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BIOCHEMICAL, ANTIGENIC AND PROTEOMIC PROPERTIES OF ISOLATES AND STRAINS OF THE CAUSATIVE AGENT OF CHICKEN INFECTIOUS CORYZA *Avibacterium paragallinarum* (Biberstein and White 1969) Blackall et al. 2005

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Abstract

Infectious coryza (haemophilus infection) of chickens is a disease reported in many countries of the world. In the Russian Federation, there is no information both about the extent of the disease spread across the poultry farms and about the serotype diversity of the agent circulating in the country. The paper for the first time demonstrates biochemical properties and specifies antigenic relatedness of new *Avibacterim paragallinarum* isolates recovered from chickens with respiratory signs in Russia and in Belarus. The paper also shows results of creation of the *Avibacterim paragallinarum* subsection in the database of tested microorganisms' mass-spectra which can be used as reference ones for the identification and protein profiling of the infectious coryza agent strains and isolates. The work aimed to determine biochemical and antigenic properties of *A. paragallinarum* isolates and to produce the species-specific mass-spectra as a tool for *Avibacterim paragallinarum* species identification and intraspecies differentiation. *A. paragallinarum* No. 29545 ATCC (serotype A1) form the collection of the Federal Centre for Animal Health (ARRIAH) served as a reference strain. Thirteen *A. paragallinarum* isolates were used in the study. The isolates were recovered from the pathological material collected from chickens with respiratory pathology (nasal exudates, contents of infraorbital sinus and conjunctival sac, lung tissues) in 2015. The isolates were inoculated onto Columbia agar supplemented with 5 % defibrinated sheep blood, together with a streak of *Staphylococcus epidermidis*. Pure cultures of the agent were grown on the serum agar containing NADP at 20 μkg/cm^3 and 5 % of horse blood serum. The bacteria were cultured for 24-72 at high CO_2 concentration and 37 °C. Bilateral antigenic relatedness of the reference strain ATCC No. 29545 and *A. paragallinarum* isolates was determined using slide agglutination test (SAT). Hemagglutination activity was assessed using hemagglutination inhibition test (HI). Homogeneity of the tested isolates' serogroup was confirmed by PCR. Amplified 800 bp DNA fragments were indicative of the presence of serogroup A *A. paragallinarum* genome, 1000-1100 bp of serogroup B, and 1500-1600 bp of serogroup C. *A. paragallinarum* identification was performed using MALDI Autoflex III Biotyper mass-spectrometer (Bruker Daltonik GmbH, Germany). The resulted mass-spectra were recorded, processed and analyzed using FlexControl 3.4 software (Bruker Daltonik Gmb», Germany) according to MALDIBiotyper 2.0. UserManual, Version 2.0 SR1, Germany, 2008. Testing of biochemical properties indicated that all 13 isolates of *A. paragallinarum* form a diverse group. The *A. paragallinarum* isolates' growth absolutely depended upon presence of blood serum in the culture medium. Saccharolytic activity of the *A. paragallinarum* isolates also varied. All isolates and the reference strain can utilize glucose and sucrose, but not lactose, trehalose and galactose. The property also varied for mannitol and mannose. As for antigenic properties, all tested isolates belonged to the same serogroup B that

was also confirmed by real-time polymerase chain reaction method. Examination of proteomic properties of *A. paragallinarum* isolates revealed typical MS peaks, m/z 4768-4770 and 5347-5349, that were similar to those for the reference strain *A. paragallinarum* ATCC No. 29545. Protein profile mass-spectra were entered into MALDI Autoflex III Biotyper database to improve the reliability of *A. paragallinarum* species identification. Basing on the examined biochemical, antigenic and proteomic properties, three *A. paragallinarum* isolates were deposited inещ the strain collection of the Federal Centre for Animal Health (ARRIAH) as *A. paragallinarum* strain No. 1818, *A. paragallinarum* strain No. 5111, and *A. paragallinarum* strain No. 1116.

