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Influence of Probiotic Microorganisms on Microbial Biofilms in Feeds

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Abstract. At different stages of feed production and storage, it is possible to contaminate both feed and their components with various pathogenic and opportunistic microorganisms that can cause infectious diseases not only among animals but also have epidemiological significance. The aim of the study was to isolate biofilm-forming strains of microorganisms from feed, as well as to study the inhibitory activity of the probiotic complex of bacteria of the genus *Bacillus* against microbial biofilms. Identification and species affiliation of isolated bacterial field isolates were performed by cultural-morphological and biochemical properties. The formation of biofilms was studied by determining the ability of isolates of microbial associations and individual species of microorganisms to adhere to the surface of a 96-well polystyrene tablet according to the method of O'Toole & Kolter, 1998. Determination of microbial contamination of 50 industrial batches of feed from 4 pig farms in two regions of Ukraine (barley, compound feed SK-31 for rearing, SK-51 for fattening pigs, EXCELL starter for pigs 15%, shop prestarter, compound feed for lactating sows). In 11 experimental batches of barley (68.8%) and 13 batches of 3 types of feed (SK-31, SK-51, feed for lactating sows) identified associations with different microorganisms *Pasteurella multocida, Corynebacterium striatum, Bacillus subtilis, Leptothrix ochracea, Haemophilus parasuis* and yeast *Candida albicans*. The association of *Actinobacillus pleuropneumonia* bacteria with *B. subtilis* was identified in 2 batches (50%) of the shop prestarter. Moderate, by optical density, biofilm formation for associations of microorganisms *P. multocida* + *C. striatum* + *C. albicans* (D_{620}=3.59) and *P. multocida* + *L. ochracea* + *C. albicans* (D_{620}=3.62). Planktonic forms of *C. striatum* and *P. multocida* showed low film-forming activity at the level (D_{620}≤1.51). Inhibitory activity of the probiotic complex of bacteria of the genus *Bacillus (B. subtilis, B. licheniformis, B. amyloliquefaciens)* was determined by isolated variations of microbial biofilms in 5 feed species, which displaced all biofilm-forming microorganisms except *H. parasuis*

Keywords: biofilm-forming microorganisms, planktonic bacteria, microbial contamination of food, probiotic complex of bacteria of the genus *Bacillus,* inhibitory activity

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INTRODUCTION

According to the EU's (EU) livestock development strategy, the biosafety of feed production and animal feeding is one of the key factors in preventing epizootics and, consequently, microbial contamination of the human food chain (Gozdzielewska *et al*., 2020). Therefore, in the EU the requirements for the sanitary quality of compound feeds are formulated almost stricter than for food products (Giraldo *et al*., 2019; More, 2020).

The quality and safety of feed for farm animals depends on the correct composition and compatibility of nutrients, technology of their processing, and the degree of contamination by pathogenic microorganisms that are introduced into finished feed with raw materials (Paliy *et al*., 2018; Przeniosło-Siwczyńska *et al*., 2020). Pathogenic and opportunistic microorganisms in feed pose a threat to human and animal health and cause economic damage. Animal feed is most often affected by microorganisms: *Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella, Clostridium perfringens, Neisseria* spp.*, Pasteurella multocida, Actinobacillus pleuropneumonia,* fungi *Aspergillus niger* and *Aspergillus candidus, Candida albicans* (Monge *et al*., 2012; Udhayavel *et al*., 2017; Gosling *et al*., 2021).

Unfortunately, feed production in Ukraine in terms of control of microbial contamination of raw materials and the final product is still regulated by old standards that do not take into account new scientific knowledge, in particular the existence of bacterial associations in the form of microbial biofilms. This is a significant problem, because today it is known that bacterial biofilms are a complex dynamic biological system for the protection of microorganisms and increase their resistance to antimicrobial drugs by 100-1000 times compared to planktonic cells (Tassinari *et al*., 2019; Uruén *et al*., 2020). Being in the attached state, bacteria in biofilms are protected from environmental factors and the action of antibacterial substances in the environment and the host organism during infection. Moreover, the ability of microorganisms to form biofilms (film-forming activity) is already considered a factor in their pathogenicity (Soll & Daniels, 2016; Goodwine *et al*., 2019; Katongole *et al*., 2020). Therefore, the study of the composition of microorganisms in feed and their biofilm-forming activity is an important area for preventing the development of associated animal diseases and, consequently, microbial contamination of the human food chain.

