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Microbial composition of livestock buildings is the basis for the creation of a biological preparation to stabilize the microbial background

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ABSTRACT

The present study investigates the microbial composition within the livestock breeding premises of a cattle breeding farm with a complete technological cycle of agricultural products at JSC "Astana-Onim, Kazakhstan. The research focuses on the quantitative and qualitative composition of the microbiocenosis, primarily comprising five genera: coliforms, bacilli, lactobacilli, staphylococci, and protozoa. The research methodology included a combination of various approaches, such as observation, analysis, and experimentation. The data collection involved careful sampling and laboratory analysis, enabling a comprehensive assessment of the microbial composition within the livestock breeding premises. The results obtained from this study contribute to a better understanding of the microbial dynamics in such settings, with implications for the sanitation and health management of livestock facilities. Moreover, the study identifies the microbial composition in areas housing both sick and healthy animals. It further identifies representatives of the indigene microflora and takes note of the reaction of lactic acid bacteria. Notably, the growth of colonies on selective media with distinct morphological and cultural properties is observed. In conlusion, species and quantitative composition of microflora in livestock facilities include various species and genera. Some notable percentages include Staphylococcus aureus (12%), Bacillus subtilis (12%), B. mucoides (19%), B. mesentericus (6%), total Bacillus genus bacteria (37%), molds (15%), Escherichia coli (5%), Lactobacillus spp. (15%), and Proteus vulgaris (7%).

Keywords: Microflora, Indigene microflora, Probiotic microorganisms, Selective media, Quantitative and Species composition.
Article type: Research Article.

INTRODUCTION

The improvement of livestock and animal productivity is essential for agricultural development. However,the presence of infectious diseases, particularly those caused by opportunistic pathogenic microflora, posessignificant challenges, particularly in young animals (Sidorov *et al.* 2000; Panin 2001). In the pursuit of disease control, the use of disinfectants to sanitize animal premises has been a common practice. Nevertheless, this approach comes with its own set of issues. One major concern with conventional disinfectants is their composition, which often includes toxic substances effective against pathogenic microflora but hazardous to both animal and human health (Sidorov *et al.* 2000). Additionally, the continuous use of these disinfectants leads to microbial resistance, making the disinfection process less effective over time (Sidorov *et al.* 2000; Panin, 2001). Moreover, the non-specific action of disinfectants, while eliminating harmful microorganisms, also eradicates beneficial ones (Sidorov *et al.* 2000; Panin 2001). Consequently, this creates a clean surface that rapidly becomes decontaminated by pathogenic bacteria, resulting in short-lived and unstable microbial reduction (Sidorov *et al.* 2000; Panin 2001). Given the

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persistent tendency of bacteria, particularly pathogenic strains, to develop resistance against disinfectants, there is a constant need to increase their concentration and frequency of application (Panin 2001). Unfortunately, this approach comes at a cost, as the chemical ingredients in these disinfectants can be harmful to both human health and the environment (Davies & Wales 2019). To address these challenges and explore alternative solutions for effective microbial control, the present study focuses on investigating the microbial composition of livestock breeding premises in a cattle breedingfarm. Specifically, the study examines the quantitative and qualitative aspects of the microbiocenosis, with a particular emphasis on five dominant genera: coliforms, bacilli, lactobacilli, staphylococci, and protozoa(Sidorov *et al.* 2000; Panin 2001). In summary, the study seeks to provide a better understanding of the current challenges posed by conventional disinfectants and highlight the importance of exploring sustainable and effective approaches to maintain a healthy microbial balance in livestock breeding premises (Usui *et al.* 2022). By identifying the specific microbial components and their interactions, this research contributes to the advancement of microbial control strategies that are both safe and environmentally friendly. The widespread use of disinfectants and antibiotics has led to concerning consequences, with pathogenic bacteria mutating and becoming even more dangerous and resistant to these treatments. This has resulted in the emergence of new pathogens, and even previously neutral bacteria have now turned pathogenic. Paradoxically, the more we rely on disinfectants and antibiotics, the worse the situation becomes, as the mutation of bacteria occurs at a faster rate than the development of new treatments. In fact, even the disinfectants and antibiotics themselves have become more aggressive and hazardous to human life and health (Martínez & Baquero, 2014; Rozman *et al.* 2021). Addressing this critical issue, scientists and microbiologists from Ghent University have adopted an intriguing approach to combat the problem. They have employed probiotics as the foundation for adisinfectant (Chaucheyras-Durand & Durand 2022). This alternative strategy holds promise in dealing with the challenges posed by traditional disinfectants and antibiotics. Given the aforementioned concerns, the research conducted in this direction holds immense relevanceand practical applicability in preventing infectious diseases in animals and ensuring the production of highsanitary quality products. By studying the microbial composition of livestock buildings, we can identify living indigene microorganisms that compete with pathogenic ones for food sources and territory. This understanding may offer novel solutions for maintaining a balanced microbial environment and mitigating the harmful effects of pathogenic bacteria in livestock breeding premises (Sidorov *et al.* 2000; Chaucheyras-Durand & Durand 2022). Interestingly, although there have been notable advancements in this field in other countries, there appearsto be limited research on this topic in Kazakhstan. This highlights the significance of conducting studies inthis direction to explore the potential of probiotic-based approaches in addressing sanitation challenges in livestock facilities in Kazakhstan.

