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ORIGINAL ARTICLE

Studies on Extracellular Phospholipase Enzyme Produced by Clinical Isolates of Fungi Isolated from Fresh Water Fishes with Reference to Jabalpur Region

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ABSTRACT
The production of fresh water fishes Catla catla trade has increased significantly over the past decade. Concerning the high demand for fresh water fishes, these freshwater fish are susceptible to fungi infection that can cause mortality to individual and fish eggs. The purpose of this study is to determine their extracellular enzymes for virulent screening. Six fishes with apparent signs of infection such as ulcerative, haemorrhages and dermal lesions were collected from Gaur River, Jabalpur (MP). Fungi were isolated from the fish's internal organs (liver and kidney) as well as external organs (mouth, fin, skin, and gills). The extracellular enzyme test such as phospholipase enzyme test was used to examine this fungus. The dense white zone of precipitation around the colonies of phospholipase positive isolates was distinctive and well defined. The test strain of fungi that was screened for this study turned out to be a productive source of enzymes that break down proteins and polysaccharides. The production of the extracellular phospholipase enzyme by Aspergillus-like species demonstrated the isolate's virulence and potential to spread infection.
Keywords: *phospholipase, haemorrhages, polysaccharides*

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INTRODUCTION

Freshwater fishes are an important protein source for people of many countries and are one of the most important groups of vertebrates which provide free economic services to human beings in several ways. The fresh water resources has a rich diversity of fresh water fishes including fish species like *Catla catla* [8]. There are more than 600 species of freshwater fungi with a greater number known from temperate, as compared to tropical, regions. Three main groups can be considered which include ingoldian fungi, aquatic ascomycetes and non-ingoldian hyphomycetes, chytrids and, oomycetes. The primary causes for fungal infections are, the change in water temperature, decreased water quality, injury due to trauma or excessive handling, or another disease caused by bacteria orprotozoan [2]. Oomycetes are sapro-phytic opportunists multiplying on fish that are physically injured, stressed or infected. Members of this group are generally considered as agents of secondary infections arising from conditions such as bacterial infection, poor husbandry practices, and infestations by parasites and social interactions. The oomycetes (fungi) are the second most damaging pathogens of freshwater fishes after bacteria. One of the most destructive oomycete pathogens of fish is *Aspergillus*. It is endemic to all freshwater habitats and is responsible for the decrease in natural fish populations. Several factors are involved in the development of fungal infections in fish. These factors may affect the fish or the fungus, and it is a combination of factors rather than any single condition, which ultimately leads to infection. It has long been considered that the fungi responsible for saprolegniasis are secondary pathogens, and lesions are commonly seen after handling and after traumatic damage to the skin, in overcrowded conditions, and conjunction with pollution or bacterial or parasitic or viral infections. The temperature has a significant effect on the development of infections. As the majority of fungal infections are secondary invaders, the fungal

infection is considered as mixed infections. Fungal infection in fishes causes damages on various parts of the body Fungal infections of fish harmed fisheries and aquaculture. Aquatic fungi surface water infects aquatic animals, causing infections such as lobomycosis, acute ulceration (ulcerative dermal necrosis) and gill rot in fish. Aquatic fungi and water molds from freshwater sources have been reported to be responsible for the infection of aquatic species. The presence of toxic aquatic fungi in water tends to infect aquatic animals, including those that are consumed by humans. Humans may be directly exposed to fungal toxins through ingestion of sea food like fish, prawns, Spirulina [7]. Fungal infection in fish has long been a concern particularly for fish culturists. Many of the fungi that affect the fishes are considered opportunistic, attacking the fish body when the fish is under stress due to unfavourable environmental conditions or attacked by any other pathogen. Fungi are known to attack fish eggs, fry, fingerlings and adult fish. Water molds infections cause losses of freshwater fishes and thin eggs in both natural and commercial fish farm The purpose of this study is to determine their extracellular enzymes for virulent screening.

MATERIAL AND METHODS

Screening of fungal isolates for phospholipase production

Screening is highly selective procedure for detection and isolation of only those microorganisms which are of interest. It gives selective information, which produces phospholipase. Study was conducted for the screening of phospholipase production by all the strain isolated from fresh water fishes. The amount of enzyme production by the strains was determined by calculating their Pz values on the 5th and 8th day of incubation. A total of 50 clinical strains were screened for its phospholipase activity on Sabouraud's Dextrose Egg Yolk Media (SEA). All the above contents were mixed together except egg yolk and the media was autoclaved for 15 min. at 15 lbs. Then the egg yolk was separated and added to the medium. Now the molten sterilized medium was poured in sterile glass petriplates aseptically. After solidification of the medium the plates were spot inoculated with actively growing colony of pure culture. Inoculations in the petriplates were done by inverting the petriplates to avoid the falling of inoculums on the entire surface of media. So that clear zone can be observed. These plates were incubated at 28±2°C and 37°C for 5 days. Then the zones around the colonies were observed. The radius of the clearing zone was measured and considered criteria for extracellular phospholipase activity of fungal isolates. Zone formation around the colonies of isolates indicates the positive activity and vice versa.