The aim of the study was to isolate biofilm-forming strains of microorganisms from feed with further study of the inhibitory activity of the probiotic complex of bacteria of the genus *Bacillus* against microbial biofilms.

MATERIALS AND METHODS

Microbiological studies of feed were conducted in the laboratory for the study of pig diseases of the National Research Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) according to modern methods (Cullen *et al*., 2021). Identification and species affiliation of isolated field isolates of bacteria were performed by cultural-morphological and biochemical properties (Goodfellow *et al*., 2012).

Pasteurella multocida was cultured on Hottinger broth and agar, on blood agar with the addition of 5% sheep blood. According to the biochemical properties of *P. multocida*, D-glucose and fructose were catabolized with the formation of acid, were oxidase- and catalase-positive, reduced nitrate to nitrite; samples with methyl red and for the formation of acetoin (Foges-Proskauer), and lysinecarboxylase, arginine dihydrolase and gelatinase were negative.

Actinobacillus pleuropneumonia was isolated on meat-peptone agar (MPA) and 5% blood agar with the addition of 10% yeast extract. *A. pleuropneumonia* catabolized D-glucose and fructose to form acid, were positive for β-galactosidase, and negative for methyl red and indole.

Neisseria sicca was grown on meat-peptone broth (MBP), IPA and 5% blood agar. According to the biochemical properties of the bacterium *N. sicca* oxidase- and catalog-positive, formed carbonic anhydrase, reduced nitrite.

Yeast-like fungi *Candida albicans* were grown on wort agar, glucose-peptone medium. To determine the species of *C. albicans*, a test for the formation of germinal (embryonic) tubes was performed. The fungi *C. albicans* fermented glucose, maltose, sucrose, galactose, did not ferment lactose and raffinose.

Corynebacterium striatum was isolated on tellurite medium, blood and serum agar. *C. striatum* decomposed glucose, fructose, mannose, maltose, starch to acid; did not decompose lactose, sucrose. Urease was not isolated, nitrates were not reduced to nitrites.

Leptothrix ochracea was isolated on nutrient medium (Meus srl, manufactured by Piove di Sacco, Italy). Bacteria from glucose formed acid without gas, did not form protein and hydrogen sulfide, did not reduce nitrates and did not dilute gelatin, catalase-negative.

Bacillus subtilis grew on BCH, IPA, potato-peptone agar. Bacteria fermented sucrose, glucose, mannitol, salicin, esculin, fructose and did not ferment lactose, maltose, rhamnose, dulcite, inositol, sorbitol, galactose, raffinose. Did not form indole, but formed hydrogen sulfide, hydrolyzed starch, casein, but did not hydrolyze tyrosine and hemolyzed sheep erythrocytes, reduced nitrates and produced catalase.

Haemophilus parasuis was cultured on 5% blood and chocolate agar. Bacteria formed indole, which did not produce urease and hemolysin, in addition, fermented with the development of xylose acid, salicin, glycine, diluting gelatin, exhibited catalase activity.

Biofilm development was studied by determining the ability of isolates of microbial associations and individual species of microorganisms to adhere to the surface of a 96-well polystyrene plate according to the

method of O'Toole & Kolter (1998). The microorganisms were cultured in meat-peptone broth (MPB) at a temperature of (37±0.5)°C for 48 hours.

According to the standard protocol, planktonic cells were removed from the wells of the plate and the microbial biofilms were stained with crystalline violet. To do this, 150.0 µl of distilled water and 20.0 µl of 1% crystal violet were added to the well and incubated for 45 min at room temperature. After washing three times with distilled water, 200.0 µl of 96% ethanol was added to the wells to extract the ink from the biofilm, and the optical density of the solution was measured on an ELISA reader at an optical wavelength of 620 nm (D_{620}). In the process of estimating the density of biofilms, film-forming microorganisms were used as an experiment, and a nutrient medium for the cultivation of biofilms was used as a control.

50 industrial batches of feed from 4 pig farms of two regions of Ukraine (barley, compound feed SK-31 for rearing, SK-51 for fattening pigs, shop prestarter, compound feed for lactating sows) were studied. Feed selection was carried out both in the feed shop and in the livestock room where the animals are kept, as well as directly from products obtained from feed mills.

Pathogenicity of isolated associations of microorganisms Dosis certa lettalis (DCL) – from 5 types of feed was determined on 60 white outbred mice (n=10), weighing 18-20 g, age (8-9) – one month of age, kept in vivarium. The animals were adapted for 15 days to the conditions of detention. 5 experimental and one control group of animals of 10 individuals of the same age in each were formed.