MATERIALS AND METHODS

The research was carried out at the Department of "Veterinary Sanitation," within the laboratory of biotechnology and experimental biology named "Astana Bioscience Business Centre," as well as in the livestock complex of JSC "Astana-Onim, Kazakhstan." Two distinct groups were considered for the studyincluding healthy and ill animals. The healthy group consisted of 400 breeding heifers of Holstein-Friesian breed, all of which were part of the production groupof the company JSC "Astana-Onim." On the other hand, the ill group comprised 26 heifers exhibiting various health conditions such as postpartum endometritis, weakened immunity due to prior illnesses, andinjuries or wounds. The research aimed to investigate the microbial composition of the livestock breeding premises at JSC "Astana-Onim" which holds a complete technological cycle of agricultural products. Both the quantitativeand qualitative compositions of the microbiocenosis were studied, with a focus on five key genera: coliforms,bacilli, lactobacilli, staphylococci, and protozoa. Moreover, the microbial composition was analyzed in various areas, including locations where healthy and ill animals were kept. The identification of representatives of indigene microflora and the reaction of lactic acid bacteria were also observed. In addition, the growth of colonies on selective media, exhibiting pronounced morphological and cultural properties, was closely monitored. The research methodology included a combination of various approaches, such as observation, analysis, and experimentation. The data collection involved careful sampling and laboratory analysis, enabling a comprehensive assessment of the microbial composition within the livestock breeding premises. The resultsobtained from this study contribute to a better understanding of the microbial dynamics in such settings, with implications for the sanitation and health management of livestock facilities. In this study, we collected samples from various areas within the livestock breeding premises, including washes from walls, feeders, bedding, drinkers, water from

drinkers, and washes from milking equipment. To ensure the integrity of the samples, they were carefully transported at controlled temperatures, up to +10 °C. Sampling from these different surfaces and equipment allows us to obtain a comprehensive representationof the microbial composition within the livestock facilities. By analyzing these samples, we can gain insights into the presence and distribution of microorganisms in various locations, which is essential for assessing the overall hygiene and sanitation conditions. Transporting the samples at controlled temperatures helps to preserve the integrity of the microbial populations and prevents any potential alterations or degradation of the microorganisms during transportation. This careful handling of the samples ensures that the data collected accurately reflects the microbial dynamics present in the different areas of the livestock breeding premises. Ultimately, this information will contribute to a better understanding of the hygiene status and potential risks within the facility and aid in the development of effective sanitation and management strategies.

Sampling and transporting samples

For sampling, we utilized sterile catheters and cotton swabs, ensuring that the samples were collected underaseptic conditions. These samples were then placed in sterile tubes containing a transport medium, enabling their preservation during transportation to the laboratory. To maintain the integrity of the samples, they were transported within a maximum time frame of 6 hours. Upon arrival at the laboratory, the samples were further processed. To prepare the samples for analysis, wemixed them with 10 mL isotonic NaCl solution and ensured thorough mixing. Subsequently, the samples were stored and transported under refrigeration conditions, maintaining a temperature of no more than + 4°C. In the laboratory, dilutions were carried out to facilitate the analysis. Specifically, 1 mL of the suspension from the previous tube was added to the next nine tubes, each containing 9 mL isotonic NaCl solution. This resulted in serial dilutions, starting from 10^{-2} in tube 2, 10^{-3} in tube 3, and so on, until reaching a dilution of 10^{-6}. For culturing, 0.1 mL of the diluted suspension was inoculated onto selective and differential media. The cultures were then incubated for various durations, i.e., 16, 24, and 36 hours, within thermostat incubators set at 30 °C, 37 °C and 42 °C. To isolate the microorganisms, we employed the Gold method, which involves selective growth conditionsto facilitate the isolation of specific microorganisms. The identification of microorganisms to theirrespective genera was based on a combination of cultural properties and microscopic examination of the growth obtained on selective media. This meticulous approach to sampling, transporting, and processing the samples allowed us to conduct a comprehensive and accurate analysis of the microbial composition within the livestock breeding premises.The results obtained from this methodological procedure are crucial for understanding the distribution and behavior of microorganisms in different areas of the facility, thereby contributing to the overall assessment of sanitation and hygiene conditions.

List of selective chromogenic nutrient media used in the work

In our research, we utilized selective chromogenic nutrient media, a cutting-edge approach that allows forrapid detection and identification of microorganisms. These media contain specific fluorogenic substrates that target enzymatic activities unique to various microorganisms, enabling their identification at the initialinoculation stage. These results in reduced testing time and swift attainment of results, eliminating the need for further biochemical tests to identify microorganisms. One of the selective chromogenic media employed in this study was ECC agar, a differential diagnostic medium recommended for preliminary identification of *Escherichia coli* and other coliform bacteria in foodand environmental samples. On this medium, *E. coli* colonies appear blue/purple, *Klebsiella pneumoniae* colonies display pink/red coloration, and *Pseudomonas aeruginosa* straw/yellow. ECC agar contains Salmon-GAL and X-glucuronide, two chromogenic substrates that undergo cleavage by microbial β-D-galactosidase and β-D-glucuronidase, respectively. This enzymatic activity leads to distinctive color changes in the colonies, facilitating the identification of specific microorganisms. The composition of ECC agar includes peptone special and yeast extract to provide essential nutrients andgrowth factors for bacteria. Lactose serves as a substrate for detecting lactose-fermenting bacteria throughthe neutral red indicator, while phosphates maintain pH stability, and sodium chloride ensures isotonicity.During testing, food samples were diluted and homogenized before being applied to the agar surface and incubated at 37 °C for 18-24 hours. The number of blue/purple colonies was then counted, and the quantityof *E.coli* per 1 gram of the sample was calculated. Notably, ECC agar should only be used for *in vitro* diagnostic purposes. Another selective and differential medium used in our study was *Pseudomonas* Isolation agar, which is employed for isolating and identifying pseudomonads from both

