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The Study of the Microbial Composition of Livestock Premises is the Basis for the Creation of a Biological Preparation for the Stabilization of the Microbial Background

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Key words: Microflora, indigenous microflora, probiotic microorganisms, selective media, quantitative and species composition

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Abstract: Studies of the microbial composition of cattle-breeding premises of the breeding farm for breeding cattle with the completed technological cycle of production of agricultural products of JSC "Astana-Onim" were conducted. The quantitative and qualitative composition of the micro-biocenosis is determined mainly by five genera: coliforms, bacilli, lactobacilli, staphylococcus, protaeus. The microbial composition in the places of sick and healthy animals was determined. Representatives of indigenous microflora were identified. The reaction of lactic acid bacteria was noted. The growth of colonies on selective media with pronounced morphological and cultural properties was observed.

INTRODUCTION

An increase in the number and productivity of animals is hampered by a number of factors, among which an important place is occupied by diseases of infectious etiology including those caused by opportunistic microflora which in recent years has played a decisive role in animal diseases, especially in young animals.

The use of disinfectants for sanitation of premises brings a number of problems that arise when using them, since they contain poisonous substances that effectively work against pathogenic microflora but they are dangerous for the health of animals and humans and they must be periodically changed because bacteria arise to them addiction and disinfection is not effective.

Also the disadvantage of all disinfectants is the nonspecific action of these substances, killing both good and harmful microorganisms as a result, a clean surface is created on which a rapid re-contamination (re-colonization) of pathogenic bacteria occurs. Disinfection gives a fast but short and unstable period of reducing the number of microorganisms[1]. In this case, bacteria, especially their pathogenic species, show a strong tendency to resistance and resistance to any substances that can damage or destroy them. In connection with the problems of resistance of pathogens to disinfectants that have arisen at the present time, their concentration and processing frequency are continuously increasing which also has a harmful effect on humans and the environment due to harmful chemical ingredients in their composition[1, 2].

The use of disinfectants and antibiotics leads to a mutation of pathogenic bacteria (they become even more dangerous and resistant to the action of antibiotics and disinfectants). There are new pathogens and neutral bacteria become pathogenic. As a result, the more people use disinfectants and antibiotics, the worse the situation

becomes! Mutation occurs faster than the development of new disinfectants and antibiotics which themselves are becoming more aggressive and dangerous for human life and health. Applying an increasingly new "chemistry" in the fight against bacteria, we only improve and it seems that this will always happen[3-6].

An interesting approach to solving this problem was used by scientists microbiologists of Ghent University. The probiotic is taken as the basis of the disinfection substance[1, 7].

In connection with the above, research conducted in this direction will be relevant and will find its practical application in the prevention of infectious diseases in animals and production of high sanitary quality, since, having studied the microbial composition of livestock premises we can isolate living indigenous microorganisms that compete with pathogenic microorganisms for power sources and territory.

Literature review: To date, there are urgent issues of sanitation at livestock facilities which solve a wide range of tasks to ensure sanitation in livestock premises.

Livestock farms daily struggle with the spread of pathogenic bacteria. To this end, many disinfectants have been developed based on various chemicals that are foreign to the animal organism. In addition to being xenobiotics polluting the environment, they have a negative impact on the animal's body, causing teratogenic, mutagenic, carcinogenic and other effects. The constant accumulation of xenobiotics in the body of animals leads to the production of environmentally unfavorable products.

Recently, there have been studies of scientists on the ability of spore-forming bacteria to exert a probiotic effect. The use of bacilli as a probiotic drug has a history of several decades throughout the world. On the basis of bacilli, a number of probiotics are produced for use in medicine and veterinary medicine as therapeutic or prophylactic preparations as well as biologically active additives[8-11].

A number of publications have been found on the development, on the basis of spore-forming bacteria, of probiotic preparations with a sanitizing effect, referred to the generation of so-called self-eliminating antagonists[11-16]. When using preparations based on probiotic cultures, the sanitizing effect is achieved by colonization of the treated surfaces with cultures of probiotic bacteria which, by creating a new microbiocenosis, suppress the development of pathogenic microflora on the basis of antagonism, competing for food and habitat[4].

Environmental probiotic organisms can be a safe and effective means to fight infection[7, 8]. In Kazakhstan, there are no works in this direction.

MATERIALS AND METHODS

The research was carried out at the Department of Veterinary Sanitation, in the Laboratory of Biotechnology and Experimental Biology Astana Bioscience Business Centre as well as in the livestock complex of JSC Astana-onim.

Animals were divided into 2 groups: healthy and sick animals (postpartum endometritis, after the transferred diseases, weakened immunity, various injuries and wounds, etc.).

A healthy group of JSC "Astana-Onim" included 400 heads of breeding heifers of the Holstein-Frisian breed that are members of the production group of the enterprise. The sick group contained 26 heads (Fig. 1a, b).

We selected flushing from the surface of the walls, animal feeders, bedding, drinking bowls, water from drinking bowls and flushing from the milking equipment. Samples were transported under conditions of a temperature regime up to +10 (Fig. 2).

Sampling and transport of samples: Sterile catheters and cotton buds were used for sampling which were placed in sterile test tubes with a transport medium and delivered to the laboratory for no >6 h.

Samples were taken from various sources, after which they were placed in tubes with 10 mL of isotonic NaCl solution, mixed and transported in a refrigerator no more than +4°C. Further, in the laboratory, dilutions were carried out, in this case, 1 mL was added in 9 consecutive tubes for each source with 9 ml of isotonic NaCl solution. from the previous test tube, thus obtaining in 2 tubes a dilution of 10-2°, in 3 test tube 10-3, etc. before obtaining a dilution of 10-6°. After receiving the dilution, 0.1 mL was inoculated suspensions into selective and differential media in parallel and the cultures were cultured for 16, 24 and 36 h in a thermostat at 30°C, 37°C and 42°C. The isolation of isolates was carried out according to the Gold method. The belonging of microorganisms to the genus was established on the basis of the cultural properties and microscopy of the grown on selective media of colonies (Fig. 3 and Table 1).

