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Bacteria of genus *Bacillus* as antagonists of pathogens in aquaculture

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Abstract. Due to the increasing resistance of aquaculture pathogens to antibiotics, the search for new approaches to the control of infectious diseases is of particular importance. A promising approach is the development of probiotic preparations based on antagonist bacteria. The paper studied the antagonistic activity of *Bacillus* strains that are promising for developing probiotics for fish. The strains were isolated from the bottom sediments of the Don River in the area of the Donskoy Fish Reserve, as well as from the intestines of cyprinids of natural populations, namely, from silver carp (*Cyprinus gibelio*), roach (*Rutilus heckelii*), and bream (*Abramis brama*). The inhibitory effect of *Bacillus* strains against causative agents of aeromonosis, pseudomonosis and bacterial hemorrhagic septicemia was studied by the method of delayed antagonism. *Aeromonas* and *Pseudomonas* species pathogenic for fish were isolated from sturgeons, cyprinids and salmons grown in pond farms and recirculating aquaculture systems in the Krasnodar Region, Rostov Region and the Republic of Adygea. It has been shown that sensitivity to the action of bacilli is not only a species, but also a strain-specific trait among bacterial pathogens of fish. Five strains were identified that exhibited the maximum antagonistic activity against the studied pathogens, and their species identification was carried out by molecular biological and mass spectrometric methods. It has been established that the most pronounced antagonism is exhibited by strains of bacilli isolated from bottom sediments. The growth of all studied strains of fish pathogenic species of *Pseudomonas* was suppressed only the *B. subtilis* R4 strain. Thus, strains of aerobic spore-forming bacteria suitable for the creation of probiotic preparations for aquaculture objects have been isolated and characterized.

Keywords: aeromonosis, probiotics, fish diseases, antagonist bacilli, aeromonads, pseudomonads, aquaculture object

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Научная статья

Бактерии р. *Bacillus* – антагонисты патогенов аквакультуры

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Аннотация. На фоне возрастающей резистентности патогенов аквакультуры к антибиотикам особое значение приобретает поиск новых подходов к контролю инфекционных заболеваний. Перспективным подходом представляется разработка пробиотических препаратов на основе бактерий-антагонистов. Изучена антагонистическая активность бактерий р. *Bacillus*, перспективных для создания пробиотиков для рыб. Штаммы выделяли из донных отложений р. Дон в районе Донского рыбного заповедника, а также из кишечника карповых рыб природных популяций, а именно от серебряного карася (*Cyprinus gibelio*), тарани (*Rutilus heckelii*), леща (*Abramis brama*). Ингибирующее действие бактерий р. *Bacillus* в отношении возбудителей аэромоноза, псевдомоноза и бактериальной геморрагической септицемии исследовали методом отсроченного антагонизма. Патогенные для рыб бактерии р. *Aeromonas* и р. *Pseudomonas* были выделены от осетровых, карповых и лососевых рыб, выращиваемых в условиях прудовых хозяйств и установках замкнутого водоснабжения Краснодарского края, Ростовской области и Республики Адыгея. Показано, что чувствительность к действию бацилл является не только видовым, но и штамм-специфичным признаком среди бактериальных патогенов рыб. Выделено 5 штаммов, проявляющих максимальную антагонистическую активность в отношении исследуемых патогенов, проведена их видовая идентификация молекулярно-биологическим и масс-спектрометрическим методами. Установлено, что наиболее выраженный антагонизм проявляют штаммы бацилл, выделенные из донных отложений. Рост всех изученных штаммов патогенных для рыб видов р. *Pseudomonas* подавлял только штамм *B. subtilis* R4. Таким образом, выделены и охарактеризованы штаммы аэробных спорообразующих бактерий, подходящие для создания на их основе пробиотических препаратов для объектов аквакультуры.

Ключевые слова: аэромоноз, пробиотики, заболевания рыб, бациллы-антагонисты, аэромонады, псевдомонады, объект аквакультуры

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Introduction

In modern conditions, aquaculture is developing in several directions, which have significant differences. These are pasture, pond and industrial aquaculture (in pools, in recirculating aquaculture systems, in fish farms using cages), as well as artificial reproduction including sturgeon, salmon hatcheries and spawning farms. For each of these directions the risk of diseases in breeding objects is quite high [1]. Diseases can be associated with a violation of biotechnological, sanitary standards for growing and quarantine measures of imported aquaculture objects. In addition, a high risk of morbidity is caused by the environmental problems due to the unsatisfactory quality of the aquatic environment, especially in cage farms concentrated in coastal zones and in freshwater reservoirs, which are more often exposed to anthropogenic pollution.

As a result, the resistance of the fish organism sharply decreases, which causes diseases from the opportunistic bacteria that constantly circulate in the aquatic environment [2, 3].

