Prospects of using adaptogens to stimulate nonspecific resistance and reduce stress factors of aquaculture facilities

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Abstract. The article considers the possibility of using adaptogens based on lactobacilli that stimulate nonspecific resistance and help reduce the impact of stress factors on aquaculture facilities as a result of the current difficult situation on the market of feed and feed additives for trout. The object of the study was authentic strains of lactobacilli – *Lactobacillus brevis* 1.5, *L. brevis* 1.7 and *L. brevis* 2.6. Under experimental conditions, the biochemical activity of lactobacilli was evaluated in relation to glucose, sucrose, mannitol, L-arabinose and glycerol; antibacterial activity and the ability to co-culture. According to the results of the study, the most promising strains were selected for use as a basis for the development of biologics of targeted adaptogens.

1 Introduction

Increasing the output of aquaculture products is an important condition for supporting Russia's food security and ensuring the socio-economic development of the regions. The development of aquaculture is especially promising for the Northwestern region (Karelia) due to favorable environmental conditions for the cultivation of valuable salmon species. Currently, the Republic of Karelia holds a leading position in cage trout farming [1]. One of the key factors for the sustainable functioning of trout farms is the timely and effective prevention of the occurrence and spread of diseases in farmed fish. It is known that the content of cultivated trout in conditions of crowding and feeding with artificial feeds provokes a decrease in immunity, the rapid spread of infections, the connection of secondary pathogens to the primary infection with the creation of associated complexes that aggravate the disease and complicate its clinical picture [2]. Under the influence of adverse environmental factors, the enzyme activity of bacteria increases, as a result of which the relations in the "parasite-host" system are disrupted, which contributes to the rapid development of associations of symbiotic bacteria that can acquire pathogenic properties. Due to the high level of susceptibility of cultivated species of aquatic organisms to diseases, deterioration of water quality and a wide range of stressful conditions (from enzymatic insufficiency to infections and the development of new living conditions), the aquaculture industry is experiencing a constant restriction in development, which significantly increases

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the importance of developing new domestic therapeutic and prophylactic drugs with the properties of adaptogens [3].

An adaptogen based on lactobacilli may be a promising biological product in this direction. Lactobacilli taxonomically belong to the phylum Firmicutes, class Bacilli, order Lactobacillales, family Lactobacillaceae: Domain Bacteria, Phila XIII. Firmicutes, Class I. Bacilli, Order II. Lactobacillales, Family I. Lactobacillaceae, Genus I. Lactobacillus, Genus II. Paralactobacillus, Genus III. Pediococcus.

Bacteria with different morphologies are found within the genus *Lactobacillus*. Most of the representatives have the form of straight sticks with rounded ends, assembled into chains of various lengths, either arranged singly or in pairs. C Among lactobacilli, there are short coccoid and convoluted forms, as well as long, filamentous rods from 0.7-1.1 to 3.0-8.0 microns in length, arranged singly or assembled into chains.

Lactobacillus are representatives of the normoflora of fish, have antagonistic activity against opportunistic bacteria, viruses and fungi that cause diseases of fish and shellfish [4].

Lactobacillus are safe microorganisms. They have GRAS status declared by the FDA, as well as QPS status assigned to them by EFSA, and can serve as biological food preservatives due to the antimicrobial activity of their metabolites, such as organic acids (mainly lactic acid and acetic acid), carbon dioxide (CO2), hydrogen peroxide (H2O2), lysozyme, phenylmolactic acid, fatty acids, antibiotics (reuterin) and bacteriocins [5]. The spectrum of inhibition by antimicrobial compounds of lactobacilli includes microorganisms that cause diseases of fish and shellfish, such as *Aeromonas salmonicida, A. hydrophila, Edwardsiella tarda, Pasteurella piscicida, Vibrio anguillarm, V. salmonicida, V. harveyi, V. parahaemolyticus and Yersinia ruckeri, Lc. garvieae, S. iniae*, conditionally pathogenic *E. coli*, fungi of the genus *Candida*, mold fungi, etc. [6]. The most studied bacteriocins of lactic acid bacteria are currently nizin, Lactacin B, Lactocin 27, Plantaricin A, Plantacin B and Helveticin, Leukocin, Saracin, Pediocin PAl/AcH, Enterocins AS-48, A, B and etc. [7-8]. They can affect bacteria by inhibiting cell wall synthesis, increasing cell permeability of target cell membranes, or inhibiting RNase or DNase activity. Lactobacillus bacteriocins are non-toxic to animals and humans, do not change the nutritional properties of food, are effective at low concentrations, and do not lose activity when cooled or heated.