Keywords: strain, isolate, *Avibacterium paragallinarum,* identification, biochemical characterization, antigenic properties, proteomic properties, mass spectrometry, agglutination test, hemagglutination inhibition assay

Infectious rhinitis of chickens caused by *Avibacterium paragallinarum* of family *Pasteurellaceae*, a gram-negative bacterium previously classified as *Haemophilus paragallinarum*, is a disease widespread in industrial poultry farms in Argentina, Africa, Australia, Bangladesh, Brazil, Bulgaria, Indonesia, India, China, Mexico, Malaysia, USA (states of California, Oregon and Alabama), Thailand, and Japan [1-4]. It is characterized by catarrhal inflammation of the mucous membranes of the nasal cavity, respiratory airways and conjunctiva, subcutaneous edema of the head, and occasionally pneumonia [5-7]. Infectious rhinitis causes significant economic damage to the poultry industry due to growth retardation in chicks and up to 40% loss of egg production in chickens [7]. The mortality rate of young chickens can reach 10% [1]. The extent of the spread of this disease in the poultry farms of the Russian Federation is unknown, as is the serotypic diversity of the pathogen, since the laboratory diagnosis of infectious rhinitis in chickens is not regulated by the relevant documents

Traditionally, the causative agent of the disease is identified by its growth, morphological and biochemical properties. Mass spectrometric methods based on the analysis of protein or lipid fractions of a microbial cell has increased the capabilities of veterinary specialists in identifying bacteria. Proteomic methods for determining the species of microorganisms are not inferior to genotypical ones in many parameters, e.g. in sensitivity and resolution, and have a clear advantage in the cost of consumables, the speed of analysis and the absence of the influence of nonspecific DNA on the result. Protein profiling by mass spectrometry is a powerful analytical tool both in fundamental fields and in clinical practice [8, 9]. The use of proteomic methods for the identification and study of the properties of *A. paragallinarum* isolates, an economically significant but still insufficiently studied pathogen with a complex antigenic structure, allows researchers to obtain new knowledge about its biology, which can be used to address practical problems in veterinary medicine.

MALDI-TOF (matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry) is a method for identifying microorganisms, which is based on analysis of a set of proteins that is unique for each species. It consists in the release and ionization of membrane proteins using laser radiation in the presence of an auxiliary substance (matrix) and subsequent separation of ions in a time-of-flight mass analyzer [10, 11]. However, in the MALDI BioTyper identification database (Bruker Daltonik GmbH, Germany), data on the protein mass spectra of *A. paragallinarum* species were previously absent, which made it impossible to use this method to identify *A. paragallinarum* isolates.

This work reports on creation of a new reference mass spectra database for identification and protein profiling of *A. paragallinarum* isolates and strains. Also, here we characterize biochemical properties and the antigenic relationship of *A. paragallinarum* strains isolated by us earlier on poultry farms of the Russian Federation and the Republic of Belarus from chickens with respiratory pathology.

The work aimed to examine biochemical and antigenic properties of *Avibacterium paragallinarum* isolates from Russia and Belarus and to obtain mass spectra specific to representatives of the species, followed by their analysis and use for identification and intraspecific differentiation.

Materials and methods. The reference *A. paragallinarum* strain No. 29545 ATCC (serotype A1) was obtained from the State Collection of Microorganism Strains (Federal Center for Animal Health, ARRIAH). The study involved 13 strains of *A. paragallinarum* isolated from infraorbital sinuses and conjunctival sac, nasal cavity exudates, and lung tissue of chickens with respiratory pathology from poultry farms of the Russian Federation (Vladimirskaya, Kostromskaya, Moscow, Orenburg, Yaroslavl, Ulyanovsk regions, Republic of Mordovia, Republic of Tatarstan) and the Republic of Belarus in 2015.

Bacteria were cultured on the following nutrient media with growth-stimulating additives: Columbia broth and agar (Columbia Columbia Broth, Columbia Agar, Becton, Dickinson and Co., USA), Mueller-Hinton agar (HiMedia Laboratories Pvt. Ltd, India), normal equine serum for cultures of microorganisms (JSC NPO Mikrogen, Russia), NADP (Bontac Bio-Engineering Co., Ltd, China), Giss medium (NPO Nutrient media, Russia).

A. paragallinarum cultures were isolated from pathological materials by plating on Columbia agar with 5% defibrinated ram blood and a streak of *Staphylococcus epidermidis* as "feeding" bacteria. Pure cultures of the pathogen were grown on serum agar containing 20 μg/cm^3 NADP and 5% horse blood serum. Bacteria were cultured for 24-72 h at 37 °C and the increased air level of CO_2.

