UDC 574.3: 579.26
© 2015

**O.M. Zhukorskiy,**
**doctor of Agricultural Science, National Ukrainian Academy of Agrarian Sciences.**
**A.V. Hulai,**
**candidate of Biologycal Sciences (PhD equivalent), Institute of Agroecology and Environmental Management of National Ukrainian Academy of Agrarian Sciences.**

# Reaction of the populations of erysipelothrix rhusiopathiae pathogenic bacteria to the influence of northern pike skin secretions

**Purpose.** The research is to establish the reaction of E. rhusiopathiae pathogenic bacteria populations to the influence of northern pike skin secretions. **Methods**. Samples of fish skin mucus were taken with the help of filter paper, extracted, sterilized, and tested on the cultures of *E. rhusiopathiae*. **Results.** The content of *E. rhusiopathiae* bacteria in the test material was bigger than in the control samples; in the 1:10 dilution samples of northern pike skin secretions it was 3.26 times bigger; in 1:100 dilutions – 2.54 times bigger; in 1:1000 samples – 1.20 times bigger.**Conclusions**. The populations of *E. rhusiopathiae* pathogenic bacteria undergo a stimulating influence produced by northern pike due to the secretion of biologically active substances in the fish environment by its skin glands

***Key words:*** *Erysipelothrix rhusiopathiae, northern pike, skin secretions, stimulating influence.*

Intensive manufacturing of agricultural produce at livestock complexes and farms often causes deterioration of the surrounding environment. Bacterial contamination of soil and water with pathogenic and potentially pathogenic microflora is especially dangerous. In this regard, control and optimization of the biogeocenosis state is very relevant in the area of livestock complexes. To successfully achieve these objectives, it is necessary to obtain information about the features of ecological interactions between pathogens of infectious diseases and the components of aquatic and groundwater ecosystems. *Erysipelothrix rhusiopathiae* bacteria are quite widespread in nature and resistant to adverse environmental factors; in addition, these microorganisms are pathogenic for both animals and humans [1]. Erysipelas, a disease caused by *E. rhusiopathiae*, leads to great losses in pig breeding. Infected and clinically healthy pigs that are bacilli carriers release pathogens into environment with faeces and urine [7, 10, 11]. Soil and water, contaminated with pus and farm runoff serve as the habitat for *E. rhusiopathiae*. Getting into natural groupings, *E. rhusiopathiae* bacteria take part in a variety of ecological relationships with the biocenosis components, are spread by food chains and distributed across the territory causing the risk of infecting humans and animals.

Water reservoirs play an important role in human agricultural activities and are actively used for public recreation. Scientific literature contains very little information about the nature and consequences of *E. rhusiopathiae* interactions with different types of aquatic organisms. We set the task to explore the features of ecological relationships of *E. rhusiopathiae* pathogenic bacteria with the components of biocenosis in fresh water – with algae [4], higher plants [5-12], and animals [3]. Fish farming is one of the most important areas of economic use of water; in this regard, the study of *E. rhusiopathiae* interactions with commercially valuable fish species is of particular interest. We know that fish can be a source of infecting humans with *E. rhusiopathiae* [8,9].

One of the most common species of freshwater (river, lake, and pond) fish in Ukraine is northern pike (*Esox lucius*). To study the relationship with *E. rhusiopathiae* bacteria, northern pike was chosen due to being

widespread in different types of water reservoirs in Ukraine and the fact that this fish species is placed at the top of most freshwater food chains [6].

The purpose of the research is to establish the reaction of *E. rhusiopathiae* pathogenic bacteria populations to the influence of northern pike skin secretions.

**Material and methods of research.**

The living fishes required for studies were bought in special stores. When choosing the fishes, skin integrity and satisfactory appearance were paid attention to. On the laboratory table, the fishes were fixed on one side. The filter paper, previously soaked with water, was placed on the skin of the upper part of the fishes. After 1 minute, the paper was removed with clean tweezers and placed in glass tubes to obtain aqueous solutions of skin secretions. The volume of water required for extraction was calculated on the basis of the ratio of 0.1 $cm^3$ of water per 1 $cm^2$ of filter paper area with fish skin secretions. After 1 hour, the water from the tubes was sterilized using bacterial filters with the pore size of <0.2 microns.

