotic showed a very high level of antagonistic properties against the test culture of *S. aureus*. This is confirmed by the diameter of the growth inhibition zones of the test bacteria at the level of 40.1 ± 0.5 mm, which significantly exceeded the determined threshold of the range for the ultra-high level of antagonism.

As revealed by the test of associated probiotic cultures, the "Biomagn" preparation showed lower antibacterial efficiency against the test culture of *P. aeruginosa*, compared with the other indicator microorganisms. The diameter of the growth inhibition zone of the pseudomonad test microorganisms was 34.1 ± 0.1 mm and was in the range of the values with high level of antagonistic activity (Table 2). In the conducted tests, the growth control of all the test cultures was characterized by intensive growth.

Therefore, according to the analysis of the results of experimental studies of the detection of the antibacterial effect using the agar block method after the interaction of the associated probiotic cultures of the preparation "Biomagn" with the test Gram-negative and Gram-positive test microorganisms *E. coli*, *S. typhimurium*, *S. aureus* and *P. aeruginosa*, the diameters of the growth inhibition zone corresponded to very high and high levels of antagonistic activity – 39.7 ± 0.3, 39.7 ± 0.3, 40.1 ± 0.5 and 34.1 ± 0.1 mm, respectively, compared with intensive growth of all test cultures in controls.

Table 2
Antagonistic activity of the "Biomagn" probiotic against the test cultures of various microorganisms using the agar block method (x ± SE, n = 12)

The test culture	Average diameters of growth retardation zones, mm
Escherichia coli ATCC 25922	39.7 ± 0.3
Pseudomonas aeruginosa ATCC 15442	34.0 ± 0.1
Salmonella typhimurium ATCC 29630	39.7 ± 0.3
Staphylococcus aureus ATCC 6538	40.1 ± 0.5

Discussion

The use of the latest production technologies, created thanks to the achievements of science, is able to solve the tasks of producing a sufficient amount of high-quality food raw materials for poultry farming, safe for human health. The dynamics of colonization of the gastrointestinal tract by microorganisms have been thoroughly researched. In the intestines of healthy poultry, in addition to indigenous bacteria, opportunistic microorganisms prevail. The species composition of conditionaly pathogenic bacteria in the gastrointestinal tract of poultry is influenced by internal and external environmental factors. The main representatives of the gastrointestinal microbiota are bifidobacteria, which are found in the parietal mucus and lumen of the large intestine; enterococci and lactobacilli, which inhabit the oral cavity, goiter, stomach, small and large intestine; *Escherichia* with pronounced enzymatic activity and no virulence, which inhabit the distal part of the small intestine and prevail in the large intestine. Therefore, the formation of a normal microbiocenosis and physiological symbiosis requires fulfillment of the energy and plastic needs of microorganisms and the poultry. Unsatisfactory conditions cause the emergence of infectious diseases of bacterial etiology in poultry. Currently, the use of antibiotic preparations in poultry farming is prohibited. According to modern data, an alternative to antibiotics for controlling the development of bacterial infections are probiotic preparations, in particular those containing combined strains of probiotic microorganisms that can correct the normal flora of the gastrointestinal tract of poultry from birth and perform enzymatic, adhesive, synthesizing and other functions (Lutgendorff et al., 2009; Klaenhammer et al., 2012; Arena et al., 2014; Ashraf et al., 2014; Kucheruk et al., 2018; Zazharskyi et al., 2020).

Research on the use of representatives of normal microflora as part of multicomponent probiotics together with their metabolites for the prevention and treatment of dysbacteriosis of various etiologies is promising for the development of probiotic preparations. Such experiments require an in-depth search of the mechanisms of the relationship between microorganisms and the macroorganism (Krysenko et al., 2010; Khyzhniak et al., 2012; Mousavi et al., 2018).

An equally important role is played by bacteriocinogeny, that is, the synthesis of protein-peptide antibiotic compounds that kill related species of microorganisms or inhibit their growth, providing a positive antimicrobial effect (Skrypnyk et al., 2020). Bacterial metabolites are also of practical importance for the normalization of microbiocenosis of the gastrointestinal tract (Macelline et al., 2017).

The absence of any negative consequences after enteral use of *B. subtilis* bacteria has been proven (Stoianovskyi et al., 2018; Shkromada et al., 2021). Studies have experimentally proven the harmlessness of probiotic strains of *Bacillus* bacteria, their antioxidant properties, higher antimicrobial and enzymatic activity with a positive influence on the immune status of the host's body, which decrease the level of cholesterol in blood (Trufanov et al., 2008; Shkromada et al., 2021).

Bacilus subtilis and *B. licheniformis* have unique peculiarities of metabolism to produce primary and secondary antibiotic scompounds. Most of these metabolites are ribosomal and non-ribosomal peptides, polyketides, terpenes and siderophores. Of the synthesized primary ribosomal metabolites of *B. subtilis* and *B. licheniformis*, bacteriocins – lantibiotics of type A (subtilin, erycin S) and type B (mersacidin) – are of primary importance. They promote the formation of pores in the cytoplasmic membrane of competing bacteria and inhibit the synthesis of the cell wall. Secondary metabolites of *B. subtilis* and *B. licheniformis* play important role in the growth of the population pressure of these bacteria on competitive microorganisms and reduce their number in the environment (Balamuralikrishnan et al., 2016; Mingmongkolchai et al., 2018).

The results of our research of the antagonistic properties of the new probiotic preparation "Biomagn" using two different methods – delayed antagonism and agar blocks – revealed its very high and high levels of antagonism after interaction with the Gram-positive and Gram-negative test bacteria *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 29630, *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442, as expressed in the size of the diameters of their growth inhibition zones.

However, there is an opinion that the pharmacotherapeutic potential of probiotic preparations against certain types of microorganisms and microbial associations needs to be substantiated (Mingmongkolchai et al., 2018). We examined the antagonistic properties of the probiotic preparation "Biomagn" based on its action towards Gram-negative and Gram-positive test cultures of pathogens. However, research by other scientists has shown that this is not enough and probiotics should be studied according to their action against field isolates of pathogens circulating in poultry farms (Stoianovskyi et al., 2018). For the effective differentiated use of probiotics, especially combined probiotic preparations and combinations of probiotics and other preparations, it is necessary to conduct more integrated research of their action against pathogenic and opportunistic pathogens (Trufanov et al., 2008; Krysenko et al., 2010). The data we obtained confirm the the synergistic action of combined probiotic preparations containing various types of probiotic strains of bacteria and their metabolites combined (Kucheruk et al., 2018; Shkromada et al., 2021).

Conclusion

The results of our study of the level of antagonistic properties of the "Biomagn" preparation, which contains a complex of probiotic microorganisms *B. subtilis*, *B. licheniformis*, *B. coagulans*, *E. faecium*, their fermentation products, natural aluminosilicates, other natural substances, performed using diffusion methods of timed antagonism and agar blocks, showed very high level of antagonistic activity against the test cultures of *E. coli*, *S. typhimurium*, *S. aureus* and high level of antagonism against *P. aeruginosa*. The parameters of the antagonistic activity of the new probiotic preparation "Biomagn" against bacterial infections indicate that it is promising for the application in the poultry industry. The new probiotic can naturally provide a therapeutic and preventive effect, correcting the intestinal microflora of the poultry from the first hours, providing replacement of pathogenic microorganisms and their elimination through antagonism.

The study was carried out under the research topic "Development of new and improvement of the existing approaches, methods and means of monitoring and laboratory research (testing) of safety indicators and individual quality indicators of objects of sanitary measures, by-products of animal origin, feed additives, premixes, feed, soil and water" (0118U100597).

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