clinical and non-clinical materials. This medium is a modification of King A medium, designed to enhance the detection and differentiation of pseudomonads. Pancreatic gelatin digestion provides nitrogenous nutrients essential for microbial growth, while glycerol acts as an energy source and contributes to the production of the piocyanin pigment characteristic of *P. aeruginosa*. Potassium phosphate further supports pigment formation, and triclosan selectively inhibits the growth of Gram-positive and Gram-negative (except *P. aeruginosa*) microorganisms. Some pyocyanin-positive strains may also produce small amounts of fluorescein, resulting in blue-green or green pigment formation. By utilizing these advanced selective chromogenic nutrient media, we were able to effectively and efficiently identify specific microorganisms within the livestock breeding premises of JSC "Astana-Onim".This approach significantly contributed to our understanding of the microbial composition in the studied areas, providing valuable insights into sanitation and hygiene conditions and facilitating the prevention of infectious diseases in animals. To enhance the hemolytic properties and achieve more distinct results, it is advisable to inoculate a small inoculum and incubate the seeding under anaerobic conditions. Another important medium used in our research is Triple Sugar Iron agar (TSI agar), which serves to differentiate pathogenic intestinal bacteria based on their ability to ferment carbohydrates and produce hydrogen sulfide. Initially proposed by Sulkinand Willet, the agar was later modified by Hajna for the differentiation of Enterobacteriaceae and is in accordance with ARHA recommendations for examining meat and food products, milk and dairy products,to confirm the presence of *Salmonella*, and for identifying Gram-negative microorganisms. The International Committee for Standardization also recommends this medium, with minor modifications for *Salmonella* identification. The components of TSI agar include peptic animal tissue digestate, casein hydrolysate, yeast and meat extracts, providing essential nitrogenous substances, sulfur, trace elements, B vitamins, and more. Sodium chloride helps maintain optimal osmotic pressure. Lactose, sucrose, and glucose serve as fermentable substrates. Sodium thiosulfate, in combination with iron ions, acts as an indicator for hydrogen sulfide production, and phenol red functions as a pH indicator.

Table 1. Sources of sampling in «Astana - Onim» JSC

Sample №	**Source of allocation**	**Group**
1	2	3
AO 001	Dry litter (straw)	Animals of the main production group, 400 heads
AO 002	Wall surface in the trough area	
AO 003	Water from a drinker	
AO 004	Dry litter (manure)	
AO 005	Drinker surface	
AO 006	Trough (main feed)	
AO 007	Trough (dry feed mixes)	
AO 008	Litter (raw straw)	
AO 009	Wall surface	
AO 010	Stable floor (concrete with mechanical cleaning system)	
AO 011	Walls in the feeding area	
AO 012	Walls in the seating area	
AO 013	The surface of the mats in the seating area (material rubber)	
AO 014	The floor of the camp (surface of the mound of sand)	
AO 015	Wall surface in the trough area	
AO 016	Water from a drinker	
AO 017	Dry litter (manure)	
AO 018	Drinker surface	
AO 019	Trough (main feed)	
AO 020	Trough (dry feed mixes)	
AO 021	Litter (raw straw)	Animals from the group of sick animals, 26 animals
AO 022	Wall surface	
AO 023	Stable floor (concrete with mechanical cleaning system)	
AO 024	Walls in the feed area	
AO 025	Walls in the seating area	

	The surface of the mats in the recreation area (material rubber)	
AO 026		
AO 027	The floor of the camp (surface of the mound of sand)	
AO 028	Carousel surface (milking machine for 50 cows)	
AO 029	Floor on the carousel	
AO 030	Floor in the milking parlor	Automatic milking room(healthy animals)
AO 031	Carousel surface (milking machine for 35 cows)	
AO 032	Wall surface in the milking parlor	
AO 033	Water from a drinker	
AO 034	Carousel surface (milking machine for 50 cows)	
AO 035	Floor on the carousel	
AO 036	Floor in the milking parlor	
AO 037	Carousel surface (milking machine for 35 cows)	Automatic milking room(ill animals)
AO 038	Wall surface in the milking parlor	
AO 039	Water from a drinker	
AO 040	Ceiling surface	

During fermentation, microorganisms that ferment glucose release multiple acids, leading to a color changein the medium from red to yellow. More acids are generated in the column (fermentation) compared to theslanted part (oxidation). Additionally, bacteria produce alkaline products during oxidative decarboxylationof peptone. The glucose/lactose (sucrose) ratio of 1:10 is of crucial significance. Phenol red, as the indicator,turns yellow at pH values below 6.8. When the initial pH is 7.4, relatively small amounts of acids are sufficient to develop yellow staining of the medium. Alkaline products can neutralize the limited acid formed in the slanted part during glucose fermentation. Therefore, an alkaline (red) slant and an acidic (yellow) butt indicate that the microorganism ferments glucose, but does not ferment lactose and/or sucrose. On the other hand, bacteria that ferment lactose and/orsucrose, in addition to glucose, produce large amounts of acids that cannot be neutralized by amines, resulting in both the slant and butt exhibiting acidity (yellow). If gas is formed during fermentation, it can be identified by bubbles and characteristic breaks in the medium. Moreover, some bacterial species have the ability to reduce thiosulfate to hydrogen sulfide, which interacts with iron ions to form an insoluble black precipitate of iron sulfide, thus aiding in further differentiation. Thiosulfate is indeed reduced only in an acidic environment, and blackening typically occurs in the columnarea of TSI agar. The reactions observed on this medium allow for valuable insights into the carbohydrate fermentation abilities of microorganisms:

An alkaline bevel and an acidic column indicate that only glucose is fermented by the microorganism.

An acidic bevel and an acidic column indicate that, in addition to glucose, lactose and/or sucrose are fermented by the microorganism. The presence of bubbles and discontinuities in the medium signifies gas formation by the microorganism. The observation of a black precipitate indicates the formation of hydrogen sulfide by the microorganism. Noteworthy, some Enterobacteriaceae and hydrogen sulfide-producing *Salmonella* may not exhibit a reaction to hydrogen sulfide on TSI agar. Instead, they may show a positive reaction on Kliegler's mediumand a negative reaction on TSI agar. This difference in reactions is attributed to the suppression of the enzymatic pathway responsible for hydrogen sulfide formation during sucrose utilization. Another selective medium used in our research is Bismuth-sulfite agar or Bismuth-sulfite modified agar. This medium is recommended for the selective isolation and preliminary identification of *Salmonella typhi*and other *Salmonella* from pathological material, wastewater, food, water, and other test materials. It effectively inhibits the growth of Gram-positive and intestinal Gram-negative microorganisms due to the presence of brilliant green and bismuth sulfite. On this medium, *S. typhi*, *S. enteritidis*, and *S. typhimurium* usually form black colonies with a metallic sheen surrounded by a blackened area, indicating hydrogen sulfide production and reduction of sulfite to black iron sulfide. On the other hand, *S. paratyphi* A forms light green colonies. Due to its high selectivity, a large inoculum is applied to Bismuth-sulfite agar, as it actively suppresses Gram-positive and coliform microorganisms. However, Notably the growth of some salmonellae can also be inhibited on this medium, and therefore, it should not be the sole selective medium used during the study. Specifically, Bismuth-sulfite agar suppresses certain types of *Shigella*

