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Microorganisms' Growth Inhibition in Poultry Meat Using *Bacillus* spp.

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ABSTRACT

Meat processing enterprises are currently seeking ways to improve the efficiency of their operations. This study aimed to assess the presence of harmful microorganisms in poultry meat treated with a probiotic complex of *Bacillus* spp. bacteria during storage. Of the 2,516 meat samples collected from broiler chickens across six poultry processing enterprises in the Dnipropetrovsk region over three years, 1,845 samples tested positive for pathogens. *Listeria* spp. were isolated in 52.7% of meat samples, S. aureus in 28.7%, *P. aeruginosa* – in 6.9%, *E. coli* in 4.2%, and *Salmonella* spp. in 7.5%. The next stage of the study was the infection of 10 samples of poultry meat with pathogens of test cultures (*Escherichia coli UNCSM* - 007, *Pseudomonas aeruginosa UNCSM* - 012, *Staphylococcus aureus UNCSM* - 017, *Listeria ivanovii UNCSM* - 042, *Salmonella* Enteritidis *UNCSM* - 081), followed by aerosol treatment with a probiotic complex of *Bacillus* spp. (1.5×10^8 in ml (0.5 Mac Farland) administered at a dose of 1 ml per sample with daily registration of colony growth. Following pathogen contamination and a single aerosol treatment with the probiotic complex of *Bacillus* spp., the growth *of E. coli* and *S. aureus* was already suppressed on the second day of meat storage. The probiotic complex of *Bacillus* spp. was able to displace *Salmonella* Enteritidis on the third day and *P. aeruginosa* on day 4, but the growth of *L. ivanovii* could be observed only on day 5. The probiotic complex of *Bacillus* spp. formed visible biofilms from the five strains of microorganisms and remained viable for five days, forming a dense biofilm with a high accumulation rate of 4.73 D620. A distinctly noticeable ability to form microbial biofilms within three days was observed in planktonic forms of *L. ivanovii* up to 2.88 D620, followed by *P. aeruginosa* at 2.28 D620. Low biofilm density was observed for *Salmonella* Enteritidis (1.77 D620) and *S. aureus* (1.76 D620). The probiotic complex of bacteria of the genus *Bacillus* spp. shows potential for use in meat processing plants to prevent the growth of harmful microbial biofilms on meat products stored under refrigeration.

Keywords: Antagonistic activity, Biofilm formation, Microbial biofilm, Pathogen, Probiotic complex of *Bacillus* spp.

INTRODUCTION

Currently, much attention is directed to the safety of food products of animal origin. To meet international quality and safety standards, these products must be free from toxic substances, as well as pathogenic and opportunistic microorganisms. It is crucial to note that poultry meat may contain harmful microorganisms, which can lead to severe food poisoning and even death in humans. Therefore, the initial processing of poultry carcasses (thawing, pinching, burning,) plays a significant role in ensuring the quality of the meat. It is especially important to avoid contamination of the carcasses with pathogenic and opportunistic microflora. Additionally, cross-contamination of meat products during primary processing may result in a higher concentration of harmful microorganisms, which can pose a risk to human health (Bridier et al., 2015; Markowiak and Śliżewska, 2018; Zazharskyi et al., 2023). The microbiota found on the surfaces of food processing plants are often diverse and include foodborne pathogens as well as food spoilage bacteria. Predominant genera in meat processing plants include *Pseudomonas*, *Acinetobacter*, *Staphylococcus*, and *Serratia* (Fegan and Jenson, 2018). One of the pathogens regularly encountered in such environments is *Listeria monocytogenes*, which causes the life-threatening disease listeriosis. Therefore, it is important to maintain viability at low temperatures. Moreover, it should be noted that their pathogenic prevalence is not reduced by chilling the meat. Increased resistance to inhibitory agents is associated with the survival of microorganisms, which is often linked to the development of biofuels (Wang et al., 2015; Puga et al., 2016). One of the pathogens regularly living in such media is *Listeria monocytogenes*. This means that illness due to listeriosis poses a more serious threat to human health (D'Ostuni et al., 2016; Fan et al., 2020; Borovuk and Zazharska, 2022).

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Biofilms of *L. monocytogenes* on food contact surfaces have been identified as an important pathway for pathogenic persistence and subsequent product contamination (Nowak et al., 2017; Pažin et al., 2018; Zazharskyi et al., 2019). The formation of *Listeria* biofilms in combination with *Pseudomonas*, *Bacillus cereus*, *Escherichia coli O157:H7*, *Salmonella* spp., *Pseudomonas* spp. *i Staphylococcus aureus* bacteria contribute to the preservation and maintenance of the bacterial population, thereby showing resistance to antimicrobial drugs, ultraviolet radiation, drying, and disinfectants (Akinbobola et al., 2017; Wang et al., 2018; Zazharskyi et al., 2020). Microbial cells are capable of detaching from the biofilm and spreading, making the biofilm a potential source of contamination in the production of meat products (Flemming and Wingender, 2010; Abdullahi et al., 2016; Lin et al., 2017). Thus, contamination of food production environments with biofilms of pathogenic bacteria and the development of effective methods to remove these biofilms from meat products, production surfaces, and equipment is a significant challenge. The use of spore-probiotic strains of *Bacillus*, which can reduce microbial contamination during the storage of meat products in processing plants, is highly relevant. Previous studies have laid the foundation for this research by identifying the antagonistic properties of probiotic preparations in destroying microbial biofilms in animal feed, which is crucial for preventing microbial contamination in the human food chain. It was established that the probiotic complex of bacteria of the genus *Bacillus* during continuous exposure to feed samples could displace biofilms of almost all types of isolated feed microflora (Kolchyk et al., 2022). To improve the sanitation and hygiene of poultry meat and extend its shelf life while ensuring safe consumption, it is imperative to explore new methods that are safe for the environment. Therefore, this study aimed to evaluate the presence of harmful microorganisms in poultry meat that had been treated with a probiotic complex of *Bacillus* spp. bacteria during the storage process.

MATERIAL AND METHODS

Ethical approval

The experiment was approved by the Bioethics Committee of the National Scientific Center, Institute of Experimental and Clinical Veterinary Medicine, Kharkiv, Ukraine. During the research on animals, manipulations were carried out in accordance with the existing documents regulating the organization of work with the use of animals in experiments and adherence to the principles of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (WorldLII, 1986).

Study conditions

The research was carried out at the Laboratory of Swine Diseases at the National Scientific Center, Institute of Experimental and Clinical Veterinary Medicine (Kharkiv, Ukraine) and the Dnipropetrovsk Regional State Laboratory of the State Service of Ukraine on Food Safety and Consumer Protection (Dnipro, Ukraine).

Sampling for laboratory research

Microbiological studies were performed on 2516 meat samples from broiler chickens obtained from six poultry processing enterprises in the Dnipropetrovsk region over three years. Immediately after slaughter, meat samples (chest and thigh muscles) were taken from each carcass, weighing 25g, and examined for the presence of *Listeria, Pseudomonas, Staphylococcus aureus, Escherichia coli, and Salmonella* spp. Pathogen's detection was carried out following current regulatory documents. Specifically, they were tested for *Escherichia coli* according to ISO/IEC 17025 (2017), *Pseudomonas* spp. – ISO 13720 (2010), *Staphylococcus aureus* – ISO 11290-1 (2017), *Salmonella* spp. – ISO 6579-1 (2017), and -ISO 6888-1 (2021), *Listeria* spp. The selection of meat samples from broiler chickens was conducted in accordance with ISO/TS 17728 (2015). Sample preparation for microbiological studies adhered to ISO 6887-2 (2014), 6887-3 (2017), and ISO 6887-2 (2017).