Bacterial morphology was examined by light microscopy of Gram stained preparations (EclipseNi-U microscope, Nikon Corporation, Japan, ×1000 magnification) [12]. Capsules in bacteria were identified by Gins's staining [12]. The biochemical properties of the isolates were assessed using commercial API®NH kit (bioMerieux S.A., France) and by plating on Giss media with glucose, sucrose, lactose, mannitol, mannose, trehalose, galactose monocarbohydrates. Catalase production was assessed on a glass slide with 3% H_2O_2solution. Oxidase activity was measured using a commercial API®NH kit (bioMerieux S.A., France) in accordance with the manufacturer's instructions.

Antigenic relationship between pairs of *A. paragallinarum* isolates and antigenic properties of the reference strain ATCC No. 29545 were determined in slide agglutination test (SAT) as proposed by L.A. Page [13]. Hemagglutinating activity was assessed in the hemagglutination inhibition test (HI) as described by P.J. Blackall et al. [14]. The degree of bilateral antigenic relationship (R) of *A. paragallinarum* isolates was calculated using the formula I. Archetti and F.L. Horsfall [15] and expressed as a percentage. The value of R ≥ 70% at 3-fold repetition of titration was considered as confirmation of the statistically significant relationship of the investigated isolates [15].

The serogroup homogeneity of *A. paragallinarum* isolates was confirmed by real-time multiplex polymerase chain reaction (RT-PCR, qPCR) (microchip nucleic acid amplifier Ari-aDNA®, Lumex LLC, Russia). Bacterial DNA was extracted using a kit for the isolation of nucleic acids (OOO Biocom, Russia). Thermocycling of the samples was carried out in the following mode: 10 min at 95 °C (1 cycle); at 30 s at 95 °C, 30 s at 56 °C, 90 s at 32 °C (35 cycles). We used four primers proposed by R. Sakamoto et al. [16] (OOO Beagle, St. Petersburg): forward primer (common for genome amplification in three serogroups of *A. paragallinarum*) and three reverse primers specific for a particular serogroup.

The amplification products were analyzed by electrophoresis in 1.7% agarose gel with ethidium bromide (15 V/cm of the gel length, 45 min) (OOO

Lumex, Russia). Electrophoregrams were visualized using TCP-26.LMX transilluminator (Vilber Lourmat, France) in ultraviolet light (λ = 254 nm). Amplified DNA fragments were identified as orange band.

The results were deemed positive when the 800 bp, 1000-1100 bp, and 1500-1600 bp amplicons were detected. The 800 bp fragments indicated the genome of *A. paragallinarum* serogroup A, 1000-1100 bp of serogroup B, and 1500-1600 bp of serogroup C. The results were considered negative if the indicated amplified DNA fragments were not detected or their size did not correspond to the given values [16, 17].

MALDI-TOF spectrometry (an Autoflex III Biotyper mass spectrometer, Bruker Daltonik GmbH, Germany) was used to identify *A. paragallinarum* bacteria. The direct application mode was used in which single colonies of a fresh culture were introduced into the wells of a metal plate using a sterile loop, and a 1 μl matrix was placed on top. A saturated solution of CHCA (HC-cyano-4-hydroxycinnamic acid) in 50% aqueous acetonitrile with 2.5% trifluoroacetic acid was a matrix. The device was calibrated before each experiment with the Bruker Bacterial Standard (Bruker Daltonik GmbH, Germany) as a calibrant. Mass spectrometric analysis of *A. paragallinarum* cultures was conducted in a linear laser mode at a frequency of 50 Hz. The analysis parameters were optimized for the m/z (mass/charge) mass range from 2000 to 20,000 Da, the resultant spectrum from summing 20 single spectra was recorded. The mass spectra were recorded and analyzed using the FlexControl 3.4 and FlexAnalysis 3.0 software (Bruker Daltonik GmbH, Germany) in accordance with MALDIBiotyper 2.0. UserManual, Version 2.0 SR1 (Germany, 2008).

The obtained mass spectra were statistically processed using the Biotyper 3.0 RTC program (Bruker Daltonik GmbH, Germany). The reliability of the identification of microorganisms was assessed by comparing Score values with the data of the mass spectra of the Biotyper 3.0 reference library. The identification at Score values < 1.7 was considered unreliable. When analyzing the degree of bilateral antigenic relationship (R, %) in replicates, the mean (*M*) and standard errors of the means (±SEM) were calculated.