List of selective chromogenic nutrient media used in the work: The test material was plated on selective chromogenic media. Chromogenic media are environments of a new generation that allow rapid detection and identification of microorganisms. Chromogenic nutrient media allow us to identify specific enzymatic activities for different microorganisms. Identification of microorganisms is possible already at the stage of primary seeding, as a result, the study time and results are shortened. Chromogenic selective media include fluorogenic substrates to detect specific enzymatic

(a)

(b)

Fig. 1(a, b): Groups of animals: (a) Healthy animals and (b) Sick animals

activities of microorganisms characteristic of the group (e.g., coliforms) or a particular species (e.g., *E. coli*) of microorganisms. The investigated microorganism contains an enzyme that metabolizes a colorless chromogenic substrate, forming a colored reaction product. The chromogenic medium contrastingly changes its color (or fluoresces) when a microorganism is detected. There is no need to carry out transplantation and further biochemical tests to identify microorganisms.

Differential-diagnostic environment ECC agar: ECC Agar Differential Diagnostic Medium is recommended for the preliminary identification of Escherichia coli and other coliform bacteria in food and environmental samples.

(a)

(b)

Fig. 2(a, b): Investigated groups of animals, (a) Healthy animals and (b) Sick animals

Fig. 3: Dilution of isotonic NaCl solution

Growth on HiCrome ECC Agar: *Escherichia coli* (blue/violet), *Klebsiella pneumoniae* (rose red) and *Pseudomonas aeruginosa* (straw-yellow).

ECC Agar is a differential diagnostic medium recommended for the preliminary identification of *Escherichia coli* and other coliform bacteria in food and environmental objects.

The chromogenic mixture contains two chromogenic substrates: Salmon-GAL and X-glucuronide. Microbial ß-D-galactosidase cleaves Salmon-Gal which leads to the

Table 1: Sources of sampling in JSC "Astana-Onim"

Example No.	Selection source	Groups
1	2	3
AO 001	Dry litter (straw)	Main production group animals, 400 animals
AO 002	Wall surface in the feeder area	
AO 003	Water from the drinker	
AO 004	Dry litter (manure)	
AO 005	The surface of the drinker	
AO 006	Feeding trough (main feed)	
AO 007	Feeding trough (dry feed mix)	
AO 008	Litter (straw damp)	
AO 009	Surface of walls	
AO 010	Paul campsite (concrete with mechanical cleaning system)	
AO 011	Walls in the feeding zone	
AO 012	Walls in the recreation area	
AO 013	Surface of mats in the rest area (rubber material)	
AO 014	Paul's camp (the surface of a mound of sand)	
AO 015	Wall surface in the feeder area	Animals from the group of sick animals, 26 heads
AO 016	Water from the drinker	
AO 017	Dry litter (manure)	
AO 018	The surface of the drinker	
AO 019	Feeding trough (main feed)	
AO 020	Feeding trough (dry feed mix)	
AO 021	Litter (straw damp)	
AO 022	Surface of walls	
AO 023	Paul campsite (concrete with mechanical cleaning system)	
AO 024	Walls in the feeding zone	
AO 025	Walls in the recreation area	
AO 026	Surface of mats in the rest area (rubber material)	
AO 027	Paul's camp (the surface of a mound of sand)	
AO 028	Surface of carousel (apparatus for milking for 50 heads)	Hall for automatic milking (healthy animals)
AO 029	Floor on the carousel	
AO 030	Floor in the parlor	
AO 031	Surface of carousel (milking machine for 35 heads)	
AO 032	Wall surface in the parlor	
AO 033	Water from the drinker	
AO 034	Surface of carousel (apparatus for milking for 50 heads)	Hall for automatic milking (sick animals)
AO 035	Floor on the carousel	
AO 036	Floor in the parlor	
AO 037	Surface of carousel (milking machine for 35 heads)	
AO 038	Wall surface in the parlor	
AO 039	Water from the drinker	
AO 040	Ceiling surface	

colony colony colony staining to orange-red. The ß-D-glucuronidase produced by the *E. coli* cleaves X-glucuronide. E. coli forms colonies colored in dark blue (to violet) color due to simultaneous splitting of Salmon-GAL and X-glucuronide.

Peptone special and yeast extract satisfy the needs of bacteria in nutrients, a complex of B vitamins and other necessary growth factors. Lactose allows detecting fermenting lactose bacteria by changing the color of the neutral red indicator. Phosphates provide a constant pH and sodium chloride isotonicity of the medium. Well dried cups with spilled media. The test sample of the food product was diluted 1: 5 or 1:10 with 0.1% (w/v) peptone water (M028) and thoroughly homogenized. Distribute 0.5 mL or 1.0 mL of the suspension on the surface of the agar with a sterile glass spatula and incubate at 37°C for 18-24 h. Calculate the number of blue/violet colonies and calculate the number of *Escherichia coli* per 1 g of sample. This medium should be used only for diagnostic purposes *in vitro*. Pseudomonas Isolation Agar this agar is used as a selective and differential medium for the isolation and primary identification of pseudo-monads from clinical and non-clinical material. This agar is a modification of the King A medium that was developed to improve the definition and differentiation of pseudo-monads. Pancreatic gelatin digestion is a source of nitrogenous nutrients substances and other substances necessary for the growth of microorganisms. Glycerin is a source of energy and contributes to the development of *Pseudomonas aeruginosa* pigment Piocyanin. Phosphate potassium also promotes pigmentation. Triclosan selectively inhibits the growth of gram-positive and gram-negative (except Pseudomonas aeruginosa) microorganisms. Some piocyanin-positive strains can also produce a small amount of fluorescein, so, a blue-green or green pigment is produced.

Azide Blood Agar Base (the basis of blood agar with sodium azide). This agar is used to isolate and

differentiate cultures of streptococci and staphylococci. This agar is recommended for counting the number of streptococci in cheese. It is reminiscent of the selective medium used by Edwards for the isolation of streptococci the causative agents of mastitis. The special peptone entering into the environment is very nutritious and therefore provides an active growth of the demanding microorganisms. Sodium azide inhibits the growth of many gram-negative microorganisms. Although proteins can grow on this environment, their swarming is suppressed. The acidity of the medium can have an overwhelming effect on the activity of sodium azide. At pH 7.2, sodium azide does not change the ability of hemoglobin for streptococci, however, on bloody azide, hemolytic activity is more pronounced in them, compared to hemolysis on non-selective blood agar.