Bacterial diseases all over the world cause significant economic damage to fish farms, both due to fish death, the need for quarantine measures, and from a decrease in the consumer attractiveness of raw materials and, accordingly, the cost of fish. Bacterial diseases of fish in ponds and farms in the Russian Federation rank second after the parasitic ones among infectious diseases. The most common are aeromonosis of salmon and cyprinid fish species, pseudomonosis of carp, myxobacteriosis of sturgeon and trout fish species [4, 5]. For the period 2014–2018 80.55% of all detected outbreaks of diseases of bacterial etiology accounted for aeromonoses of cyprinids and salmon fish [4]. It should be noted that cyprinid and salmon aeromonoses are included in the list of especially dangerous quarantine diseases [6]. In addition, many fish diseases (vibriosis, aeromonosis, pseudomonosis, citrobacteriosis) are characterized by natural foci [7]. Certain environmental factors are favorable for the persistence of certain types of pathogens. The most common pathogens for the water bodies of the North Caucasus and the southern region as a whole are aeromonads. It should be noted that bacteria of the genus *Aeromonas* are constantly present in the aquatic environment, and their epizootic significance is determined by abundance and virulence [8]. Mild climatic conditions contribute to the long-term persistence of virulent aeromonads in the aquatic environment. A major factor for the high incidence of aeromonosis in fish is an increase in water temperature above 14°C. At the same time, the percentage of death (10-100%) depends on the conditions of detention and the load per unit area of the reservoir of each particular farm. Outbreaks of aeromonosis can occur in the form of epizootics, affecting species of all age groups. However, in the acute form, the disease is more often recorded in fry and yearlings of sturgeon, salmon and some species of cyprinids (crucian carp, grass carp, mirror carp) [4]. For the Rostov region, cyprinid aeromonosis is the most dangerous because it spreads not only in pond farms, but also in natural reservoirs. Aeromonosis cases were recorded in the Don, Bolshaya Kalancha, Mius and Aksai rivers. A growing number of aeromonads in natural water bodies, especially strains with pathogenicity factors in the summer–autumn period, increases the risks for the development of infection or colonization of aquatic organisms by these bacteria [8]. *Pseudomonas* bacteria are the causative agents of pseudomonosis and this disease is rather rarely detected in aquaculture objects in the farms of the southern region. However, recently the incidence of diseases, caused by the associations of gram-negative microorganisms (aeromonads, pseudomonads, enterobacteria, etc.) has increased. In particular, a more severe course of the disease is observed and an acute form of the disease develops more often if the clinical signs of aeromonosis are accompanied by the presence of pseudomonads, enterobacteria, moraxella and other opportunistic bacteria [4]. In this case, it is difficult to choose effective drugs to combat the disease.

The existing complex of veterinary-sanitary and fish-breeding and reclamation measures cannot fully provide protection against bacterial infections in aquaculture objects. This is largely due to the resistance of pathogens to drugs used in fish farming.

The use of probiotics based on spore-forming bacteria of the *Bacillus* genus is a modern trend in the prevention and treatment of infectious diseases in aquaculture. The presence of probiotic effects in spore-forming microorganisms has led to the development of preparations based on them, belonging to the group of "self-eliminating antagonists". Probiotic preparations offered on the market differ not only in price, but also in composition, quality, method and dose of application. *Bacillus* based probiotics are suitable candidates for the development of preparations for use in aquaculture. They exhibit antagonistic activity against pathogenic microorganisms and are also nontoxic to fish [9, 10]. At the same time, the ability of probiotic bacilli to manifest antagonism is a rather variable trait and depends predominantly not on the species, but on the spectrum of antimicrobial metabolites secreted by a particular strain [11]. Therefore, to combat fish diseases, it is necessary to find species-specific bacterial antagonists. In addition, it is important to consider their ability to multiply effectively and exhibit probiotic properties under suboptimal temperature conditions. The effectiveness of the prevention and treatment of bacterial diseases in aquaculture objects is associated with the development of new probiotics, including those based on spore-forming bacteria. This approach will reduce the economic damage from the death of aquaculture objects and reduce the prevalence of antibiotic-resistant strains of pathogens.

In this regard, the aim of the work was to search for new natural probiotic *Bacillus* strains and evaluation of their antagonistic activity against pathogens of bacterial diseases relevant to aquaculture objects (pseudomonosis, aeromonosis, bacterial hemorrhagic septicemia) by the method of co-cultivation.

Materials and methods

The object of the study were *Bacillus* strains isolated from silver carp (*Cyprinus gibelio*), roach (*Rutilus heckelii*), bream (*Abramis brama*) as well as from bottom sediments of the lower reaches of the Don River in the area of the Donskoy Fish Reserve.

Inoculation of bacteria from bottom sediments and imprints of samples of fish gills and intestines was carried out on wort-agar. The species were identified by MALDI-TOF MS (matrix-associated laser desorption/ionization time-of-flight mass spectrometry) on an Autoflex speed III device with Biotyper software (Bruker Daltoniss, Germany). For more precise species identification, DNA was isolated from bacterial cells and the 16S rRNA gene was sequenced. The conditions for DNA isolation and purification, as well as for sequencing, are similar to those described in our previous works [12].

The strains with no hemolytic and DNAse activity were included in the study.