Results. On blood agar with a "feeding bacterium" (a source of V-growth factor), the *A. paragallinarum* isolates after 24 hours of culture looked like small (0.1-0.5 mm) satellite colonies in zone 0.5-1.5 cm from the streak of *S. epidermidis*. With the distance from the feeding culture, the size of the colonies decreased until the complete disappearance of growth. Satellite colonies were gray-white, had a smooth convex surface without a hemolysis zone and a rounded shape with smooth edges.

When comparing isolates with the reference *A. paragallinarum* strain No. 29545 ATCC, we assessed the culture growth on blood serum-free nutrient media, under an increased carbon dioxide in the atmosphere, the ability to utilize carbohydrates and to produce various enzymes and metabolites. The results allowed conclusion that the isolates and the reference strain form a heterogeneous group in terms of growth properties and enzymatic activity (Table 1). The reference *A. paragallinarum* strain and all the isolates were NAD-dependent though the NAD-independent isolates of the pathogen are also known [18]. All *A. paragallinarum* isolates can reduce nitrates to nitrites and did not produce oxidase, α-fructosidase, β-galactosidase, indole, hydrogen sulfide, urease, and catalase. We found the dependence of the growth on the blood serum in the nutrient medium to be absolute. All *A. paragallinarum* strains we tested failed to grow on serum-free medium even at the optimal V-factor concentration. There is a viewpoint that an increased carbon dioxide in the atmosphere is obligatory for

A. paragallinarum growth [18-20]. In our test, only 9 of 13 isolates (Nos. 1, 4, 5, 7, 8, 9, 10 and 11) showed such dependence. The morphology and size of the colonies in the four isolates grown in a normal air atmosphere did not differ in any way from those in the colonies under increased CO_2 concentration. Ability of the *A. paragallinarum* strains to metabolize sugars was also unequal.

1. Growth and biochemical traits of the reference strain *Avibacterium paragallinarum* No. 29545 ATCC and *A. paragallinarum* isolates (Russia and Belarus, 2015) from chickens with respiratory pathology

Свойства бактерий	Strain, isolate No.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	29545
Need in:														
V growth factor	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CO_2	+	–	–	+	+	–	+	+	+	+	+	–	+	+
blood serum	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reduction of nitrates	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Production:														
indole	–	–	–	–	–	–	–	–	–	–	–	–	–	–
hydrogen sulfide	–	–	–	–	–	–	–	–	–	–	–	–	–	–
urease	–	–	–	–	–	–	–	–	–	–	–	–	–	–
catalase	–	–	–	–	–	–	–	–	–	–	–	–	–	–
oxidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–
α-fructosidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–
β-galactosidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fermentation:														
glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
lactose	–	–	–	–	–	–	–	–	–	–	–	–	–	–
mannitol	+	+	+	+	–	+	+	–	+	–	–	–	+	+
mannose	+	–	–	+	+	–	+	+	–	+	–	+	–	–
trehalose	–	–	–	–	–	–	–	–	–	–	–	–	–	–
galactose	–	–	–	–	–	–	–	–	–	–	–	–	–	–

N o t e. «+» means positive test, «–» means negative test.

All isolates and the reference strain fermented sucrose and glucose, but not trehalose, lactose and galactose. The trait was variability toward mannose and mannitol, as it was also reported by other researchers [20-22].

For the identification of *A. paragallinarum isolates*, it was important to determine the antigenic relationship in SAT. The test did not reveal the antigenic relationship of the reference strain and the isolates. The *A. paragallinarum* isolates formed a homologous group with the exception of Nos. 6, 7, 10, and 11. The antigens of these isolates showed only one-sided relationship with the tested sera, which did not allow conclusion that they belong to different serological groups.

We also determined the antigenic relationship of *A. paragallinarum* isolates in the hemagglutination inhibition test (Table 2) to compare the results of serotyping for agglutination and hemagglutination [23]. Our data indicate that all the studied isolates were a homogeneous in hemagglutinin. The minimum value of two-sided relationship (R) was 78.4%. That is, it can be assumed that all isolates belonged to one serogroup and one serotype.

