Biochemical activity of the microflora of a free-living bird

Zoya Litvinova¹,* , Nikolay Mandro¹, and Olga Yakubik¹

¹Far Eastern State Agrarian University, 86, Politeknicheskaya Str., Blagoveschensk, Russia

Abstract. Free-living birds have a wide range of habitats, can be present in the wild, near human homes, livestock and poultry complexes, farms. As a result of migration and migration of birds, the probability of spreading infectious diseases increases. In the body of birds, the pathogen can persist, multiply, be released into the external environment, and then enter the body of a healthy animal or bird, becoming the cause of new cases of the disease. Detection of pathogenic microflora in the body of free-living birds, determination of their biochemical activity is one of the aspects of studying pathogens in the parasitic phase of existence. The identification of biologically active bacteria allows us to predict the probability of free-living poultry participating in the epizootic process, and to plan appropriate preventive measures in poultry and animal husbandry. The purpose of the study is to study the species composition and biochemical activity of the microflora of the free-living birds of the Amur region. For research on the territory of the Amur region, impregnations from beaks and cloaks. The study identified such microorganisms as Enterococcus, Pseudomonas, Acinetobacter, Citrobacter, Actinobacillus, Escherichia, Micrococcus, Aspergillus, Enterobacter, Salmonella, Proteus, and Staphylococcus. Biochemical activity was detected in microorganisms of Enterococcus faecalis, Acinetobacter iwoffii, Actinobacillus species, Enterobacter aecum, Staphylococcus kloossi, Staphylococcus hyicus, Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus saprophyticus. Enzymatic properties of Pseudomonas aeruginosa (6.8%), Pseudomonas species (6.4%), and Micrococcus candidus (1.2%) had weakly expressed enzymatic properties.

1 Introduction

The bird fauna of the Amur region is diverse and includes more than 320 species. About 60% of the avifauna are birds of the order sparrows. This group includes migratory, nomadic, and sedentary birds. Representatives of this Zoological group have a wide range of habitats, can be present in the wild, near human homes, livestock and poultry complexes, farms [1].

As a result of migration and migration of birds, the probability of spreading infectious diseases increases. In the body of birds, the pathogen can persist, multiply, be released into the external environment, and then enter the body of a healthy animal or bird, becoming the cause of new cases.

* Corresponding author: litvinova-08@mail.ru
of the disease. Detection of microflora in the body of free-living birds, determination of its biochemical activity is one of the aspects of studying microorganisms in the parasitic phase of existence [2, 3].

The works of many authors are devoted to the study of the body of free-living birds as a reservoir of infectious diseases [4-10]. Many publications describe modern methods of diagnostics of pathogenic and opportunistic microorganisms [11-13]. Studies of the species composition and properties of the microflora of free-living birds, taking into account the territorial nature, are not enough.

Relevance, scientific and practical significance determined the choice of the topic, goals and objectives of the study.

The scientific hypothesis is that the identification of biologically active bacteria will make it possible to predict the probability of free-living poultry participating in the epizootic process, and to plan appropriate preventive measures in poultry and animal husbandry.

In this regard, the purpose of our study was to study the species composition and biochemical activity of the microflora of the free-living bird of the Amur region.

2 Materials and methods

The object of the study was captured on the territory of the Amur region birds of the order of sparrows of different species in the number of 122 individuals. The material was selected from blue magpie (Cianopica ciana), common lentil (Caprodacus erithrinus), common hlycatcher (Scutigera coleoptrata), thick-billed warbler (Acrocephalus aedon), gray-headed bunting (Emberiza melanocephala), brown warbler (Phylloscopus fuscatus), siberian stingray (Lanius cristatus), nightingale common (Luscinia Luscinia), house sparrow (passer domesticus), magpie (pica pica), common tit (Parus Major); crows (Corvus corax); blue dove (Columba livia). The birds were caught in the spring and summer with the help of fishing nets.

The material was selected from the beaks and cloaks of birds by impregnating sterile cotton swabs with the contents of mucous membranes and further immersing them in test tubes with a sterile saline solution in the volume of 0.5 ml. The selected material was sown on nutrient media (meat-peptone agar, meat-peptone broth), placed in a thermostat and kept for 24 hours at a temperature of 37°C.

Morphological properties of microorganisms by microscopy. Smears prepared from daily cultures were colored using the method of Gram, Romanovsky-Gimz, Peshkov, Kozlovsky, and ZIL-Nielsen.