and salmonellae, such as *S. sendai*, *S. berta*, *S. gallinarum*, and *S. abortus-equi* (Jain 2006). Sabouraud agar with chloramphenicol and Sabouraud agar with glucose are both essential for the selective cultivation of yeasts and molds. The latter is a modification of the original prescription proposed by Sabouraud and is specifically used for cultivating fungi, particularly those associated with skin infections. Additionally, it is recommended by the American Pharmacopoeia for testingthe presence of microorganisms. To isolate pathogenic fungi from materials heavily contaminated with fungi or bacteria, antibiotics like chloramphenicol are often added to this medium. The combination of mycotic peptone, casein hydrolysate, and animal tissue peptic digestion serves as a source of essential nutrients for fungal growth, while glucose acts as a source of energy (Samanta & Samanta, 2015; Mathews 2018). Chloramphenicol, being an effective antibiotic, suppresses the growth of a wide range Gram-positive and Gram-negative microorganisms, which imparts selectivity against fungi to the medium (Lancini & Parenti 2013; pavlova *et al.* 2022). Moreover, the low pH value of the medium promotes fungal growth while suppressing the growth of contaminating bacteria in clinical material. Noteworthy, some pathogenic fungi have the ability to form spores that can easily be carried by air currents, making it crucial to perform tests in a laminar flow box toprevent laboratory contamination. Another medium commonly used for culturing lactobacilli is Agar or MRS (deMan, Rogosa, and Sharpe) broth. This medium is slightly modified from the original prescription and is highly effective in promoting the growth of lactobacilli derived from various sources, including the mouth, dairy products, other foods, feces, and other materials. The components of this medium include proteosopeptone and meat extract as a source of essential nutrients,glucose as a fermentable substrate and an energy source, and yeast extract providing B vitamins. Additionally, Tween-80 is included to provide necessary fatty acids for the growth of lactobacilli. Sodiumacetate and ammonium citrate play a crucial role in inhibiting the growth of streptococci, mold fungi, and many other microorganisms. For cultures of the genus *Bacillus*, their growth ability on common nutrient media such as potato-sugar agar, meat-peptone broth, and agatized wort is typically used for determination. These media create optimal conditions for the growth and identification of lactobacilli and *Bacillus* species, enabling us to study and isolate these microorganisms effective. The methodology for determining the total number of bacteria is based on counting colonies of mesophilicaerobic and facultative aerobic microorganisms that grow on dense nutrient agar at a temperature of (30 ± 1) °C for 72 hours. The amount of inoculated product was determined based on the most likely microbial infestation. To calculate the total number of bacteria, we selected dilutions that resulted in the growth of at least 30 and not more than 300 colonies when inoculated on the agar plate. Each sample was sown on two or three plates to ensure accuracy. For the testing, 1 cm^3 of each dilution was placed in a pre-marked Petri dish, which was then filled with (14 ± 1) cm^3 of nutrient medium previously melted and cooled to a temperature of 40-50 °C. This was performed to determine the total number of bacteria following the guidelines of GOST 9225-84. In some cases, the test product was sown on petri dishes using 1 cm^3 and 0.1 cm^3 from the same dilution. After pouring the agar and ensuring it solidified, the petri dishes were inverted and placed in a thermostat incubator set at 30 ± 1 °C for 72 hours to allow the colonies to grow. The number of colonies on each dish was countedwhile holding it upside down against a dark background, using a magnifying glass with a magnification of4-10 times. Each counted colony was marked on the bottom of the petri dish with ink for clarity. In cases where the number of colonies was high and evenly distributed, the bottom of the petri dish was divided intofour or more identical sectors. The number of colonies in two or three sectors (but not less than 1/3 of the surface area) was counted, and the arithmetic mean number of colonies was determined. This average valuewas then multiplied by the total number of sectors on the entire petri dish, giving us the total number of colonies grown on one dish. To calculate the total number of bacteria in 1 cm^3 or 1 g of the product (X) in units, the formula 1 was used:

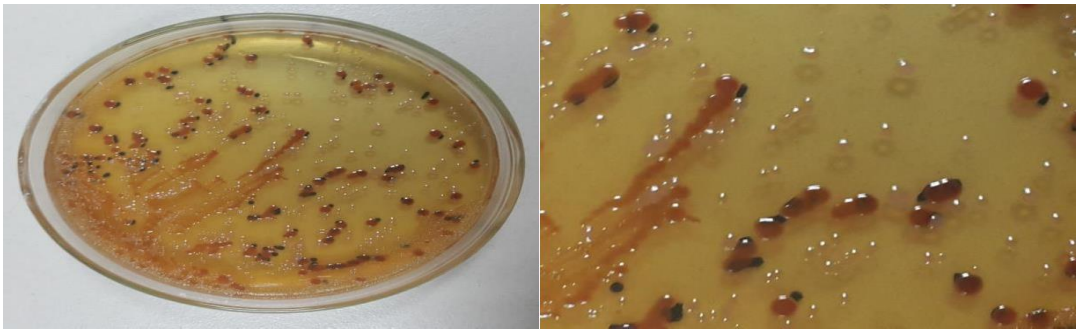
$$X = n \times 10^m \quad \textbf{(1)}$$

