Original Article

Molecular Detection of Fungi from Fish Lakes in Wasit Province

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Abstract

This study will look for the fungus Saprolegnia parasitica from fish in several lakes in the Wasit Province that are associated with fish farming (carp). Sixty samples were collected from several organs, including the gills, skin, and eyes. Which bears the following signs (skin blisters, gills that have turned dark gray from necrosis, and a blow to the eyes led) were collected from lakes in the Wasit Province during the period (October 2021- December 2021). It might take a lot of time and effort to isolate and identify fungi using traditional procedures. The fish parasite Saprolegnia parasitica may be identified using DNA-based fungal detection techniques (carp). According to the results of this PCR study, fish do not harbor this fungus. As a result, it manifests as deficiencies. It therefore shows itself as inadequacies. Poor management, including a poor feed, insufficient oxygen application, and unclean water, were to blame for the dead fish.

Keywords: Molecular Detection, Fish lakes, Fungi.

INTRODUCTION

Poor water quality is the main culprit, poor fish health is not uncommon. Healthy fish are more likely to reside in clean water. Fungal infections (also referred to as mycoses) among the most prevalent illnesses affecting fish from temperate regions. Fungus spores, which can harm stressed fish, are present in every fish pond. A healthy fish population may become more susceptible to fungal diseases due to poor water quality. Only a few fungal infections infect the fish's internal organs, with the majority of fungal infections largely affecting the external tissues of the fish (1). Fungal infections are possible as a result of inadequate cleanliness, bad water quality, and fish that have been injured, The pond is filled with numerous dead fish and decaying organic matter (1). The world's top dietary protein source is fish, particularly in countries in Africa. Like other vertebrates, aquatic animals are susceptible to a number of harmful organisms, such as fungus (2). Aquaculture has emerged as a significant way to fill the hole left by the rapidly diminishing natural fisheries by providing a necessary and required source of protein for human consumption (3). Unfavorable conditions in aquaculture can promote the spread of fish diseases. Infectious fungal infections in particular have diminished fish productivity and caused financial losses (4).

Fungal infections in fish are thought to be a secondary cause of illness because fungi that affect fish are opportunistic, attacking the fish only when they are agitated or immune-compromised (5). Despite their significance, fungal diseases are poorly known because they are hard to spot and cause the proliferation of saprophytic organisms after fish have perished (6). Saprolegnia, Achlya, and Aphanomyces, which are members of the Saprolegniales and contain those three fungi, were responsible for the well-known fungal infection. The eggs of the rare host species that saprolegniosis infects have a severe negative impact on the freshwater ecology (7).

The saprolegniaceous fungus, commonly known as water molds, is a typical element of the aquatic environment and is capable of year-round development and reproduction. Aims this study used molecular identification fungi Saprolegnia parasitica from fish (carp).

Material and methods

1- Collection of Fish Samples:

A total of 60 samples of fish (carp) with skin lesions and ulcers had been gathered, and their necrosis had turned their gills and eyes a rich gray color. For the duration of the time from selected lakes in Wasit province that have significant fish mortality (October 2021 - December 2021). After collecting the infected samples, the molecular identification of fish samples were immediately transported to the laboratory, Figure (1).

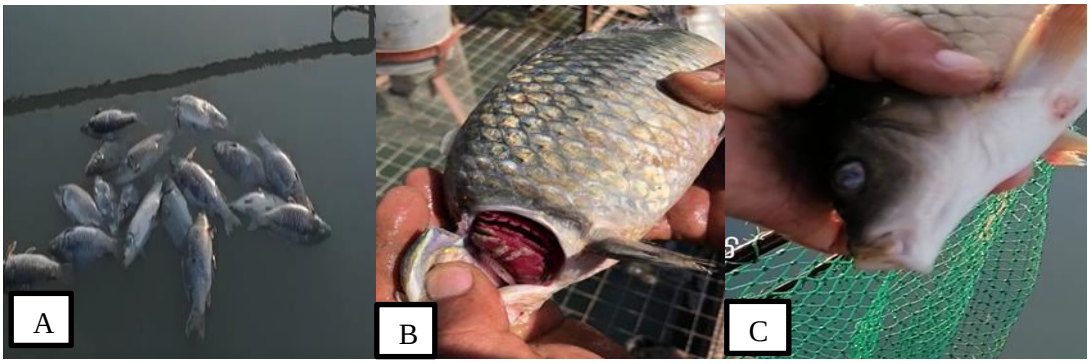

Figure (1): A- Dead fish , B- Gray gills, C- Blow gray eye.

Methods and Workflow:

A- DNA extraction

Using ABIOpure, fungal genomic DNA was isolated from tissue samples. DNA extraction kit using the manufacturer's instructions.

Add 1 ml of Lysis Mix to the sterile microcentrifuge tube in which you have placed the tissue sample. To mix, make sure the tissue is well submerged in the Lysis Buffer Vortex for 10 to 15 seconds. 1.0 ml was centrifuged at 13000 rpm for 2 minutes. The supernatant was discarded, and the pelleted cells (tissue) were suspended in 200 µl of Buffer CL with 20 µl of proteinase K added to each tube and well mixed using a vortex mixer. The tubes were then incubated for 10 min at 56 oC, with mixing occurring every 3 min. Each tube was then filled with 200 µl of BL cell lysis buffer, which was combined using a pulse-vortex mixer. All tubes were then incubated at 70 °C for 60 minutes while being stirred every three minutes. After that, 200 µl of pure ethanol were added to the lysate (shaking vigorously). The mixture was transferred to the spin column, together with any precipitate, and centrifuged there for one minute at 6000 rpm. Instead of the filtrate, the spin column was placed in a brand-new 2 ml collecting tube together with 600 µl of buffer BW. The collecting tube was once again used after the spin column had been centrifuged for a second time for one minute at 6000 rpm. Each column was treated with 700 µl of wash buffer, which was then added before it was centrifuged once for 1 minute at 6000 rpm, removing the flow through, and then inserting the column into a fresh 2 ml collection tube. This process was done several times to dry the column membrane. The spin column was placed in a brand-new 1.5 ml tube, and 100 µl of the buffer AE was poured straight over the membrane. After one minute at room temperature of incubation, the spin column was centrifuged for one minute at 13,000 rpm. After allowing each tube to stand for one minute to ensure the elution buffer was absorbed by the matrix, all of the tubes were centrifuged at 5000 rpm for 30 seconds to elute the pure DNA. Using a Nano Drop spectrophotometer and the following calculations, the concentration of the extracted DNA was calculated: 1OD260=50ng, purity=260/280.

