UDC 574.3:579.26

THE IMPACT OF ALLOCHTHONOUS FISH SKIN SECRETIONS ON THE *ERYSIPELOTHRIX RHUSIOPATHIAE* PATHOGENIC BACTERIA

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Received on July 22, 2015

Aim. To find out the impact of allochthonous fish skin secretions on the *E. rhusiopathiae* pathogenic bacteria cultures. **Methods**. The skin of live fishes was covered with filter paper, which was removed after 1 minute; then soluble components at the rate of 0.1 cm^3 of water per 1.0 cm^2 of paper area were extracted. The resulting solutions of fish skin secretions were sterilized by vacuum filtration through filters with < 0.2 μm pore diameter. The test samples contained *E. rhusiopathiae* cultures and fish skin secretions in dilutions of 1:10, 1:100, 1:1,000 and 1:10,000. The control samples contained a similar ratio of sterilewater and the bacteria under study. **Results**. The presence of freshwater fish skin secretions in water-soluble extracts causes the increase in *E. rhusiopathiae* culture density. The intensity of the revealed stimulation effect on *E. rhusiopathiae* depends on the concentration of fish skin secretions in test samples. **Conclusions**. Freshwater fish (*Hypophthalmichthys molitrix, Ctenopharyngodon idella)* skin secretions contain substances that exert a stimulating effect on the *E. rhusiopathiae* culture. The ability of the bacteria under study to use fish skin secretions indicates the formation of interspecific trophic links. To prevent and eliminate the foci of infection caused by *E. rhusiopathiae*, one has to consider the possibility of the pathogenic bacteria persistence on the skin of freshwater fish.

Keywords: *Erysipelothrix rhusiopathiae,* fish skin secretions, a stimulating effect.

DOI: 10.15407/agrisp2.03.022

INTRODUCTION

Successful modern management of agriculture is impossible without any consideration and application of a whole system of complicated interspecific connections, formed both in natural and anthropogenically modified ecosystems.

High demand for fish products and the profitability of culturing fish for sale determine great perspectives for optimal economic application of freshwater ecosystems. However, their efficient and safe use is impossible without a sufficient amount of data, reflecting the system of ecologic relations between different groups of hydrobionts.

It is known that a number of pathogenic microorganisms are capable of existing in water bodies for a long time, and the infection transmission to people and animals via water is the most dangerous mode. One of the pathogenic microorganism kinds, which can be transmitted in the abovementioned way, is *E. rhusiopathiae* – gram-positive bacteria in the form of straight or slightly bent rods, which do not form spores and capsules and have high resistance against unfavorable impact of environment [1]. The bacteria of this kind cause a disease of humans, domestic and wild animals and birds, called *Erysipelas*. *E. rhusiopathiae* were also isolated for a number of ectothermic animals, including cancriforms and fish, for which these bacteria are

saprophytes [2]. High prevalence, a considerable number of possible hosts of *E. rhusiopathiae*, as well as significant economic loss due to erysipelas conditioned the necessity of detailed study of ecologic connections of this kind of pathogenic bacteria with freshwater ecosystem components, in particular, with fish.

E. rhusiopathiae are capable of penetrating a human organism via microinjuries of hands, received while fishing or handling fish. It is believed that bacteria are located in the slime matrix, covering the surface of fish [3–5]. At the same time there are no results of experiments on studying the impact of skin secretions of freshwater fish regarding *E. rhusiopathiae* in the scientific literature.

The aim of the work was to study and to estimate the secretions of cutaneous glands of freshwater fish on *E. rhusiopathiae* cultures.

MATERIALS AND METHODS

The most common and economically important fish species were selected for the study: *Hypophthalmichthys molitrix* Valenciennes, 1844 and *Ctenopharyngodon idella* Valenciennes, 1844. Naturally both species live in the basin of Amur River. *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* were brought to many countries of Europe, North America and Asia and naturalized in the middle of the last century, *i. e.* they are typical allochthonous species for this territory. At present *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* are common objects of aquaculture, cultured on industrial scale.

The samples of skin secretions were isolated from living fish (n = 20) which were fixed sideways to the table. Slightly watered filter paper was placed on the upper surface of fish. After 1 minute exposition the paper was removed and covered with water for 1 h to extract water-soluble components of the secretions at the rate of 0.1 cm^3 of water per 1.0 cm^2 of filtration paper area. The aqueous solutions of fish skin secretions were sterilized by vacuum filtration through bacterial filters with < 0.2 μm pore diameter (Minisart, Germany).