The study was based on the cultures of *E. rhusiopathiae* bacteria (BP-2 strain), which were grown during 48 hours at the temperature of +36.7±0.3ºC in the brain and heart infusion broth (AES Chemunex, France). Experiment prototypes were prepared using the method of serial dilutions; after introducing the test cultures of bacteria these prototypes contained fish skin secretions in the following dilutions: 1:10; 1:100; 1:1000; 1:10000. For control, the ratio of sterile water and bacteria cultures, similar to the experimental one, was used. The initial density of bacteria in the samples was the same because culture inoculates of *E. rhusiopathiae* were selected from one tank and were identical in volume.

Determination of *E. rhusiopathiae* population density was carried out after 48 hours by seeding 0.1 $cm^3$ of test samples in $1\times10^{-3}$ and $1\times10^{-4}$ consecutive dilutions on the surface of brain and heart agar (AES Chemunex, France). The samples were cultured for 72 hours at the temperature of +36.7±0.3ºC, followed by counting the grown colonies and calculating the average number of the colonies of forming units (CFU) of bacteria on 1cm3.

**The results of the study.**

The results of studying the reaction of *E. rhusiopathiae* populations to the influence of northern pike skin secretions are given in the table.

**The content of *E. rhusiopathiae* in experimental and control samples under the influence of northern pike skin secretions ($\times10^6$ CFU / $cm^3$)**

| No. of experiment | Experiment (dilution of secretions) | | | | Control |
|---|---|---|---|---|---|
| | ***1:10*** | ***1:100*** | ***1:1000*** | ***1:10 000*** | |
| 1 | 19.70 | 15.50 | 7.50 | 6.20 | 6.00 |
| 2 | 20.10 | 14.80 | 7.70 | 6.50 | 6.40 |
| 3 | 20.50 | 15.30 | 7.10 | 6.40 | 5.90 |
| 4 | 19.40 | 16.00 | 6.90 | 6.10 | 6.20 |
| 5 | 19.00 | 15.40 | 7.60 | 6.30 | 6.00 |
| 6 | 20.60 | 15.80 | 7.00 | 6.20 | 6.10 |
| M* | 19.88 | 15.47 | 7.30 | 6.28 | 6.10 |
| σ | 0.63 | 0.42 | 0.34 | 0.15 | 0.18 |
| m | 0.28 | 0.18 | 0.15 | 0.06 | 0.08 |
| For dilution 1:10 | | t = 47.02 | $t_{кр}$ = 4.59; | | P ≤ 0.001 |
| For dilution 1:100 | | t = 46.07 | $t_{кр}$ = 4.59; | | P ≤ 0.001 |
| For dilution 1:1000 | | t = 6.97 | $t_{кр}$ = 4.59; | | P ≤ 0.001 |
| For dilution 1:10 000 | | t = 1.77 | $t_{кр}$ = 2.23; | | P ≤ 0.05 |

* Note. Hereinafter: M –arithmetic mean; σ - standard quadratic deviation;

m - average error; t - Student factor; $t_{cr}$ = critical parameter t; P - probability level.

In case of the high content of fish skin secretions in the experimental samples (dilution index 1:10), the density of *E. rhusiopathiae* populations was 3.26 times bigger than in the control samples. The increase in the degree of diluting skin gland secretions in the experimental samples of the studied fish species to 1:100 and 1:1000 marked the reduction in the content of *E. rhusiopathiae* in the experimental samples compared with the control samples by 2.54 and 1.20 times respectively. The existence of the stimulating influence of northern pike on E. *rhusiopathiae* populations can be statistically proved by the significant difference in the average content of bacteria in the experimental and control samples. However, in case of 1:10,000 dilutions of the skin secretions of experimental fish species, the stimulating influence on *E. rhusiopathiae* populations disappears, as indicated by the lack of a statistically significant difference between the content of bacteria in the experimental and control samples.

