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To cite this article: A E Droshnev *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **315** 072010

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Biotechnology method of salmon protection against vibriosis

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Abstract. Our country has all potential for development of an aquaculture as one of sectors of agriculture, due to a huge number of fresh water areas and the extended line of the seas. Vibriosis is a bacterial salmon disease, it is one of the reasons of the volumes of the fish which is grown up in a mariculture constraining increase. Modern trends on refusal of use in agriculture of antibiotic drugs, to creation of eco-friendly productions and decrease in economic expenses in fish breeding set a world task of use of biotechnology methods of protection of fishes against infectious diseases, in particular from vibriosis. In the present article results of designing of vaccine from domestic strains of vibrios are provided. It is shown that immunization of fish antigens of the tested strains of vibrios (*Listonella anguillarum*) in an inoculative dose 7×10^8 m.c. with adjuvant ensures safety of a livestock to 97%, does not cause allergic reactions, the host defense remains not less than 12 months.

1. Introduction

The aquaculture is an artificial culture of aquatic organisms (fishes, mollusks, crustaceans, seaweed), in Russia it has complex diversified character due to use of the world's largest fund of internal reservoirs and coastal water areas of the seas. Now it is 22.6 million ha of lakes, 5.2 million ha of water reservoirs, 1 million ha of irrigational and agricultural reservoirs of complex appointment, more than 150 thousand ha of ponds, 450 thousand km of river systems that creates enormous opportunities for development of freshwater fish breeding. Also Russia has the most extended line of the sea coast - about 60 thousand km, and the total area of shallow water of the adjacent seas (without the Arctic and subarctic zones) is 0,39 million km² that objectively contributes to the broad development of a mariculture [1].

In many countries of the world in a mariculture vibriosis - an infectious disease salmon, eels and other species of fish, caused by a bacterium from the Vibrionaceae family, the genus *Vibrio* is widespread [2]. In Russia vibriosis meets at wild fish and also in the salmon and acne farms using sea or salty water for cultivation of fishes and located in pools Baltic, Barents, White and the Far East seas. The disease proceeds enzootically, is followed by mass death of fishes and demands considerable costs of improvement of unsuccessful farms.

Occurrence of the disease is promoted by high temperature of water (above 15°C), pH more than 8.0, the low oxygen content, water pollution by organic substances (biochemical oxygen demand (BOD) is higher than 2 mg/l) and nitric connections (content of nitrogen more than 1 mg/l), a handling. At cultivation in sea delts loss of fishes from the disease reach 70-100%. At industrial fish breeding on emergence and



disease the great influence is exerted by conditions of keeping. Unbalanced, low-quality forages, uneven-aged a planting stock, recompactation and water pollution are the factors increasing susceptibility of fishes [3,4,6].

Among representatives of the genus *Vibrio* there are species constituting potential health hazard of the person in connection with ability to cause food toxicoinfections [5].

Studying of the genus *Vibrio*, in particular species of *Vibrio* (*Listonella*) *anguillarum* is necessary for a modern ichtiopathology. It was noted that the juveniles of an iridescent trout and Atlantic salmon are most susceptible to *Vibrio anguillarum* infection, clinical signs of a disease which it is characterized by a septicaemia, defeat of tissues of kidneys, a liver, digestive tract, disturbance of a water salt metabolism and intoxication are shown in 30 - 40 days after delivery of not vaccinated fish in a sea water, at a temperature of 15 - 20°C, and for a short time disease passes into an acute form with mass death. In the absence of therapy of loss reach 90%. An antibiotic therapy does not allow to carry out 100% decontamination of an organism of fish from vibrios, the disease passes into a chronic form, there are repeated outbreaks.

The world aquaculture refused use of the antibiotic drugs which are negatively influencing the immune status of an organism of fish and the end user of products - the person. For the last twenty years, in the countries which are engaged in a salmon farming there was obligatory a universal inoculation of fishes against vibriosis, works on designing and testing of vaccines and in our country, on the basis of domestic strains of vibrios are actively conducted [8,9].