The identification of pathogenic microorganisms involved several main stages: Preliminary enrichment, selective enrichment, sowing on selective media, and confirmation of suspicious colonies. For preliminary enrichment of *Listeria* spp., the meat sample was minced and mixed with the Half Fraser broth (HiMedia, India) in a 1:10 ratio and incubated at (30.0 ± 0.5) °C for 24 hours. Then, 0.1 ml was transferred to Complete Fraser broth (HiMedia, India.) and incubated at a temperature of (37.0 ± 0.5) °C for 48 hours. It was sown on selective agar media (Agar Listeria) according to Ottaviani and Agosti (ALOA, HiMedia, India) and Polymyxin Acriflavine Lithium Chloride Ceftazidime Aesculin Mannitol (PALCAM, HiMedia, India). Plates were incubated at a temperature of 37.0 ± 0.5 °C for 48 hours. After incubation, colonies of Listeria spp., which had typical morphology (blue-green colonies with or without a black center on ALOA or gray-green with a black halo on PALCAM) were assessed.

For the isolation of *Salmonella* spp, the meat sample was ground and mixed with buffered peptone water (BPW) in a ratio of 1:10, and incubated at a temperature of 37.0 ± 0.5 °C for 24 hours. Two selective media were used for

additional enrichment: soy peptone broth Rappaport-Vassiliadis (RVS, HiMedia, India), and tetrathionate broth (TT, Farmactiv, Ukraine). While the RVS broth was incubated at 41.5 ± 0.5 °C for 24 hours, the TT broth was incubated at 37.0 ± 0.5 °C for 24 hours. The samples were then plated on selective agar media: Xylose, lysine, and desocholate agar (XLD, HiMedia, India), and hexanoate-lysine agar (HE, HiMedia, India). The plates were incubated at a temperature of 37.0 ± 0.5 °C for 48 hours. After incubation, the plates were examined and suspicious colonies were counted. Typical Salmonella colonies on XLD agar displayed black centres with a red border, while on HE agar, blue-green colonies with black centres were observed.

For the isolation of *S. aureus*, 10 g of meat cut was crushed and homogenized in 90 ml of a phosphate buffer solution corresponding to a dilution of 1:10. The sample was sown on the surface of Beard-Parker agar (Ukraine), enriched with egg yolk and potassium tellurite, and using a micropipette, it was evenly distributed with a spatula. It was then incubated at a temperature of 37.0 ± 0.5 °C for 24 hours. After incubation, colonies with typical appearance of coagulase-positive staphylococci were counted. Typical colonies on Beard-Parker agar were black or gray-black with a distinct zone of lecithinase activity (opalescent zone).

The identification of *Pseudomonas* spp. was carried out using a meat sample weighing 10 g, which was homogenized in 90 ml of a phosphate buffer solution diluted 1:10. A 0.1 ml aliquot of the homogenized sample was applied to the surface of Cetrimide, Fucidin, and Cephaloridine (CFC, Merck, Germany) agar with a micropipette and evenly distributed using a sterile spatula. The plates were incubated at a temperature of 25.0 ± 0.5 °C for 24 hours. After incubation, the agar was examined and colonies with a typical appearance of *Pseudomonas* spp. (round, smooth, opalescent colonies) were counted.

E. coli was isolated from a meat sample weighing 10 g, which was homogenized in 90 ml of a phosphate buffer solution with a dilution of 1:10. The sample was plated on Endo and Levin agar (Farmaktiv, Ukraine), and incubated at a temperature of 37.0 ± 0.5 °C for 24 hours. After incubation, the agar was examined and the *E. coli* colonies on Endo had a characteristic metallic sheen, while on Levin agar, they appeared black with a metallic sheen.

According to the results, 1845 samples tested positive for pathogens out of 2516 research samples. *Listeria* spp. was isolated in 52.7% of the samples, *S. aureus* in 28.7%, *P. aeruginosa* in 6.9%, *E. coli* – 4.2%, and *Salmonella* spp. in 7.5% of the broiler chicken meat samples.

The pathogenicity of the isolated microorganisms (*Listeria* spp., *P. aeruginosa*, *S. aureus*, *E. coli*, *Salmonella* spp.) was determined using the Dosis Certa Lettalis (DCL) on 70 white outbred mice (n=10), weighing 18-20 g and aged 8-9 weeks, kept in a vivarium for a month. The animals were adapted to the conditions of detention for 15 days. Six experimental groups and one control group, each consisting of 10 animals of the same age, were formed. While mice from the six experimental groups were injected intraperitoneally with a certain type of bacteria in a dose of 0.5 ml (at a concentration of 4.0 MacFarland) isolated from meat samples, mice in the control group were injected with 0.5 ml of physiological solution. The behavioural reactions and the physiological state of mice were monitored for 10 days. In five experimental groups, 100% mortality of the animals was recorded on the second to third day of observation, indicating the pathogenicity of the field isolates. No deaths occurred in the control group (Schlegel, 1987).

Scheme of the experiment

At a poultry processing enterprise, immediately after the slaughter of broiler chickens, meat samples (muscles of the chest and thigh) were taken from 20 broiler chickens: Ten of the samples were stored on moist napkins treated with a probiotic complex of *Bacillus* spp., while the other ten samples were stored on untreated napkins (control) within 5 days. Throughout the experiment, the meat samples were kept at a temperature of + 4°C without being covered with food film. Microbial contamination of the napkins was examined when processed and unprocessed meat samples were stored on them for five days after storage. Additionally, an organoleptic assessment of broiler chicken meat was carried out on a 9-point scale with sensorial specifications (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely) (Moradi et al., 2019).

The next phase of the study was the contamination of 10 poultry meat samples with the test cultures of pathogens (*Escherichia coli UNCSM* – 007, *Pseudomonas aeruginosa UNCSM* – 012, *Staphylococcus aureus UNCSM* – 017, *Listeria ivanovii UNCSM* – 042, *Salmonella* Enteritidis *UNCSM* – 081), followed by aerosol treatment with a probiotic complex of *Bacillus* spp. The test cultures *Escherichia coli UNCSM* – 007, *Pseudomonas aeruginosa UNCSM* – 012, *Staphylococcus aureus UNCSM* – 017, *Listeria ivanovii UNCSM* – 042, and *Salmonella* Enteritidis *UNCSM* – 081 were purchased from the State Scientific Control Institute of Biotechnology and Strains of Microorganisms of Ukraine and used to contaminate the 10 poultry meat samples and the polymer base.

First, a suspension was prepared from five test cultures including *Escherichia coli UNCSM* – 007, *Pseudomonas aeruginosa UNCSM* – 012, *Staphylococcus aureus UNCSM* – 017, *Listeria ivanovii UNCSM* – 042, and *Salmonella*

Enteritidis *UNCSM* – 081. To create this suspension, 0.1 ml of each of the five reference test cultures was inoculated onto a liquid differential nutrient medium and incubated at a temperature of 30.0 to 37.0°C. After cultivation, 1.0 ml of each pathogen was added to the test tube and adjusted with phosphate buffer to the density of the suspension according to the MacFarland test standard of 0.5 units. Following this, 100 ml of sterile water and 10 ml of a daily test culture were added to sterile glass jars with a volume of 500 ml. Next, ten samples of poultry meat, each weighing 100 g, were immersed in this solution containing five pathogens, with a microbial body concentration of 1.5×10^8 /cm^3 (0.5 MacFarland) for 1 minute, according to its improved methodology. The samples were subsequently dried for 10 minutes at room temperature (20.0 ± 2.0) °C. Following the contamination of the poultry meat samples, a one-time aerosol treatment of each sample of meat products was carried out using a probiotic complex of *Bacillus* spp. bacteria. The probiotic complex contained the five strains of *Bacillus subtilis UNCSM - 020, Bacillus licheniformis UNCSM - 033, Bacillus amyloliquefaciens ALB 65, Bacillus pumilus UNCSM – 026*, and *Bacillus subtilis variant mesentericus UNCSM – 031* all purchased from the State Scientific Control Institute of Biotechnology and Strains of Microorganisms of Ukraine. For the aerosol treatment of poultry meat and moisture-retaining napkins, a probiotic complex of bacteria of the genus *Bacillus* with a concentration of microbial bodies of 1.5×10^8 in each cm^3 (0.5 MacFarland) was used. Samples of treated and untreated meat were stored for five days at a temperature of +4 °C and organoleptic studies were performed daily, assessing the appearance of the samples (appearance, texture, smell, and juiciness), total microbial contamination levels, and the presence of pathogenic microflora growth using methods specified by ISO 6887-2 (2017).