Cultivation of bacteria was carried out on the following nutrient media: meat-peptone agar, meat-peptone broth, meat-peptone gelatin, Endo, Levina, Ploskireva, bismuth sulfite agar. To determine the cultural properties of microscopic fungi, Chapek and Saburo agar media were used. The growth pattern of colonies, color, edges, shape, profile, consistency, and structure were taken into account. The color change of colonies, the ability to hemolysis, and the growth pattern were observed in differential diagnostic media. In liquid nutrient media, the presence of sediment, its quantity, the presence of a film, thickness and consistency, and the degree of turbidity of the medium were noted [14, 15].

The study of saccharolytic properties was determined by seeding microorganisms on GIS nutrient media with sucrose, lactose, glucose, maltose, mannitol, and dulcite. When evaluating the results, color changes and the presence of gas were taken into account [14, 15].

Proteolytic properties were determined by determining the ability of microorganisms to liquefy gelatin. The degree of proteolysis was studied by determining the ability of microorganisms to release gases (hydrogen sulfide, ammonia and indole). The presence of gases was determined by using indicator papers impregnated with a 12% solution of oxalic acid, 10% solution of lead acetic acid. The results were taken into account after 18-24 hours [14, 15].
To identify microbial cultures, chromogenic media were used, which allow determining the type of bacteria by detecting highly specific enzymes in the desired microorganisms [16]. The result of biochemical studies was compared with standard indicators of the determinant of bacteria Bergi.

The experimental material was processed using mathematical methods of variational statistics using the student's t-test.

3 Results

In the course of bacteriological studies, only 516 samples were studied. The largest number of microorganisms was isolated from the cloacal cavities, which accounted for 54.5% of the total number of isolated microorganisms.

The study identified such microorganisms as Enterococcus, Pseudomonas, Acinetobacter, Citrobacter, Actinobacillus, Escherichia, Micrococcus, Aspergillus, Enterobacter, Salmonella, Proteus, and Staphylococcus.

Microbial contamination of bird beaks with Enterococcus faecalis microorganisms was recorded to the greatest extent in common flycatchers and thick-billed warblers (20.7%), and to the smallest extent in siberian zhulan, common nightingale and blue magpie (6.9%).

Microorganisms of the species Pseudomonas aeruginosa, Enterococcus gallinarum and Pseudomonas species were more often detected in the thick-billed Warbler in 37.5%, 66.7% and 28.6% of cases, respectively. The least amount of Pseudomonas aeruginosa isolated from a brown leaf Warbler (12.5%); Enterococcus gallinarum - the flycatcher common (33.3%); Pseudomonas species - the common brown flycatchers and warblers (7.1%).

The type of microorganism Acinetobacter iwofi were isolated from leaf warblers, common rosefinch, and thick-billed warbler – 35.7%, 28.8%, 21.4% of the cultures respectively.

Citrobacter freundii was recorded in 4 bird species: common lentil, thick-billed Warbler, gray-headed bunting and brown warbler in 25% of cases.

Cultures of Actinobacillus species revealed thick-billed Warbler (23.1%), gray - headed bunting (15.4%), common lentils and brown warblers (17.2%); less often-in blue magpies (3.5%).

Escherichia coli and Micrococcus species are not isolated from the beak cavities. Cultures of Aspergillus fumigatus were isolated from the beak cavities of common flycatcher (50%) and thick-billed Warbler (50%).

When studying the material of their cloaca cavities, Enterococcus faecalis was more often isolated in brown Warbler (24.1%), blue magpie (27.6%), common lentils and Siberian zhulana (13.8%); Pseudomonas aeruginosa - in thick – billed Warbler (60.0%) and blue magpie (40.0%); Enterococcus gallinarum (figure 1) - in brown warbler (100.0%); Pseudomonas species-in the siberian zhulana (26.3%), brown warbler (21.1%).

Microorganisms of the species Acinetobacter iwofi (figure 2) were determined in the thick-billed warbler (38.9%); Citrobacter freundii - in the thick-billed warbler (75.0%); Actinobacillus species-in the thick-billed warbler (22.2% ), brown warbler (19.4%), siberian beetle (16.7%), common lentil (13.9%), gray-headed bunting (13.9%).

Escherichia coli microorganisms were detected in common lentils (50.0%) and blue magpies (50.0%), Micrococcus was isolated in common lentils (33.3%), brown warblers (33.3%), siberian zhulana (33.3%).