Based on the obtained results of the similarity of the isolates in growth, antigenic and morphological properties, for further work 10 most promising isolates were selected, which retained the stability of hemagglutinating, antigenic, virulent and immunogenic properties for 20 consecutive passages.

The *A. paragallinarum* serotyping by PCR analysis was shown by R. Sakamoto et al. [16] and V.V. Patil et al. [17]. In our work, the homogeneity of the serogroup of the isolates from birds with respiratory pathology was confirmed using multiplex qPCR (Fig. 1). In all isolates, we revealed 1000-1100 bp amplicons which correspond to the PCR amplification product characteristic of *A. paragallinarum* serogroup B.

2. Antigenic relationship (R, %) **of *Avibacterium paragallinarum* isolates (Russia and Belarus, 2015) from chickens with respiratory pathology** (hemagglutination inhibition test, $n = 3$, $M \pm$SEM)

No. of isolate	Serum specific to antigen No.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1	100	100	96.8±2.0	86.8±2.0	92.4±1.2	86.4±2.6	88.8±2.3	92.4±2.3	96.7±1.2	96.2±0.2	87.7±2.2	97.7±1.1	90.4±0.6
2	92.6±2.0	100	94.6±0.8	92.6±1.8	98.2±0.4	94.6±3.0	86.6±2.6	100	90.2±0.9	82.4±1.8	85.2±0.8	89.4±1.0	89.1±0.9
3	98.6±1.2	96.4±1.2	100	100	84.2±1.0	94.8±0.5	90.4±1.2	98.4±1.2	86.2±1.5	84.8±1.2	86.6±2.0	87.9±0.4	86.4±1.6
4	100	94.8±2.0	95.2±3.0	100	94.2±1.6	88.4±2.8	96.8±0.5	100	94.6±1.2	98.8±0.1	88.0±2.0	91.6±0.4	100
5	87.6±2.0	93.0±2.0	87.4±2.8	97.8±2.0	100	98.2±1.2	84.6±2.0	90.2±2.2	97.2±1.0	100	79.9±1.9	92.5±0.8	96.2±0.8
6	90.2±1.3	100	79.6±2.1	96.2±2.0	86.2±1.0	100	88.2±2.2	92.6±1.8	100	96.4±0.8	89.4±1.3	92.7±0.6	94.3±0.2
7	94.6±2.1	96.6±2.4	91.3±3.4	100	96.8±0.5	100	100	98.2±1.0	99.3±0.5	86.8±1.8	94.4±1.0	94.8±0.2	82.3±0.3
8	88.2±0.6	100	96.4±1.0	96.3±1.7	98.4±1.0	92.8±2.0	96.8±1.2	100	100	88.2±1.6	100	90.0±1.5	88.6±0.2
9	86.6±0.5	90.8±1.8	87.8±2.6	78.4±0.2	92.6±0.8	94.8±0.5	94.2±1.3	96.2±0.9	100	100	92.2±0.9	80.2±1.6	84.3±0.4
10	96.6±2.0	92.4±2.2	92.8±2.0	86.4±1.0	88.4±2.6	86.2±0.2	98.0±1.0	100	98.4±0.4	100	91.8±0.6	79.8±0.2	92.0±1.5
11	78.9±1.3	89.3±3.0	92.5±0.5	89.9±1.1	94.4±2.4	100	79.0±2.0	88.3±0.4	90.2±2.0	97.2±0.4	100	81.6±0.2	85.5±0.5
12	80.3±2.2	93.5±0.5	88.5±2.4	94.2±2.0	89.6±0.5	88.5±0.6	84.2±2.1	92.3±1.3	100	91.8±0.8	88.3±0.9	100	89.9±0.5
13	86.2±1.9	84.2±0.2	87.3±2.7	98.8±0.8	88.1±1.9	78.6±1.0	88.4±2.0	87.2±2.0	94.3±1.6	94.3±0.5	91.1±1.1	95.1±0.6	100