B- Primer Design

In this investigation, PCR primers were designed using the NCBI-GenBank database. Traditionally, the primer set was made up of specific sense primers matching to the sequences. S. parasitica primer was chosen based on the alignment of the ITS1 and ITS2 regions of S. parasitica. Primer sets with a certain vibe that match the sequences according to a reference, S.parasitica -F (5'AGAGCAAATCGGTAGTTT-3') and S.parasitica -R (5'-AGAAATGCACCAGCATACCA-3') are both 127 bp in size (8).

C- PCR amplification

PCR master mix reaction preparation is used (GoTag Green Master Mix kit protocol). According to the manufacturer's instructions, 20 µl of nuclease-free water, one µleach of forward and reverse primers (10 pmol), and three µl of template DNA were added to the master mix. For 30 cycles, the PCR machine was configured as shown in (table 1). PCR results were run for an hour at 100 volts on an agarose gel electrophoresis, where DNA bands were seen with a UV transilluminator and captured on camera.

Table (2): PCR program setting for fungal

Step	Temperature	Time	Cycles
Initial denaturation	95°C	5 min	1
Denaturation	95°C	30 sec	30
Annealing	*Saprolegnia parasitica* 60°C	30 sec	
Extension	72°C	30 sec	
Final extension	72°C	7 min	1
Hold	10	10 min	

Results and Discussion

In this work, attempts to isolate the fungus Saprolegnia parasitica from fish yielded no results (carp). There was no isolation.

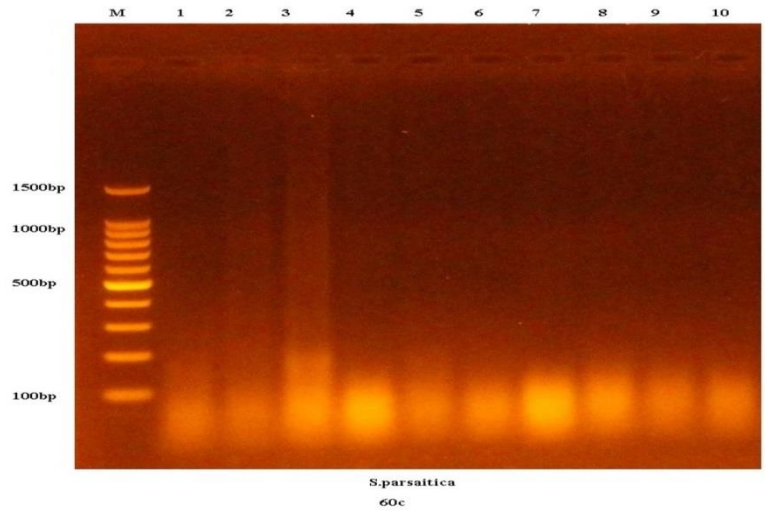

Figure (2): Pathogenic fungus PCR product analysis is shown in an agarose gel electrophoresis image. Lane M Marker Ladder, Lanes (1-10) (1500 bp), isolate of Saprolegnia parasitica having 127 base pairs.

New opportunities to address ecological dynamics for species that are tough to identify using conventional approaches are being created by the rapid expansion of the use of DNA to identify target species (9). None of the samples utilized in our investigation confirmed the presence of S. parasitica, the most commonly identified parasite, at amounts equal to those seen in (8). However, this study cannot be regarded as definitive due to the limited number of samples and the current lack of pathogens in fish lake water.

The most current research contradicts results made by (10), who said that three fungus taxa, Saprolegnia, Aphanomyces, and Branchiomyces, were found in cutaneous lesions and fish specimens with ill gills. Major fish egg illnesses include saprolegnia and aphanomyces, which have been connected to ulcer-type lesion sickness and branchiomyces, which has been linked to gill rot disease..

Culture, serology, and histology, which are traditional techniques for detecting fish illnesses, are probably slower and less precise than molecular technology. Molecular techniques have been used increasingly regularly during the past 15 years or more to diagnose fish infections. The Polymerase Chain Reaction (PCR), restriction enzyme digestion, probe hybridization, in situ hybridization, and microarray are a few of these methods. Pathogens may be found in asymptomatic fish utilizing molecular diagnostic methods, enabling the halting of an outbreak of sickness. Lessening the use of antibiotics can help prevent the emergence of bacteria that are resistant to them. The potential uses for molecular methods for fish illness detection are discussed in this study along with an assessment of the methods' current state. employing cutting-edge techniques as a common tool in a diagnostic lab (11 and 12).

Conclusion:

There is currently insufficient epidemiological information on fish diseases caused by fungi in the Wasit province. In this work, attempts to isolate the fungus Saprolegnia parasitica from fish yielded no results (carp). There was no seclusion. As a result, it manifests as deficiencies. The deceased fish were a result of inadequate management, which also included a subpar meal, insufficient oxygen application, and dirty water.

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