The relevance of the research is due to the fact that to date, the molecular mechanisms of stress in salmon fish have been poorly studied, therefore, the development of scientific approaches to the use of adaptogens to stimulate nonspecific resistance and reduce stress factors of aquaculture facilities is especially important due to the difficult situation with feed and additives for trout. According to the above, the aim of the study is to assess the possibility of using adaptogens based on authentic strains of lactobacilli isolated from the gastrointestinal microflora of rainbow trout to stimulate nonspecific resistance and reduce stress factors of aquaculture facilities.

To achieve this goal, lactobacilli were identified in the autoflora of rainbow trout, their biological properties such as enzymatic activity and antagonism towards typical test cultures were studied.

2 Materials and methods

3 strains were selected as the object of the study *Lactobacillus brevis* 1.5, *L.brevis* 1.7 and *L. brevis* 2.6 from the Collection of authentic strains of intestinal microflora of trout of the Laboratory of Microbiology of the Scientific Research Center for Aquaculture of the Institute of Biology, Ecology and Agrotechnology of Petrozavodsk State University. Microorganisms were isolated into a pure culture from the intestinal microflora of rainbow trout aged 1+, which was kept in cage cultivation conditions. According to the taxonomic profile *Lactobacillus brevis* they are assigned to a department Firmicutes, class Bacilli,

family Lactobacillaceae, genus Lactobacillus [9]. Genome-wide sequencing was performed using Sanger [10]. Determination of the most closely related 16S rRNA gene sequences from available databases using online services RDP/Classifier (http://rdp.cme.msu.edu/classifier/hierarchy.jps) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). According to the results of the BLAST analysis, the studied samples of the intestinal microflora of rainbow trout turned out to be the closest to the typical strain *Lactobacillus brevis* strain ATCC 14869 (NR_044704.2), the level of similarity with the type strain was 99.53%.

The biochemical activity, antibacterial activity and ability to co-culture were evaluated in the selected strains. To determine the biochemical activity of lactobacilli, a series of experiments on the oxidation of sugars (glucose, sucrose, L-arabinose) and alcohols (mannitol, glycerol) in an environment with bromocrisol purple was carried out. These sugars were chosen because of their species-specific fermentation by lactic acid bacteria and belonging to monosaccharides, disaccharides, hexatomic alcohol, peptose and triatomic alcohol. The strains were cultured for 24 hours, after which their ability to oxidize the selected substrates was recorded. The MRS medium was previously prepared without sugar content. A stock solution of bromocresol purple in a dilution of 1:100 was added to the resulting medium after autoclaving. For each sugar and alcohol, a falcon with a nutrient medium in a volume of 10 ml was prepared, after which a sample of the studied substrates was added to obtain a 1% solution, which was passed through a PVDF syringe filter with a pore size of 0.22 microns. The resulting medium was poured 200 µl per well into a 96-well plate, after which the studied strains were inoculated in a volume of 50 µl per 1 well. The tablets were incubated for 24 hours at a temperature of 37˚C. The results were recorded using a Tecan tablet reader at a wavelength of 212 nm. *Escherichia coli* K12 ATCC209P and *Staphylococcus aureus* ATCC 209P strains from the Collection of the VKPM were used as test cultures to assess the antagonistic activity of the "Cross-Streak" method and the possibility of co-cultivation.

Statistical processing of the results was performed using Microsoft Office Excel 2016 for Windows 10.

3 Results and Discussion

As a result of a series of experiments on the biochemical activity of authentic strains of the intestinal microflora of trout, it was found that the *L. brevis* 1.5 strain was characterized by the oxidation of glucose, sucrose and mannitol, L-arabinose and glycerol were not absorbed by this culture of lactobacilli. The L. brevis 1.7 strain oxidized the entire selected pool of substrates, except glycerol. Lactobacillus autostamps number 2.6 are capable of oxidizing glucose, sucrose and glycerol (Table 1).

