THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212
April, 2023; 3(04), 527-530

Popular Article

Saprolegnia infection: a hurdle in aquaculture

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https://doi.org/10.5281/zenodo.7874712

Introduction

Saprolegnia is a genus of oomycetes or water mold that can infect fish causing a disease known as saprolegniasis. Oomycetes is a class of fungus-like organisms but they are not true fungi. They differ from fungi in the composition of cell wall and chromosome content in the vegetative stage. Unlike fungus, they have non-septate hyphae and are more closely related to algae. Taxonomically, Oomycetes was previously divided into six orders which have been expanded in recent years. One of the order, Saprolegniales contains three main genera, *Saprolegnia*, *Achlya* and *Aphanomyces*. *Saprolegnia* species are primarily considered as saprobes which feed on decayed organic matter, though some can cause secondary infection in fish depending on the immune status of the host. However, under right circumstances, species like *Saprolegnia parasitica* can act as primary pathogens especially on catfish, salmon and trout species. The infected fish are lethargic and show loss of appetite. A very prominent sign of *Saprolegnia* infection is presence of white patches on the body surface of infected fish. The organisms also produce white hyphae which forms visible cotton-like mycelial growth at the site of infection especially fins, gills or skin. As the disease progress, the hyphal growth spread throughout the body and ultimately the infected fish die due to osmoregulatory failure. *Saprolegnia* species can infect different life stages of fish, right from egg to adult fish causing huge economic loss in terms of millions of dollars in aquaculture. Economic loss may be due to

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Published 28.04.2023

decreased growth rate, poor quality of fish, high mortality, expensive medications and additional labour cost. *Saprolegnia* species is endemic to all the fresh water habitats worldwide and are also considered responsible for decline in wild population of fish and amphibians.

Isolation and identification

Saprolegnia species can be isolated from the site of infection. For isolation, mycelia or tuft of hyphae are collected and cultured on suitable media such as potato dextrose agar. They produce white hyphae which grow radially on agar. The hyphae are aseptate which can be clearly observed microscopically. There are two types of hyphae; i) rhizoidal which penetrates into the substratum to anchor and absorb nutrition, ii) extrametrical which extends above the surface and bears reproductive organs. *Saprolegnia* reproduces both asexually and sexually. In asexual reproduction, there is release of primary zoospores which further encyst after few minutes and releases secondary zoospore, the infective stage. In sexual reproduction, there is production of gametangium, antheridia and oogonia which fuses to form morphologically distinct oospore. Traditionally, species identification largely relies upon characteristics of the sexual reproductive structures. However, defining species based on these morphological characteristics is not consistent and often impossible because these structures do not usually form on lesions. Sometimes, the morphological characteristics of different species are similar which may lead to misidentification. Further, many isolates fail to develop these structures in laboratory culture condition and it requires an expert taxonomist for species identification. So, an easier way to identify *Saprolegnia* species is through molecular approach. Most commonly, amplification of internal transcribed spacer (ITS) region followed by sequencing is practiced for species identification in *Saprolegnia*. ITS region is the spacer DNA situated between the small subunit and large subunit ribosomal DNAs, where ITS1 is located between 18S rDNA and 5.8S rDNA followed ITS2 and 28S rDNA in the eukaryotic genome. This region has highly variable nucleotide sequence and has been recommended as the barcode for fungal identification. Other added advantages of using ITS region is its small size flanked by conserved sequences and its high copy number making it easy to detect even from small quantities of genomic DNA. PCR amplification of this region can be done by using universal primers; ITS1 (forward) and ITS4 (reverse). This will give amplicons of 750 bp approximately that can be easily visualized in gel electrophoresis. Further, we have also developed a multiplex PCR protocol using primers targeting a hypothetical protein gene in addition to ITS1/ITS4 for identification of *S. parasitica*. This will amplify two target genes in one

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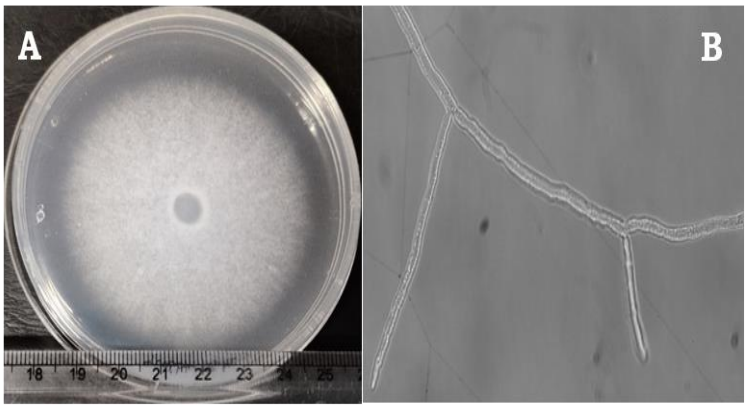
reaction producing amplicons of 750 bp and 365 bp in case of *S. parasitica* whereas the 365 bp PCR products will not be there in other *Saprolegnia* species. Generally, more accurate species identification can be achieved through combined approach using both morphological and molecular approach. Correct identification of the causative agent is important because different species may exhibit variable susceptibility towards anti-oomycete agent.



A- *Saprolegnia parasitica* on potato dextrose agar
B- Aseptate and branched hypha of *S. parasitica*

Treatment

Earlier, *Saprolegnia* infections were successfully controlled by using malachite green. Since malachite green poses significant health risk, it has been banned for use in aquaculture. This has led to recurrence of *Saprolegnia* as an economically important fish pathogen. At present, there is no drug as effective as malachite green for control of saprolegniasis. Many research publications reported the trial of various chemicals for their anti-*Saprolegnia* activity. One of them is formalin which is reported to be effective against *Saprolegnia* but associated with issues of environmental pollution, accumulation in flesh and health hazard. Other chemicals such as hydrogen peroxide, boric acid, potassium permanganate, peracetic acid and sodium chloride have also demonstrated anti-*Saprolegnia* activity. Some have used ozone treatment of water but this cannot be used for curing infected fish. We have also evaluated the effect of chlorhexidine gluconate against different *Saprolegnia* species. It has broad antimicrobial activity and can kill microbes rapidly. In our study, it was found that the drug can disrupt cell membrane of *Saprolegnia*, ultimately killing it. Some researchers have also tried plant derived compounds against *Saprolegnia* and found complete inhibition *in vitro*. Future research may focus on development of rapid identification protocol and effective compounds for use against *Saprolegnia* infection.

Prevention and control

In addition to use of chemicals for control of saprolegniasis, environmental management is equally important to prevent the spread of infection. It is essential to maintain good water quality to

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prevent *Saprolegnia* infection. This includes maintaining the water temperature, pH, dissolved oxygen at optimum levels to reduce stress on the fish. Excessive stocking and rough handling should be avoided to keep the fish in good condition. Rearing of fish of similar size and sex can be practiced if possible, to prevent fighting and injuries which may invite infectious diseases. Further, good farm management practices such as proper disinfection of equipments and quarantine of new stock before introduction to the existing populations should be carried out with utmost sincerity to prevent the spread of infection. Another important aspect to make the farmed fish healthy is the proper nutrition. A balanced and high-quality diet can improve the immune system of fish and can defend against infection. Overall, prevention and control of *Saprolegnia* infection requires good management practices in farm environment and early detection and treatment to prevent further spread of infection.

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