Bacteriophages could also be present in the selected samples, which was undesirable. A biosample was taken to check the possibility of the latter. One part of the obtained volume of smears was sterilized in the autoclave, the other – filtered through filters with the pore diameter of ≤ 0.2 μm, which intercepted bacteria but could not detain bacteriophages. Then the smears were added to *E. rhusiopathiae* cultures. The samples, containing the smears after the autoclave, were used as control, and the ones with the smears after the filtration – as the experiment. The content of bacteria in the experiment and the control was compared 12, 24, and 48 h later. The presence of bacteriophages in the test samples was evident due to the cell lysis and much lower content of bacteria compared to the control. If the described effect was revealed, the smears, received from the corresponding specimen of fish, were not used in further studies.

The cultures of *E. rhusiopathiae* bacteria were cultivated at 36.7 ± 0.3 ºC for 48 h with brain-heart infusion broth (AES Chemunex, France).

The method of serial dilutions was used to create the concentration gradient in the test samples – 1:10; 1:100; 1:1,000; 1:10,000. The control samples contained sterile water and bacterial cultures. The same initial content of *E. rhusiopathiae* in the experiment and the control was achieved, using the inoculates from one bacterial culture for each series of tests. The prepared samples were kept in the temperature range of 18−20 °C for 48 h.

The registration of results was made after plating the samples from test and control samples in the dilutions of $1 \cdot 10^{-3}$, $1 \cdot 10^{-4}$, $1 \cdot 10^{-6}$ on the surface of the brain-heart agar (AES Chemunex). The number of grown

Table 1 The content of *E. rhusiopathiae* in the test and control samples in conditions of the effect of cutaneous secretions of *H. molitrix* (× 10^6 CFU/cm^3)

No. of experiment	Experiment (dilution of secretions)				Control
	1:10	1:100	1:1000	1:10 000	
1	6.40	5.30	2.54	2.29	2.16
2	6.20	4.80	2.75	2.11	2.09
3	5.90	5.20	2.66	1.98	2.21
4	6.50	5.10	2.49	2.06	2.19
5	6.60	5.30	2.59	2.15	2.27
6	6.10	4.90	2.71	2.00	2.13
M	**6.28**	**5.10**	**2.62**	**2.10**	**2.18**
σ	0.26	0.21	0.10	0.11	0.06

For the dilution 1:10 − t = 33.85 at t_{cr} = 4.59, P ≤ 0.001; 1:100 − t = 29.86 at t_{cr} = 4.59, P ≤ 0.001; 1:1,000 − t = 8.43 at t_{cr} = 4.59, P ≤ 0.001; 1:10,000 − t = 1.32 at t_{cr} = 2.23, P ≤ 0.05.

Note. Here and in Table 2: M – mean arithmetic; σ – mean square deviation; m – mean deviation; t – Student's coefficient; t_{cr} – critical value of the index t; P – probability level.

colonies were counted after the cultivation at 36.7 ± ± 0.3 ºC for 72 h, and the average number of colony-forming units (CFU) of bacteria per 1 cm^3 estimated.

RESULTS AND DISCUSSION

The estimation and subsequent comparison of the density of *E. rhusiopathiae* cultures in the test and control samples demonstrated that the secretions of cutaneous glands of *H. molitrix* exert stimulating effect on the cultures of bacteria (Table 1). The highest stimulating effect was observed in the samples with low dilution (1:10) of cutaneous secretions of the fish species under study. On average the density of *E. rhusiopathiae* cultures in the test samples with this dilution was 2.89-fold higher than that for the control samples.

The index of the stimulating effect on *E. rhusiopathiae* cultures was considerably lower in the series of experiments with the dilution of skin secretions of *H. molitrix* 1:100 i 1:1,000, the density of cells was 2.34 and 1.21 times higher respectively, compared to the control. In the samples with the dilution of fish secretions of 1:10,000 the difference of the density of bacterial cultures in the test and control samples was not reliable, which is proven by the data of the statistical data processing.

The content of skin secretions of *C. idella* in the test samples for the dilution of 1:10 conditioned the 3.06-fold increase in the CFU number for *E. rhusiopathiae* compared to the control (Table 2). The number of bacteria in the samples with the 1:100 and 1:1,000 dilution of cutaneous gland secretions of the fish species under study was 2.22 and 1.08 times higher respectively compared to the control. The statistically reliable difference in the CFU content of bacteria in the test and control samples in the dilution range of 1:10–1:1,000 proves the presence of the stimulating effect of the skin secretions of *C. idella* on *E. rhusiopathiae* cultures. However, the evident stimulating effect on *E. rhusiopathiae* in absent in the samples with the 1:10,000 dilution of the secretions of the species under study, which is confirmed by the absence of statistically reliable difference of the content of bacteria in the test and control.