2. Materials and methods

Fish. In work used a trout - 560 samples, weighing from 10 to 500 g

Strains of vibrios. In total in work used fifty five strains of vibrios: *Vibrio anguillarum* VUR-19 (from the VGNKI collection); fifty four field strains, including thirty eight, allocated with authors of article from the iridescent trout which is grown up in the conditions of the White Sea: from them *V.anguillarum* - 26 samples, of *V.alginolyticus* - 7 samples, *V.ordalii* - 5 samples; twelve strains from parenchymatous organs of a humpback salmon: *V.anguillarum* - 8 samples of *V.alginolyticus* - 3 samples, *V.ordalii* - 1 sample; four strains from a catfish, 2 samples of *V.anguillarum* and *V.ordalii* – were characterized earlier [6].

Media and solutions for microbiological work. Fluid, semi-fluid and dense media of MPA, MPB, a rybopeptonny salt agar of RPSA and RPSB, MPPA, MPG, Giss medium, Hugh-Leyfson medium, Kligler medium, Clark's medium, triptozo-soy broth / agar (TSA/TSB) with sodium chloride, DDA, TCBS, the main alkaline peptone.

Anesthesia. Before vaccination it is necessary to enter fish into a condition of an anesthesia benzocaine solution use in ethyl alcohol or ethyl ether of the benzoic acid [7].

3. Results

Morphological, cultural, biochemical and tinctorial properties of the vibrios allocated during the period autumn of 2007 - summer of 2012 are studied. The vibrios allocated from water and aquatic organisms have similar morphological and tinctorial properties. On a halophilic vibrios are subdivided into three biotypes. Two species of *V.parahaemolyticus* and *V.alginolyticus*, belong to I and II biotypes (the maintenance of NaCl of 3% and 7% respectively) that can constitute potential health hazard of the person, in connection with ability to cause food toxicoinfections. The cultures of *V. anguillarum*, *V.ordalii*, *V.vulnificus* met in a sea water, less than 10 are more rare in a desalinated water with salinity. Such distribution of these types is caused by their tolerance to salt content. Some species of vibrios are capable to breed in conditions of keeping of NaCl salts up to 16%.

It is shown that vibrios of a species of *V.parahaemolyticus* have high hemolytic activity, these microorganisms should be considered as indicators of a veterinary and sanitary condition of the environment.

In experiences of infection of an iridescent trout with halophilic vibrios of species of *V. anguillarum* and *V. ordalii* it is established that for *V. anguillarum* the minimum LD₅₀ makes 200 million m.c., whereas *V. ordalii* makes 600-800 million m.c..

At repeated browning of microorganisms of "VBF", "BBM", "BOK/07", "AF3/4" is established lack of dissociation throughout more than 60 passages.

Optimum accumulative mediums are RPSA, RPSB, TSA, TSB with addition of 3-5% of NaCl.

At the following stage of researches the selected strains of vibrios (*Listonella anguillarum*) - "VBF", "BOK/07", tested "AF3/4" in experiences of direct infection of an iridescent trout at different temperatures, entered intraperitoneally from 50 to 500 million m.c., with a step 50, of 0.2 cm³.

The greatest virulence had two strains - "VBF" and "AF3/4", LD₅₀ at 8°C makes 200 million m.c., at 15-18°C - 100 million m.c.. In the made experiments of heat stability of *Vibrio anguillarum* antigens of different origin noted destruction of N-antigen when boiling, but with maintaining the agglutinating ability somatic (O) antigen. Introduction of boiled suspension of a bacterial mass in different doses of an iridescent trout did not cause forming of permanent protection. Infection of fishes later was followed 21 days by development of clinical signs of a vibriosis and death within 10 - 14 days of 40-80% of an experimental trout. The titer of agglutinins made 1:16 - 1:32.