To enhance hemolytic properties and obtain more precise results, it is recommended to inoculate a small inoculum and incubate the crop under anaerobic conditions.

Triple sugar iron agar: This agar is used to differentiate pathogenic intestinal bacteria by their ability to ferment carbohydrates and form hydrogen sulphide. Proposed originally by Sulkin and Willet, this agar was then modified by Hajna for differentiation of enterobacteria. This prescription corresponds to the recommendations of the ARNA on the study of meat and food products, milk and dairy products, to confirm the presence of Salmonella and to identify Gram-negative microorganisms. With minor changes (concerning the identification of Salmonella), this medium is recommended by the International Committee for Standardization. Peptic digest of animal tissue, casein hydrolyzate, yeast and meat extracts are a source of nitrogenous substances, sulfur, microelements, B vitamins, etc. Sodium chloride maintains optimal osmotic pressure. Lactose, sucrose and glucose are fermentable substrates. Sodium thiosulfate in combination with iron ions is an indicator for hydrogen sulphide, phenolic red is a pH indicator.

The microorganism, fermenting glucose, promotes the formation of many acids, changing the color of the medium from red to yellow. More acid is released in the column (during fermentation), compared to the sloping part (oxidation). Bacteria also form alkaline products (during oxidative decarboxylation of peptone). Of fundamental importance is the ratio of glucose/lactose (sucrose) = 1:10. The phenolic red indicator turns yellow at pH values below 6.8. At a starting pH of 7.4, a relatively small amount of acids is required to develop a yellow color of the medium. Alkaline products can neutralize a small amount of acid formed in the sloping portion during fermentation of glucose. Thus, an alkaline (red) bevel and an acidic (yellow) bar indicate that the microorganism ferments glucose but does not ferment lactose and/or sucrose. Bacteria that ferment lactose and/or sucrose in addition to glucose form a large number of acids which can not be neutralized by amines, so the bevel and column will be acidic (yellow). If a gas forms during fermentation, it can be determined from bubbles and characteristic ruptures of the medium. Some species of bacteria reduce thiosulfate to hydrogen sulphide which, interacting with iron ions, forms an insoluble black precipitate of iron sulphide. The reduction of thiosulphate occurs only in an acidic medium and the blackening usually occurs in the zone of the column:

- Alkaline slant/acid column = only glucose is fermented
- Sour beet/sour stalks = except for glucose, lactose and/or sucrose is fermented
- There are bubbles and ruptures in the medium = formation of gas
- Black precipitate is noted = formation of hydrogen sulphide

Some enterobacteria and hydrogen sulfide-producing salmonella on this medium may not respond to hydrogen sulphide. Some bacteria react positively to hydrogen sulphide on Kligler's medium and negatively on the Tri-sugars iron-containing agar. This is due to the suppression of the enzymatic pathway for the formation of hydrogen sulphide during the utilization of sucrose.

Bismuth-sulphite agar/Bismuth-sulphite agar modified. This medium is recommended for the selective isolation and preliminary identification of Salmonella typhi and other salmonella from pathological material, sewage, food, water and other test material. It is recommended for selective isolation and preliminary identification of Salmonella from pathological material, sewage, food, water and other test material. Brilliant green and bismuth sulphite suppress the growth of gram-positive and intestinal gram-negative microorganisms. *Salmonella typhi*, *Salmonella enteritidis* and *Salmonella typhimurium* usually form on this medium black colonies with a metallic luster surrounded by a blackening zone as a result of the production of hydrogen sulphide and the reduction of the sulphite to iron sulphide having a black color. *Salmonella paratyphi* a forms light green colonies. In view of the high selectivity of the medium, a large inoculum is applied to it (gram-positive and coliform microorganisms are actively suppressed). In this environment, growth of some salmonella can be inhibited, so it should not be the only selective medium in the study. On bismuth-sulfite agar, the growth of Shigella and Salmonella such as *S. sendai*, *S. berta*, *S. gallinarum*, *S. abortus*-equi is suppressed.

Sabouraud chloramphenicol/dextrose agar: The Sabouraud with chloramphenicol is recommended for the

selective cultivation of yeast and mold fungi and the Saburoa agar with glucose is also used for the cultivation of acid-loving bacteria. The glucose agar is a modification of the Sabouraud Sylvestre for the cultivation of fungi, in particular those that are associated with skin infections. Agar Saburo with glucose is recommended by the US Pharmacopeia for the test for the presence of microorganisms. To isolate pathogenic fungi from materials abundantly contaminated with fungi or bacteria, antibiotics, for example chloramphenicol are often added to this medium. Mycological peptone, casein hydrolyzate and peptic digest of animal tissue serve as a source of necessary nutrients for growth. Glucose is a source of energy. Chloramphenicol inhibits the growth of a wide range of Gram-positive and Gram-negative microorganisms, giving the medium selectivity against fungi. Low pH contributes to the growth of fungi and suppresses the growth of bacteria that contaminate clinical material. Since some pathogenic fungi can easily form spores that are easily entrained by air currents, it is recommended to conduct research in a laminar box to prevent laboratory infections.

Agar/Broth MRS for lactobacilli: These media are recommended for the cultivation of lactobacilli. This environment is given by the deMan, Rogosa and Sharpe prescriptions with a slight modification. Lactobacilli from the oral cavity, dairy products, other food products, feces and other material 25 OBI are abundantly growing on it. Proteose-peptone and meat extract are a source of necessary nutrients, glucose is a fermentable substrate and a source of energy. The yeast extract provides the vitamins of the B group. Tween-80 is the source of the fatty acids necessary for the growth of lactobacilli. Sodium acetate and ammonium citrate suppress the growth of streptococci, mold fungi and many other microorganisms. Cultures of the genus Bacillus were determined by their ability to grow on common nutrient media: potato-sucrose agar, meat-peptone broth and agarized wort (Fig. 4 and 5).