The next stage aimed to investigate how the probiotic complex of bacteria belonging to the *Bacillus* spp. genus affects the pathogenic microflora of poultry meat. For this purpose, a comparative analysis of the probiotic complex's *Bacillus* spp. film formation dynamics and the multispecies biofilm of pathogenic microorganisms *(E. coli UNCSM* – 007, *P aeruginosa UNCSM* – 012, *S. aureus UNCSM* – 017, *L. ivanovii UNCSM* – 042, *S.* Enteritidis *UNCSM* – 081*)* on poultry meat was conducted. The formation of biofilms of microorganisms was studied by determining the ability of isolates of microbial associations and individual types of microorganisms to adhere to the surface of a 24-well polystyrene plates according to the method outlined by O'Toole and Kolter (1998). To obtain biofilms, 24-well plates were used, into which the nutrient medium was poured, and 1 ml of each strain (*Bacillus subtilis UNCSM - 020, B. licheniformis UNCSM - 033, B. amyloliquefaciens ALB 65, B. pumilus UNCSM – 026, B. subtilis variant mesentericus UNCSM – 031)* and the probiotic complex of the genus *Bacillus* spp., as well as 10 CFU of a mixture of five pathogenic bacteria were added separately. A separate plate was used for each microorganism. Cultivation was carried out at a temperature of 37.0 ± 0.5 ^{0}C for 5 days.

After culturing the bacteria, the medium with planktonic cells was carefully collected from the wells of the plate. To remove the remaining planktonic cells, the wells with biofilms were washed for 2 minutes with sterile phosphate-buffered saline (pH 7.2-7.4) in a volume of 5 ml, and the buffer was completely removed. Then, 4 ml of filtered 0.1% gentian violet solution was added to each well of the 24-well plate. The biofilms were incubated with the dye for 15 minutes at room temperature (20.0 ± 2.0 °C). The dye was removed from the well, and unbound dye was thoroughly washed off with buffer. The plates were inverted onto filter paper and dried. A 95% ethanol solution (4 ml) was added to each well for dye elution. The solvent was collected and placed in clean flat-bottomed plates, and the optical density was measured at a wavelength of 620 nm. During the evaluation of the density of biofilms, the film-forming microorganisms served as the experimental groups, while a nutrient-differential diagnostic medium for the cultivation of biofilms was used as the control.

The probiotic complex of bacteria of the genus *Bacillus* spp. has been introduced in veterinary medicine for various applications, including aerosol disinfection, cleaning of water supply systems, and the stimulation and regulation of digestive processes (Alrubay et al., 2020; Valeris-Chacin et al., 2021). Similar commercial preparations of probiotic bacilli are widely used for these purposes in veterinary medicine. Therefore, the probiotic complex of Bacillus bacteria was used to treat poultry meat samples and to influence the pathogenic microflora. It has antimicrobial activity associated with the ability to synthesize antibiotic-like substances with a wide spectrum of action, thereby suppressing pathogenic and opportunistic bacteria, as well as fungal flora, and stimulating the protective functions of animals (Wu et al., 2018). The protective functions of the animal body present a promising area for utilizing this probiotic complex in extending the long-term storage of poultry carcasses.

Statistical analysis

The results of the study were statistically analyzed using Microsoft Excel 2016 (for Windows XP). The probability of the extracted results was assessed using the Student's criterion. Differences in data were considered significant at $p < 0.05$.

RESULTS

In ten meat samples of broiler chickens during the three-day storage period on an untreated napkin, no organoleptic signs of meat spoilage were detected and no pathogenic microphores were isolated. They were evaluated for 7 points on the 3rd day of storage. However, on the 4th and 5th days, the growth of *E. coli* (7.20 and 12.30 CFU/g), an unpleasant smell, the decomposition of tissues, and the appearance of dark spots on the surface were observed in the meat samples, which amounted to 2 points (Table 1). When storing meat products on a napkin treated with a probiotic complex of Bacillus spp. bacteria, signs of spoilage and pathogenic microorganisms were not detected within 5 days. The meat samples were rated at 8 points, which indicated inhibition of microflora growth.

Twenty-four hours after contamination with pathogens of poultry meat followed by aerosol treatment with a probiotic complex of *Bacillus* spp., the growth of pathogenic agents including *L. ivanovii, S. enteritidis, E. coli, P. aeruginosa,* and *S. aureus* was noted (Table 2). Complete suppression of the growth of *E. coli* and *S. aureus* microorganisms by the probiotic complex *Bacillus* spp. was already observed on the second day of meat storage. While the probiotic complex of bacteria of the genus *Bacillus* spp. displaced *S.* Enteritidis on the third day by 57.9% and P. *aeruginosa* on the fourth day by 65.4%, the growth of *Listeria spp*. was observed until the fifth day (6.7 CFU/g). Therefore, *L. ivanovii* can form a biofilm in monoculture, and in a consortium with microorganisms *S. enteritidis, E. coli, P. aeruginosa*, and *S. aureus*, which also have a species-specific ability to form a film, the biofilm-forming properties are strengthened and form a biofilm resistant to the action of disinfectants.

Bacterial contamination of meat treated with probiotic *Bacillus* spp. was lower on each day of storage compared to untreated control samples ($p < 0.05$). The probiotic complex of bacteria of the genus *Bacillus* spp. caused inhibition of the growth activity of the studied pathogenic microorganisms, which can form biofilms of different densities due to their high intensity of film formation and antagonistic effect. In this case, the antagonistic activity of *Bacillus* spp. manifested itself later in *L. ivanovii* and *P. aeruginosa* on days 4 and 5 due to the formation of joint dense biofilms by them.

The count of mesophilic aerobic and facultatively anaerobic microorganisms in poultry meat treated with a probiotic complex of *Bacillus* spp. was 18.2% lower after a day, 32.7% on the third day, and 27.8% on the fifth day compared to the value of pure meat in the control (Figures 1 and 2).

Table 1. Determination of the level of microbial contamination after aerosol treatment with a probiotic complex of bacteria of the genus *Bacillus* spp. in the meat of broiler chickens

Meat storage period (day)	Meat with untreated napkin		Meat with a napkin treated with the probiotic *Bacillus* spp.	
	Contamination (CFU/g)	Quality (points)	Contamination (CFU/g)	Quality (points)
1	Growth is absent	9	Growth is absent	9
2	Growth is absent	7	Growth is absent	8
3	Growth is absent	7	Growth is absent	8
4	7.20±0.20* E. coli	*3*	Growth is absent	8
5	12.30±0.26* E. coli	*2*	Growth is absent	8

*The difference in the values of the indicators is reliable at $p < 0.05$ relative to the corresponding indicators

Table 2. Experimental infection of poultry meat with test strains of pathogens followed by treatment with a probiotic complex of *Bacillus* spp.

Meat storage period, (day)	Bacterial contamination						
	Control pure meat, (CFU/g)	Meat, aerosol treated with probiotic *Bacillus* spp., (CFU/g)	Meat, treated with probiotic *Bacillus* spp., isolated pathogenic microorganisms, (CFU/g)				
			L. ivanovii *UNCSM* – 042	***S.* Enteritidis** *UNCSM* – 081	***E. coli*** *UNCSM* – 007	***P. aeruginosa*** *UNCSM* – 012	***S. aureus*** *UNCSM* – 017
1	111.10 ± 0.59	90.10 ± 0.55*	33.50 ± 0.58 *L. ivanovii*	*27.30* ± 0.26 *S. enteritidis*	17.20 ± 0.20 *E. coli*	26.20 ± 0.36 *P. aeruginosa*	14.10 ± 0.35 *S. aureus*
2	559.60 ± 0.45	322.20 ± 0.70*	24.60 ± 0.37* *L. ivanovii*	*11.50* ± 0.22* *S. enteritidis*	solid growth *Bacillus* spp.	24.20 ± 0.25* *P. aeruginosa*	solid growth *Bacillus* spp.
3	981.50 ± 0.60	656.0 ± 2.67*	16.10 ± 0.53* *L. ivanovii*	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.	9.00 ± 0.26 *P. aeruginosa*	solid growth *Bacillus* spp.
4	1481.00 ± 0.65	953.00 ± 3.67*	6.70 ± 0.26* *L. ivanovii.*	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.
5	1801.9 ± 0.59	1312,00 ± 4.42*	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.	solid growth Bacillus spp.	solid growth *Bacillus* spp.	solid growth *Bacillus* spp.