White mice from 5 experimental groups were injected intraperitoneally for 24 hours with a certain association of bacteria at a concentration of 1 billion tons of food isolated from each type of food. Mice in the control group were injected with 0.5 ml of saline. The criterion of avirulence was the absence of infectious pathology and death of mice within 10 days. Behavioral responses and physiological status of mice were monitored.

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 (1986).

To study the inhibitory activity of the probiotic complex of bacteria of the genus *Bacillus,* namely *B. subtilis, B. licheniformis, B. amyloliquefaciens* (abbreviated *Bacillus spp.*) On isolated biofilm-forming associations of feed microorganisms, samples of contaminated feed with a period of 24 hours stirring at a temperature of $(37.0\pm0.5)^0$C with a probiotic complex of bacteria of the genus *Bacillus*, taken in final concentrations of 100, 50 and 10 thousand spores per 1 ml of sterile saline, at a dose of 0.5 ml.

Probiotic complex of bacteria of the genus *Bacillus,* which includes 3 cultures of *B. subtilis, B. licheniformis, B. amyloliquefaciens* is used in poultry and pig breeding for aerosol disinfection, water purification, to stimulate and regulate digestive processes. It has antimicrobial activity associated with the ability to synthesize antibiotic-like substances with a broad spectrum of action, thereby suppressing pathogenic and opportunistic bacteria, fungal flora and stimulates the protective functions of animals and birds.

RESULTS AND DISCUSSION

According to the results of microbiological studies, feed of plant origin for pigs, regardless of the place of sampling, had a high level of microbial contamination, and their species composition was very diverse (Table 1).

***Table 1**. Microorganisms that were isolated from industrial batches of feed for pigs*

Type of feed	In total, parties	Isolated microorganisms		Contaminated batches
		Feed breeding 10^{-5}	feed breeding $10^{-1.5}$	
Barley	16	*C. striatum*	*P. multocida* *C. striatum* *C. albicans*	11
Compound feed SK-31 for rearing piglets	7	*B. subtilis*	*P. multocida* *B. subtilis*	3
Compound feed SK-51 for fattening pigs	15	*P. multocida*	*P. multocida* *L. ochracea* *C. albicans*	6
Compound feed for lactating sows	8	–	*P. multocida* *N. sicca* *H. parasuis* *C. albicans*	4
Shop prestarter	4	*B. subtilis*	*A. pleuropneumonia* *B. subtilis*	2

All 50 batches of feed, which were studied at a dilution of 1:50 ($10^{-1.5}$), contained different types of microorganisms that formed stable associations, because during 3 consecutive passages of association with 2-3 microbial agents, in particular pathogenic for pigs, were not dissociated. At the same time, almost all feed samples (except for one batch of feed SK-51, samples of which were taken in the pigsty, as well as directly from the factory packages of the feed manufacturer), tested in accordance with current regulations in Ukraine at a dilution of 1:100000 (i.e. $10^{-5.0}$) in experiments on white laboratory mice did not have virulence properties (did not contain pathogens). During the entire observation period (10 days) after the introduction of a certain association of bacteria intraperitoneally, all animals remained viable, ate food well, no changes in the fur cover were observed. Mice were active, physiological discharges were not disturbed, behavioral reactions were normal. No clinical signs of toxicosis were observed.

Field isolate of *P. multocida* isolated from compound feed SK-51 for fattening pigs caused the death of 60% of white mice (out of 10 infected white mice killed 6 individuals) by 2 day with signs of depression, refusal of food, lack of activity, indicating its virulence.

In 11 experimental batches of barley (68.8%) and 13 batches of 3 types of feed S-31, SC-51 and for lactating sows (43.3%) isolated *Pasteurella multocida* in association with *C. striatum, B. subtilis, L. ochracea, N. sicca, H. parasuis* and yeast fungi *C. albicans*.

Two batches (50%) of the shop prestarter were contaminated with the bacterial association of *A. pleuropneumonia* with *B. subtilis*. At the next stage of research, the ability of microorganisms isolated from feed to form biofilms was studied (Table 2).