Table 1. Oxidation of sugars and alcohols by the studied *L. brevis* autostamps

	1.5	1.7	2.6
Glucose	+	+	+
Sucrose	+	+	+
Mannitol	+	+	-
L-arabinose	-	+	-
Glycerol	-	-	+

As a result of studying the antibacterial activity of *L. brevis* "Cross-Streak" autostamps against test cultures of *St. aureus* ATCC209P (gram-positive), *E. coli* K12 (gram-negative), which were cultured together on a dense modified MRS nutrient medium:LB with lactobacillus autostamps, it was found that all strains have antimicrobial activity. *L. brevis*

1.5 strains in relation to *St. aureus* ATCC209P caused the formation of an inhibition zone with a size of 2.1 = 0.15 cm, and in relation to *E. coli* K12 -1.3 = 0.14 cm. For the lactobacillus autostamps number 1.7, these indicators corresponded to 1.2 = 0.4 cm and 1.1 = 0.5 cm. In the variant of the experiment with the lactobacillus autostams number 2.6, the inhibition zones were 1 = 0.1 cm, 1.5 = 0.17 cm (Fig. 1).

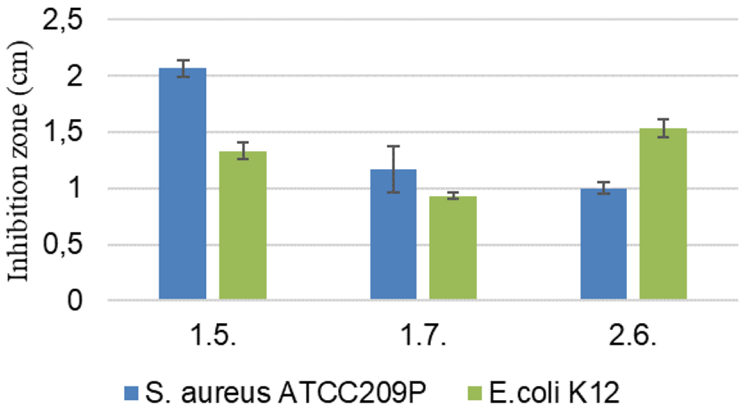

Fig. 1. Pathogen growth inhibition zones obtained by the "Cross-Streak" method during co-cultivation

4 Conclusion

As a result of the performed study, the biological properties of *Lactobacillus brevis* strains isolated from the intestinal microflora of rainbow trout were studied. These are metabolic activity against glucose, sucrose, mannitol, L-arabinose, glycerol; antimicrobial activity; the possibility of co-cultivation with *Escherichia coli*, *Staphylococcus aureus* and the effect of exogenous compounds isolated by the studied strains during planktonic cultivation. Variable biochemical and antibacterial activity has been established. When co-cultured on a dense medium, inhibition zones were recorded for all test cultures studied, in the variant of the experiment on co-culturing in a liquid medium, this strain showed antibacterial activity for both gram-positive and gram-negative bacteria, but when studying the supernatant, this effect was shown only for gram-positive cultures. Strain 1.7 in the study of the antimicrobial activity of "Cross-Streak" by the method on a dense medium caused the formation of inhibition zones against *Staphylococcus* and *E. coli*, however, when co-cultured on a liquid medium, no decrease in CFU of test microorganisms was recorded. For strain 2.6, due to the use of the "Cross-Streak" method to study antibacterial activity, inhibition zones were also recorded for all test cultures. Co-cultivation with *St. aureus* and *E. coli* stimulated the growth of microorganisms. As a result of the analysis of the data obtained on the antimicrobial activity of lactobacillus autostamps and the effect of exogenous compounds isolated by the studied strains during planktonic cultivation, it can be said that the most promising for research purposes related to the search for promising adaptogens to stimulate nonspecific resistance and reduce stress factors of objects are considered to be lactobacillus autostamps *L. brevis* 1.5 and *L. brevis 2.6.*

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