7 species of bacteria were used as test cultures. Species of *Aeromonas* genus - *Aeromonas salmonicida* (7 strains), *Aeromonas veronii* (30), *Aeromonas caviae* (14), *Aeromonas eucrenophila* (4), *Aeromonas ichthiosmia* (3), *Aeromonas bestiarum* (3), *Aeromonas hydrophila* (5), of which 14 are deposited in collections of microorganisms of Federal State Budgetary Institution "The Russian State Center for Animal Feed and Drug Standardization and Quality" (FGBU "VGNKI") (Table 1); 3 species of *Pseudomonas* genus - *P. putida* (2 strains), *P. fluorescens* and *P. aureofaciens* by 1 strain from each were also used to assess the antagonistic activity of bacilli.

Table 1

Deposited test strains of aeromonads from the collection of microorganisms of the FGBU "VGNKI"

Bacterial species of *Aeromonas* genus	Registration number at depositing	Fish species / sampling area
Aeromonas caviae	VKSHM-B-297M	Acipenseridae, Seversky district of the Krasnodar Region
	VKSHM-B-300M	Sterlet, Krasnodar
Aeromonas veronii	VKSHM-B-296M	Cyprinids, Dinskoy district of the Krasnodar Region
	VKSHM-B-299M	Cyprinids, Krasnodar
	VKSHM-B-305M	Cyprinids, Adygea
	VKSHM-B-302M	Cyprinids, Krasnodar
Aeromonas salmonicida	VKSHM-B-295M	Koi carp, Krasnodar
	VKSHM-B-293M	Russian sturgeon, village Starominskaya, the Krasnodar Region
	VKSHM-B-307M	Acipenseridae, village Bryukhovetskaya, the Krasnodar Region
Aeromonas eucrenophila	VKSHM-B-294M	Acipenseridae, village Starominskaya, the Krasnodar Region
	VKSHM-B-298M	Acipenseridae, Korenovsky district of the Krasnodar Region
Aeromonas ichthiosmia	VKSHM-B-303M	Cyprinids, Dinskoy district of the Krasnodar Region
	VKSHM-B-304M	Acipenseridae, village Starominskaya the Krasnodar Region
	VKSHM-B-306M	Cyprinids, Shcherbinovsky district of the Krasnodar Region

Strains of *Aeromonas* and *Pseudomonas* were isolated from parenchymal organs and skin ulcers of fish with bacterial hemorrhagic septicemia and aeromonosis. The study included fish from 3 families: cyprinids (*Cyprinidae*) – common carp (*Cyprinus carpio*), koi carp (*Cyprinus carpio haematopterus*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*); sturgeons (*Acipenseridae*) – Russian sturgeon (*Acipenser gueldenstaedtii*), sterlet (*Acipenser ruthenus*), stellate sturgeon (*Acipenser stellatus*); salmonids (*Salmonidae*) – rainbow trout (*Salmo gairdneri irideus*) and brook trout (*Salmo trutta morpha fario*) grown in pond farms and recirculating aquaculture system in the Krasnodar Region, Rostov Region, Republic of Adygea. Pathological anatomical autopsy, primary bacteriological inoculation of samples of fish organs and tissues, and isolation of bacteria were carried out in accordance with regulatory documents [13, 14].

To assess the antagonistic activity of *Bacillus* strains the method of delayed antagonism was used (method of perpendicular streaking) in accordance with MUK 4.2.2602-10.

Inoculation of antagonist strains on the rich nutrient medium (nutrient agar is used for cultivating microorganisms) was carried out using a loop with a diameter of (3.5 ± 0.5 mm) with a straight streaking along the diameter of a Petri dish. It was incubated for 3 days, after which a suspension of the overnight test culture (not less than 10^9 CFU/ml) was inoculated perpendicularly to the grown streak of the antagonist strain using a loop with a diameter of 2 mm. The cultures were incubated for 24 h at a temperature of (26 ± 1°C), followed by measurement of the growth inhibition zone of the test cultures.

Results and discussion

As a result of the screening of 28 strains, 5 promising strains of *Bacillus* were selected, of which 3 strains (R1, R4, R5) were isolated from cyprinids of natural populations and 2 strains (G5, G6) from bottom sediments.

The results of mass spectrometric analysis showed that strains R4, G5, G6 belong to *Bacillus subtilis*, R1 to *Bacillus mojavensis*, R5 to *Bacillus sp*. To clarify the species identification, an analysis of the 16S rRNA gene was carried out, as a result of which strain R5 belonged to the species *Bacillus velezensis*.

It was shown that the level of antagonistic activity of *Bacillus* strains varies not only in relation to the species of aeromonads, but also in relation to the strains of these species (Table 2).