***Table 2**. Estimation of density of biofilms of microorganisms isolated from different types of feed*

Bacterial associations	Biofilm growth time, hours	Relative density of microbial biofilm (D_{620})	
		Experiment	Control
P. multocida *C. striatum* *C. albicans*	48	3.59±0.05	0.06±0.005
P. multocida *B. subtilis*	48	3.46±0.08	0.21±0.02
P. multocida *L. ochracea* *C. albicans*	24	3.62±0.19	0.05±0.003
P. multocida *N. sicca* *H. parasuis* *C. albicans*	48	3.52±0.51	1.47±0.12
A. pleuropneumonia *C. perfringens* *B. subtilis*	48	3.57±0.59	1.54±0.12
P. multocida	24	1.51±0.11	0.23±0.05
B. subtilis	24	2.86±0.35	0.22±0.06
	48	3.96±0.15	0.08±0.006

***Note**: the difference between the values of the indicators of experimental animals (group 1) is probable at $p \leq 0.05$ relative to the corresponding indicators in the control (group 2). Scale for assessing film-forming activity: Optical density of biofilms up to ≤ 2x OD – low; up to x 4x OD – moderate; ›4x OD – expressed*

The most pronounced, in terms of optical density, biofilm formation for associations of microorganisms *P. multocida* + *C. striatum* + *C. albicans*, D_{620}=3.59 and *P. multocida* + *L. Ochracea* + *C. albicans,* D_{620}=3.62. This indicates the important role of the yeast fungi *C. albicans* in the formation of polymicrobial biofilms – possibly as a "biofilm matrix" that serves as a framework for the planktonic forms to which they attach and hold (Lohse *et al.*, 2018).

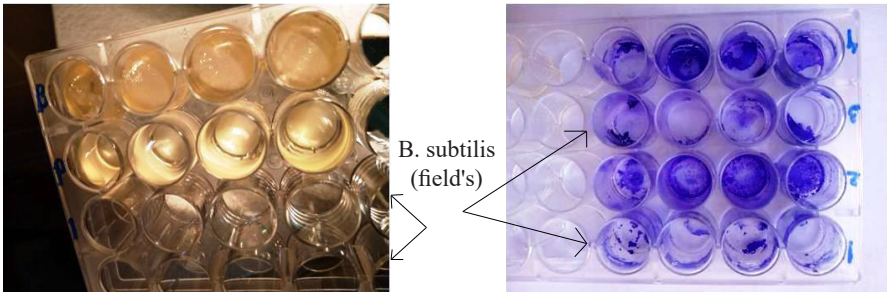
All field isolates with significant biofilm activity were isolated from a 1:50 feed extract dilution. Conversely, the vast majority of planktonic forms of these field isolates were isolated from feed extracts diluted 1:100,000, i.e. in accordance with the requirements of veterinary and sanitary control of feed in Ukraine.

Planktonic forms of *C. striatum* and *P. multocida were* formed at the level of $D_{620} \leq 1.51$ (low film-forming activity) and were found in feeds exclusively as part of polymicrobial biofilms. At the same time, field isolates of "feed" bacteria *B. subtilis* in all cases dominated in polymicrobial biofilms, the optical density of which was at the level of D_{620}=2.86±0.35 (moderate film-forming activity). These microbial associations with high biofilm-forming activity did not dissociate during successive 3-fold passage on agar media.

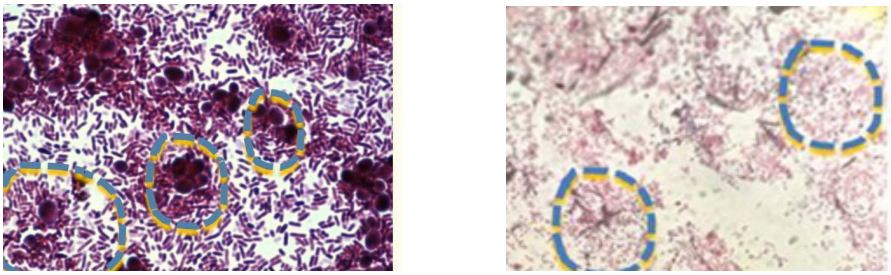
At the same time, during the passage on liquid

microbiological media, these associations dissociated into separate components. This phenomenon was especially evident in the presence of *Bacillus subtilis* in the association of forage microflora. This indicates the existence of biofilm-specific mechanisms of stability in the environment – at least for "solid-phase" polymicrobial biofilms compared to planktonic bacteria ("suspension forms").