Table 2. The content of *E. rhusiopathiae* in the test and control samples in conditions of the effect of cutaneous secretions of *C. idella* ($\times 10^6$ CFU/cm^3)

No. of experiment	Experiment (dilution of secretions)				Control
	1:10	1:100	1:1000	1:10 000	
1	1.85	1.41	0.70	0.61	0.66
2	1.81	1.39	0.67	0.58	0.62
3	1.97	1.43	0.65	0.62	0.58
4	1.88	1.35	0.69	0.57	0.60
5	1.93	1.40	0.68	0.61	0.65
6	1.99	1.31	0.66	0.59	0.63
M	**1.91**	**1.38**	**0.68**	**0.60**	**0.62**
σ	0.07	0.04	0.02	0.02	0.03
m	0.03	0.02	0.01	0.01	0.01

For the dilution of 1:10 – t = 37.45 at t_{cr} = 4.59, P ≤ ≤ 0.001; 1:100 – t = 31.80 at t_{cr} = 4.59, P ≤ 0.001; 1:1,000 – t = 3.26 at t_{cr} = 2.23, P ≤ 0.05; 1:10,000 – t = 1.66 at t_{cr} = 2.23, P ≤ 0.05.

The study of the experimental data obtained using the correlation analysis [6] testifies to the fact that the index of bacteria content in the test samples is directly related to the concentration of the secretions of cutaneous glands of fish. For instance, the coefficient value of the correlation between the abovementioned indices is 0.81 for *H. molitrix* and 0.87 – for *C. idella*.

Higher density of bacterial cultures in the test samples compared to the control is explained by the fact that the secretions of cutaneous glands of fish species under study contain nutrients, which can be actively used by *E. rhusiopathiae*. These data testify to the formation of interspecific trophic relations between the *E. rhusiopathiae* bacteria and *H. molitrix* and *C. idella* fish. Therefore, one should not neglect the probability of the presence of *E. rhusiopathiae* on the surface of fish in conditions of different types of natural and artificial water bodies.

To prevent the infection of erysipelas during the work, related to catching and handling freshwater fish, the workers should protect skin on their hands from possible injuries, and apply antibacterial agents in case of injuries immediately.

CONCLUSIONS

The presence of secretions of cutaneous glands of *H. molitrix* and *C. idella* fish in the environment up to the dilution of 1:10,000 (*in vitro*) determines the stimulating effect in the cultures of *E. rhusiopathiae* bacteria.

The direct correlation between the density of *E. rhusiopathiae* cultures and the concentration of fish skin secretions in the test samples was revealed.

The capability of *E. rhusiopathiae* bacteria to use the substances, located in the secretions of cutaneous glands of *H. molitrix* and *C. idella*, indicates the formation of interspecific trophic relations.

To prevent and eliminate the foci of infection caused by *E. rhusiopathiae*, one has to consider the possibility of the pathogenic bacteria persistence on the skin of freshwater fish.

Вплив секретів шкірних залоз алохтонних видів риб на культури патогенних бактерій *Erysipelothrix rhusiopathiae*

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Мета. З'ясувати вплив секретів шкірних залоз алохтонних видів риб на культури патогенних бактерій *E. rhusiopathiae.* **Методи.** На шкірні покриви живих риб поміщали фільтрувальний папір, через 1 хв його знімали і екстрагували водорозчинні компоненти з розрахунку 0,1 см^3 води на 1,0 см^2 площі паперу. Одержані розчини секретів шкірних залоз риб стерилізували методом фільтрації під вакуумом через фільтри діаметром пор < 0,2 мкм. Дослідні зразки містили культури *E. rhusiopathiae* та шкірні виділення риб у розведеннях 1:10, 1:100, 1:1000 та 1:10000. Контроль – аналогічні співвідношення стерильної води та культур піддослідного виду бактерій. **Результати.** Присутність у середовищі водорозчинних екстрактів шкірних виділень прісноводних риб спричиняє зростання щільності культур *E. rhusiopathiae*. Виразність виявленого ефекту стимуляції *E. rhusiopathiae* прямо залежить від концентрації секретів шкірних залоз риб у дослідних зразках. **Висновки.** Секрети шкірних залоз прісноводних риб **(***Hypophthalmichthys molitrix, Ctenopharyngodon idella*) містять речовини, які чинять стимулювальний вплив на культури *E. rhusiopathiae.* Здатність піддослідного виду бактерій використовувати виділення шкірних залоз риб вказує на формування міжвидових зв'язків трофічного типу. При проведенні заходів, направлених на профілактику та ліквідацію вогнищ інфекцій, обумовлених *E. rhusiopathiae,* слід обов'язково враховувати можливість персистенції цих патогенних бактерій на покривах прісноводних риб.

Ключові слова: *Erysipelothrix rhusiopathiae*, шкірні виділення риб, стимулювальний вплив.

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