Method for determining the total number of bacteria: The method is based on the counting of colonies of mesophilic aerobic and facultative aerobic microorganisms growing on dense nutritive agar at (30+1)°C for 72 h. The quantity of the planted product was established taking into account the most probable microbial contamination. To determine the total number of bacteria selected those dilutions, when sown on a cup grows no <30 and no >300 colonies. From each sample we sow two or three cups. Each of the dilutions was inoculated in an amount of 1 cm^3 in one Petri dish with a pre-marked cap and poured (14+1) cm^3 of molten and cooled to 40-50°C nutrient medium to determine the total number of bacteria according to GOST 9225-84. The seed

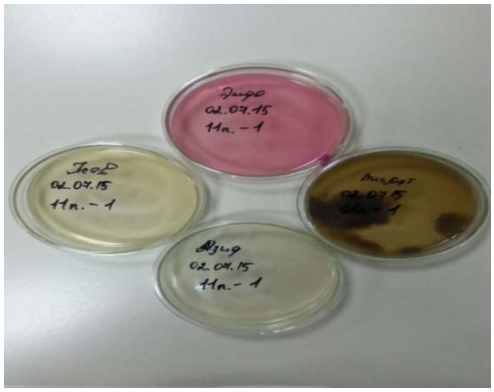


Fig. 4: Differential-diagnostic environments

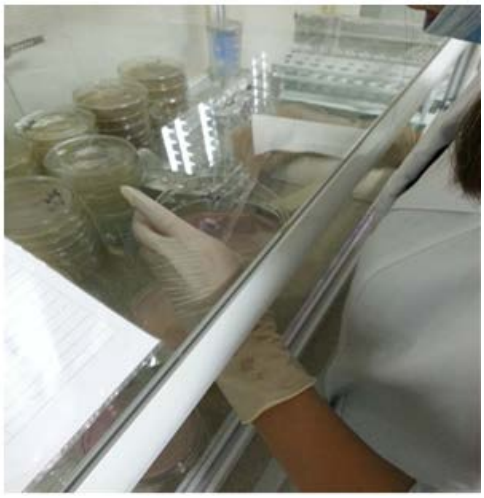


Fig. 5: Crops on differential diagnostic environments

of the test product on a Petri dish from the same dilution in an amount of 1 and 0.1 cm^3. Immediately after the agar is poured, the contents of the Petri dish are thoroughly mixed by gentle rotational rocking to uniformly distribute the seed. After the agar was congealed, Petri dishes were pierced with lids and placed in such a form in a thermostat with a temperature of (30+1)°C for 72 h. The number of grown colonies was counted on each cup, placing it upside down on a dark background, using a magnifying glass with 4-10 times. Each counted colony was marked on the bottom of the cup with ink. With a large number of colonies and a uniform distribution of the colonies, the bottom of the Petri dish was divided into four or more identical sectors, the number of colonies in two or three sectors was counted (but not <1/3 of the surface of the cup), the arithmetic mean of the colonies was found and multiplied by the total number sectors of the entire cup. Thus, the total number of colonies grown on one cup was found.

The total number of bacteria in 1 cm^3 or 1 g of product (X) in units was calculated by Eq. 1:

$$X = n*10\ m \quad (1)$$

Where:
n = The number of colonies counted per petri dish
m = The number of tenfold dilutions

The final result of the analysis was taken as the arithmetic average obtained for all the dishes. Definitions of enzyme catalase lactic acid bacteria. The 1 mL of a 1% solution of hydrogen peroxide was applied to the surface of a microbial culture grown on a dense nutrient medium MPC-4 in a Petri dish so that it covered the surface of the culture with a thin layer. The appearance of gas bubbles in the layer of applied liquid indicates the formation of oxygen as a result of the decomposition of hydrogen peroxide under the action of catalase. Investigation of morphological and cultural properties of microorganisms Primarily studied material was studied under a microscope in Gram stain smears to reveal morphology of microbes-shape, size, location of cells, Gram staining and motility ratio; Gram staining was studied using the "Motic" laboratory microscope BA310 using conventional techniques. We studied macromorphology, describing the nature and structure, the size of the colonies.

RESULTS AND DISCUSSION

Selection of samples for bacteriological studies from livestock facilities of JSC "Astana-Onim". Isolates were isolated from the samples of the premises with the content of patients and healthy groups of animals (Table 2).

On selective media intended for the isolation and identification of various groups of microorganisms, growth of colonies with pronounced morphological and cultural properties (different types of staining, forms) was observed. According to the morphological properties of individual isolates we were grouped, since, they had some similarity in the shape, size and color of the colonies and also when the cells were microscopically revealed similar morphological features.

Table 3 shows the qualitative and quantitative characteristics of the microbial composition of animal sites (see annexes).

Chromogenic ECC agar was used to identify the bacteria of the *E. coli* group. When 0.1 mL of the suspension was diluted from a dilution of 10-6 and incubated at a temperature of 36-38°C for 18-20 h, a clear differentiation of *E. coli* colonies was observed on all the seeded dishes which were colored black (Fig. 6 and 7).

Dextrose agar Saburo was used for fungi and yeast. Incubated at a temperature of (30±1)°C for (48±3) h,

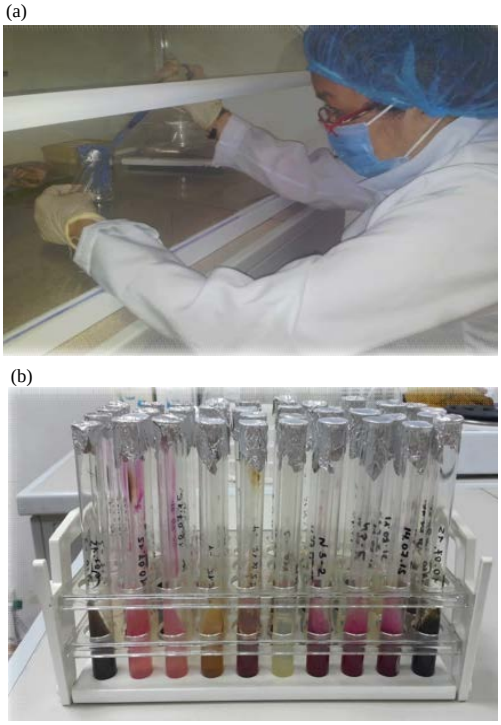


Fig. 6(a, b): Process of preparation of species differentiation of isolates on selective chromatid media