According to preliminary data from bacterioscopy, the level of film-forming activity of bacteria in microbial feed associations correlated with the phenomenon of "bacterial swarming", which manifested itself in the development of associations of different microbial species in the form of "coacervates": in smears 1). Most of these "coacervates" formed around the cells of fungi and yeast.



a) Typical results of biofim-forming test with field bacterial isolates



b) 48 hrs "coacervates" of forage microflora

Figure 1. *Bacterial film formation (a), including microscopy (b). Swarming of film-forming bacteria, with the development of "coacervates" (object magnification × 1100)*

The phenomenon of dissociation of forage microflora under the influence of *B. subtilis* in its composition motivated us to further study the mechanism of influence of probiotic bacteria on biofilm-forming associations of microflora isolated from feed (Table 1) using strains specially selected for use in probiotics – potential tool for the "sanitation" of feed. The results of three series of treatment with a probiotic complex of bacteria of the genus *Bacillus* (*B. subtilis, B. licheniformis, B. amyloliquefaciens*) feed samples containing microbial biofilms are presented in Table 3.

Table 3. *Associations of bacteria isolated from pig feed samples treated with a probiotic complex of bacteria of the genus Bacillus*

Type of feed	Isolated microorganisms	Optical density at wavelength, D_{620}
Barley	*Bacillus* spp.	2.936±0.42
Compound feed SK-31 for rearing piglets	*Bacillus* spp.	3.16±0.51
Compound feed SK-51 for fattening pigs	*Bacillus* spp.	2.69±0.27
Compound feed for lactating sows	*Bacillus* spp. *H. parasuis*	3.35±0.59
Shop prestarter	*Bacillus* spp.	3.22±0.23

It was found that the probiotic complex of bacteria of the genus *Bacillus* during exposure to feed samples displaced into biofilms of almost all species of isolated feed microflora. The exception was the biofilm-forming bacteria *Haemophilus parasuis of the* microbial association of one of the batches of compound feed for lactating sows (their association with *P. multocida, N. sicca* and *C. albicans*). As shown in Table 3, under the conditions of the experiment (final concentration of spores 100 thousand), the biofilm of *Bacillus spp.* with an optical density in the range from D_{620}=2.69 to D_{620}=3.22 displaced from the feed microbial associations microorganism and

P. multocida, N. sicca, C. perfringens and fungi *C. albicans*. However, in this mode, they did not show competitive activity against the bacterium *H. parasuis*.

The obtained results forced us to look for an explanation of the peculiarities of the interaction of the probiotic complex of bacteria of the genus *Bacillus* with the causative agent of polyserositis/Glacier disease of pigs. For this purpose, comparative studies of the dynamics of film formation of the probiotic complex of bacteria of the genus *Bacillus* as such and in the presence of "feed" isolate of *H. parasuis* (polybacterial biofilms) were performed. Figure 2 presents data on the film-forming activity of these bacteria in a period of 10 days. It is obvious that *Bacillus* spp. under the same conditions of cultivation, even at the peak of its growth (up to the 3rd day) by 13-15% lagged behind the growth intensity of the polybacterial biofilm *Bacillus* spp. + *H. parasuis*. Moreover, already on the 8th day after sowing the density of the biofilm of the probiotic complex of bacteria of the genus *Bacillus* in four replicates began to decrease significantly and on the 10th day the optical density reached values of D_{620}=2.34. At the same time, the polybacterial biofilm of *Bacillus* spp. + *H. parasuis* for 10 days, according to optical indicators, practically did not lose the volumes reached in 24-28 hours after sowing D_{620}=3.55.

Figure 3 presents data on the film-forming activity of the same bacteria, but in the period of 24 hours after sowing.