where n represents the number of colonies counted per petri dish, and m is the number of tenfold dilutions.By using this formula, the total number of bacteria in the given sample can be accurately determined. For the analysis, the arithmetic mean of all dishes was calculated and taken as the final result. To determine the catalase enzyme activity of lactic acid bacteria, a procedure was followed. A microbial culture grown on dense nutrient medium MRS-4 in a petri dish was used for the test. 1 mL of 1% hydrogenperoxide solution was applied to the surface of the microbial culture in the petri dish, creating a thin layer covering the culture. The appearance of gas bubbles in the liquid layer indicated the formation of oxygen as a result of hydrogen peroxide decomposition under the catalytic action of catalase enzyme. Furthermore, the morphological and cultural properties of the microorganisms were investigated. Initially,the studied material was observed under a microscope using Gram-stained smears to reveal

the morphologyof the microorganisms. This included observing the shape, size, cell arrangement, Gram-staining, and motility ratios. The examination was performed using a Motic BA310 laboratory microscope following standard procedures. Additionally, the macromorphology of the colonies was studied, describing their character, structure, and size.

RESULTS AND DISCUSSION

Sampling for bacteriological research was conducted in livestock facilities of "Astana-Onim" JSC. The research aimed to isolate and identify various microorganisms present in the premises where both sick andhealthy groups of animals were kept. The sampling process involved collecting samples from different areas within the livestock facilities, including areas where sick and healthy animals were kept. The samples were collected using proper sterile techniques to avoid contamination. The collected samples were then processed and plated on selective nutrient media to encourage the growthof specific types of microorganisms. The goal was to isolate and identify different bacterial species present in the environment, especially those that might be relevant to the health and productivity of the animals. Once the microorganisms were isolated on the nutrient media, they were further characterized based on their morphological and cultural properties, including colony appearance, color, size, shape, and growth patterns. Additionally, microscopy was used to study the microorganisms' cellular characteristics, such as Gram-staining and cell arrangements. Overall, the bacteriological research and isolation of isolates from the samples collected were essential steps in analyzing the microbial composition and studying its correlation with the health and productivity of the animals in "Astana-Onim" JSC's livestock facilities. Based on the selective media used for isolation and identification of various groups of microorganisms, distinct colonies with specific morphological and cultural properties were observed. We grouped individual isolates based on their morphological properties, such as shape, size, and color of the colonies. Additionally, cell microscopy revealed similar morphological features among these grouped isolates. To identify bacteria belonging to the *E. coli* group, we used chromogenic ESS agar. This selective medium allows for the differentiation of *E. coli* colonies. When a 0.1 mL suspension with a dilutionof 10^{-6} was inoculated on the ESS agar plates and incubated at a temperature between 36-38 °C for 18-20hours, *E. coli* colonies stained black, which enabled their clear identification on all the inoculated plates. These observations and identification methods are crucial for understanding the microbial landscape in the animal housing sites and can help determine the presence of specific pathogens or beneficial microorganisms that may impact the health of the animals. The *E. coli* identification are essential for further analysis and research on the microbiological aspects of "Astana- Onim" JSC's livestock facilities. Sabouraud dextrose agar (SDA) was utilized for the isolation and identification of fungi and yeasts. The incubationwas performed at a temperature of 30 ± 1°C for a period of 48 ± 3 hours. During this incubation period, distinct colonies were formed, allowing for the differentiation of yeasts and mold fungi based on their colony characteristics. Yeasts formed small colonies with a clear yellow-brown edge on SDA. Mold fungi formed large, flat colonies with a dark center and a diffuse edge on SDA. To detect staphylococci, the isolates were cultured on Levin's medium, on which, staphylococci formed purple colonies with a metallic sheen. This specific colony appearance on this medium is a characteristic feature of staphylococci, aiding in their identification. For the detection of lactose-negative Enterobacteriaceae, isolates were cultured on Levin-GRM medium. On this medium, lactose-negative Enterobacteriaceae formed clear and colorless colonies, which is anothercharacteristic feature allowing for their differentiation. These selective media and colony characteristics are important tools for the accurate identification of specific microorganisms, such as fungi, yeasts, staphylococci, and lactose-negative Enterobacteriaceae, which are likely relevant to the bacteriological research conducted in the livestock facilities of "Astana- Onim" JSC. Proper identification of these microorganisms can provide valuable insights into the microbialcomposition and potential health implications for



the animals in the facility.

Fig. 1. Differentiation of isolates on selective chromatid media.

Based on the characteristics observed in the isolates, we divided them into different groups of microorganisms. One such group was identified as representatives of the genus *Pseudomonas*. This identification was achieved by culturing the isolates on specific media, namely Luria-Bertani medium andPseudomonas agar, and incubating them for 16 hours at a temperature of 37 °C. The colonies that formed on these media exhibited specific characteristics that were indicative of the genus *Pseudomonas*. The colonies of *Pseudomonas* isolates had smooth edges, and their color varied from beige to green. These features, along with other identifying traits, helped distinguish them as members of the *Pseudomonas* genus. During the analysis of the research results, we found that the qualitative composition of the microbiocenosis (microbial community) in the livestock facilities was mainly represented by five genera. These genera were:

- Coliforms
- Bacilli
- Lactobacilli
- Staphylococci
- Protists

This suggests that these five genera of microorganisms were the dominant groups present in the samples collected from the livestock premises during the bacteriological research. Each of these genera may includevarious species with different roles and characteristics, and their abundance and interactions could have implications for the overall health and hygiene conditions of the livestock facility. Understanding the composition of the microbiocenosis is essential for assessing the health and environmental conditions of the livestock facility and can help in devising appropriate measures for maintaining the well-being of the animals and ensuring food safety. The provided information outlines the average species and quantitative composition of microflora isolatedin livestock buildings. Here are the percentages of different microorganisms in the overall composition:

A. *Staphylococcus aureus*: 12%
B. *Bacillus subtilis*: 12%
C. *Bacillus mucoides*: 19%
D. *Bacillus mesentericus*: 6%
E. Total Bacillus bacteria: 37%
F. Mold spores: 15%
G. *Escherichia coli*: 5%
H. *Lactobacillus* spp.: 15%
I. *Proteus vulgaris*: 7%