Table 2

Species composition of aeromonad strains sensitive to antagonist bacilli

Strain	*Bacillus* species	Source of *Bacillus* strains isolation	Antagonism towards aeromonad test strains	Proportion of test strains sensitive to antagonist bacilli, %
R1	*B. mojavensis*	Intestines of carp fish	*A. salmonicida*	28
			A. eucrenophila	62
R4	*B. subtilis*	Intestines of carp fish	*A. salmonicida*	28
			A. caviae	100
			A. eucrenophila	100
			A. hydrophila	80
			A. bestiarum	33
R5	*B. velezensis*	Intestines of carp fish	*A. salmonicida*	14
			A. caviae	100
			A. hydrophila	100
G5	*B. subtilis*	Sediments	*A. salmonicida*	86
			A. eucrenophila	100
G6	*B. subtilis*	Sediments	*A. salmonicida*	86
			A. caviae	100
			A. eucrenophila	100
			A. bestiarum	100
			A. hydrophila	100

Thus, the antagonistic activity of bacilli differed significantly against the strains of immobile aeromonads *Aeromonas salmonicida*. The most pronounced antagonistic activity was found in bacilli isolated from the bottom sediments (more than 35 mm). Antagonistic bacilli isolated from fish exhibited a strong inhibitory effect only against 2 deposited strains (growth inhibition zone > 35 mm), the remaining 5 test cultures of aeromonads had a weak response (growth inhibition zone from 1 to 3 mm).

During co-cultivation of bacilli with motile aeromonads *A. eucrenophila*, *A. bestiarum*, and *A. ichthiosmia*, their growth inhibition zones varied widely. All antagonist bacilli (except *B. velezensis* R5) showed a good inhibitory effect on *A. eucrenophila* test cultures (more than 35 mm). In relation to cultures of *A. bestiarum*, the effect of bacilli differed significantly (zones of growth inhibition from 1 to 35 mm). At the same time, strains of *Bacillus subtilis* R3 and G6 (more than 35 mm) showed the greatest antagonistic activity, while *B. subtilis* R4 and *B. velezensis* R5 inhibited the growth of the tested cultures to a lesser extent (growth inhibition zone 3-5 mm). The weakest antagonistic activity of bacilli was noted against *A. ichtiosmia* (from 1 to 3 mm).

It is known that mobile species *A. caviae* and *A. hydrophila* are most often found as causative agents of aeromonosis and bacterial hemorrhagic septicemia. The inhibitory effect of bacilli to 5 cultures of *A. hydrophila* and 14 test cultures of *A. caviae* showed significant differences in the severity of antagonism (growth inhibition zones from 1 to 35 mm). However, the most effective antagonists were bacterial *Bacillus* strains R3, R4, R5, G6.

The inhibitory effect of 4 strains of antagonist bacilli against pathogenic for fish *Pseudomonas* species was studied. 3 species of *Pseudomonas* (*P. putida*, *P. fluorescens*, *P. aureofaciens*) isolated from fish with bacterial hemorrhagic septicemia were used as test cultures. It was found that most of the tested antagonist bacilli (*B. mojavensis* R1, *B. velezensis* R5) did not show a pronounced inhibitory effect on *P. fluorescens* and *P. aureofaciens* species. The growth of *Pseudomonas* test cultures was suppressed only by *B. subtilis* R4. Although *P. putida* strains differed in a number of biochemical properties and were isolated from different fish species (trout and Russian sturgeon), the antagonistic effect of bacilli against these strains was identical. *B. subtilis* R4 and *Bacillus velezensis* R5 strains suppressed the growth of *P. putida* test cultures. The results of the antagonistic activity of bacilli against *Pseudomonas* are presented in Table 3.

Table 3

Evaluation of the antagonistic activity of *Bacillus* bacteria against strains of *Pseudomonas* species pathogenic for fish

Test strains of pseudomonads / Bacteria *Bacillus*	Zone of *Pseudomonas* bacteria growth inhibition, mm			
	***P. putida* 100**	***P. putida* 101**	***P. fluorescens* 75**	***P. aureofaciens* 107**
R1 *B. mojavensis*	absent*	absent	not defined	absent
R4 *B. subtilis*	10	10	more than 40	5
R5 *Bacillus velezensis*	over 40	over 40	20	absent

*Zone of growth inhibition is absent.

The genus *Bacillus* is one of the most commonly used genera of probiotics in aquaculture due to its ability to produce bacteriocins, influence the growth rates, host immune system and resistance to pathogens [15].

It has been shown that in vivo conditions *B. subtilis*, *B. velezensis*, *Bacillus amyloliquefaciens*, *Bacillus circulans*, *Bacillus thuringiensis* and *Bacillus aerius* increase the resistance of aquaculture objects to pathogenic bacteria, including *Streptococcus*, *Aeromonas*, *Vibrio*, *Enterococcus* and *Lactococcus* [16]. Representatives of the genus *Bacillus* are a promising group for searching the new bacteria that inhibit quorum sensing in pathogens [17].

In addition, probiotics based on bacteria of the genus *Bacillus*, in particular *B. subtilis* and *B. licheniformis*, can be used to purify water and bottom sediments in fish ponds by removing toxic substances, such as ammonia, nitrites, nitrates and carbon dioxide, as well as by competing with opportunistic bacterial species [15, 18].