Enterococcus faecalis was isolated from thick – billed Warbler and gray-headed bunting-3 cultures each (20.3%), Pseudomonas species - from common flycatcher (5.3%), thick - billed warbler and blue magpie (11.1%) and common flycatcher (5.6%), Citrobacter freundii-from common lentil, blue magpie (12.5%).

Actinobacillus species was rarely isolated in blue magpies (8.3%) and common flycatchers (5.6%).
The high content of microorganisms in the cavities of the beaks was found in thick-billed reed warbler-28 cultures (27.7%), common lentils – 18 cultures (17.8%), gray-headed oatmeal and brown warbler-17 cultures each (16.8%). Minimal microbial contamination was detected in the siberian zhulan – 5 cultures (5.0%), blue magpie – 3 cultures (2.9%) and common nightingale – 2 cultures (2.0%).

Significant microbial contamination of cloacal cavities was observed in thick-billed warbler-29 cultures (23.9%), brown warbler – 22 cultures (18.2%), common lentils and blue magpies - 19 cultures for each species (15.70%). The lowest microbial contamination of cloaca was found in birds of the species gray-headed bunting – 12 cultures (9.9%) and common flycatcher – 4 cultures (3.3%).

![Fig. 1. Smear of a daily agar culture of Enterococcus gallinarum isolated from the cloaca cavity of a brown warbler (Gram color, magnification x 100).](image1)

![Fig. 2. Smear of a daily agar culture of Acinetobacter iwoffii isolated from the cloaca cavity of a thick-billed Warbler (Gram color, magnification x 100).](image2)

The study of biochemical properties showed that the bacteria of the species Lactobacillus species (18.6%) fermented glucose to form acid, maltose, sucrose, lactose, did not break down dulcite, mannitol. Proteolytic properties were shown in the ability to release gas (hydrogen sulfide).
Enterococcus faecalis (15.7%) cleaved glucose, maltose, sucrose and lactose.
Escherichia coli (13.9%) changed glucose and lactose with the formation of acid and gas, curtailed milk, and isolated indole.

The enzymatic properties of Acinetobacter iwoffi (12.8%) were shown in the fermentation of glucose, maltose, sucrose, lactose; proteolytic properties - in the ability to release gas (ammonia).

The enzymatic properties of Pseudomonas aeruginosa (6.8%) were poorly expressed. Microorganisms did not change glucose, sucrose, maltose, or lactose.

Enterococcus gallinarum microorganisms (1.4%) had the ability to ferment glucose, maltose, sucrose, lactose, and dilute gelatin.

The biochemical properties of Pseudomonas species (6.4%) were poorly expressed. The micro-organisms did not change glucose, lactose, maltose, or sucrose; they liquefied gelatin and converted nitrates into nitrites.

Citrobacter freundii (2.3%) had the ability to ferment glucose and lactose with the formation of acid and gas, to dilute gelatin and curdled milk, and to release hydrogen sulfide and ammonia.

Micrococcus candidus (1.2%) had weakly expressed enzymatic properties. The ability to ferment glucose with the formation of acid has been established.

Citrobacter agglomerans microorganisms (0.6%) fermented glucose, maltose, lactose and mannitol with the formation of acid and gas; indole, hydrogen sulfide were not isolated, and gelatin was slowly liquefied.

Salmonella enteritidis bacteria (6.3%) did not ferment sucrose or lactose. Fermented with the formation of acid and gas glucose, mannitol. They did not form indole, but isolated hydrogen sulfide.

Enterobacter cloacae (0.8%) fermented glucose, lactose, maltose, and mannitol. Hydrogen sulfide, ammonia, and indole were not isolated.

Enterococcus aerogenes microorganisms (1.4%) fermented glucose, maltose, lactose, mannitol, and slowly liquefied gelatin.

Proteus mirabilis (0.4%) changed glucose to form acid, lactose and dulcite, and isolated hydrogen sulfide.

Enterococcus aecum (0.4%) was fermented with the formation of acid glucose, maltose, sucrose and lactose.

All kinds of microorganisms of the genus Staphylococcus fermented glucose, lactose, sucrose and maltose, except for the types Staphylococcus kloosii and Staphylococcus aureus – mannitol is changed. Hydrogen sulfide and ammonia were isolated.

Streptococcus mutli bacteria fermented glucose, maltose, sucrose and lactose.

The results of biochemical studies are presented in table 1.

The results of the research showed that 172 cultures (33.4%) out of 516 cultures were active in biochemical terms. Proteolytic activity was established based on the ability of microorganisms to secrete indole (13.9%), hydrogen sulfide (29.3%) and ammonia (23.3%).