Further analysis of the results revealed changes in the quantitative composition of several groups of microorganisms between places where healthy and sick animals were kept.Specifically, there were significant differences in the levels of pseudomonads and *E. coli*. In the group with sick animals, the level of pseudomonads increased from 7% to 13%, and the level of *E. coli* increased from 5% to 7%. On the other hand, the representatives of indigenous microflora, specifically *Lactobacillus* and *Bacillus*, decreased in the group with sick animals. The total number of the latter microorganisms decreased from 37% in the healthy group to 28% in the group with sick animals. Similarly, lactic acid bacteria (*Lactobacillus* spp.), which are integral to the gastrointestinal tract of cattle, also showed a decrease in activity from 15% to 12% in the group with sick animals. Based on these results, it can be inferred that the species composition of microorganisms in livestock buildings is directly and inversely correlated with the physiological state of the animals. The changes in themicrobial composition in the presence of sick animals indicate that the health and well-being of the animalsmay influence the abundance and types of microorganisms present in their living environment.

We conducted seeding and identification using selective nutrient media, which allowed us to study the spectrum of microorganisms present and their degree of prevalence (insemination) in differentareas of the livestock facility. This type of analysis is crucial for understanding the relationship between microorganisms and the health and productivity of the animals, as well as for implementing appropriate measures to maintain a healthy and hygienic environment for livestock. We aimed to isolate probiotic cultures and create a probiotic preparation. To achieve this, we isolated specific bacterial isolates from the samples collected. These isolates were Gram-positive, non-

sporous, immobile bacilli with rounded ends. They showed active growth on MRS-1 medium, but no growth on meat-peptone agar, and were catalase-negative. These characteristics were used to select microorganisms belonging to the genus *Lactobacillus*. Based on the initial studies, it was evident that the cultures collected consisted of bacilliform species of lactic acid bacteria, specifically *Lactobacillus* spp. Further experiments involved culturing the selected isolates for different durations (16, 24, and 36 hours) at varying temperatures (30 °C, 37 °C, and 42 °C) in a thermostat incubator. On petri dishes with selective medium, colonies of various colors such as white, beige, yellow, orange, and green grew. These colonies had different morphologies with even and wavy edges, and they varied in shine (shiny, dull, and floury). Some colonies exhibited antagonistic activity, visible through the presence of a lysis zone around them, with sizes ranging from 0.5 to 2 mm. To ensure purity, the cultures were further purified using the Gold method by sowing on agarized nutrient media. In total, 93 isolates were selected in the first stage, and 137 in repeated selections, indicating multiple rounds of selection to obtain desirable isolates. Next, we microscopically examined the obtained isolates. The lactic acid cultures studied were found to be represented by bacilli, cocci, and varying forms in terms of length, thickness, and arrangement. We further studied the morphological and cultural features of the lactobacilli. Based on their growth on solid nutrient media, all lactobacilli cultures studied were divided into 2 groups:

The first group showed growth on MRS-4 agarized medium as superficial round colonies with clear edges,white or gray in color, and varying in size from small to small. The second group, which represented less than 20% of the cultures studied, formed colonies with irregularedges, gray in color, and often with a compacted center. Growth on liquid nutrient media for this group wascharacterized by either turbidity of the medium or no turbidity formation. The detailed characterization and classification of these lactobacilli isolates are essential for their potentialuse as probiotic cultures and in the development of a probiotic preparation for various applications, including promoting animal health and enhancing productivity.

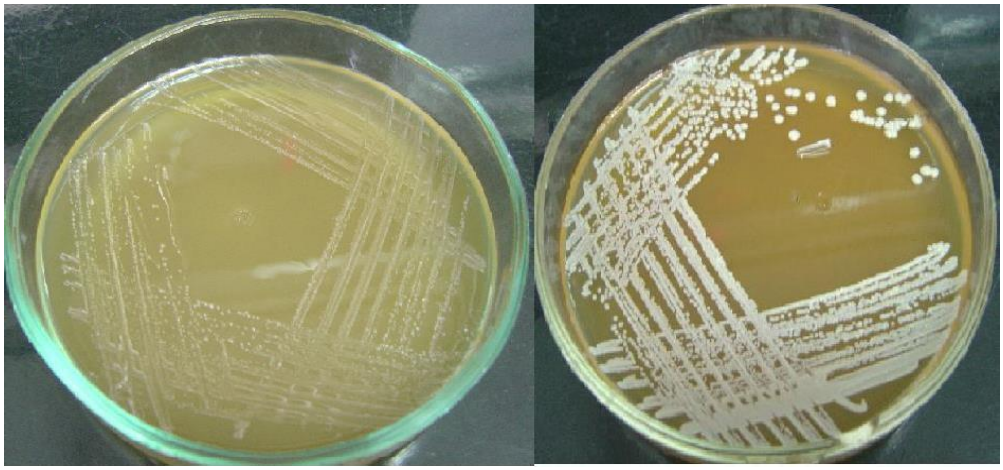


Fig. 2. Colony growth of isolated lactobacilli on MRS-4 medium.

The second group of lactobacilli was predominantly represented by long, thick bacilli that were arranged singly or in some places by short chains. These characteristics are important for distinguishing them from the lactobacilli in the first group. Fig. 5 provides visual examples of the microscopic variants of lactobacilli characteristic of the first group. The cultures studied in this group mainly consist of bacilli that vary in length, thickness, and arrangement. Some bacilli appear wrapped in rings, which might be a distinct and noteworthy feature of this group. In Fig. 5A, the bacilli are arranged both singly and in chains, suggesting that there might be different arrangements within this group. In Fig. 5B, the bacilli are shorter and appear to be arranged in clustersor packs. These microscopic observations and variations are valuable for the identification and classification of different lactobacilli strains within the first group, which could have implications for their potential applications as probiotics or in the development of probiotic preparations. Understanding the morphology and arrangement of lactobacilli is crucial in characterizing their properties and determining their suitabilityfor specific purposes, such as promoting animal health and productivity in livestock facilities. In the cultural and morphological properties of lactobacilli when grown in MRS broth, there are three types of growth observed, and in all cases, a precipitate is formed. The growth types refer to the appearance of the broth after incubation and may provide insights into the

metabolic activities and fermentation patterns of the lactobacilli strains. These properties are valuable in characterizing and distinguishing different strains of lactobacilli for various applications, including probiotic development and promoting animal health in livestock facilities.

