Saprolegnosis: dissemination in aquaculture and control methods

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Abstract. Saprolegnia spp, the causative agent of which is aquatic mold fungi, affects fish and spawn during factory hatchery and is one of the problems when growing avaculture in industrial conditions. The review describes control methods of Saprolegnia spp. Traditional methods of saprolegnia control are chemical compounds such as malachite green, purple "K" and formalin, which have carcinogenic and mutagenic effects. Safer but somewhat outdated methods are the treatment of spawn with incubation of boric acid and hydrogen peroxide, as well as water ozonization. Alternatives to these methods of control may be natural biologically active substances: thyroxine and cortisol, bark extract Drimys winteri, chitosan. Besides, various isolates of microorganisms are used as biological methods of saprolegnosis control in aquaculture, namely: bacteria of the genus Pseudomonas, Aeromonas, Bacillus subtilis, Pantoea agglomerans. The use of marine algae containing sulfated polysaccharides, including fucoidan, to control saprolegnosis has been confirmed. Fucoidan has been shown to have wide biocidal activity, but its antimicrobial properties have not been studied. In this regard, the study of the influence of the structure of fucoidan on its antimicrobial properties is a promising trend in the search for new safe means of saprolegnosis control.

1. Introduction
Worldwide, aquaculture remains one of the fastest-growing animal food industries. FAO projects that by 2023, the share of farmed fish in total production will reach 49 percent. Particularly relevant to aquaculture is the farming of valuable sturgeon species, whose numbers are constantly declining in natural bodies of water. One of the problems in growing avaculture in industrial conditions is the damage of fish and spawn during the factory hatchery with saprolegnia spp., the causative agent of which is aquatic mold fungi Saprolegnia spp. Saprolegnia spp. affect all fish, but especially fry. In the absence of treatment, saprolegnia can lead to the death of infected fish. During incubation, mold fungi, usually affecting damaged and unfertilized eggs, are gradually transferred to live eggs. By covering the surface of eggs with hyphae, the mushrooms make it difficult for eggs to breathe and lead to their death.

2. Overview of saprolegnosis treatments
Chemical compounds such as malachite green, purple "K" and formalin are the main means of fighting saprolegnosis. However, the use of these drugs is limited because of their possible carcinogenic and mutagenic effects [1], and the use of malachite green in fish farms abroad is prohibited [2]. It has been revealed that the use of hydrogen peroxide as a drug to treat saprolegnia in...
the period of sturgeon spawn incubation is promising, but more research is needed to find solutions with optimal concentrations, as well as to develop new methods of the drug application. [3, 4].

As part of the search for safer substances in vitro, the activity of compounds such as benzoic acid, acetic acid, iodine acid, copper sulphate, sodium per carbonate was tested on strains Saprolegnia parasitica and Saprolegnia delica. Only sodium percarbonate proved to be ineffective at any concentration [5]. Boric acid can also be used to control saprolegia. In in vitro experiments it can reduce the activity of Saprolegnia spores and mycelium growth up to complete inhibition of germination and growth, in vivo experiments it also inhibited the development of saprolegnosis on spawn under continuous or periodic exposure [4]. Also boric acid was shown to be efficient in treating Saprolegnia infection in Nile tilapia, considering its negative impact on the environment [6]. To improve the health of carps in fish farms on the discharge channels of power plants infected with associative agents of aeromonosis and saprolegnosis, fish were given furazolidon, simultaneously adding the organic dye malachite green to the water [7]. Application of iodine-containing preparation Monclavit-1 significantly reduced damage of rainbow trout spawn by saprolegniun [8].

Ozonators are used as a physical method of water disinfection to prevent saprolegia, but studies have shown that ozonization has a positive effect only with additional treatment of water with ultraviolet, which is extremely energy consuming [9].

Natural biologically active substances may act as an alternative to chemicals and physical control methods. It has been shown that short-term treatment of sturgeon (Acipenser gueldenstaedtii Brandt) at the stage of transition to intensive feeding with hormonal preparations thyroxine and cortisol increases resistance of larvae to saprolegnosis infection [10]. The immunomodulatory effect of the biologically active component (polygodial) of Drimys winter bark extract - the Chilean medicinal tree - has been revealed as an anti immunosuppressive effect of Saprolegnia parasitica and applied as a protection against this infection of oomycetes in fish [11]. A study of Chaga extract has shown its marked antimicrobial effect on Saprolegnia fungi developing on goldfish spawn [12]. Antifungal activity of chitosan polysaccharide and its various modifications against Saprolegnia parasitica has been established [13].