• The difference in the values of the indicators is reliable at $p < 0.05$ relative to the corresponding indicators (between the values of columns 2 and 3);
*- the difference in the values of the indicators is reliable at $p < 0.05$ relative to the corresponding indicator of first day of storage

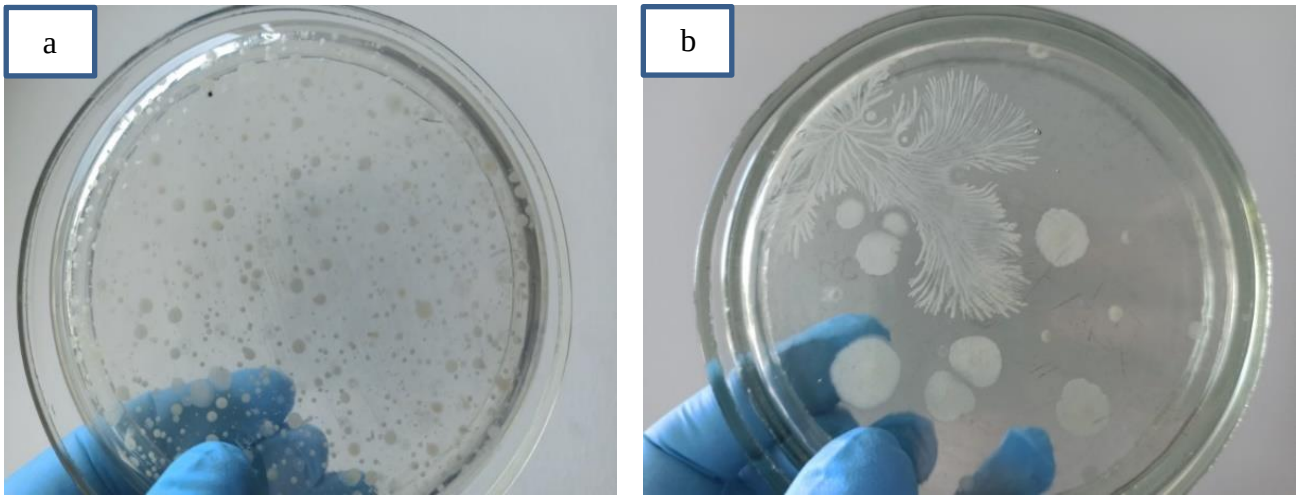

Figure 1. A swab from poultry meat on the third day after infection. **a**: Not treated with a probiotic complex of bacteria of the genus *Bacillus* spp., **b**: Treated with a probiotic complex of bacteria of the genus *Bacillus* spp.

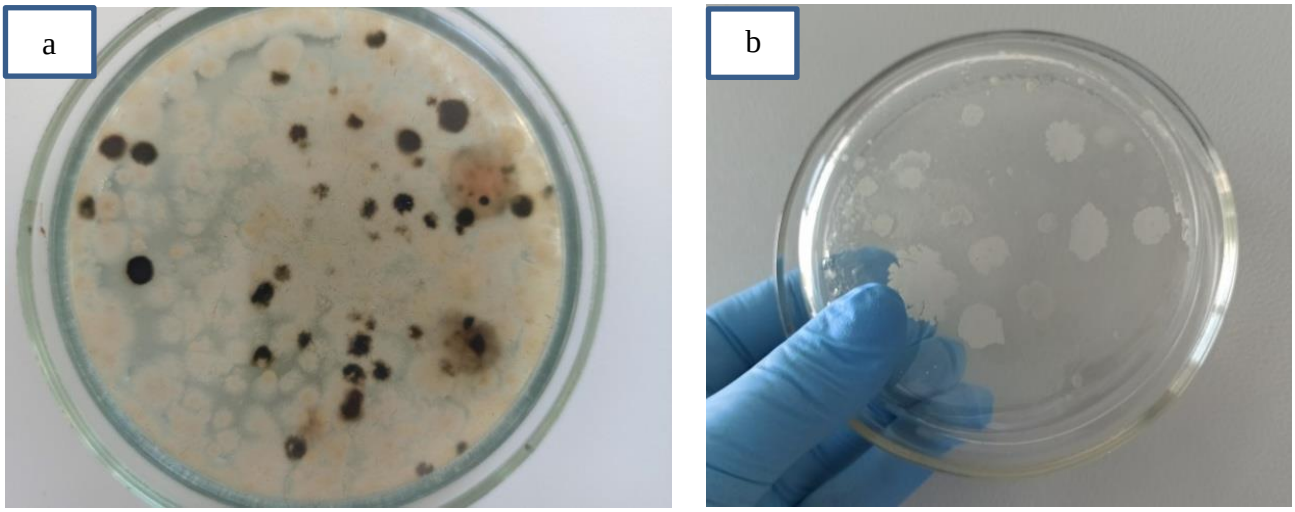

Figure 2. A swab from poultry meat on the fifth day after infection. **a:** Not treated with a probiotic complex of bacteria of the genus *Bacillus* spp., **b**: Treated with a probiotic complex of bacteria of the genus *Bacillus* spp.

According to the studies conducted, it has been established that each strain of the *Bacillus* spp. probiotic bacteria complex exhibits a significant biofilm formation ($p < 0.05$) intensity during a 5-day cultivation period. *Bacillus subtilis* formed a biofilm in only 24 hours, with an optical density reaching 3.25 ± 0.16 $D_{620,}$ which increased by 19.7% within 5 days (Figure 3). The growth intensity of biofilms of *B. pumilus, B. amyloliquefaciens, B. subtilis variant mesentericus,* and *B. licheniformis* also increased by the fifth day, and the density of biofilms was recorded at the level of 3.74 to 3.97 D_{620}, representing increases of 15.7 and 24.5% compared to the initial values. The difference between the accumulation of microbial biofilms among the five strains was noted to be insignificant ($p < 0.05$).

On the first day of cultivation, probiotic cells of the genus *Bacillus* spp. transit from a planktonic to a stationary state for the formation of a microbial biofilm. As they develop, stationary cells stick to each other and the surface, releasing an extracellular matrix that has properties contributing to the survival of the five strains of bacilli in the biofilm. The probiotic complex of bacteria of the genus *Bacillus* spp. developed visible biofilms from the five strains of microorganisms. The concentration of bacteria of the genus *Bacillus* spp. maintained its viability for 5 days and accumulated at a high rate, thereby forming a pronounced dense biofilm at - 4.73 D_{620}.

After conducting a series of experiments aimed at assessing the intensity of biofilm formation in pathogenic microorganisms that contaminate meat products, the following results were obtained (Figure 4). A pronounced ability to form microbial biofilms within 3 days was noted in planktonic forms of *Listeria* spp. (1.78 - 2.88 D620) and P. *aeruginosa* (1.95-2.28-2.28 D620), while low biofilm densities were observed in *S.* Enteritidis (1.38-1.77), *S. aureus* (1.33-1.76), and *E. coli* (1.49-1.91 D620). At the same time, in all planktonic forms of bacteria, a decrease in the density of biofilms was recorded from day 4 to day 5 by 4.2% in *Listeria* spp., 6.9% in *P. aeruginosa*, 6.8% in *S. enteritidis*, 14.8% in *S. aureus*, and 7.3% in *E. coli* compared to the values on day 3.