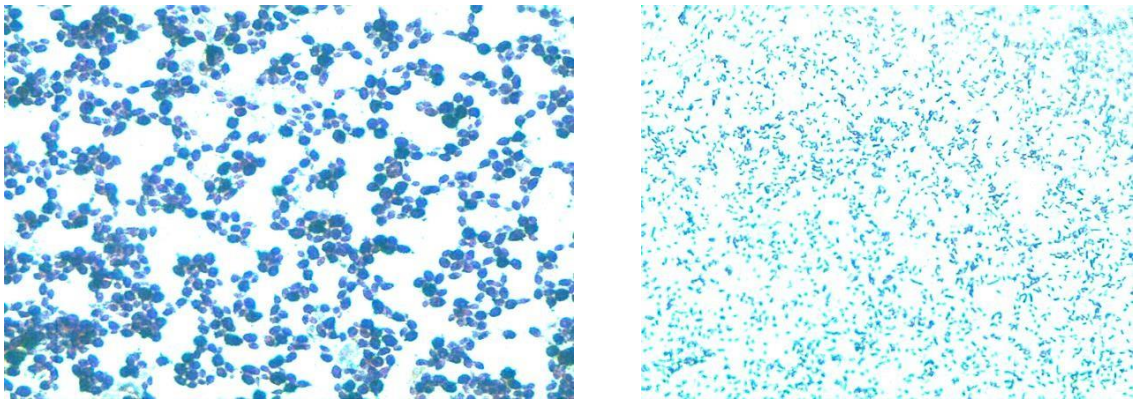


Fig. 5. Variants of the microscopic pattern characteristic of the studied isolates.

Based on the morphological and cultural properties, the isolated lactobacilli can be divided into two main groups, as mentioned before. Additionally, spore-forming cultures were represented, presumably belongingto the genus *Bacillus*. A summary of the characteristics of the different groups are as follows:

Lactobacilli

Group 1. Shows growth on MRS-4 agar as superficial round colonies with clear edges, white or gray in color. The growth in MRS broth can display uniform turbidity along the column or with a transparent ring on top, and a precipitate is formed.

Group 2. Represents less than 20% of the cultures studied. It forms colonies with irregular edges and is gray in color, often with a compacted center. Growth on liquid nutrient media is characterized by turbidityof the medium or no turbidity formation. Microscopic variants include long, thick bacilli arranged singly or in short chains.

Spore-forming cultures (presumably Bacillus)

These cultures form flat, dry colonies of dense consistency with a white granular plaque on meat-peptone agar after 24 hours of incubation at 37 °C. The colonies have a diameter of 2.5 mm and slightly indented edges. In meat-peptone broth, they give abundant growth, forming a thin, leathery film of white on the wallsof the test tube. On agarized wort, the colonies are dirty white, round, with an irregular edge. Notably, these characterizations are based on the phenotypic features of the isolates. For amore comprehensive identification and classification of the bacteria, further molecular and genetic analyses may be required.

Fig. 6. Characteristic morphological and cultural picture for the studied spore-forming cultures.

The information provided indicates that when the isolates are incubated in a high-grade liquid medium, they display superficial growth. This means that the growth occurs on the surface of the liquid medium, andthe bacteria do not sink or form clumps at the bottom. Instead, they tend to stay suspended and form a visible layer on the liquid surface. Similarly, when these isolates are inoculated by injection into complete 0.7 agar, growth is observed on thesurface of the agar. This indicates that the bacteria do not penetrate deep into the agar but rather grow on top of it. The characteristic of superficial growth in both liquid and solid media is consistent with the phenotypic

properties of the bacteria and provides valuable information for their identification and classification. However, to determine the exact species and further characterize these isolates, additional analyses, such as genetic and biochemical testing, may be required. The characterization of the level of bacterial infestation in the "Ill" and "Healthy" groups of animals revealed differences in the microbial composition between the two groups, suggesting that the microbiotapresent in the animals' living environment varies depending on their health status. In the "Ill" group of animals, certain groups of microorganisms showed an increase in abundance comparedto the "Healthy" group. Specifically, the level of pseudomonads increased significantly from 7% to 13% ofthe total composition, and the proportion of *E. coli* also from 5% to 7%. These changes indicate that there might be an overgrowth of potentially harmful bacteria in the environment of the "Ill" group. Conversely, some beneficial microorganisms showed a decrease in abundance in the "Ill" group. Representatives of indigenous microflora, such as *Lactobacillus* and *Bacillus*, decreased in their levels. Forexample, the total number of *Bacillus* in the "Healthy" group was 37%, while in the "Ill" group, reduced to 28%. Lactic acid bacteria, which are essential components of the gastrointestinal tract of cattle, also decreased in activity from 15% to 12%.

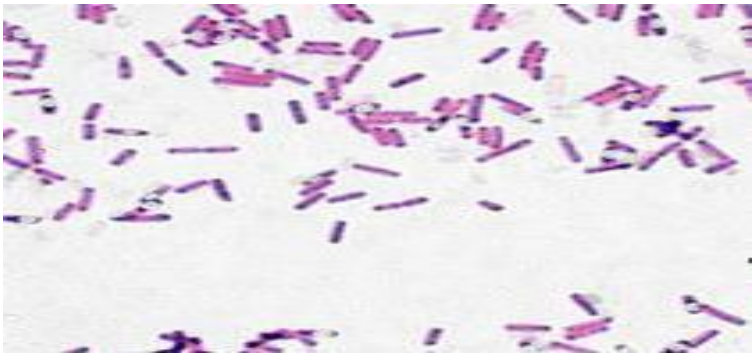


Fig. 7. Microscopic picture of bacteria of the genus *Bacillus*.