Different bacterial isolates are proposed as new biological methods to combat saprolegnosis in aquaculture. For example, live inoculants of aquatic strains Pseudomonas significantly reduced the mortality of salmon roe caused by Saprolegnia diclina in vivo experiments; the surfactant produced by them inhibited the growth of saprolegnia, but did not provide significant protection against saprolegnosis [14]. Isolates of bacteria Pseudomonas fluorescens LE89 and Pseudomonas fluorescens LE141, inhibiting Saprolegnia parasitica in vitro, were investigated on fish with experimental S. parasitica infestation when added to water and fodder. A protective effect was observed when the isolates were introduced into water, but neither of the two isolates prevented S. parasitica infection when introduced into food [15]. It was found that the bacteriotic-like inhibitor (BLIS) produced by the strain Aeromonas media A199 inhibited the growth of Saprolegnia sp. in vitro, and in vivo experience promoted the subsequent rapid recovery of affected fish from the invasion of this parasite [16]. In [17] it was found that in carp fish, which received the probiotic Bacillus subtilis, immunity to saprolegnia increased significantly. An antifungal agent containing the strain of Bacillus sp. was also suggested. CSM11143R, which has a high level of productivity of siderophores with high iron capture ability, and thus inhibits the growth of pathogenic bacteria and Saprolegnia sp [18]. From the skin surface of wild species of trout Salmo trutta L. and Oncorhynchus mykiss Walbaum isolates of aboriginal skin microflora were obtained and studied for Saprolegnia growth inhibition. The isolates identified as Aeromonas piscicola, A. sobria, Pantoea agglomerans, and Pseudomonas fluorescens showed the highest inhibiting capacity in in vitro experiments because they reduced the adhesion coefficient of Saprolegnia to the fish skin surface [19]. The results [20] show that fungi of genus Trichoderma are able to inhibit the growth of Saprolegnia diclina in aquaculture, because they have mycoparasitic effect on it.

In addition to therapeutic methods, preventive measures against saprolegnosis are currently being developed. An experimental vaccine against Saprolegnia parasitica based on the protein SpSsp1
present in the antibodies of healthy rainbow trout Oncorhynchus mykiss was developed in [21], but more research is needed on the possibility of using such a vaccine.

3. The therapeutic potential of brown algae extracts in the treatment of saprolegniosis

Recently, marine algae extracts with antimicrobial activity have attracted increased attention as preventive and therapeutic agents in aquaculture [22]. Among them, brown algae extracts containing fucoidan polysaccharide have the widest range of biological activity, including antimicrobial activity [22].

**Table 1. Antibacterial activity of fucoidan**

| Source                    | Degree of hydrolysis | Microorganisms with respect to which activity is observed                                                                 | References |
|---------------------------|----------------------|--------------------------------------------------------------------------------------------------------------------------|------------|
| Sargassum wightii         | not hydrolized       | Escherichia coli, Klebsiella pneumoniae, Vibrio cholerae, Proteus proteus, Shigella sonnie, Pseudomonas aeruginosa, Salmonella typhi | [23]       |
| Sargassum polycystum      | not hydrolized       | Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli, Salmonella typhimurium, Enterococcus faecalis, Streptococcus mutans, Streptococcus oralis, Streptococcus sobrinus, Streptococcus sanguinis | [25]       |
| Fucus vesiculosus         | not hydrolized       | Aeromonos hydrophila                                                                                                        | [26]       |
| Spatoglossum asperum      | not hydrolized       | Escherichia coli, Pseudomonas aeruginosa                                                                                     | [28]       |
| Nizamuddinia zanardinii   | not hydrolized       | Did not show antibacterial activity                                                                                           | [29]       |
| Laminaria japonica        | not hydrolized       | Escherichia coli, Staphylococcus aureus                                                                                      | [29]       |
| Laminaria japonica        | Hydrolyzed to oligosaccharides | Did not show antibacterial activity                                                                                           | [29]       |

It is known that fucoidans of brown algae Sargassum wightii and Sargassum polycystum show antibacterial action on human and animal pathogens, including fish [23]; fucoidan Fucus vesiculosus is active in the relations of plaque bacteria and food pathogenic bacteria [25]. Fucoidan from Spatoglossum asperum algae shows antimicrobial activity in relation to Aeromonos hydrophila bacteria, one of the main pathogens of fish [24]. It is important to note that antimicrobial activity depends on the type of algae, method of fucoidan production, chemical composition of polysaccharide, number of sulfates, molecular weight, and structural and conformation features of the structure [26]. Fucoidans obtained by different methods of extraction from brown algae had differences in the molar percentage of sugars in their composition, molecular weight, and exhibited antimicrobial activity with respect to different bacteria [27]. Partial depolymerization of fucoidan Fucus vesiculosus led to a change in the severity of antimicrobial effect [28], and hydrolysis of fucoidan from Laminaria japonica, which does not exhibit antibacterial action, led to the formation of products of different degree of polymerization with a pronounced antimicrobial activity [29]. Thus, the antibacterial properties of fucoidans have been studied quite extensively, with little information on their antifungal activity. According to the report [30] commercial fucoidan shows a pronounced fungicidal effect on Candida albicans. Data on antifungal activity of brown algae fucoidans with respect to Saprolegnia spp. is not available.

4. Conclusion

The search for new biological methods to address saprolegnosis in aquaculture is currently an acute issue. Natural biologically active substances can act as an alternative to chemical substances and physical methods of saprolegnosis control. An important direction of research is to obtain and study...
the biocidal properties of natural polymers. Carbohydrates are more complex biopolymers for synthesis and structural characteristics than nucleic acids and proteins, the development of antifungal preparations based on carbohydrates is only being developed. Of particular scientific and practical interest in this regard is fucoidan, which has a number of biological activities and valuable properties (non-toxicity, biocompatibility, biodegradability). Despite the fact that a large number of studies have been devoted to the biocidal activity of fucoidan, the mechanisms of biocidal activity of this biopolymer at the cellular and molecular levels have not been disclosed. The relationship between the chemical structure of fucoidan and its biological effect on cells of microorganisms is still not fully established. There are conflicting information about the influence of the molecular mass of fucoidan on its biocidal action. In this regard, studies on the structure of cyclic oligosaccharides and brown algae fucoidan, in particular, are relevant and form the basis for the development of new drugs with antimicrobial properties.

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