Planktonic forms of the above-mentioned microorganisms join each other within 4 hours to form strong microcolonies. During the next 12 hours, the number of planktonic forms decreases and mature colonies of a bacterial film with an optical density of 2.96 D_{620} are formed. The intensity of film formation of a multispecies biofilm reaches a maximum moderate density at the level of 3.24 D_{620} on the third day of cultivation, and then dispersion of planktonic forms into the nutrient medium occurred on day 5, resulting in a decrease of biofilm density by 11.5%.

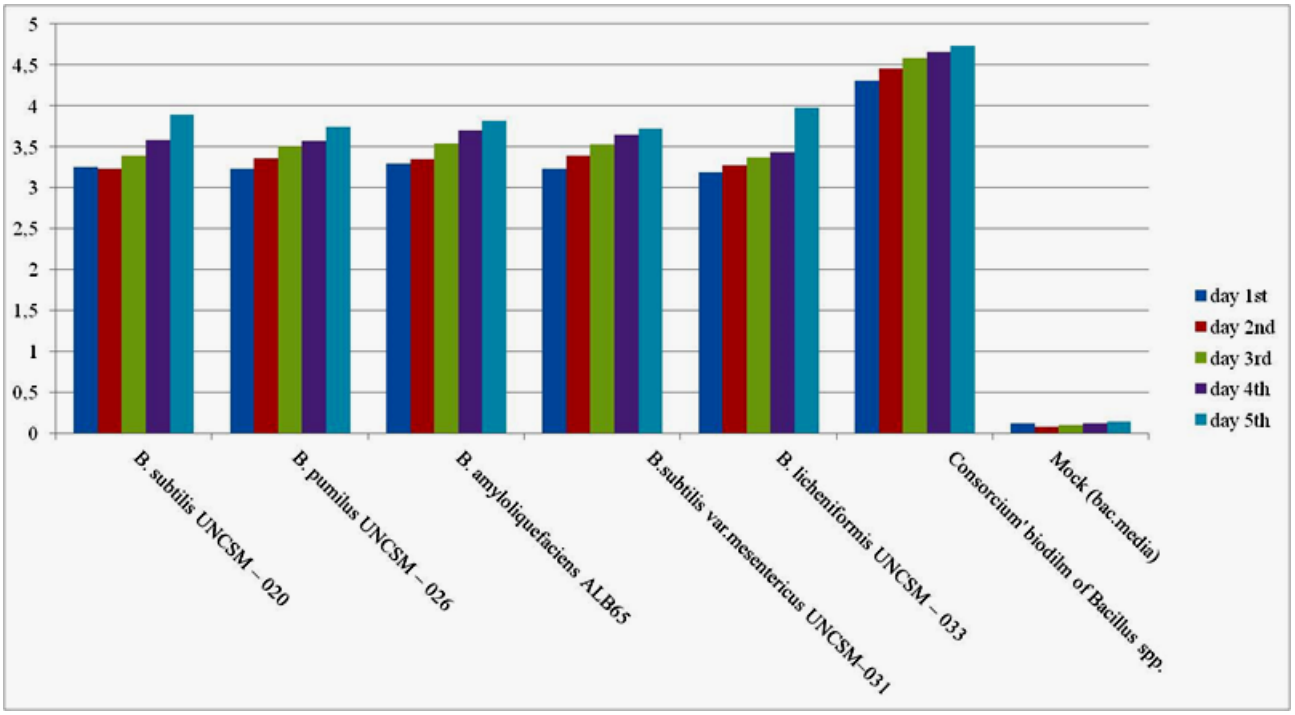

Figure 3. The intensity of biofilm formation of 5 strains of the probiotics complex *Bacillus* spp. on polystyrene plates during 5 days (n = 9, $p < 0.05$). Note: The optical density of biofilms up to $D_{620} < 2.0$ is low; $2.0 \leq D_{620} \leq 4.0$ – moderate; $D_{620} > 4x$ OD – expressed

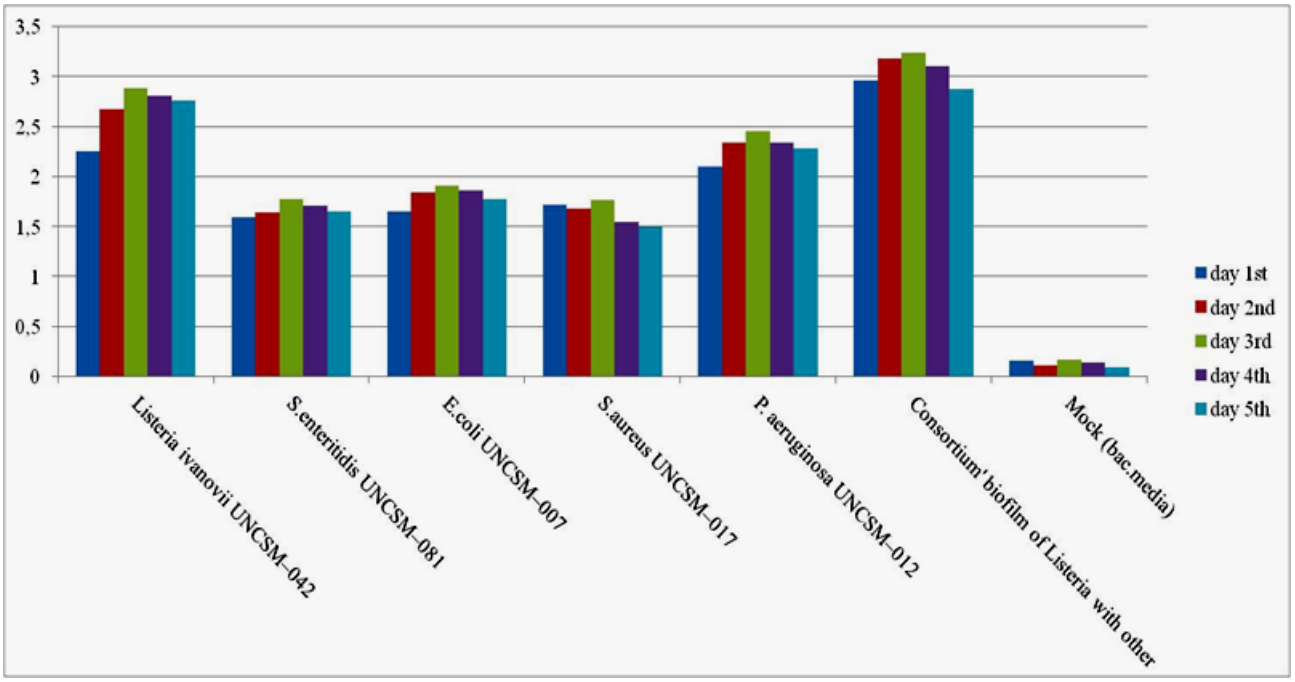

Figure 4. The intensity of biofilm formation of pathogenic microorganisms on polystyrene plates during 5 days (n = 7, $p < 0.01$). Note: The optical density of biofilms up to $D_{620} < 2.0$ is low; $2.0 \leq D_{620} \leq 4.0$ – moderate; $D_{620} > 4x$ OD– pronounced

DISCUSSION

The results of the present study indicated that *L. ivanovii* on poultry meat has the ability to adhere with the subsequent formation of biofilms of varying intensity, both in planktonic form and in association with pathogenic microorganisms *S. enteritidis*, *E. coli*, *S. aureus*, and *P. aeruginosa*, which interact as part of a mixed biofilm with one another, demonstrating a mutualistic relationship. The formation of a mixed biofilm was accompanied by the formation of an exopolysaccharide matrix by *L. ivanovii*, which holds the entire colony of pathogenic microorganisms capable of forming microbial biofilms on the surface of meat products, contributing to their preservation and maintenance.