Bacillus strains also have a beneficial effect on the microbiome of the gastrointestinal tract of aquaculture objects, which leads to improved feed conversion and accelerates the growth of aquatic organisms [16]. Their mechanisms of action, however, are still the subject of active discussion and study.

Probiotic strains of bacilli affect the process of intestinal colonization by other types of microorganisms, including pathogens, due to the mechanisms of both direct competition and influence on the adhesion process [15, 16].

Among the specific properties of bacillary probiotics, the immunostimulating properties are also worth mentioning. Thus, it was demonstrated that bacilli can modulate the mechanisms of the innate immune response of fish and molluscs, for example, phagocytic and lysozyme, antiprotease and peroxidase, superoxide dismutase and myeloperoxidase activities, and respiratory burst [15, 19]. In addition, probiotics based on *Bacillus* strains can cause changes in animal cell physiology, in particular, affect neutrophil migration, plasma bactericidal activity and increase the ability of neutrophils to attach, which ultimately can lead to an improvement in the immune response, for example, an increase in complement activity, immunoglobulin production in the gut-associated lymphoid fish tissues [16, 19–21].

Spore-forming bacteria have the ability to synthesize a wide range of compounds that inhibit the growth of other microorganisms. In particular, *B. velezensis* was shown to be capable of synthesizing bacilizin, which shows activity against opportunistic Gram-negative bacteria [22]. The ability to synthesize a wide range of biologically active compounds, including peptides, lipopeptides, siderophores, etc., makes it possible to effectively use probiotics based on *Bacillus* strains against bacteria pathogenic for aquaculture objects, such as representatives of *Aeromonas*, *Vibrio*, *Pseudomonas*, and others [23]. In particular, it has recently been shown that the *B. coagulans* strain had a pronounced antagonistic effect against *P. aeruginosa* isolated from diseased carps and also reduced the death of fish in the in vivo experiment [24]. Similar data were obtained for *B. velezensis* WLY23 which effectively suppressed 20 fish pathogenic bacteria, including *A. schubertii*, *A. jandaei*, *A. hydrophila*, *A. veronii*, *A. aquariorum*, *P. shigelloides*, *N. seriolae*, *S. agalactiae* and *S. iniae* [25]. It should be noted that in our study, the *B. velezensis* R5 strain was also characterized by the widest spectrum of antimicrobial activity.

Conclusion

Thus, the conducted studies make it possible to identify a group of natural strains of bacilli with antagonistic activity against aeromonads and pseudomonads pathogenic for fish, which may be promising for developing the probiotic preparations for aquaculture. It has been found that sensitivity to the antagonistic action of *Bacillus* is not only a species, but also a strain trait among bacterial pathogens of fish.

The strains selected in the course of this work can be used to develop drugs that are active against pathogens which are of current importance for aquaculture.

References

1. Rudakova S. L. Obespechenie ikhtiopatologicheskogo blagopoluchiia ob"ektov i khoziaistv akvakul'tury Rossii [Ensuring ichthyopathological well-being of objects and farms of aquaculture in Russia]. *Trudy VNIRO*, 2016, vol. 162, pp. 104-115.

2. Bogachev A. I. Rossiiskii sektor akvakul'tury: sostoianie i znachenie dlia ekonomiki [Russian aquaculture sector: state and importance for economy]. *Vestnik Voronezhskogo gosudarstvennogo agrarnogo universiteta*, 2018, no. 2, pp. 227-236.

3. Basankina V. M., Prutsakov S. V., Kruzhnov N. N. Uslovno-patogennaia mikroflora kak vozbuditeli zabolevaniia u ryb [Conditionally pathogenic microflora as pathogens in fish]. *Teoriia i praktika sovremennoi agrarnoi nauki*, 2018, pp. 405-409.

4. Basankina V. M. *Epizooticheskie osobennosti aeromonoza ryb v usloviiakh regiona Severnogo Kavkaza. Dissertatsiia ... kand. vet. nauk* [Epizootic features of fish aeromonosis in conditions of North Caucasus region. Diss ... Cand. Vet. Sci.]. Krasnodar, 2020. 23 p.

5. Golovin P. P., Golovina N. A., Romanova N. N. *Kadastr lechebnykh preparatov, ispol'zuemykh i aprobirovannykh v akvakul'ture Rossii i za rubezhom* [Inventory of medicinal preparations used and tested in aquaculture in Russia and abroad]. Moscow, Rosinformagrotekh Publ., 2005. P. 5.

6. *Ob utverzhdenii perechnia zaraznykh, v tom chisle osobo opasnykh, boleznei zhivotnykh, po kotorym mogut ustanavlivat'sia ogranichitel'nye meropriiatiia (karantin): prikaz Minsel'khoza Rossii № 476 ot 19 dekabria 2011 g.* [On approval of the list of contagious, including especially dangerous, animal diseases, for which restrictive measures (quarantine) can be established: order of the Ministry of Agriculture of Russia No. 476 dated December 19, 2011]. Available at: https://rsnadzor.ru/f/perechen_476.pdf (accessed: 16.02.2022).