Gram-staining

These findings highlight the importance of a balanced and diverse microbiota for the health and well-beingof animals. The changes in microbial composition observed in the "Ill" group might be indicative of an imbalance in the microbiota, which could be contributing to the health issues observed in these animals. Further analysis and investigation would be necessary to understand the specific mechanisms behind thesechanges and their potential impact on the animals' health and productivity.

Table 4. Microbial landscape of the groups in the analysis.

Indicators	**Ill animals**			**Healthy animals**		
	06.06.2015	04.07.2015	14.08.2015	06.06.2015	04.07.2015	14.08.2015
E. coli	8.39 ± 0.26	6.14 ± 0.22	5.46 ± 0.24	8.3 ± 0.23	4.39 ± 0.22	3.76 ± 0.26
Enterococcus	5.00 ± 0.20	4.96 ± 0.23	4.84 ± 0.21	4.9 ± 0.20	4.3 ± 0.21	3.21 ± 0.19
Bacillus	2.3 ± 0.12	3.7 ± 0.17	2.7 ± 0.12	3.6 ± 0.12	6.43 ± 0.16	9.21 ± 0.15
Lactobacillus	6.98 ± 0.16	7.62 ± 0.17	7.24 ± 0.24	6.7 ± 0.14	9.7 ± 0.17	8.63 ± 0.23
Bifidobacteria	5.22 ± 0.22	6.65 ± 0.16	8.12 ± 0.15	6.32 ± 0.22	8.42 ± 0.16	8.73 ± 0.15
Staphylococcus	1.79 ± 0.22	1.53 ± 0.25	1.26 ± 0.17	1.2 ± 0.21	1.3 ± 0.23	1.2 ± 0.17
Proteus	1.56 ± 0.26	1.72 ± 0.22	1.32 ± 0.18	1.32 ± 0.23	1.21 ± 0.21	1.1 ± 0.22

The results of the studies have revealed that the qualitative composition of the microbiocenosis in the healthy groups of animals is represented by 7 families of microorganisms: E. *coli*, *Enterococcus*, *Bacillus* spp.,

Lactobacillus spp., *Bifidobacterium* spp., *Staphylococcus* spp., and *Proteus* spp. These findings were in agreement with Egorova *et al.* (2016) and Neseem *et al.* (2021). The comparison between the healthy and sick groups of animals showed significant differences in the composition of different groups of microorganisms. Specifically, in the healthy group, the level of representatives of lactic acid bacteria was found to be significantly higher. Lactic acid bacteria, such as *Lactobacillus* and *Bifidobacterium*, are known for their beneficial effects on gut health and overall well- being. Their higher abundance in the healthy group suggests a well-balanced and healthy microbiota in those animals. Conversely, in the group with sick animals, the levels of certain species, such as *Proteus* spp. and *E. coli*, were found to be increased. *Proteus* spp. and certain strains of *E. coli* are known to be opportunistic pathogens and can cause infections and health issues in animals, particularly when theirpopulations are not kept in check by a balanced microbiota. These findings indicate that the health status of the animals is correlated with the composition of their microbiota. A higher abundance of beneficial microorganisms, like lactic acid bacteria, in the healthy groupsuggests a more favorable microbial environment that can support good health. On the other hand, an increase in potentially harmful bacteria in the group with sick animals may indicate an imbalance in the microbiota, which could be contributing to their health problems. Understanding these relationships between microbiota composition and animal health can provide valuable insights into ways to promote and maintain animal health through targeted probiotic or prebiotic interventions to restore a balanced microbiota and prevent the overgrowth of harmful microorganisms. However, further research and studies are necessary to fully elucidate the mechanisms underlying these correlations and to develop effective strategies for promoting animal health through microbiota management. Based on the information provided in the study, several key findings have been identified: The qualitative composition of microbiocenosis (Gilbride *et al.* 2006; Gaire, 2020; Lepesteur 2022): The microbial community in the livestock buildings is mainly represented by five genera: coliforms, bacilli, lactobacilli, staphylococci, and protopedes. These groups of microorganisms have distinct morphological and cultural properties.

Changes in microbial composition in sick animals. A comparison between the microbial composition of healthy and sick animals revealed significant changes in the quantitative composition of several groups of microorganisms. Pseudomonads increased from 7% to 13% of the total composition, and *E. coli* bacteria increased from 5% to 7% in the group with sick animals.

Decrease in indigenous microflora. Representatives of indigenous microflora, particularly the genera *Lactobacillus* and *Bacillus*, decreased in the group with sick animals. The total number of *Bacillus* genus bacteria in the healthy group was 37%, whereas in the group with sick animals, reduced to 28%.

Decrease in lactic acid bacteria. Lactic acid bacteria, a group of beneficial microorganisms, showed a decrease in activity from 15% to 12% in the group with sick animals.

CONCLUSION

Species and quantitative composition of microflora. The average composition of microflora isolated in livestock buildings includes various species and genera. Some notable percentages include *Staphylococcusaureus* (12%), *Bacillus subtilis* (12%), *Bacillus mucoides* (19%), *Bacillus mesentericus* (6%), total *Bacillus*genus bacteria (37%), molds (15%), *Escherichia coli* (5%), *Lactobacillus* spp. (15%), and *Proteus vulgaris*(7%).

Distinct morphological and cultural properties. Colonies with distinct morphological and cultural properties, such as different staining and shapes, were observed on selective media intended for the isolationand identification of various groups of microorganisms. These findings provide valuable insights into the composition and dynamics of the microbiota in livestock facilities and its relationship with the health and well-being of the animals. Understanding these relationships can aid in the development of strategies to improve animal health through targeted probiotic interventions and better management of microbial communities in livestock settings.

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