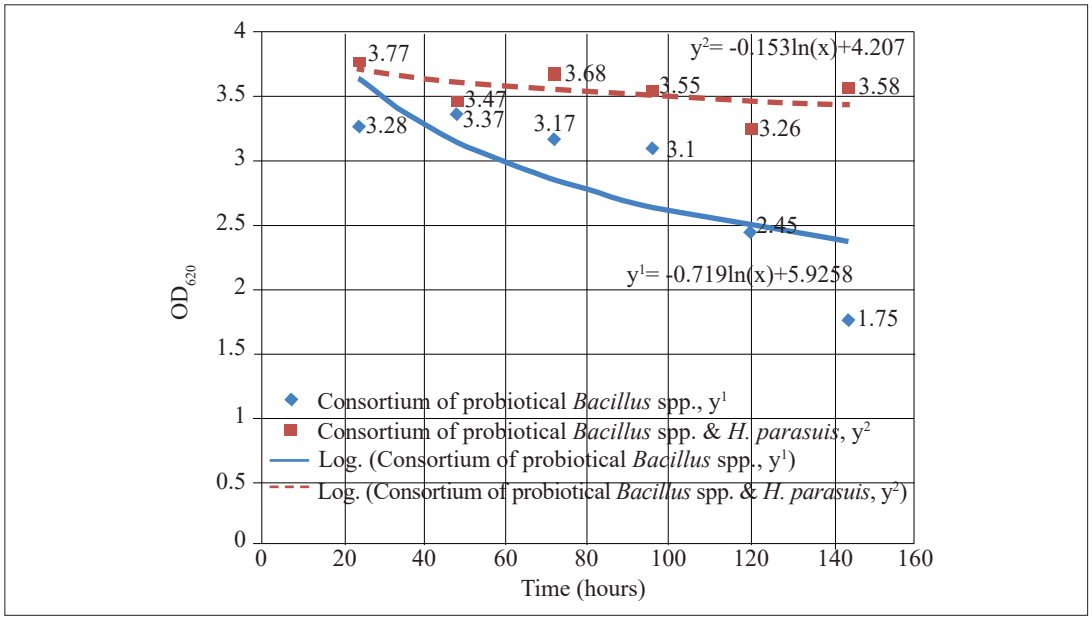


***Figure 2**. Comparison of the dynamics of film development of the probiotic complex of bacteria of the genus Bacillus separately and in association with H. parasuis for 10 days*

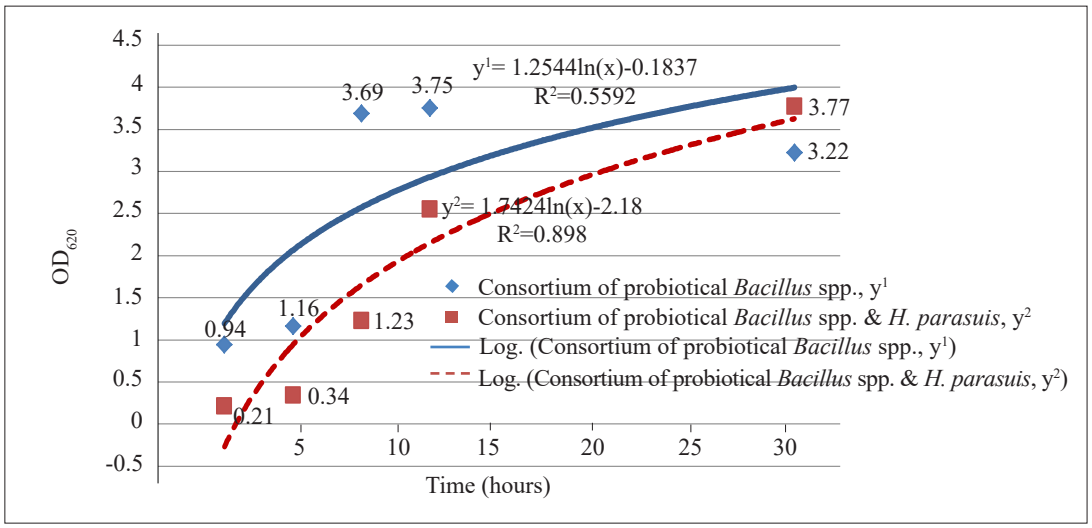


***Figure 3**. Comparison of the dynamics of film development of the probiotic complex of bacteria of the genus Bacillus separately and in association with H. parasuis on the first day*

The obtained data indicate a significantly higher (more than 70%) growth rate of the probiotic complex of bacteria of the genus *Bacillus* under the same cultivation conditions during the first 18-20 hours after sowing, compared to its polybacterial biofilm *Bacillus* spp. + *H. parasuis*. Only after 24-26 hours of cultivation, judging by the obtained growth curve, the biofilms of these bacterial associations reached their maximum parameters according to the optical density of their samples in the test of control of film-forming activity (Fig. 2).

Therefore, it is established that the associations of bacteria that contaminate feed for pigs are characterized by high film-forming activity. This indicates the probable formation of microbial biofilms of different composition and physical properties on the surface of grain, granules and other feed components, as well as on the surfaces of containers, feeders, etc., which may contain and promote the survival of infectious agents dangerous to pigs (Abdullahi *et al.*, 2016; Kukhtyn *et al.*, 2019).

