Comparative analysis of proteomes of bacteria representatives of the genus *Bordetella*

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Abstract. The article is devoted to the study of proteomes of poorly studied representatives of genus *Bordetella*. According to the results of the study, molecular masses of and protein masses of bacteria of genus *Bordetella* were extracted. In the NCBI system, in accordance with the data obtained by comparing genomes of *Bordetella* genus, an in-silico analysis of the correspondence of annotated proteomes was performed and the idea was identified by proteome of these species.

1. Introduction

Based on phenotypic and genotypic data, the bacteria of *Bordetella* genus, as well as the species *Alcaligenes* and *Achromobacter* are part of the family *Alcaligenaceae*, belonging to the group of beta-proteobacteria [1-2]. The bacteria of *Bordetella* genus are the pathogens of wide range of lung and bronchial infections, from asymptomatic colonization to lethal pneumonia, both in humans and animals, so they have received considerable attention in medicine and veterinary medicine. Genus *Bordetella* includes 16 species, among which three species: *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* are of very great biomedical importance. *B. holmesii*, *B. avium* *B. petrii*, *B. hinzii*, *B. pseudohinzii*, *B. trematum*, *B. ansorpii*, *B. bronchialis*, *B. flabilis*, *B. sputigena*, *B. muralis*, *B. tumbae*, *B. tumulicola* have also been added to this genus in recent times [2-11].

Biological characteristics of bacteria are the basis for the development of their identification methods. Currently, bacterial classification is undergoing constant changes due to the emergence of new methods, tools and principles of classification. The most modern and generally accepted methods are molecular-genetic methods of species identification - They are all based on the characteristics of the uniqueness of particular genome. However, they do not allow us to take into account phenotypic features of genus, species, group or strain. Methods of protein (proteomic) profiling have these features.

The most modern methods of profiling using mass spectrometry are, however, rather expensive equipment, qualification requirements and need for specific software do not allow to use it in routine studies. For these purposes, there are methods for separating biological high-molecular compounds (including proteomes) in electric field.
2. Materials and methods

For the research we used reference-strain B. holmesii ATCC 51541, B. avium ATCC BAA-1003, B. hinzii ATCC ATCC 51784, B. trematum ATCC 700309, B. petrii ATCC BAA-461, taken from the museum «Department of Microbiology, Virology, Epizootology and Veterinary and Sanitary Expertise FSBEI HE «Ulyanovsk SAU».

The Bordet Gengou (Becton and Dickinson, USA) was used to accumulate bacterial mass of the strains; to remove extracellular proteases and proteins of flying medium, cells were centrifuged at 5000g during 3 minutes and suspended in potassium-phosphate buffer at 37 °C. The second step was to add a hot (95 °C) SDS buffer to the sediment and mix it thoroughly (ELMI CM-50, Czech Republic). The destruction of bacterial cells was carried out on the Soniprep 150 ultrasonic homogenizer (MSE, UK). The samples were incubated at 95 °C for 5 minutes. The samples were cooled to 20 °C and 250 µl of 2x buffer SDS-PAGE sample was added, and incubated for 20 min at room temperature. To obtain bacterial proteins in supernatant, the samples were centrifuged at 125000g for 30 minutes.

Electrophoresis was performed in a vertical electrophoresis chamber (Mini-protean tetra, Bio-rad) in 8-16% polyacrylamide gel (Criterion TGX,Bio-Rad). For further analysis, the molecular weight marker is 3.5-245 kDa (Abcam, UK). Further in-silico analysis was performed using GelAnalizer2010a.

3. Results

As the result of our study, we obtained an electrophoregram of representatives of genus Bordetella (figure 1). We have established the molecular weights of proteins that are part of bacterial cells of these species.

Figure 1. Electrophoretogram. The result of proteomic separation of representatives of genus Bordetella. Strains: 1-2 Bordetella avium, 3-4 Bordetella petrii, 5-6 Bordetella trematum, 7-8 Bordetella holmesii ATCC 51541, 9 Bordetella bronchiseptica, 10 Bordetella hinzii, 11 protein-marker of molecular weight.
As a result of in-silico analysis, we established molecular weights of proteins of *B. holmesii* bacteria, which were 210, 174, 150, 38, 129, 113, 100, 88, 72, 59, 51, 43, 36, 31, 29, 25, 23, 20 kDa, *B. hinzii* - 234, 206, 171, 119, 93, 87, 77, 67, 52, 43, 40, 39, 37, 33, 30, 25 kDa, *B. trematum* - 181, 119, 102, 85, 77, 67, 54, 45, 38, 34, 30, 29, 28, 27, 26, 25, 23 kDa. In bacteria *B. petrii* as a result of the analysis we found protein molecules of the following: 174, 158, 143, 133, 120, 104, 88, 77, 69, 52, 43, 40, 34, 28, 26, 25, 24, 23, 22, 21, 20 kDa.

In NCBI system, in accordance with the data obtained by comparing genome of *B. hinzii*, in-silico analysis of concordance with annotated proteomes was carried out, 4522 proteins were identified, and 5574 proteins were identified in *B. avium* bacteria. At the same time, 4006 proteins were found in bacteria of *B. trematum* genus. The smallest number was annotated in bacteria of *B. holmesii* genus - 2841. In bacteria of *B. petrii* genus 5695 proteins were found in the NCBI system as a result of analysis.

4. Discussion
As a result of the study, proteomes of bacteria of *Bordetella* genus were profiled, and its biological characteristic was given according to electrophoregram and in-silico analysis. According to the results of the analysis, it becomes obvious that each of identified proteins can correspond to antigen. Thus, this study can be used in further work of studying antigenic structure and developing appropriate serological diagnostics.

5. Conclusion
As a result of carried out research, we gave a comparative analysis of proteomes of bacteria of *Bordetella* genus. As a result of the study, we obtained electrophoregram, and molecular weights of proteins that are part of bacterial cells of these species were established. In NCBI system, in accordance with obtained data by comparing genome of *B. hinzii*, in-silico analysis of compliance with annotated proteomes was performed, 4522 proteins were identified, and 5574 proteins were identified in *B. avium* bacteria. At the same time, bacteria of *B. trematum* species were found to have 4006 proteins. The smallest number was annotated in bacteria of *B. holmesii* species - 2841. In bacteria of *B. petrii* species 5695 proteins were found as a result of the analysis in NCBI system.

According to the results of the analysis, it becomes obvious that each of identified proteins can correspond to an antigen. Thus, this study can be used in further study of antigenic structure and development of appropriate serological diagnostics.

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