According to the literature, interactions with other bacteria in biofilms explain the persistence of pathogens such as *L. monocytogenes* in food production environments (Gilbert et al., 2002). In multispecies biofilms, *L. monocytogenes* can interact directly with one or more other species in the biofilm (Elias and Banin, 2012; Jamal et al., 2018). *L. monocytogenes* can grow and survive in multispecies biofilms (*Psychromonas*, *Shewanella*, *Yersinia*, and *Lactobacillus*) formed from bacteria belonging to the background microbiota isolated in meat processing plants. In addition, it has been shown (Puga et al., 2018) that *L. monocytogenes* can colonize biofilms formed by *P. fluorescens* in co-culture experiments, which leads to an increase in bacterial cell density in vitro.

It is known that spore-forming bacteria of the genus *Bacillus* spp. can secrete bacteriocins and mycotoxins (antibiotic-like substances) and exhibit a sufficiently pronounced antibacterial effect against both opportunistic and pathogenic microflora including *Listeria*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Milyan et al, 2014; AlGburi et al., 2017).

Probiotic bacteria of the genus *Bacillus* spp. are characterized by their antimicrobial properties, which allow them to destroy (lyse) certain bonds in the peptidoglycan structure of the cell walls of a mixed biofilm (Khochamita et al., 2015; Petrova and Sauer, 2016; Kolchyk et al., 2020). It is known that spore-forming bacteria of the genus *Bacillus* spp. synthesize stable biofilms on various surfaces and show lytic sensitivity even in *Listeria* spp. and *P. aeruginosa*, suggesting high resistance to antimicrobial drugs due to their ability to form biofilms (Branda et al., 2004; Irkitova et al., 2018; Kolchyk et al., 2022).

The mechanism of the antagonistic activity of bacteria *Bacillus* spp. consists in the synthesis of non- polypeptide antibiotics (surfactin, bacillisin, bacitracin) and ribosomal synthesized peptides (subtilin, ericin S, mersacidin), which are synthesized by a bacterial cell in the stationary phase of growth (Cutting, 2011; Khardziani et al., 2017; Kotowicz et al., 2019). Different strains of *Bacillus* bacteria secrete a different set of antimicrobial substances, thereby exerting an inhibitory effect on a wide range of gram-positive and gram-negative bacteria (Baruzzi et al., 2011). In the mechanism of the selective antagonistic action of bacteria from the genus *Bacillus*, bacilli play a significant role in reducing the antilysozyme and adhesive activity of pathogenic bacteria, which determine the persistent properties of these microorganisms and their ability to parasitize intracellularly (Sorokulova, 2013; Rusaleyev et al., 2019; Chechet et al., 2022).

The obtained experimental results are consistent with the existing literature on the use of a probiotic complex of bacteria of the genus *Bacillus* spp. consisting of the five strains including *B. subtilis UNCSM* - 020, *B. licheniformis UNCSM* - 033, *B. amyloliquefaciens ALB* 65, *B. pumilus UNCSM* - 026, and *B. subtilis variant mesentericus UNCSM* - 031, which complement each other in the spectrum of antagonistic activity, as well as enzyme and amino acid production.

After the action of the probiotic complex of bacteria of the genus *Bacillus* spp. on the microbial biofilm of pathogenic microorganisms, *E. coli* and *S. aureus* cells were lysed to a greater extent (on the second day), and *S. enteritidis*, *P. aeruginosa*, and *Listeria* spp. (on days 3 and 4). This led to the destruction of the mixed biofilm and the dispersal of microorganisms, occurring due to a decrease in the number of essential substances for the body, after which they switched to the planktonic state with damage to their cell walls.

It should be noted that *Bacillus* spp. bacteria are capable of forming their biofilms, a type of cooperative existence in natural conditions. Bacilli form long chains of immobile cells that adhere to each other and the surface, secreting an extracellular matrix that provides rigidity and forms a stable biofilm (Wilking et al., 2012; Patel and DuPont, 2015). Spore-forming bacteria of the genus *Bacillus* spp. synthesize stable biofilms on various surfaces and show lytic sensitivity even in *Listeria* spp. and *P. aeruginosa*, which indicated high resistance to antimicrobial drugs due to the ability to form biofilms (Branda et al., 2004; Irkitova et al., 2018; Kolchyk et al., 2022).

Colonies of each strain of the probiotic complex of bacteria of the genus *Bacillus* spp. formed biofilms of moderate density: *B. subtilis* at the level of 3.89 D_{620}, *B. pumilus* at 3.72 D_{620}, *B. subtilis var. mesentericus* at 3.72 D_{620}, *B. amyloliquefaciens* at 3.81 D_{620}, and *B. licheniformis* at 3.97 D_{620}. In contrast, a consortium of the five strains synthesized a dense biofilm at 4.73 $D_{620,}$ with maximum preservation on day 5 of cultivation or intrageneric coaggregation.

As the biofilm expanded and matured, the production of the extracellular matrix continued, leading to the formation of folds (wrinkles) in the bacilli biofilms. These folds contributed to the formation of a complex network of channels in the biofilm, which facilitated the circulation of fluid and the distribution of nutrients. In this context, the biofilm of the probiotic complex of bacteria of the genus *Bacillus* spp. exhibited strong adhesion to the surface of meat products and co-aggregated pathogenic bacteria that multiplied and synthesized their biofilm. This process led to the dispersion of *L. ivanovii*, *S. enteritidis*, *E. coli*, *S. aureus*, and *P. aeruginosa* microorganisms and disrupted the growth mechanism of the microbial biofilm.

The obtained results are consistent with those in the literature in that the expression of *B. subtilis* biofilm genes is induced during co-culture with other members of the *Bacillus* genus (Shank et al., 2011; Bleich et al., 2015). *B.*

amyloliquefaciens ANT1 and its culture supernatant exhibit antibacterial activity against *B. cereus ATCC* 17778, *P. aeruginosa ATCC* 15442, *Aeromonas hydrophila ATCC* 7966 and *Aspergillus niger ATCC* 9642, all of which can form biofilms (Elias and Banin, 2012; Song et al., 2016).

Furthermore, bacteriocin BaCf3, isolated from *Bacillus amyloliquefaciens* BTSS3, can reduce biofilms by up to 80% even at low concentrations against strong food-borne biofilm producers such as *S. typhimurium, C. perfringens, E. faecalis,* and *P. aeruginosa* (Bindiya et al, 2019).

CONCLUSION

The results of the study showed that the probiotic complex of bacteria of the genus *Bacillus* spp completely inhibited the growth of pathogens *E. coli, S. aureus, S. enteritidis,* and *P. aeruginosa* during 2-4 days. However, the growth of *L. ivanovii, Listeria* spp. was observed up to day 5 after treatment, thereby reducing the number of pathogenic bacteria by about 11.5%. The probiotic complex containing *Bacillus* spp. exhibits antagonistic properties against pathogenic microflora and is capable of forming a biofilm, which facilitates the elimination of foodborne pathogens existing in the mixed biofilm on the surface of meat products. The broad specificity of bactericidal action and the high antagonistic activity of the probiotic complex of bacteria of the genus *Bacillus* spp. (*B. subtilis, B. licheniformis, B. amyloliquefaciens, B. pumilus,* and *B. subtilis variant. mesentericus*) against a consortium of pathogens (*L. ivanovii, S. enteritidis, E. coli, S. aureus,* and *P. aeruginosa)* opens prospects for practical application in meat processing and the storage of meat products. Based on the obtained results, it is possible to plan the development of spore-forming probiotic preparations for the neutralization of pathogenic microflora in meat products at meat processing enterprises in the future.

DECLARATIONS

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Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Authors' contribution

Andriy Buzun and Tetiana Illarionova conceived and designed the experiment, and Olena Kolchyk and Iryna Borovuk conducted bacteriological studies and analyzed the obtained results. Olena Kolchyk, Andriy Buzun, and Nadiia Zazharska interpreted and wrote the article, edited and reviewed the manuscript. All authors have read and approved the final draft of the manuscript for publication in the journal.