Saccharolytic activity is established by determining the fermentation of carbohydrates by microorganisms. Decomposed glucose 84.9% of bacterial cultures; maltose-82.5%; sucrose-61.8%; mannitol-41.4%; dulcite-0.8%; lactose-69.7%. Milk was curdled by 17.3% of microorganisms, gelatin was liquefied by 33.4% of cultures.

Biochemical activity was detected in microorganisms of Enterococcus faecalis, Acinetobacter iwoffi, Actinobacillus species, Enterobacter aecum, Staphylococcus kloosii, Staphylococcus huisis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophuticus.
Table 1. Results of biochemical studies.

| Study                   | Result |
|-------------------------|--------|
|                         | positive | negative |
|                         | number of samples | % | number of samples | % |
| Formation of indole     | 72       | 13.9     | 443     | 86.0 |
| Formation of hydrogen sulfide | 151      | 29.3     | 364     | 70.7 |
| Formation of ammonia    | 120      | 23.3     | 395     | 76.7 |
| Fermentation of carbohydrates: |         |        |         |      |
| Glucose                 | 437      | 84.9     | 78      | 15.2 |
| Maltose                 | 425      | 82.5     | 90      | 17.5 |
| Sucrose                 | 318      | 61.8     | 197     | 38.3 |
| Mannitol                | 213      | 41.4     | 302     | 58.6 |
| Dulcite                 | 4        | 0.8      | 511     | 99.2 |
| Lactose                 | 359      | 69.7     | 156     | 30.3 |
| Curdling milk           | 89       | 17.3     | 426     | 82.7 |
| Dilution of gelatine    | 172      | 33.4     | 344     | 66.6 |

4 Discussion

Birds break off sparrows have a large habitat range. This group includes families that are divided into migratory, nomadic and sedentary birds. As a result of migration and migration of birds, the probability of spreading infectious diseases increases. The species composition and its microflora of free-living birds of the Amur region are insufficiently studied. In this regard, the goal was set and the tasks of investigations were defined.

When investigating 13 species of free-living birds poison sparrows isolated Enterococcus, Pseudomonas, Acinetobacter, Citrobacter, Actinobacillus, Escherichia, Micrococcus, Aspergillus, Enterobacter, Salmonella, Proteus, Staphulococcus.

The bird that found the degree of contamination of the beak cavities was microorganisms of Enterococcus faecalis and Actinobacillus species. These types of microorganisms had stable properties of their own.

Cultures of Actinobacillus species (29.8%) were most often isolated from the cloacal cavities.

Enterococcus faecalis (23.9%) was isolated in 6.6% of the bird species blue magpie and 5.8% - brown Warbler.

Less frequently, micro-organisms of the species Micrococcus candidus (2.5%), Escherichia coli (1.7%) and Enterococcus gallinarum (0.8%) were isolated.

A large degree of contamination of cloacal cavities is represented by cultures of microorganisms of Actinobacillus species and Enterococcus faecalis. These types of microorganisms were isolated from the cavities of beaks and cloaks, which corresponds to the level of their circulation in the gastrointestinal tract of birds. When determining the correlation coefficient between microbial contamination of the oral cavity and the cloaca of wild birds, it was found that this coefficient is equal to + 0.97, which indicates a strong relationship.

Biochemical activity was detected in microorganisms of Enterococcus faecalis, Acinetobacter iwoffii, Actinobacillus species, Enterobacter aecum, Staphulococcus kloossi, Staphulococcus huisis, Staphulococcus aureus, Staphulococcus epidermidis,
Staphylococcus saprophuticus. The enzymatic properties of Pseudomonas aeruginosa (6.8%), Pseudomonas species (6.4%), and Micrococcus candidus (1.2%) were poorly expressed.

All isolated microorganisms had stable properties and were assigned to Actinobacillus species (17.4%), Enterococcus faecalis (15.7%), Escherichia coli (12.4%), Acinetobacter iwoffii (12.2%), pseudom Aeroginosa (6.8%), Pseudomonas species (6.4%), Staphylococcus kloossi (4.3%), Salmonella enteritidis (2.7%), bacterium Cocciformes (2.5%), Citrobacter freundii, Staphylococcus aureus, Bacillus subtilis (2.3%), Streptococcus multi (1.7%), Klebsiella oxutoca (1.6%), Enterococcus gallinarum, Enterobacter aerogenes (1.4%), Micrococcus candidus (1.2%), Enterobacter cloacae, Staphylococcus huisis (0.8%), Enterobacter agglomerans, Bacillus retiformes (0.6%), Aspergillus fumugatus, Proteus mirabilis, Enterobacter accum, Staphylococcus epidermidis, Staphylococcus saprophuticus, Bacillus brachiosporum, Mucor (0.4%).