7. Lartseva L. V., Obukhova O. V., Lisitskaia I. A. *Mikroflora ryb i drugikh gidrobiontov: uchebnoe posobie* [Microflora of fish and other aquatic organisms: textbook]. Astrakhan', Astrakhanskii un-t, 2008. 107 p.

8. Iukhimenko L. N., Koidan G. S., Bychkova L. I., Smirnov L. P. Biologicheskie svoistva aeromonad i ikh rol' v patologii ryb [Biological properties of aeromonads and their role in fish pathology]. *Rybnoe khoziaistvo. Seriia: Bolezni gidrobiontov v akvakul'ture*. Moscow, Izd-vo VNIERKh, 2001. Iss. 1. Pp. 1-10.

9. Abrosimova N. A., Abrosimova K. S., Abrosimova E. B., Morozova M. A. *Kormovoe syr'e i biologicheski aktivnye dobavki dlia rybnykh ob"ektov akvakul'tury: uchebnoe posobie* [Feed raw materials and biologically active additives for aquaculture fish objects: textbook]. Saint-Petersburg, Lan' Publ., 2019. 151 p.

10. Golovko G. V., Chistiakov V. A., Sazykina M. A, Zipel't L. I., Kolenko M. A., Satarov V. V., Shipilo V. Iu. Ispol'zovanie probioticheskoi dobavki na osnove *Bacillus subtilis* «V-1895» v akvakul'ture [Use of probiotic supplement based on *Bacillus subtilis* "V-1895" in aquaculture]. *Rybnoe khoziaistvo*, 2009, no. 5, pp. 60-64.

11. Morozova M. A., D'iachenko, M. A., Chistiakov, V. A., Parkhomenko Iu. O., Stepanova Iu. V. Otsenka chuvstvitel'nosti aeromonad k antibakterial'nym preparatam i sporovym probiotikam [Evaluation of sensitivity of aeromonads to antibacterial drugs and spore probiotics]. *Aktual'nye voprosy rybolovstva, rybovodstva (akvakul'tury) i ekologicheskogo monitoringa vodnykh ekosistem: materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii, posviashchennoi 90-letiiu Azovskogo nauchno-issledovatel'skogo instituta rybnogo khoziaistva (Rostov-na-Donu, 11–12 dekabria 2018 g.)*. Rostov-on-Don, Izd-vo Azov. nauch.-issledovat. in-ta ryb. khoz-va, 2018. Pp. 70–75.

12. Gorovtsov A. V., Vasilchenko N. G., Prazdnova E. V., Chistyakov V. A., Kukharenko L. E. The influence of soil type and preceding crop on the suppression of fusarium by indigenous spore-forming bacteria. *Periodico Tche Quimica*, 2019, vol. 16, no. 33, pp. 225-240.

13. *Instruktsiia o meropriiatiiakh po bor'be s aeromonozom karpovykh ryb (utv. Minsel'khozprodom RF 17 avgusta 1998 g. № 13-4-2/1366)* [Instruction on measures to combat aeromonosis of cyprinids (approved by the Ministry of Agriculture and Food of the Russian Federation on August 17, 1998 No. 13-4-2 / 1366)]. Available at: https://sv.yanao.ru/documents/active/22710/ (accessed: 24.01.2022).

14. *Metodicheskie ukazaniia po laboratornoi diagnostike psevdomonoza ryb MU № 13-4-2/1403 ot 22 sentiabria 1998 g. (utv. Departamentom veterinarii Ministerstva sel'skogo khoziaistva Rossiiskoi Federatsii)* [Guidelines for laboratory diagnosis of fish pseudomonosis MU No. 13-4-2 / 1403 of September 22, 1998 (approved by the Department of Veterinary Medicine of the Ministry of Agriculture of the Russian Federation)]. Available at: https://e-ecolog.ru/docs/zh49kSe1HNrKqhT4CwedP (accessed: 24.01.2022).

15. Soltani M., Roy S., Lymbery A., Kumar V., Ringoe E. Genus *Bacillus*, promising probiotics in aquaculture: Aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. *Reviews in Fisheries Science & Aquaculture*, 2019, vol. 27 (3), pp. 331-379.

16. Ringoe E., Soon Ho Lee, Hoseinifar S. H., Harikrishnan R., Hien Van Doan, Soltani M., Seong Song. Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture. *Journal of Applied Microbiology*, 2020, vol. 129, no. 1, pp. 116-136.

17. Chen B., Peng M., Tong W., Zhang Q., Songet Z. The quorum quenching bacterium *Bacillus licheniformis* T-1 protects zebrafish against *Aeromonas hydrophila* infection. *Probiotics Antimicrob Proteins*, 2019, vol. 12 (1), pp. 160-171.

18. Kewcharoen W., Srisapoome P. Probiotic effects of *Bacillus* spp. from Pacific white shrimp (*Litopenaeus vannamei*) on water quality and shrimp growth, immune responses, and resistance to *Vibrio parahaemolyticus* (AHPND strains). *Fish Shellfish Immunol.*, 2019, vol. 94, pp. 175-189.