Competing interests

The authors have declared that no competing interests exist.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

REFERENCES

Abdullahi UF, Igwenagu E, Mu'azu A, Aliyu S, and Umar MI (2016). Intrigues of biofilm: A perspective in veterinary medicine. Veterinary World, 9(1): 12-18. DOI: https://www.doi.org/10.14202/vetworld.2016.12-18

Akinbobola AB, Sherrya L, Mckay WG, Ramage G, and Williams C (2017). Tolerance of *Pseudomonas aeruginosa* in *in-vitro* biofilms to high-level peracetic acid disinfection. Journal of Hospital Infection, 97(2): 162-168. DOI: https://www.doi.org/10.1016/j.jhin.2017.06.024

Alrubaye AAK, Ekesi NS, Hasan A, Koltes DA, Wideman RF Jr, and Rhoads DD (2020). Chondronecrosis with osteomyelitis in broilers: further defining a bacterial challenge model using standard litter flooring and protection with probiotics. Poultry Science, 99(12): 6474-6480. DOI: https://www.doi.org/10.1016/j.psj.2020.08.067

AlGburi A, Volski A, Cugini C, Walsh EM, Chistyakov VA, Mazanko MS, Bren AB, Dicks LMT, and Chikindas ML (2016). Safety properties and probiotic potential of Bacillus 68 subtilis KATMIRA1933 and Bacillus amyloliquefaciens B-1895. Advances in Microbiology, 6(6): 432-452. DOI: https://www.doi.org/10.4236/aim.2016.66043

Baruzzi F, Quintieri L, Morea M, and Caputo L (2011). Antimicrobial compounds produced by Bacillus spp. and applications in food. In: A. Méndez-Vilas (Editor), Science against microbial pathogens: Communicating current research and technological advances, pp. 1102-1111. Available at: https://bdigital.ufp.pt/bitstream/10284/9889/1/Metals_BookChapter_AFVinha_2011.pdf

Bindiya ES, Tina KJ, Sasidharan RS, and Bhat SG (2019). BaCf3: highly thermostable bacteriocin from *Bacillus amyloliquefaciens* BTSS3 antagonistic on food-borne pathogens. 3 Biotech, 9(4): 136. DOI: https://www.doi.org/10.1007/s13205-019-1639-2

Bleich R, Watrous JD, Dorrestein PC, Bowers AA, and Shank EA (2015). Thiopeptide antibiotics stimulate biofilm formation in *Bacillus subtilis*. Proceedings of the National Academy of Sciences, 112(10): 3086-3091. DOI: https://www.doi.org/10.1073/pnas.1414272112

Borovuk I and Zazharska N (2022). Evaluation of broiler meat in experimental listeriosis. Journal of Advanced Veterinary and Animal Research, 9(1): 155. DOI: https://www.doi.org/10.5455/javar.2022.i580

Branda SS, González-Pastor JE, Dervyn E, Ehrlich SD, Losick R, and Kolter R (2004). Genes involved in formation of structured multicellular communities by *Bacillus subtilis*. Journal of Bacteriology, 186(12): 3970-3979. DOI: https://www.doi.org/10.1128/jb.186.12.3970-3979.2004

Bridier A, Sanchez-Vizuete P, Guilbaud M, Piard JC, Naitali M, and Briandet R (2015). Biofilm-associated persistence of food-borne pathogens. Food Microbiology, 45: 167-178. DOI: https://www.doi.org/10.1016/j.fm.2014.04.015.

Cutting SM (2011). Bacillus probiotics. Food Microbiology, 28(2): 214-220. DOI: https://www.doi.org/10.1016/j.fm.2010.03.007

Chechet OM, Kovalenko VL, Horbatyuk OI, Gaidei OS, Kravtsova OL, Andriyashchuk VO, Musiets IV, and Ordynska DO (2022). Study *in vitro* of antagonistic activity of *Bacillus* isolates and selection of promising probiotic strains. Scientific and Technical Bulletin of State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives and Institute of Animal Biology, 23(1): 219-227. DOI: https://www.doi.org/10.36359/scivp.2022-23-1.2

D'Ostuni V, Tristezza M, De Giorgi MG, Rampino P, Grieco F, and Perrotta C (2016). Occurrence of *Listeria monocytogenes* and *Salmonella* spp. in meat processed products from industrial plants in Southern Italy. Food Control, 62: 104-109. DOI: https://www.doi.org/10.1016/j.foodcont.2015.10.025

Elias S and Banin E (2012). Multi-species biofilms: Living with friendly neighbours. FEMS Microbiology Reviews, 36(5): 990-1004. DOI: https://www.doi.org/10.1111/j.1574-6976.2012.00325.x

Fan Y, Qiao J, Lu Z, Fen Z, Tao Y, Lv F, Zhao H, Zhang C, and Bie X (2020). Influence of different factors on biofilm formation of *Listeria monocytogenes* and the regulation of cheY gene. Food Research International, 137: 109405. DOI: https://www.doi.org/10.1016/j.foodres.2020.109405

Fegan N and Jenson I (2018). The role of meat in foodborne disease: Is there a coming revolution in risk assessment and management?. Meat Science, 144: 22-29. DOI: https://www.doi.org/10.1016/j.meatsci.2018.04.018

Flemming HC and Wingender J (2010). The biofilm matrix. Nature Reviews Microbiology, 8(9): 623-633. DOI: https://www.doi.org/10.1038/nrmicro2415

Gilbert P, Maira-Litran T, McBain AJ, Rickard AH, and Whyte FW (2002). The physiology and collective recalcitrance of microbial biofilm communities. Advances in Microbial Physiology, 46: 203-256. DOI: https://www.doi.org/10.1016/s0065-2911(02)46005-5

Irkitova AN, Grebenshchikova AV, and Matsyura AV (2018). Antagonistic activity of *Bacillus subtilis* strains isolated from various sources. Ukrainian Journal of Ecology, 8(2): 354-364. DOI: https://www.doi.org/10.15421/2018_354

ISO 11290-1 (2017). Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 1: Detection method.

ISO 13720 (2010). Meat and meat products — Enumeration of presumptive *Pseudomonas* spp.

ISO/TS 17728 (2015). Microbiology of the food chain — Sampling techniques for microbiological analysis of food and feed samples.

ISO 6579-1 (2017). Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Part 1: Detection of *Salmonella* spp.

ISO 6887-2 (2017). Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 2: Specific rules for the preparation of meat and meat products.

ISO 6887-3 (2017). Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 3: Specific rules for the preparation of fish and fishery products.

ISO 6888-1 (2021). Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Method using Baird-Parker agar medium.

ISO/IEC 17025 (2017). General requirements for the competence of testing and calibration laboratories.

Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussain T, Ali M, Rafiq M, and Kamil MA (2018). Bacterial biofilm and associated infections. Journal of the Chinese Medical Association, 81(1): 7-11. DOI: https://www.doi.org/10.1016/j.jcma.2017.07.012

Kolchyk OV, Buzun AI, Paliy AP, Nalivayko LI, Chekan OM, Grebenik NP, Bondarenko IV, Rebenko HI, Kushnir VY, and Todorov NI (2020). Biofilms of pathogenic bacteria in pig industry. Ukrainian Journal of Ecology, 10(4): 202-209. DOI: https://www.doi.org/10.15421/2020_189

Kolchyk O, Illarionova T, Buzun A, Paliy A, and Palii A (2022). Influence of probiotic microorganisms on microbial biofilms in feeds. Scientific Horizons, 25(1): 41-50. DOI: https://www.doi.org/10.48077/scihor.25(1).2022.41-50

Khardziani T, Sokhadze K, Kachlishvili E, Chistyakov V, and Elisashvili V (2017). Optimization of enhanced probiotic spores production in submerged cultivation of *Bacillus amyloliquefaciens* B-1895. Journal of Microbiology, Biotechnology and Food Sciences, 7(2): 132-136. DOI: https://www.doi.org/10.15414/jmbfs.2017.7.2.132-136

Kotowicz N, Bhardwaj RK, Ferreira WT, Hong HA, Olender A, Ramirez J, and Cutting SM (2019). Safety and probiotic evaluation of two *Bacillus* strains producing antioxidant compounds. Beneficial Microbes, 10(7): 759-772. DOI: https://www.doi.org/10.3920/bm2019.0040