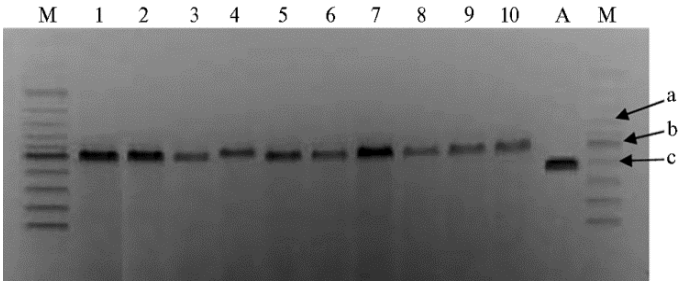

Fig. 1. Serotyping reference strain *Avibacterium paragallinarum* No. 29545 ATCC and *A. paragallinarum* isolates (Russia and Belarus, 2015) from chickens with respiratory pathology by multiplex qPCR: a — serogroup C (1500-1600 bp), b — serogroup B (1000-1100 bp), c — serogroup A (800 bp); 1-10 — isolates Nos., A — strain *A. paragallinarum* No. 29545, M — DNA fragment length marker (Zao Rvrogen, Russia).

Based on the morphological, biochemical and antigenic characteristics, three isolates of *A. paragallinarum* were deposited in the State Collection of Microorganism Strains (ARRIAH), isolate No. 4 as strain No. 1818, isolate No. 8 as strain No. 5111, and isolate No. 12 as strain No. 1116.

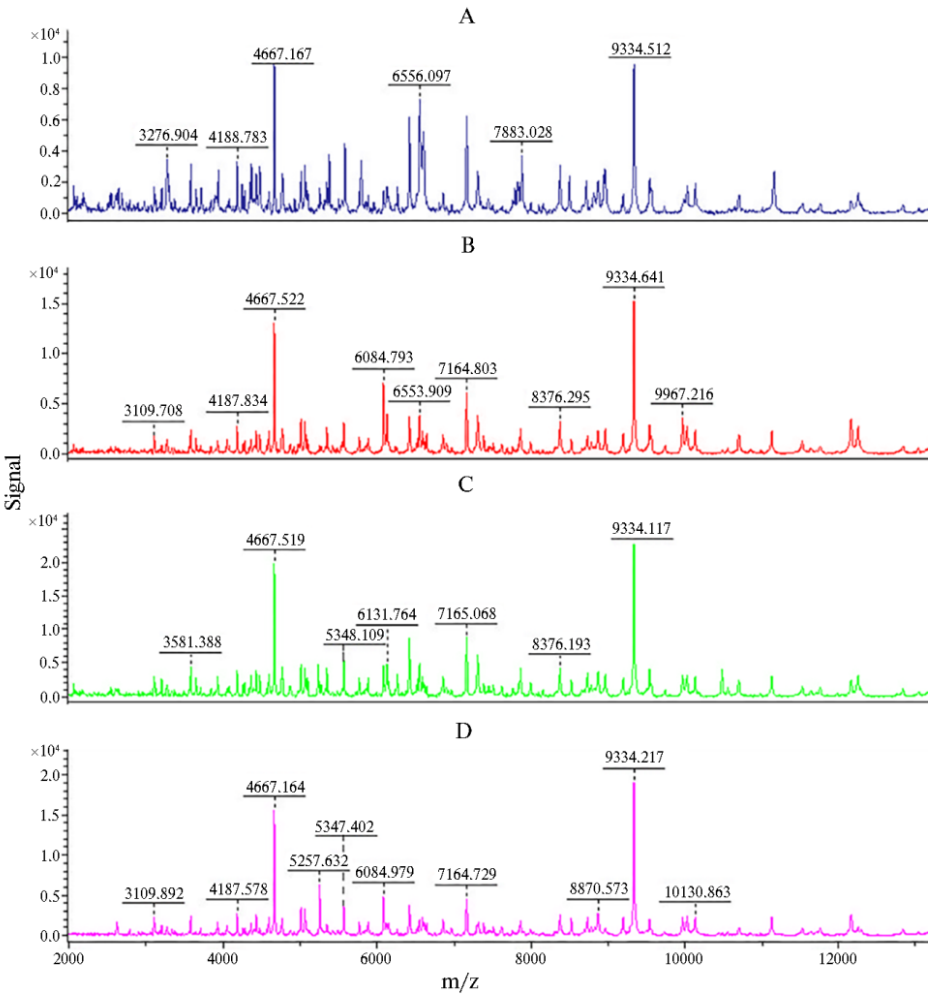

Fig. 2. Mass-spectra of individual colonies of reference strain *Avibacterium paragallinarum* No. 29545 ATCC (A) **accession strains Nos. 1818** (B)**, 1116** (C)**, 5111** (D) **derived from *A. paragallinarum* isolates (Russia and Belarus, 2015) from chickens with respiratory pathology** (peaks of m/z 4667 and 9334 were common for all strains of *A. paragallinarum*).