The results show that the current norms of feed contamination control in Ukraine do not provide an objective assessment, as they do not take into account the presence of strong microbial biofilms in feeds, from which it is almost impossible to isolate a reliable concentration of microflora so that it can be detected 10^{-5}. Obviously, this is beneficial to feed producers, but it poses a danger to pig farming and the human food chain.

A large number of opportunistic pathogens in the feed of decomposition are nutrients, which leads to metabolic disorders, beriberi, toxicosis, decreased overall resistance, productivity of animals (Vestby *et al.*, 2020; Kim *et al.*, 2021). 34% of feed samples received from pig farms had a high level of microbial contamination. This indicates technological violations during production in factories, and during their storage, in the absence of proper veterinary and sanitary control in their use in animal feed.

Even the saprophytic microflora of feed under favourable conditions for its reproduction transport or storage in critical concentrations can affect the consumption and value of feed – because it decomposes nutrients, and sometimes with the development of toxic products of catabolism (Ramírez-Castillo *et al.*, 2018; Suarez *et al.*, 2019; Burgos-Morales *et al.*, 2021). The presence of pathogenic microorganisms in feed is usually associated with microbial contamination (MS) of raw materials. After all, the slightest contamination of feed materials, in particular premixes, with pathogenic microflora leads to favorable conditions for its reproduction to MH of ready-made feed in the feeder, with corresponding epizootic consequences (Makovcova *et al.*, 2017; Guzmán-Soto *et al.*, 2021). Prior to the discovery of a strategy to effectively protect bacteria from adverse environmental factors through the formation of microbial biofilms (Donlan, 2011), feed microbiology could not explain the mechanism of microbial contamination of feed (MSC) as a dynamic process. Therefore, modern criteria for the assessment of MFC in the former USSR and now in Ukraine are based almost exclusively on data from biological control of feed by its producer, while microbial biofilms are formed mainly during transportation and storage (Magana *et al.*, 2018; Muhammad *et al.*, 2020; Zhang *et al.*, 2020).

Bacterial biofilms are formed by the adhesion of microbial cells almost exclusively on the solid surfaces of polymers (both biological and abiological) with their inclusion in the polymer matrix (Yuan *et al.*, 2020; Apiwatsiri *et al.*, 2021; Skandalis *et al.*, 2021). Under favourable conditions, microbial biofilms are formed in raw materials, feed, food, various organs and tissues in humans and animals, on the surface of equipment when food comes into contact with technological surfaces (Callaway *et al.*, 2021; Jiang *et al.*, 2021).

In the joint cultivation of opportunistic pathogens with bacteria of the genus *Bacillus*, the latter form antibiotic substances and thus have a bactericidal and bacteriostatic effect on microorganisms that form biofilms, namely yeast-like fungi *C. albicans, Escherichia coli*, *Streptococcus, Staphylococcus, Staphylococcus, Salmonella, Klebsiella, Proteus, Pseudomonas aeruginosa* (Bai *et al.*, 2017). Different strains of bacteria of the genus *Bacillus* secrete different sets of antimicrobial substances. For example, one strain of *B. subtilis* secretes subtilin, which has antibiotic properties against gram-positive bacteria, another strain of *B. subtilis* secretes the antibiotic ericin *S*, which has the same spectrum of activity as subtilin (Stein *et al.*, 2002). Antimicrobial peptides secreted by *B. subtilis* have a huge advantage over traditional antibiotics because they are close to antimicrobial peptides released by the animal and are part of its natural immunity (Teixeira *et al.*, 2013). Similar substances have been identified in a large number of tissues and epithelial surfaces, including skin, eyes, ears, mouth, intestines, immune, nervous and urinary systems. Such as defensins, lysozyme, cathelicidin, dermtsidine, lectin, histatine and others (Wang, 2014). *B. subtilis* secrete similar substances, so resistance to them is rare, side effects are usually absent (Guariglia-Oropeza & Helmann, 2011). Lack of resistance to antimicrobial peptides of animals and *B. subtilis* is associated with the fact that their action is often aimed at the development of membrane pores, which leads to the death of bacteria. *B.subtilis* due to the release of antimicrobial substances inhibits the development of pathogenic microflora, which creates the conditions for filling the vacated niches and normal bacteria (Sumi *et al.*, 2015).