Data analysis conducted with the help of correlation analysis indicates that the reduction in the amount of bacteria in the experimental samples is associated with a decrease in the concentration of the secretions of fish skin glands ($r = 0.83$) [2].

The study results indicate that the products of pike skin glands secretions are a favorable environment for the existence of *E. rhusiopathiae* pathogenic bacteria. We can assume that in vivo *E. rhusiopathiae* may accumulate on the skin of freshwater fish in case of bacterial contamination of water.

Secreting mucus in the environment of *E. rhusiopathiae*, northern pike is thus changing the conditions of bacteria existence, which results in the formation of the topical type of biocenotic connections between these species.

The obtained data should be considered when assessing the ways of *E. rhusiopathiae* circulation in environmental objects and for the prevention of erysipelas disease in humans and animals. In particular, in case of massive catch and processing of freshwater fish, the workers should protect their hands with rubber gloves and treat the injured skin areas with antibacterial agents.

**Conclusions**.

The populations of *E. rhusiopathiae* pathogenic bacteria undergo a stimulating influence produced by northern pike due to the secretion of biologically active substances in the fish environment by its skin glands.

**Bibliography**


1. Borisovich, Ju.F. & Kyrillov L.V. (2003) Infekcionnye bolezni zhivotnyh: Spravochnyk – M.: Agropromizdat.
2. Brandt, Z. (2003) Analyz dannyh. Statisticheskie i vychislytel'nye metody dlja nauchnyh rabotnikov i inzhenerov. – M.: Mir, OOO "Yzdatel'stvo AST".
3. Hulai, A.V. & Zhukorskiy, O. M., & Hulai, V.V. (2014) Krjakva (Anas platyrhynchos L.) kak yzstochnik vozbudytelja rozhi // Bioraznoobrazie nazemnyh i vodnyh zhivotnyh. Zooresursy. Materialy II Vserosyjskoj nauchnoj Ynternet konferencii s mezhdunarodnym uchastiem. Kazan', 39-41.
4. Gulay, A. V., & Zhukorskiy, O. M. (2013). Biotychni zviazky patohennykh bakterii Erysipelothrix rhusiopathiae ta synozelenykh vodorostei Microcystis pulverea. Biolohiia tvaryn, 15 (3), 9-16.
5. Gulay, A. V., & Zhukorskiy, O. M. (2013). Formuvannia ekolohichnykh zviazkiv Erysipelothrix rhusiopathiae z Riccia fluitans u hidrobiotsenozakh. Rybohospodarska nauka Ukrainy, 4, 17-24.
6. Shcherbukha, A. Ya. (1981). *Ryby nashykh vodoim*. Kyiv: Radianska shkola.
7. Cussler, K., & Balks, E. (2001) 100 years of erysipelas prophylaxis: significance and reduction of animal experiments // ALTEX, 18(1), 29–33.

8. Opriessnig, T., & Shen, H. G., & Bender, J. S. (2013) Erysipelothrix rhusiopathiae isolates recovered from fish, a harbour seal (Phoca vitulina) and the marine environment are capable of inducing characteristic cutaneous lesions in pigs // Journal of Comparative Pathology, 148 (4). 365–372.
9. Lehane, L, &. Rawlin, T. (2000) Topically acquired bacterial zoonoses from fish // Medical Journal of Australia, 173 (5). 25–29.
10. Yamamoto, K. & Kijima, M. & Takahash, T. (1999) Serovar, pathogenicity and antimicrobial susceptibility of erysipelothrix rhusiopathiae isolates from farmed wild boars (Sus scrofa) affected with septicemic erysipelas in Japan // Res. Vet. Sci. 67 (3), 301–303.
11. Wood, R.L. & Beran, G. W. (1994) Erysipelothrix infections //in: Handbook of Zoonoses, 2 ed. CRC Press. 83–91.
12. Zhukorskiy, O. M. & Gulay O. V & Gulay V. V. & Tkachuk, N. P. (2014) Changes in the Population Density of Pathogenic Microorganisms in Response to the Allelopathic Effect of Thypha Latifolia // Agricultural sciens and practice, 1. 31–36.