The next stage of our research was generation of a database of mass spectra for accession strains *A. paragallinarum* No. 5111, No. 1818 and No. 1116 and the reference strain ATCC 29545, which are supposed for future automated identification and profiling representatives of this species based on similarities/differences in mass spectrometric characteristics.

For strains of *A. paragallinarum*, protein profiles were constructed (Fig. 2) and mass lists were formed to determine characteristic peaks (Table 3). We compared the positions of the peaks, their frequency and intensity to identify strains and isolates of *A. paragallinarum* as per the protocol by J.H.K Chen et al. [24] and N. Takeuchi et al. [25].

Analysis of the obtained spectra revealed that they were in the range of m/z 2000-10000. Peaks for m/z 4667 and 9334 turned out to be common for all strains and isolates of *A. paragallinarum*. A unique feature of the MALDI Biotyper program is the option to replenish the personal database with new spectra and analyze mass spectra based on comparing the obtained protein profiles with a personal library of reference spectra. A result of this work is creation of the corresponding subsection of this database of mass spectra at the level of *A. paragallinarum* species, which can be used in the future to identify strains and isolates of the infectious rhinitis pathogen in chickens.

3. Proteomic characterization of *Avibacterium paragallinarum* strains and isolates (Russia and Belarus, 2015) from chickens with respiratory pathology

Peaks, m/z	S/N intensity	Peak frequency, %	Presence or absence of the peak in *A. paragallinarum* ATCC 29545/ratio of S/N
2067-2069	4.4-12.4	43	–
3109-3111	4.1-5.9	36	–
3208-3210	4.0-8.6	57	–
3580-3582	4.0-10.1	71	+/6.2
3930-3932	4.3-9.5	14	–
4186-4188	4.3-8.1	71	+/6.6
4278-4280	4.6-11.7	36	–
4433-4435	4.4-7.6	57	+/5.1
4480-4482	4.0-7.9	28	+/6.1
4667	9.2-39.7	100	+/19.3
4768-4770	4.2-7.6	57	+/5.2
5011	4.9-8.8	86	+/5.7
5065	4.8-9.6	78	+/6.4
5098-5060	8.7-16.7	57	–
5347-5349	4.3-5.3	28	+/4.2
5542-5543	5.1-7.7	21	–
5567-5569	4.4-7.7	64	–
6084	5.0-14.7	78	–
6261-6263	4.2-17.7	50	–
6420	6.4-24.7	86	+/13.9
6552-6553	4.3-16.8	36	+/16.8
6643-6645	4.3-5.7	21	–
6855-6857	4.3-5.1	28	–
7164	10.7-22.7	93	+/15.6
7308-7310	7.6-11.2	50	–
7864-7866	5.9-12.2	57	–
8373-8375	6.9-10.7	64	–
8522-8524	4.4-6.7	28	–
9317-9319	10.0-10.7	14	–
9334	24.0-62.3	100	+/33.2
9536-9538	7.2-12.3	71	–
9964-9965	10.1-13.0	50	–

Thus, the revealed biochemical properties indicate a heterogenicity of *Avibacterium paragallinarum* isolates from chickens with respiratory pathology from poultry farms in regions of Russia and Belarus, while the hemagglutination inhibition test indicates their belonging not only to one serogroup, but also to

one serotype. The homology of the isolates at the group level is confirmed by multiplex qPCR-based serotyping. We identified characteristic peaks (m/z 4768-4770 and 5347-5349) in proteomes of the isolates similar to those in the reference *A. paragallinarum* ATCC strain No. 29545. These peaks are added to the mass spectrometer database. This expands the existing database with new spectra and profiles obtained for the tested strains, which improves the reliability of species identification. Based on the studied biochemical, antigenic and proteomic characteristics, three isolates of *A. paragallinarum* were deposited in the State Collection of Microorganism Strains of the Federal Center for Animal Health as strain No. 1818, strain No. 5111, and strain No. 1116.

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