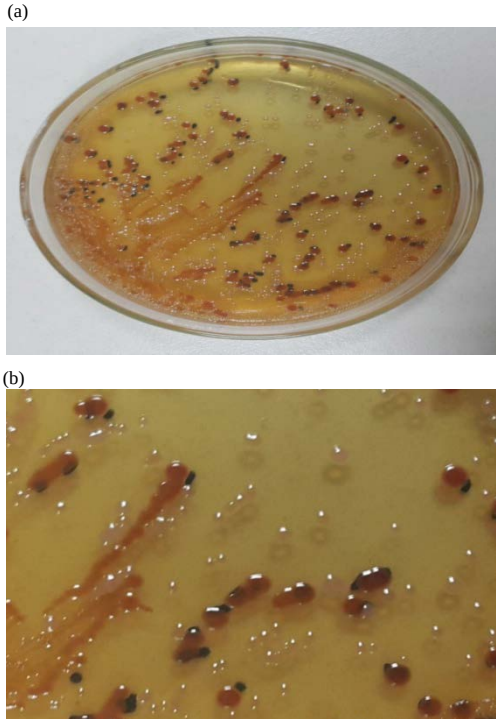


Fig. 7(a, b): Differentiation of isolates on selective chromatid media

Table 2: Identification of microorganisms

Triple	Mano Cattail Environment (MCE)	Bismuth	Endo	Azide	Microscopy
Colonies milky-pink, convex, the edges are even, the surface is smooth, fine-grained	Colonies are white, the edges are even, in the center are transparent, round, coarse	Colonies of dark brown color, convex, shining, the edges are even, the surface is smooth, fine-grained	Colonies of crimson color, flat, shiny, smooth edges, fine-grained	Colonies of milk color, flat, shining, the edges are even, fine-grained	Gram-positive rods, clusters
Colonies of yellow color, matte, convex, small	There is no growth	Colonies of milky color, shiny, convex, the edges are even, medium-grained	Colonies of milky color, shiny, convex, the edges are even, medium-grained	Colonies of milky color, shiny, convex, edges uneven fine-grained	Gram- positive sticks with irregular margins, there are also single sticks with clusters
Colonies of dairy-pink, convex, the edges are even, the surface is smooth, fine-grained	Colonies are white, the edges are even, in the center are transparent, round, coarse	Colonies of dark brown color, convex, shiny, the edges are even, the surface is smooth, fine-grained	The colonies are crimson, flat, shiny, the edges are even, fine-grained	Colonies of milky color, flat, shining, the edges are even, fine-grained	Gram-positive rods, clusters
Colonies of pink color, shiny, convex, edges uneven, medium-grained	There is no growth	Colonies of pink color, shiny, convex, the edges are even, coarse	There is no growth	There is no growth	Gram-positive, round, solitary and clustered
Colonies are pink, shiny, convex, edges are uneven, medium-grained	There is no growth	Colonies of pink color, shiny, convex, the edges are even, coarse	There is no growth	There is no growth	Gram-positive, round, solitary and clusters
Colonies of milky color, matte, convex, the edges are even, medium-grained	There is no growth	Colonies of dark brown color, shiny, convex, uneven, coarse-grained	There is no growth	Colonies of pink color matte, convex, fine-grained	Gram-positive, round, single and there are clusters
Colonies of pink color, shiny, convex, edges uneven, medium-grained	There is no growth	Colonies of pink color, shiny, convex, the edges are even, coarse	There is no growth	There is no growth	Gram-positive, round, solitary and clusters
Colonies of milky color, matte, convex, the edges are even, medium-grained	There is no growth	Colonies of dark brown color, shiny, convex, uneven, coarse-grained	There is no growth	Colonies of pink color, matte, convex, fine-grained	Gram-positive, round, single and there are clusters
Colonies of dairy pink, convex, the edges are even, the surface is smooth, fine-grained	Colonies are white, the edges are even, in the center are transparent, round, coarse	Colonies of dark brown color, convex, shiny, the edges are even, the surface is smooth, fine-grained	The colonies are crimson, flat, shiny, the edges are even, fine-grained	Colonies of milky color, flat, shining, the edges are even, fine-grained	Gram-positive rods, clusters
Colonies are yellow, matte, convex, small	There is no growth	Colonies are milky, shiny, convex, edges are even, medium-grained	Colonies pink, matte, convex	Colonies are milky, shiny, convex, edges are uneven, fine-grained	Gram-positive rods with irregular edges, single there are also clusters
Colonies milky, matte, convex, smooth edges, medium grain	There is no growth	Colonies are dark brown, shiny, convex, edges are uneven, coarse grains	There is no growth	Colonies pink, matte, convex, fine-grained	Gram-positive, round, single there are also clusters
Solid growth, pink and brown colonies, smooth matte edges	There is no growth	Colonies are milky, shiny, convex, edges are even, medium-grained	Colonies pink, matte, convex	Colonies are milky, shiny, convex, edges are uneven, fine-grained	Gram-positive rods with irregular edges, single there are also clusters
Colonies pink, shiny, convex, edges uneven, medium-grained	There is no growth	Pink colonies, shiny, convex, smooth edges, coarse-grained	There is no growth	There is no growth	Gram-positive, round, solitary and clustered
Colonies pink, shiny, convex, edges uneven, medium-grained	There is no growth	Pink colonies, shiny, convex, smooth edges, coarse-grained	There is no growth	There is no growth	Gram-positive, round, solitary and clustered

