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STUDY OF *B. ANTHRACIS* UA-07 VACCINE STRAIN PATHOGENICITY FACTORS' CHANGES AFTER LYOPHILIZATION

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A lot of scientific papers of the national and international literature devoted to characterization of the biological properties of anthrax. It is well-known fact that in natural conditions along with the typical strains, atypical forms of anthrax agent are found quite often. It is believed that reproduction of anthrax in the soil is accompanied by the irreversible loss of its pathogenic properties. But there is also a view that some of these atypical strains in certain experimental conditions are able to repair and even enhance its pathogenicity. We therefore decided to investigate the changes of anti-lysozyme activity and toxigenicity titer of B. anthracis UA-07 culture after lyophilization and passage through the culture media and laboratory animals. It was found that the lyophilized vaccine strain of the anthrax pathogen is capable repair anti-lysozyme activity and increase toxin titer.

Anthrax, properties, anti-lysozyme activity, toxigenicity.

Problem statement, analysis of recent publications on its solution. Anthrax is a disease common to humans and animals, does not lose its relevance today. The relevance of studying the properties of this agent, improvement of its isolation and identification methods issued with potential threat of biological terrorism acts.

Analysis of OIE reports testifies about the prevalence of anthrax in many countries of Europe and Asia, as well as its considerable distribution among different species of animal.

A lot of research papers and recommendations in the national and international literature devoted to characterization of the biological properties of anthrax. It is well-known fact that in natural conditions along with the typical strains, atypical forms of anthrax agent are found quite often to characterize which using only traditional laboratory methods of research is not enough. [10, 11]

The issue about the ecology of anthrax pathogen in the soil can not be considered solved, as in this regard there are different points of view. It is believed that reproduction of the anthrax pathogen in soil is accompanied by the irreversible loss of its pathogenic properties.

This statement is supported by the evidence of isolation from old cattle burial grounds atypical *Bacillus anthracis* cultures, which are not able to form the

capsule, as well as cultures forming mucoid colonies in the air. [7] Recently, however, the data have appeared showing that some of these atypical strains in certain experimental conditions are able to repair or even increase their virulence with a corresponding change of the genotype. [2]

One of the signs of pathogenicity and ability to persistence of *B. anthracis* in macroorganism is the production of substances that inactivate factors of nonspecific anti-infectious protection such as lysozyme, complement, interferon, as well as specific protection – immunoglobulins, etc. The presence of such factors as lysozyme activity in bacteria provides them with the benefits of growth and reproduction in the living organism. The study of anti-lysozyme activity (ALA) allows to define *B. anthracis* pathogenicity level in relation to the body defences.

Anthrax pathogen is able to produce and secrete exotoxin, which consists of three factors: the protective, edema and lethal. [4, 12]

Field and vaccine culture of *B. anthracis* are very different by toxicity level and quantity of toxin. Field isolates produce exotoxin in small quantities but it is highly aggressive when 70% of its composition contains lethal and edema factors and only 30% – protective.

Vaccine strains differ from isolates by the intensity and quantities of produced exotoxin, the protective factor of exotoxin prevailing in them. [1, 8, 13]

In our work, we decided to investigate the variation of some factors of pathogenicity of the vaccine strain of the anthrax pathogen after lyophilization and after passage through the culture media and laboratory animals.

The purpose and objectives of the study is to identify changes of ALA and toxigenicity titer of *B. anthracis* UA-07 reference culture after lyophilization and after passage through the culture media and laboratory animals.

Materials and methods. We used the reference unencapsulated spore-forming *B. anthracis* UA-07 strain.

ALA was determined by microbiological method: lyophilised cultures of microorganisms cultured in a nutrient medium which contains lysozyme in concentration from 0.2 to 25 g/cm^3. The inactivation of lysozyme was determined by growth of micrococcus indicator culture on nutrient medium. [3, 6, 9]

To determine the toxin production disk precipitation reaction was used modified by Zaviriukha A.I. and Stepanjuk O.P. [5]

Data on ALA and toxigenicity definition were determined after lyophilization and in 1 month after its passage through the medium (meat-peptone broth, plain agar) and laboratory animals (outbred guinea pig, n = 3).

The results of research. After *B. anthracis* UA-07 pathogen released from the lyophilic protective environment by planting in meat-broth with subsequent passages on plain agar it demonstrated a sufficiently high ALA – 15 g/cm^3 (Figure 1).

After month of culture passages through the culture media, as well as laboratory animals, this strain increased ALA to 26 mg/cm^3. That is, research culture ALA has increased in 1.7 times compared with the culture, freed from protective lyophilic medium.

We have also determined toxigenicity of *B. anthracis* UA-07 strain. Studies have shown that after lyophilization the anthrax causative agent toxins level in disk precipitation reaction was 1 : 16. In a month of culturing on media, the titer inreased to 1 : 68, indicating an increase of toxin production by the culture.

Conclusions

It was found that the lyophilized vaccine strain of *B. anthracis* UA-07 is capable to repair ALA after cultivation on nutrient media and passage through animals (26 mg/cm^3). During this time, the titer of the strain toxigenicity decreased from 1: 16 to 1: 68, which indicates an increase of the toxin production by this culture and is an important indicator for the vaccine strains.

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Характеристике биологических свойств возбудителя сибирской язвы в отечественной и зарубежной литературе посвящено немало научных работ и рекомендаций. Известно, что в природных условиях вместе с типичными штаммами довольно часто встречаются атипичные. Существует мнение, что размножение возбудителя сибирской язвы в почве сопровождается необратимой утратой его патогенных свойств. Но также существует мнение, что некоторые из таких атипичных штаммов в определенных условиях эксперимента способны восстанавливать и даже повышать свою патогенность. Поэтому мы решили исследовать изменения уровня антилизоцимной активности и титра токсигенности референтной культуры B. anthracis UA-07 после лиофилизации и пассирования через питательные среды и лабораторных животных. Установлено, что лиофилизированный вакцинный штамм возбудителя сибирской язвы способен восстанавливать антилизоцимную активность и понижать титр токсинообразования.

Сибирская язва, свойства, антилизоцимная активность, токсигенность

Характеристиці біологічних властивостей збудника сибірськи у вітчизняній і зарубіжній літературі присвячено чимало наукових робіт та рекомендацій. Відомо, що за природних умов разом з типовими штамами досить часто зустрічаються атипові. Існує думка, що розмноження збудника сибірки в ґрунті супроводжується необоротною втратою його патогенних властивостей. Але також існує інша думка, що деякі з таких атипових штамів за певних умов експерименту здатні відновлювати і навіть підвищувати свою патогенність. Тому ми вирішили дослідити зміни рівня антилізоцимної активності і титру токсигенності референтної культури B. anthracis UA-07 після ліофілізації і пасажування через живильні середовища і лабораторних тварин. Встановлено, що ліофілізований вакцинний штам збудника сибірки здатний відновлювати антилізоцимну активність і знижувати титр токсиноутворення.

Сибірка, властивості, антилізоцимна активність, токсигенність