19. Yi Y., Zhang Z., Zhao F., Liu H., Yu L., Zha J., Wang G. Probiotic potential of *Bacillus velezensis* JW: Antimicrobial activity against fish pathogenic bacteria and immune enhancement effects on *Carassius auratus*. *Fish Shellfish Immunol.*, 2018, vol. 78, pp. 322-330.

20. Zhou S., Song D., Zhou X., Mao X., Zhou X., Wang S., Wei J., Huang Y., Wang W., Xiao Su-M., Qin Q. Characterization of *Bacillus subtilis* from gastrointestinal tract of hybrid Hulong grouper (*Epinephelus fuscoguttatus × E. lanceolatus*) and its effects as probiotic additives. *Fish Shellfish Immunol.*, 2018, vol. 84, pp. 1115-1124.

21. Di J., Chu Z., Zhang S., Huang J. Evaluation of the potential probiotic *Bacillus subtilis* isolated from two ancient sturgeons on growth performance, serum immunity and disease resistance of *Acipenser dabryanus*. *Fish Shellfish Immunol.*, 2019, vol. 93, pp. 711-719.

22. Nannan C., Vu H. Q., Gillis A., Caulier S., Nguyen Thuy T. T., Mahillon J. Bacilysin within the *Bacillus subtilis* group: Gene prevalence versus antagonistic activity against Gram-negative foodborne pathogens. *Journal of Biotechnology*, 2021, vol. 327, pp. 28-35.

23. Van Doan H., Soltani M., Ringoe E. In vitro antagonistic effect and in vivo protective efficacy of Gram-positive probiotics versus Gram-negative bacterial pathogens in finfish and shellfish. *Aquaculture*, 2021, vol. 540, pp. 736-581.

24. Ji T., Cao Y., Cao Q., Zhang Y., Yang H. The antagonistic effect and protective efficacy of gram-positive probiotics *Bacillus* coagulans to newly identified pathogens *Pseudomonas aeruginosa* in crucian carp *Carassius auratus gibelio*. *Aquaculture Reports*, 2022, vol. 24, pp. 101-126.

25. Zhang D. F., Xiong X. L., Wang Y. J., Gao Y. X., Ren Y., Wang Q., Shi C. B. *Bacillus velezensis* WLYS23 strain possesses antagonistic activity against hybrid snakehead bacterial pathogens. *Journal of Applied Microbiology*, 2021, vol. 131 (6), pp. 3056-3068.