References

1. V. A. Nechaev, T. V. Gamova Birds of the Russian Far East (annotated catalog). Russian Academy of Sciences, far Eastern Department, *Biology and soil Institute* **564** (2009)

2. A. E. Losaberidze, A. A. Lysenko, Y. U. Ponamarenko Analysis of the epizootic state of poultry farming in the Russian Federation, *Veterinary Medicine of Kuban* **2**, 89 (2014)

3. D. L Williams. Research in infectious disease in wild birds, *International International Journal of Avian & Wildlife Biology* **4**, 2, 74 (2019)

4. N. V. Pimenov, A. I. Laishevtcev Modern methods of epizootic and epidemiological monitoring in the poultryindustry on the example of salmonella infection. *Russian journal of agricultural and socio-economic sciences* **64**, 4, 257-269 (2017)

5. P. I. Baryshnikov, A. I. Bondarev, B. V. Novikov, B. B. Razumovskaya Associated course of infectious diseases in wild birds of the forest-steppe region of the Altai territory, *Agau Bulletin* **11**, 72-74 (2012)

6. O. L. Asmolova, N. I. Zemlyanskaya Microflora of wild and synanthropic birds, objects of poultry farms in a comparative aspect. *AVU* **139**, 9, 32-35 (2015)

7. A. P. Savchenko, P. A. Savchenko, I. A. Savchenko, V. I. Yemelyanov, N. V. Karpova Bird species-the main carriers and vectors of influenza a viruses in Eastern Siberia, *Acta Biomedica Scientifica* **104**, 4, 102-111 (2015)

8. Zdražilová Dubská, Lenka, Ivan Literák, Kocianova E, Taragelova V, Sverakova V, Oldřich Sychra and M Hromadko Synanthropic Birds Influence the Distribution of Borrelia Species: Analysis of Ixodes ricinus Ticks Feeding on Passerine Birds. *Applied and Environmental Microbiology*, USA: *AMER SOC MICROBIOLOGY*, **77**, 3, 1115-1117 (2011)

9. Cavalcanti, Isabella Macário Dissemination of Multidrug-Resistant Bacteria in Birds. *Approaches in Poultry, Dairy & Veterinary Sciences*, **3**, 4, 1-3 (2018)

10. De Luca, Carlotta & Niero, Giulia & Cattarossi, Diego & Bedin, Marco & Piccirillo, Alessandra Pet and Captive Birds as Potential Reservoirs of Zoonotic Bacteria, *Journal of Exotic Pet Medicine*, **27**, 1, 17-20 (2017)

11. Kammon Abdulwahab Salmonella infections in poultry farms of Libya, *Journal of the American Veterinary Medical Association*, **1**, 9 (2010)
12. Wei, Bai & Kang, Min & Jang, Hyung-Kwan Genetic characterization and epidemiological implications of Campylobacter isolates from wild birds in South Korea, *Transboundary and Emerging Diseases*, 66, 56-65 (2019)

13. Ricklefs, Robert & Medeiros, Matthew & Ellis, Vincenzo & Svensson-Coelho, Maria & Blake, John & Loiselle, Bette & Soares, Leticia & Fecchio, Alan & Outlaw, Diana & Marra, Peter & Latta, Steven & Hellgren, Olof & Bensch, Staffan & Valkiunas, Gediminas Avian migration and the distribution of malaria parasites in New World passerine birds. *Journal of Biogeography*, 44, 5, 1113-1123 (2016)

14. N. M. Kolychev, V. N. Kislenko *Handbook of Microbiology and immunology*. Novosibirsk, 255 (2010)

15. V. V. Menshikov Methods of clinical laboratory research. Clinical Microbiology, bacteriological research, mycological research, parasitological research, infectious immunodiagnostics, molecular research in the diagnosis of infectious diseases, 3, 879. (2009)

16. Methods of microbiological control of environmental objects and food products using petrifilms: guidelines: MUC 4.2.2884-11: Federal service for supervision of consumer rights protection and human welfare. - Moscow: Federal service for supervision of consumer rights protection and human welfare, p 23.