Table 2: Continue

Triple	Mano Cattail Environment (MCE)	Bismuth	Endo	Azide	Microscopy
Solid growth, in the center of red at the edges of white, transparent, convex, smooth edges, coarse-grained	There is no growth	Flat growth, milky, dull	Colonies are dark crimson, flat, shiny, smooth edges, fine-grained	Milky colonies, round, shiny, flat, slightly convex, medium-grained	Gram-positive elongated sticks, in chains
Solid growth, in the center of red at the edges of white, transparent, convex, smooth edges, coarse-grained	There is no growth	Flat growth, milky, dull	Colonies are dark crimson, flat, shiny, smooth edges, fine-grained	Milky colonies, round, shiny, flat, slightly convex, medium-grained	Gram-positive elongated sticks, in chains
Solid growth, in the center of red at the edges of white, transparent. Rovn	There is no growth	Flat growth, milky, dull	Colonies are dark crimson, flat, shiny	Colonies are milky, round, shiny, flat, medium	Gram-positive elongated sticks, in chains
No growth	There is no growth	Colonies are milky, shiny, convex, solid growth	There is no growth	There is no growth	Gram-positive sticks with rounded edges
Colonies are yellow, matte, convex, small	There is no growth	Colonies are milky, shiny, convex, edges are even, medium-grained	Colonies are pink, matte, convex, edges are even, fine-grained	Colonies are milky, shiny, convex, edges are uneven, fine-grained	Gram-positive rods with irregular edges, single there are also clusters
No growth	There is no growth	No growth	There is no growth	There is no growth	Gram-positive, round, solitary and clustered
Colonies of milky pink, convex, smooth edges, smooth surface, fine-grained	There is no growth	Colonies are dark brown, convex, shiny, smooth edges, smooth surface, fine-grained	Crimson colonies, flat, shiny, smooth edges, fine-grained	Milky colonies, flat, shiny, smooth edges, fine-grained	Gram-positive elongated rods, single and in clusters
Colonies pink, shiny, convex, edges uneven, medium-grained	There is no growth	Pink colonies, shiny, convex, smooth edges, coarse-grained	There is no growth	There is no growth	Gram-positive, round, solitary and clustered
Colonies pink, shiny, convex, edges uneven, medium-grained	There is no growth	Pink colonies, shiny, convex, smooth edges, coarse-grained	There is no growth	There is no growth	Gram-positive, round, solitary and clustered
Colonies pink, shiny, convex, edges uneven, medium-grained	There is no growth	Pink colonies, shiny, convex, smooth edges, coarse-grained	There is no growth	There is no growth	Gram-positive, round, solitary and clustered
Colonies are milky, matte, convex, edges are even, medium-grained	There is no growth	Colonies are dark brown, shiny, convex, edges are uneven, coarse-grained	There is no growth	Colonies pink, matte, convex, fine-grained	Gram-positive, round, solitary and clustered
Colonies are yellow, 26 matte, convex, small	There is no growth	Colonies are dark brown, shiny, convex, edges are uneven, coarse-grained	There is no growth	Colonies pink, matte, convex, fine-grained	Gram-positive rods with irregular edges, single there are also clusters
Colonies are milky, matte, convex, edges are even, medium-grained	There is no growth	Colonies are dark brown, shiny, convex, edges are uneven, coarse-grained	There is no growth	Colonies pink, matte, convex, fine-grained	Gram-positive, round, solitary and clustered
Solid growth, in the center of red at the edges of white, transparent, convex, coarse-grained	There is no growth	Flat growth, milky, dull	Colonies are dark crimson, flat, shiny, smooth edges, fine-grained	Milky colonies, round, shiny, flat, slightly convex, medium-grained	Gram-positive elongated sticks, in chains
No growth	There is no growth	Colonies are milky, shiny, convex, solid growth	There is no growth	There is no growth	Gram-positive sticks with rounded edges
Colonies are yellow, matte, convex, small	There is no growth	Colonies are milky, shiny, convex, edges are even, medium-grained	Colonies are pink, matte, convex, edges are even, fine-grained	Colonies are milky, shiny, convex, edges are uneven, fine-grained	Gram-positive rods with irregular edges, single there are also clusters

Table 2: Continue

Triple	Mano Cattail Environment (MCE)	Bismuth	Endo	Azide	Microscopy
Colonies are yellow, matte, convex, small	There is no growth	Colonies of milky color, shiny, convex, smooth edges, medium-grained	Colonies are pink, matte, convex, edges are even, fine-grained	Colonies are milky, shiny, convex, edges are are uneven, fine-grained	Gram-positive rods with irregular edges, single there are also clusters
Colonies of milky pink, convex, smooth edges, smooth surface, fine-grained	Colonies are white, the edges are even, transparent in the center, round, coarse-grained	Colonies are dark brown, convex, shiny, smooth edges, smooth surface, fine-grained	Crimson colonies, flat, shiny, smooth edges, fine-grained	Milky colonies, flat, shiny, smooth edges, fine-grained	Gram-positive rods, clusters
Colonies are yellow, matte, convex, small	There is no growth	Colonies of milky color, shiny, convex, smooth edges	Colonies are pink, matte, convex, edges are even, fine-grained	Colonies are milky, shiny, convex, edges are uneven, fine-grained	Gram-positive rods with irregular edges, single there are also clusters
Pink colonies, shiny, convex, edges uneven, medium-grained	There is no growth	Pink colonies, shiny, convex, smooth edges, coarse-grained	No growth	There is no growth	Gram-positive, round, solitary and clustered
Colonies pink, shiny, convex, edges uneven, medium-grained	There is no growth	Pink colonies, shiny, convex, smooth edges, coarse-grained	No growth	There is no growth	Gram-positive, round, solitary and clustered
Colonies are pink, shiny, convex, edges are uneven, medium-grained	There is no growth	Pink colonies, shiny, convex, smooth edges, coarse-grained	No growth	There is no growth	Gram-positive, round, solitary and clustered
Colonies of milky37 color, matte, convex, smooth edges, medium-grained	There is no growth	Colonies are dark brown, shiny, convex, edges are uneven, coarse-grained	No growth	Colonies pink, matte, convex, fine-grained	Gram-positive, round, solitary and clustered
Colonies are yellow,38 matte, convex, small	No growth	Colonies are milky, shiny, convex, edges are even, medium-grained	Colonies are pink, matte, convex, edges are even, fine-grained	Colonies are milky, shiny, convex, edges are uneven, fine-grained	Gram-positive rods with irregular edges, single there are also clusters
Colonies are milky, matte, convex, edges are even, medium-grained	There is no growth	Colonies are dark brown, shiny, convex, edges are uneven, coarse-grained	There is no growth	Colonies pink, matte, convex, fine-grained	Gram-positive, round, solitary and clustered
Solid growth, there are colonies of pink and brown, color, matte, smooth edges	There is no growth	Milk colonies are also black, convex, matte, edges are uneven, medium-grained	Colonies pink, shiny, convex, smooth edges, medium-grained	Colonies of milky color, matte, convex, smooth edges, fine-grained	Gram-positive sticks with rounded edges, clusters