Список источников

1. Рудакова С. Л. Обеспечение ихтиопатологического благополучия объектов и хозяйств аквакультуры России // Тр. ВНИРО. 2016. Т. 162. С. 104–115.
2. Богачев А. И. Российский сектор аквакультуры: состояние и значение для экономики // Вестн. Воронеж. гос. аграр. ун-та. 2018. № 2. С. 227–236.
3. Басанкина В. М., Пруцаков С. В., Кружнов Н. Н. Условно-патогенная микрофлора как возбудители заболевания у рыб // Теория и практика современной аграрной науки. 2018. С. 405–409.
4. Басанкина В. М. Эпизоотические особенности аэромоноза рыб в условиях региона Северного Кавказа: дис. ... канд. вет. наук. Краснодар, 2020. 23 с.
5. Головин П. П., Головина Н. А., Романова Н. Н. Кадастр лечебных препаратов, используемых и апробированных в аквакультуре России и за рубежом. М.: Росинформагротех, 2005. С. 5.
6. Об утверждении перечня заразных, в том числе особо опасных, болезней животных, по которым могут устанавливаться ограничительные мероприятия (карантин): приказ Минсельхоза России № 476 от 19 декабря 2011 г. URL: https://rsnadzor.ru/f/perechen_476.pdf (дата обращения: 16.02.2022).
7. Ларцева Л. В., Обухова О. В., Лисицкая И. А. Микрофлора рыб и других гидробионтов: учеб. пособие. Астрахань: Астрахан. ун-т, 2008. 107 с.
8. Юхименко Л. Н., Койдан Г. С., Бычкова Л. И., Смирнов Л. П. Биологические свойства аэромонад и их роль в патологии рыб // Рыбн. хоз-во. Сер.: Болезни гидробионтов в аквакультуре. М.: Изд-во ВНИЭРХ, 2001. Вып. 1. С. 1–10.
9. Абросимова Н. А., Абросимова К. С., Абросимова Е. Б., Морозова М. А. Кормовое сырье и биологически активные добавки для рыбных объектов аквакультуры: учеб. пособие. СПб.: Лань, 2019. 151 с.
10. Головко Г. В., Чистяков В. А., Сазыкина М. А, Зипельт Л. И., Коленко М. А., Сатаров В. В., Шипило В. Ю. Использование пробиотической добавки на основе *Bacillus subtilis* «В-1895» в аквакультуре // Рыбное хозяйство. 2009. № 5. С. 60–64.
11. Морозова М. А., Дьяченко, М. А., Чистяков, В. А., Пархоменко Ю. О., Степанова Ю. В. Оценка чувствительности аэромонад к антибактериальным препаратам и споровым пробиотикам // Актуальные вопросы рыболовства, рыбоводства (аквакультуры) и экологического мониторинга водных экосистем: материалы Междунар. науч.-практич. конф., посвящ. 90-летию Азов. науч.-исследоват. ин-та рыб. хоз-ва (Ростов-на-Дону, 11–12 декабря 2018 г.). Ростов н/Д.: Изд-во Азов. науч.-исследоват. ин-та рыб. хоз-ва, 2018. С. 70–75.
12. Gorovtsov A. V., Vasilchenko N. G., Prazdnova E. V., Chistyakov V. A., Kukharenko L. E. The influence of soil type and preceding crop on the suppression of fusarium by indigenous spore-forming bacteria // Periodico Tche Quimica. 2019. V. 16. N. 33. P. 225–240.
13. Инструкция о мероприятиях по борьбе с аэромонозом карповых рыб (утв. Минсельхозпродом РФ 17 августа 1998 г. № 13-4-2/1366). URL: https://sv.yanao.ru/documents/active/22710/ (дата обращения: 24.01.2022).
14. Методические указания по лабораторной диагностике псевдомоноза рыб МУ № 13-4-2/1403 от 22 сентября 1998 г. (утв. Департаментом ветеринарии Министерства сельского хозяйства Российской Федерации). URL: https://e-ecolog.ru/docs/zh49kSe1HNrKqhT4CwedP (дата обращения: 24.01.2022).
15. Soltani M., Roy S., Lymbery A., Kumar V., Ringoe E. Genus *Bacillus*, promising probiotics in aquaculture: Aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish // Reviews in Fisheries Science & Aquaculture. 2019. V. 27 (3). P. 331–379.
16. Ringoe E., Soon Ho Lee, Hoseinifar S. H., Harikrishnan R., Hien Van Doan, Soltani M., Seong Song. Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture // Journal of Applied Microbiology. 2020. V. 129. N. 1. P. 116–136.
17. Chen B., Peng M., Tong W., Zhang Q., Songet Z. The quorum quenching bacterium *Bacillus licheniformis* T-1 protects zebrafish against *Aeromonas hydrophila* infection // Probiotics Antimicrob Proteins. 2019. V. 12 (1). P. 160–171.
18. Kewcharoen W., Srisapoome P. Probiotic effects of *Bacillus* spp. from Pacific white shrimp (*Litopenaeus vannamei*) on water quality and shrimp growth, immune responses, and resistance to *Vibrio parahaemolyticus* (AHPND strains) // Fish Shellfish Immunol. 2019. V. 94. P. 175–189.
19. Yi Y., Zhang Z., Zhao F., Liu H., Yu L., Zha J., Wang G. Probiotic potential of *Bacillus velezensis* JW: Antimicrobial activity against fish pathogenic bacteria and immune enhancement effects on *Carassius auratus* // Fish Shellfish Immunol. 2018. V. 78. P. 322–330.
20. Zhou S., Song D., Zhou X., Mao X., Zhou X., Wang S., Wei J., Huang Y., Wang W., Xiao Su-M., Qin Q. Characterization of *Bacillus subtilis* from gastrointestinal tract of hybrid Hulong grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) and its effects as probiotic additives // Fish Shellfish Immunol. 2018. V. 84. P. 1115–1124.
21. Di J., Chu Z., Zhang S., Huang J. Evaluation of the potential probiotic *Bacillus subtilis* isolated from two ancient sturgeons on growth performance, serum immunity and disease resistance of *Acipenser dabryanus* // Fish Shellfish Immunol. 2019. V. 93. P. 711–719.
22. Nannan C., Vu H. Q., Gillis A., Caulier S., Nguyen Thuy T. T., Mahillon J. Bacilysin within the *Bacillus subtilis* group: Gene prevalence versus antagonistic activity against Gram-negative foodborne pathogens // Journal of Biotechnology. 2021. V. 327. P. 28–35.
23. Van Doan H., Soltani M., Ringoe E. In vitro antagonistic effect and in vivo protective efficacy of Gram-positive probiotics versus Gram-negative bacterial pathogens in finfish and shellfish // Aquaculture. 2021. V. 540. P. 736–581.
24. Ji T., Cao Y., Cao Q., Zhang Y., Yang H. The antagonistic effect and protective efficacy of gram-positive probiotics *Bacillus coagulans* to newly identified pathogens *Pseudomonas aeruginosa* in crucian carp *Carassius auratus gibelio* // Aquaculture Reports. 2022. V. 24. P. 101–126.
25. Zhang D. F., Xiong X. L., Wang Y. J., Gao Y. X., Ren Y., Wang Q., Shi C. B. *Bacillus velezensis* WLYS23 strain possesses antagonistic activity against hybrid snakehead bacterial pathogens // Journal of Applied Microbiology. 2021. V. 131 (6). P. 3056–3068.

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