Obtaining quality and safe animal feed is impossible without compliance with the basic veterinary and sanitary and technological aspects of production (Muckey *et al.*, 2020; Paliy *et al.*, 2021a) . The main attention should be focused on the destruction of opportunistic, pathogenic microflora (Rodionova *et al.*, 2021) and parasitic insects (Paliy *et al.*, 2021b). Only a comprehensive approach to solving pressing problems of feed production and animal feeding will allow obtaining high quality livestock products.

CONCLUSIONS

In violation of the technology of feed production and storage of feed for farm animals, they can be contaminated with various microorganisms (*P. multocida, C. striatum, B. subtilis, L. ochracea, N. sicca, H. parasuis, C. albicans, A. pleuropneumonia*), which have epizootological and sanitary-hygienic significance. The ability of isolated microorganisms to form biofilms has been studied.

It is proved that the growth rate of the probiotic complex of bacteria of the genus *Bacillus* in biofilms (*Bacillus* spp.) Increases by the 8th day and gradually begins to decrease to the level of D_{620}<2.34 on the 10th day, while the polybacterial biofilm of *Bacillus* spp.+ *H. parasuis* maintains the intensity of growth up to the 10th day at the level of the index D_{620}<3.55.

The probiotic complex of bacteria of the genus *Bacillus (B. subtilis, B. licheniformis, B. amyloliquefaciens)* is promising for the formation of microbial biofilms in feed for pigs, which do not allow to take root in most of the above species dangerous to the human food microflora. Indicators of the dynamics of the development of probiotic biofilms are of prognostic value for the analysis of antagonistic-symbiotic activity of bacteria – both in the first two days (hourly) and in a weekly period of time.

The prospect of further research is to develop an innovative scheme for storing feed for farm animals, taking into account microbiological risks depending on regional climatic conditions.

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Вплив пробіотичних мікроорганізмів на мікробні біоплівки у кормах

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Анотація. На різних етапах виробництва кормів та їх зберігання можливе обсіменіння як кормів, так і їх компонентів різними патогенними та умовно-патогенними мікроорганізмами, що можуть бути причиною виникнення інфекційних захворювань не тільки серед тварин, а й мати епідеміологічне значення. Метою роботи було виділення біоплівкоутворюючих штамів мікроорганізмів із кормів, а також вивчення інгібуючої активності пробіотичного комплексу бактерій роду *Bacillus* відносно мікробних біоплівок. Ідентифікацію та видову належність виділених польових ізолятів бактерій проводили за культурально-морфологічними та біохімічними властивостями. Утворення біоплівок вивчали за допомогою визначення здатності ізолятів мікробних асоціацій та окремих видів мікроорганізмів до адгезії на поверхні 96-лункового полістиролового планшету за методикою O'Toole & Kolter, 1998. Проведено визначення мікробної забрудненості 50 промислових партій кормів з 4-х свиногосподарств двох областей України (ячмінь, комбікорм СК-31 для дорощування, СК-51 для свиней відгодівлі, EXCELL стартер для свиней 15 %, цеховий предстартер, комбікорм для лактуючих свиноматок). У 11 дослідних партіях ячменя (68,8 %) та 13 партіях 3 видів комбікормів (СК-31, СК-51, комбікорм для лактуючих свиноматок) виділяли асоціації з різними мікроорганізмами *Pasteurella multocida, Corynebacterium striatum, Bacillus subtilis, Leptothrix ochracea, Neisseria sicca, Haemophilus parasuis* та дріжджоподібні гриби *Candida albicans.* У баквисівах 2 партій (50 %) цехового предстартеру ідентифікували асоціацію бактерій *Actinobacillus pleuropneumonia* з *B. subtilis.* Встановлено помірне, за оптичною щільністю, *біоплівкоутворення* для асоціацій мікроорганізмів *P. multocidam* + *C. striatum* + *C. albicans* (D_{620}=3,59) та *P. multocida* + *L. ochracea* + *C. albicans* (D_{620}=3,62). Низьку активність плівкоутворення демонстрували планктонні форми бактерій *C. striatum* та *P. multocida* на рівні (D_{620}≤1,51). Визначено інгібуючу активність пробіотичного комплексу бактерій роду *Bacillus (B. subtilis, B. licheniformis, B. amyloliquefaciens)* на виділені варіації мікробних біоплівок у 5 видах кормів, який витіснив усі біоплівкоутворюючі мікроорганізми, окрім *H. parasuis*

Ключові слова: біоплівкоутворюючі мікроорганізми, планктонні бактерії, мікробна забрудненість корму, пробіотичний комплекс бактерій роду *Bacillus*, інгібуюча активність