Table 3: Cultural-morphological properties of lactobacilli

Parameters	Growth on MRS-1	Growth on MRS-4	Microscopy
Group 1	Uniform turbidity along the column, sediment turbidity of the medium with a transparent ring on top, sediment parietal growth, sediment is formed the medium remains transparent, the sediment	Colony diameter 0.1-0.2 cm white, gray, smooth edges	Gr+short, long sticks, sometimes with rounded ends, coccoid, arranged in different ways
Group 2	Turbidity of the medium with a transparent ring on top, sediment the medium remains clear, the sediment uniform turbidity along the column, sediment	Colony diameter 0.1 cm, gray, uneven edges, sometimes the center is highlighted	Gr+large sticks, arranged in chains, bent, wrapped

colonies were formed: in yeast small colonies with a clear edge of yellow-brown color, molds formed large with a dark center and with a diffuse edge, flat colonies. To identify staphylococci, the isolates were cultured on Levin's medium on which staphylococci formed violet-colored colonies with metallic luster. Lactose-negative enterobacteria formed transparent, colorless colonies. Staphylococci formed small colonies

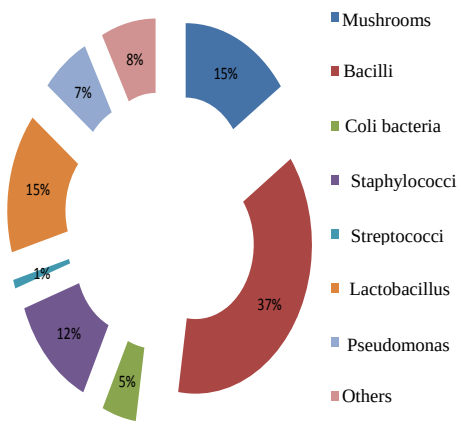


Fig. 8: Microbial composition in the places of keeping of healthy animals

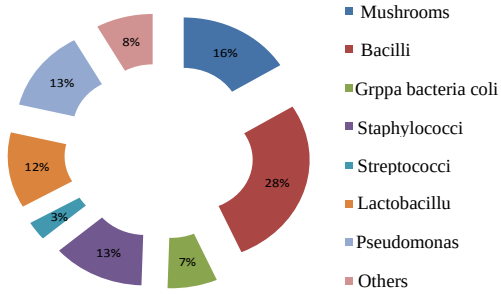


Fig. 9: Microbial composition in places of sick animals

with a dark center on Levin-RMM medium (Fig. 7). According to the above characteristics, the isolates were divided into groups of microorganisms. Representatives of the pseudomonas genus were identified by culturing isolates on Luria-Bertani and Pseudomonas agar for 16 h at 37°C, the colonies formed had smooth edges ranging from beige to green.

During the analysis of the obtained results, we found that the qualitative composition of the microbiocenosis is mainly represented by five genera: coliforms, bacilli, lactobacilli, staphylococci, protaeus (Fig. 8 and 9).

On average, the species and quantitative composition of microflora isolated in livestock houses was 12%, *Bacillu subtilis* 12%, *Bacillus mucoides* 19%, *Bacillus mesentericus* 6% total bacteria of the genus Bacillus 37% mold spores 15%, *Esherichia coli* 5 %, *Lactobacillus* spp. 15% and *Proteus vulgaris* 7%.

Further analysis of the results obtained made it possible to determine the correlation between the species composition, quantitative characteristics of microorganisms and health and the productivity of animals. The microbial composition in the places of sick animal's content compared to the healthy group showed changes in the quantitative composition of a number of groups of microorganisms. Thus, the level of representatives of pseudomonads has increased significantly from 7-13% of the total composition and bacteria of the *E. coli* group from 5-7%. At the same time, representatives of the indigenous microflora represented by the genera Lactobacillus and Bacillus were reduced, so the total number of microorganisms of the genus Bacillus in the healthy group was 37% while in the group with sick animals their level was 28%. Lactic acid bacteria which are an integral part of the gastrointestinal tract of cattle, also reacted with a decrease in activity from 15-12%.

Based on the results obtained, it can be seen that the species composition of the microorganisms of livestock premises directly and back correlates with the physiological state of the animals.

We sowed and identified with selective culture media, studied the spectrum of microorganisms and the degree of dissemination To isolate probiotic cultures and also to create a probiotic preparation, work was carried out to isolate isolates from the obtained samples.

To obtain microorganisms of the genus Lactobacillus, isolates were selected which were Gram-positive, fixed-free fixed sticks with rounded ends, showed active growth on medium MRS-1, lack of growth on meat-peptone agar and were catalase negative.

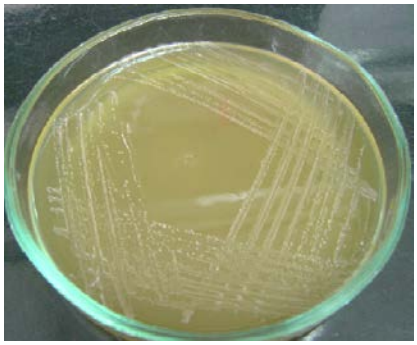
Already from the results of the first studies it was obvious that the cultures we collected have rod-shaped lactic acid bacteria of the species *Lactobacillus* spp. Cultures were cultured for 16, 24 and 36 hours in a thermostat at 300°C, 370°C and 420°C. Petri dishes with selective medium grew colonies of white, beige, yellow, orange and green; with smooth and wavy edges; Shine, dull and mealy colonies were revealed by gloss and transparency. Some colonies had antagonistic activity which was evident from the lysis zone which in some strains was 0.5-2 mm. If necessary, the cultures were further purified by the Gold method by seeding on agarized nutrient media. A total of 93 isolates were selected in the first stage and 137 with repeated screenings.