Khochamita N, Siripornadulsila S, Sukonb P, and Siripornadulsil W (2015). Antibacterial activity and genotypic–phenotypic characteristics of bacteriocin-producing *Bacillus subtilis* KKU213: Potential as a probiotic strain. Microbiological Research, 170 (1): 36-50. DOI: https://www.doi.org/10.1016/j.micres.2014.09.004

Lin S, Yang L, Chen G, Li B, Chen D, Li L, and Xu Z (2017). Pathogenic features and characteristics of food borne pathogens biofilm: Biomass, viability and matrix. Microbial Pathogenesis, 111: 285-291. DOI: https://www.doi.org/10.1016/j.micpath.2017.08.005

Milyan VO, Harhota MA, and Nechipurenko OO (2014). Research of probiotic properties of *Bacillus* spp. 1.1. and *B. amyloliquefaciens* УКМ B-5113 strains. ScienceRise, 5(1): 15-22. DOI: https://doi.org/10.15587/2313-8416.2014.32023

Markowiak P and Śliżewska K (2018). The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathogens, 10(1): 1-20. DOI: https://www.doi.org/10.1186/s13099-018-0250-0

Moradi M, Tajik H, Mardani K, and Ezati P (2019). Efficacy of lyophilized cell-free supernatant of *Lactobacillus salivarius* (Ls-BU2) on *Escherichia coli* and shelf life of ground beef. Veterinary Research Forum, 10(3): 193-198. DOI: https://www.doi.org/10.30466/vrf.2019.101419.2417

Nowak J, Cruz CD, Tempelaars M, Abee T, van Vliet AHM, Fletcher GC, Hedderley D, Palmer J, and Flint S (2017). Persistent *Listeria monocytogenes* strains isolated from mussel production facilities form more biofilm but are not linked to specific genetic markers. International Journal of Food Microbiology, 256: 45-53. DOI: https://www.doi.org/10.1016/j.ijfoodmicro.2017.05.024

O'Toole GA and Kolter R (1998). Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: A genetic analysis. Molecular Microbiology, 28(3): 449-461. DOI: https://www.doi.org/10.1046/j.1365-2958.1998.00797.x

Patel R and DuPont HL (2015). New Approaches for bacteriotherapy: prebiotics, new-generation probiotics, and synbiotics. Clinical Infectious Diseases, 60(suppl 2): S108-S121. DOI: https://www.doi.org/10.1093/cid/civ177

Pažin V, Jankuloski D, Kozačinski L, Dobranić V, Njari B, Cvrtila Ž, Lorenzo JM, and Zdolec N (2018). Tracing of *Listeria monocytogenes* contamination routes in fermented sausage production chain by pulsed-field gel electrophoresis typing. Foods, 7(12): 198. DOI: https://www.doi.org/10.3390/foods7120198

Petrova OE and Sauer K (2016). Escaping the biofilm in more than one way: Desorption, detachment or dispersion. Current Opinion in Microbiology, 30: 67-78. DOI: https://www.doi.org/10.1016/j.mib.2016.01.004

Puga CH, SanJose C, and Orgaz B (2016). Biofilm development at low temperatures enhances *Listeria monocytogenes* resistance to chitosan. Food Control, 65: 143-151. DOI: https://www.doi.org/10.1016/j.foodcont.2016.01.012

Puga CH, Dahdouh E, SanJose C, and Orgaz B (2018). *Listeria monocytogenes* colonizes *Pseudomonas fluorescens* biofilms and induces matrix over-production. Frontiers in Microbiology, 9: 1706. DOI: https://www.doi.org/10.3389/fmicb.2018.01706

Rusaleyev VS, Pruntova OV, and Vasilyev DA (2019). Cultural morphological and biochemical characteristics of *Bacillus subtilis* strains. Veterinary Science Today, 1: 58-62. DOI: https://www.doi.org/10.29326/2304-196X-2019-1-28-58-62

Shank EA, Klepac-Ceraj V, Collado-Torres L, Powers GE, Losick R, and Kolter R (2011). Interspecies interactions that result in *Bacillus subtilis* forming biofilms are mediated mainly by members of its own genus. Proceedings of the National Academy of Sciences, 108(48): E1236-1243. DOI: https://www.doi.org/10.1073/pnas.1103630108

Schlegel G (1987). General microbiology: Translated from German. Moscow, pp. 329-336

Song B, Wang Y, Wang G, Liu GL, Li W, and Yan F (2016). The lipopeptide 6-2 produced by Bacillus amyloliquefaciens anti-CA has potent activity against the biofilm-forming organisms. Marine Pollution Bulletin, 108(1-2): 62-69. DOI: https://www.doi.org/10.1016/j.marpolbul.2016.04.062

Sorokulova I (2013). Modern status and perspectives of bacillus bacteria as probiotics. Journal of Probiotics & Health, 1(4): e106. DOI: https://www.doi.org/10.4172/2329-8901.1000e106

Valeris-Chacin R, Pieters M, Hwang H, Johnson TJ, and Singer RS (2021). Association of broiler litter microbiome composition and *Campylobacter* isolation. Frontiers in Veterinary Science, 24(8): 654927. DOI: https://www.doi.org/10.3389/fvets.2021.654927

Wang H, Zhang X, Zhang Q, Ye K, Xu X, and Zhou G (2015). Comparison of microbial transfer rates from *Salmonella* spp. biofilm growth on stainless steel to selected processed and raw meat. Food Control, 50: 574-580. DOI: https://www.doi.org/10.1016/j.foodcont.2014.09.049

Wang H, Cai L, Li Y, Xu X, and Zhou G (2018). Biofilm formation by meat-borne Pseudomonas fluorescens on stainless steel and its resistance to disinfectants. Food Control, 91: 397-403. DOI: https://www.doi.org/10.1016/j.foodcont.2018.04.035.

Wilking JN, Zaburdaev V, De Volder M, Losick R, Brenner MP, and Weitz DA (2012). Liquid transport facilitated by channels in Bacillus subtilis biofilms. Proceedings of the National Academy of Sciences, 110(3): 848-852. DOI: https://doi.org/10.1073/pnas.1216376110

World legal information institute (WorldLII) (1986). Council of Europe treaty series - explanatory reports. Council of European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Available at: http://www.worldlii.org/int/other/treaties/COETSER/1986/1.html

Wu T, Zhang Y, Lv Y, Li P, Yi D, Wang L, Zhao D, Chen H, Gong J, and Hou Y (2018). Beneficial impact and molecular mechanism of Bacillus coagulans on piglets' intestine. International Journal of Molecular Sciences, 19(7): 2084. DOI: https://www.doi.org/10.3390/ijms19072084

Zazharskyi V, Parchenko M, Fotina T, Davydenko P, Kulishenko O, Zazharska N, and Borovik I (2019). Synthesis, structure, physicochemical properties and antibacterial activity of 1,2,4-triazoles-3-thiols and furan derivatives. Voprosy Khimii i Khimicheskoi Tekhnologii, 6: 74-82. DOI: https://www.doi.org/10.32434/0321-4095-2019-127-6-74-82

Zazharskyi V, Parchenko M, Parchenko V, Davydenko P, Kulishenko O, and Zazharska N (2020). Physicochemical properties of new S-derivatives of 5-(5-bromofuran-2-yl)-4-methyl-1,2,4-triazol-3-thiols. Voprosy Khimii i Khimicheskoi Tekhnologii, 6: 50-58. DOI: https://www.doi.org/10.32434/0321-4095-2020-133-6-50-58

Zazharskyi VV, Alifonova KV, Brygadyrenko VV, Zazharska NM, Goncharenko VP, and Solomon VV (2023). The ability of *Sitophilus oryzae* (Coleoptera, Curculionidae) to transmit Mycobacterium bovis: Morphology, cultural biochemical properties of the bacteria. Regulatory Mechanisms in Biosystems, 14(3): 476-486. DOI: https://www.doi.org/10.15421/10.15421/022368