Therefore further experiments were made with the integral microbic cells inactivated by formalin in a dose from 200 million m.c., with a step 100 which entered intraperitoneally, into the area of belly fins of 0.1 cm³. Forming of an immune response at an iridescent trout weighing 30 g was controlled at different water temperatures: 5, 10, 15 °C. The received results demonstrate that the lowest titers of agglutinins observed at 5 °C and dose from 200 to 400 million m.c. are 1:8 - 1:16, at increase in a dose the titer did not exceed 1:32. At a temperature of 10 - 15 °C and dose from 100 to 500 million m.c. titers averaged - 1:128, at increase in a dose up to 600-900 million m.c. - 1:256.

Availability of antibodies in RA was observed in 10 days after an injection, the titer reached the maximum values for 21 days and remained within 12 months of observation.

Specific protection was studied by method of direct intraperitoneal infection of the vaccinated fish with the two-day cultures of a strain of "VBF" in a dose of 200 million m.c., at the temperature of 10-15°C. As control used an intact trout of the same standard. This temperature band was chosen because the natural course of a vibriosis at delts cultivation is observed under such circumstances.

The made experiments showed that in control groups 100% of fishes perished, and in experienced - depending on an inoculative dose, from 4 to 8 samples perished that made from 13 to 27%, at the same time respectively, 83-97% of fishes survived.

Thus, it was established that an inoculative dose from 200 to 500 million m.c. it is not rather effective - protection at the level of 83%. Doses from 600 to 900 million m.c. ensured safety of a livestock to 97%. Use of concentration of antigen over 1x10⁹ m.c. did not lead to increase in antibody formation that defined expediency of use of inoculative concentration within 7x10⁸ m.c. and addition of adjuvants.

4. Conclusion

The vibrios allocated from water and aquatic organisms have similar morphological and tinctorial properties, on degree of a halophilic are divided into three biotypes. The vast majority of cultures treats the first and second biotype that can constitute potential health hazard of the person, in connection with ability to cause food toxicoinfections. At industrial fish breeding on emergence and disease the great influence is exerted by conditions of keeping and contamination by vibrios of species of *V. anguillarum*, *V. ordalii*, *V. alginolyticus*. The etiological agent of a vibriosis of salmon in most cases is *V. anguillarum*.

For designing of vaccinal drug 4 strains of *V. anguillarum* are picked up: "VBF", "BBM", "BOK/07" and "AF3/4" as a result of which repeated browning (more than 60 passages) is established lack of dissociation. These cultures are undemanding to composition of nutrient mediums, optimum accumulative

mediums are a tryptic soy agar (RPSA) and broth (RPSB), a tryptose soy agar and broth (TSA, TSB) with addition of 3-5% of NaCl. At cultivation do not change cultural and biochemical properties.

As model for definition of virulence of vibrios it is reasonable to use parenteral infection of an iridescent trout with weight from 30 g. For halophilic vibrios the dependence of a lethal dose on water temperature is established, the higher the water temperature, the lower the contaminating dose is.

The inactivation of bacteria by creation of concentration of 0.35% of formalin allows to keep antigenic properties and provides sterility. On thermotolerance of *Vibrio anguillarum* has thermostable somatic O-antigen and flagellar thermolabile N-antigen.

Use of whole antigen in an inoculative dose $6-9 \times 10^8$ m.c. creates immunity to a vibriosis to 97% of a livestock of fishes.

For upgrading of an end main product due to increase in tension of immunity it is necessary to use immunostimulators on the basis of oil and adsorptive adjuvants.

Acknowledgment

Work is performed within the Program of Basic scientific research of the state academies of Sciences for 2019-2020, the subject (project) No. 0578-2014-0016 "To develop the adjuvant-vaccine against vibriosis of salmon allowing to carry out mass processing and prevention of fishes" without attraction of additional sources of financing.

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