Further, microscopy of the obtained isolates was carried out. The investigated lactic cultures were represented by chopsticks, cocci, differing in length, thickness and nature of location. Morphological and cultural features of lactobacilli were studied. By the nature of growth on solid nutrient media, all the cultures of lactobacilli studied were divided into 2 groups:

The first group was characterized by growth on the MCS-4 agar medium in the form of superficial round colonies with distinct edges, white or gray, ranging in size from small to small.

The second group which was in the percentage ratio of <20% of the studied cultures, formed colonies with uneven edges, gray, often with a densified center. Growth in a liquid nutrient medium was characterized by a

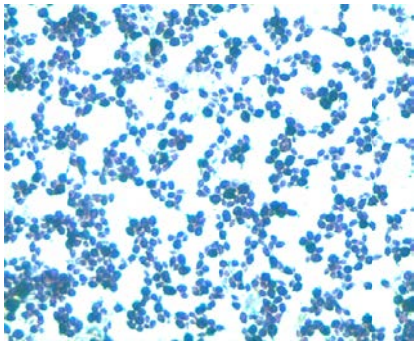
(a)



(b)

Fig. 10(a, b): Growth of colonies of isolated lactobacilli on medium MPC-4

(a)



(b)

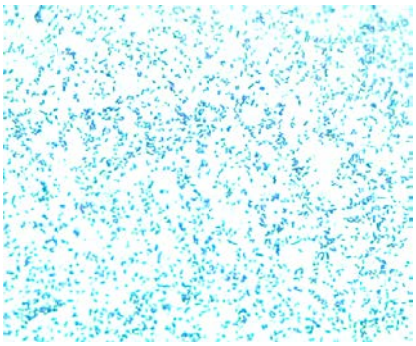


Fig. 11(a, b): Variants of the microscopic pattern characteristic of the isolates under study

turbidity of the medium or a lack of formation of turbidity (Fig. 10). The second group is predominantly represented by long, thick rods arranged singly, sometimes in short chains (Fig. 11). Figure 11 shows the variants of the microscopic picture of lactobacilli, characteristic of the first group. The cultures studied are mainly represented by rods that differ in length, thickness and nature of the arrangement and sticks that wrap themselves in the rings are often found. In Fig. 11a the rods are located singly and in chains, in Fig. 11b the rods are short, arranged in clusters, in bundles.

With the growth of this group of bacteria in the MRC broth, the medium forms three types of growth: uniform turbidity along the column or with a transparent ring from above, the medium remains transparent, near-wall growth. In all cases, a precipitate forms (Table 2).

Thus, according to the morphological and cultural properties of lactobacillus, it can be conditionally divided into two main groups, differing in the nature of growth on dense and liquid nutrient media and also in the microscopic picture.

As a result, according to the phenotypic characteristics, 24 isolates of cultures were assigned to the genus Lactobacillus.

Spore-forming cultures were presumably born by the genus Bacillus. On meat-peptone agar, after 24 h of incubation in a thermostat at 37°C, they formed flat, dry colonies of dense consistency with a characteristic white granular coating easily removed from the agar, the diameter of the colonies being 2.5 mm. The edges are almost completely cut which corresponds to the literature data (24.25</s>).

Also, cultures were isolated that, when grown on meat-peptone broth, gave abundant growth, forming a thin, leathery white film that grew on the walls of the tube. On the agarized wort the colonies are dirty white, round with an uneven edge (Fig. 12).

When incubated in a complete liquid medium, there is a superficial increase. When planting with a prick in a high-grade 0.7 agar growth on the surface (Fig. 13).

As a result, according to phenotypic traits, 19 isolates of cultures were assigned to the genus Bacillus. Characterization of the level of bacterial contamination of groups in the analysis of “Patients” and “Healthy” groups of animals showed differences in microbial composition (Table 4).

During the research it was established that the qualitative composition of the microbiocenosis is represented by 7 families (*E. coli*, *Enterococcus*, *Bacillus* spp., *Lactobacillu* spp., *Bifidobacteria* spp., *Staphylococcuspus*, *Proteus* spp.).

Based on the data obtained, the dependence of the composition of various groups of the microorganism in the safe groups of animals on the disadvantaged was

Table 4: Microbial landscape of groups in analysis

	Sick animals			Healthy animals		
Indicators	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

Fig. 12: Characteristic morphological and cultural pattern for the spore-forming cultures under study

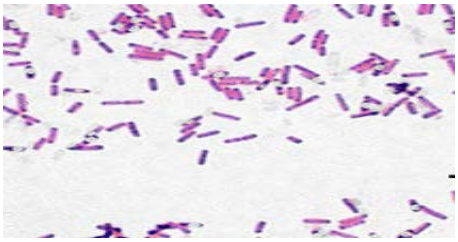


Fig. 13: Microscopic picture of bacteria of the genus Bacillus, gram staining

revealed. Thus, it was found that in the healthy group, the level of the representatives of the group of lactic acid bacteria was significantly higher, whereas in the group with sick animals the indices of such species as proteins and bacteria of the *E. coli* group were increased.

CONCLUSION

In the course of the study, we found: the qualitative composition of the microbiocenosis is mainly represented by five genera: *Coliforms*, *Bacilli*, *Lactobacilli*, *Staphylococci*, *Protaeus*. Microbial composition in the areas of sick animals compared with a healthy group showed changes in the quantitative composition of several groups of microorganisms. Significantly increased the level of pseudomonads from 7-13% of the total composition and bacteria of the *E. coli* group from 5-7%. Representatives of the indigenous microflora represented by the genera *Lactobacillus* and *Bacillus* were reduced, the total number of microorganisms of the genus Bacillus in the healthy group was 37%, in the group of sick animals their level was 28%.

Lactic acid bacteria reacted with a decrease in activity from 15-12%. On average, the species and quantitative composition of the microflora isolated in livestock houses was 12%, Bacillusubtilis 12%, Bacillusmucoides 19%, *Bacillus mesentericus* 6% total bacteria of the genus *Bacillus* 37% mold spores 15%, *Esherichia coli* 5%, *Lactobacillus* spp. 15% and *Proteus vulgaris* 7%. On selective media intended for the isolation and identification of various groups of microorganisms, growth of colonies with pronounced morphological and cultural properties (different types of staining, forms).

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