Measurement and Calculation of zone of phospholipase activity Pz

The visible precipitation zones of the test solutions were measured after 3rd and 5th day of incubation. The zone estimation was done by using the zone reader (Himedia). Test medium was translucent. The dense white zone of precipitation around the colonies of phospholipase positive isolates was distinctive and well defined. Phospholipase activity measured in terms of the ratio of diameter of the colony to the total diameter of colony plus (+) zone of precipitation. According to different Pz values strains were classified into 5 groups:-Negative Pz group (-) i.e. very much low phospholipase production [Greater than 1.0]. Very high Pz group (+) i.e. very low phospholipase production [below 0.90-1.0]. High Pz group (++) i.e. low phospholipase production [below 0.89-0.80]. Low Pz group (+++) i.e. high phospholipase production [below 0.79-0.70]. Very low Pz group (++++) i.e. very high phospholipase production (Pz < 0.69). Isolated fungal strains including *Aspergillus niger, Aspergillus fumigates* and *Aspergillus flavus* were screened for its phospholipase activity on Sabouraud's Dextrose Egg Yolk Media (SEA). The dense white zone of precipitation around the colonies of phospholipase positive isolates was distinctive and well defined.

RESULTS AND DISCUSSION

In our results we calculated Pz value and mean of *Aspergillug niger, Aspergillus fumigatus* and *Aspergillus flavus* obtained from fish samples screened for phospholipase production on 5th and 8th day of incubation. Following strains showed very low Pz group (++++): O 19, O 78, O115, O120, O125, O128, all of them shows very high phospholipase production with range (less than 0.69) i.e. very high phospholipase production (Pz < 0.69). Mean was calculated for each strain on the basis of Pz value on 5th and 8th day. On the basis of mean calculated, O128 strain shows very high phospholipase production with mean 0.24 for *Aspergillug niger*. For *Aspergillus fumigatus* these strain showed low Pz group(++++):O13, O44, O45, O74, O85, and O107, all of them shows very high phospholipase production with range (less than 0.69) i.e. very high phospholipase production (Pz< 0.69). Mean was calculated for each strain on the basis of Pz value on 5th and 8th day. On the basis of mean calculated, O 136 strain shows very high phospholipase production with mean 0.24. Similar studies were conducted by [2], extracellular enzyme activity forms a clear zone, allowing the organism to consume protein from the agar [2] In addition, the

lipolytic activity demonstrates the ability of the fungi to hydrolyse lipids [4]. Proteolytic enzymes can cause massive tissue damage in the host, which facilitates an infection [5]. Protease can cleave peptide bonds and functions in the pathogenesis and virulence of specific illnesses [3]. Meanwhile, for amylase activity that involve polysaccharide degrading enzymes, the production of this enzyme often relates to the ability of the organism to protect itself from being killed by various environmental condition [6] and also able to degrade.

Table 1: Pz value and mean of *Aspergilllus niger* obtained from Fish samples screened for phospholipase production on 5thand 8thday of incubation

S.No.	Strain description	strains	Source	Diameter of colony	Pz value (5thday)	Pz value (8thday)	Mean
1.	O19	*Aspergillus niger*	Fish	10mm	0.41	0.31	0.36
2.	O78	*Aspergillus niger*	Fish	10mm	0.28	0.35	0.31
3.	O115	*Aspergillus niger*	Fish	10mm	0.41	0.37	0.39
4.	O120	*Aspergillus niger*	Fish	10mm	0.35	0.35	0.35
5.	O125	*Aspergillus niger*	Fish	10mm	0.23	0.41	0.32
6.	O128	*Aspergillus niger*	Fish	10mm	0.25	0.23	0.24

Table2: Pz value and mean of *Aspergillus fumigates* obtained from Fish samples screened for phospholipase production on 5thand 8thday of incubation

S.No.	Strain description	strains	Source	Diameter of colony	Pz value (5thday)	Pz value (8thday)	Mean
1.	O13	*Aspergillus fumigatus*	Fish	10mm	0.37	0.38	0,37
2.	044	*Aspergillus fumigatus*	Fish	10mm	0.26	0.29	0.275
3.	O45	*Aspergillus fumigatus*	Fish	10mm	0.23	0.25	0.24
4.	074	*Aspergillus fumigatus*	Fish	10mm	0.31	0.26	0.285
5.	O85	*Aspergillus fumigatus*	Fish	10mm	0.28	0.35	0.315
6.	O107	*Aspergillus fumigatus*	Fish	10mm	0.41	0.41	0.41

Table3:Pz value and mean of *Aspergillus flavus* obtained from Fish samples screened for phospholipase production on 5thand 8thday of incubation

S.No.	Strain description	strains	Source	Diameter of colony	Pz value (5thday)	Pz value (8thday)	Mean
1.	O38	*Aspergillus flavus*	Fish	10mm	0.41	0.31	0.36
2.	O59	*Aspergillus flavus*	Fish	10mm	0.28	0.35	0.315
3.	O63	*Aspergillus flavus*	Fish	10mm	0.4	0.37	0.385
4.	O89	*Aspergillus flavus*	Fish	10mm	0.35	0.35	0.35
5.	O101	*Aspergillus flavus*	Fish	10mm	0.23	0.41	0.32
6.	O136	*Aspergillus flavus*	Fish	10mm	0.25	0.23	0.24

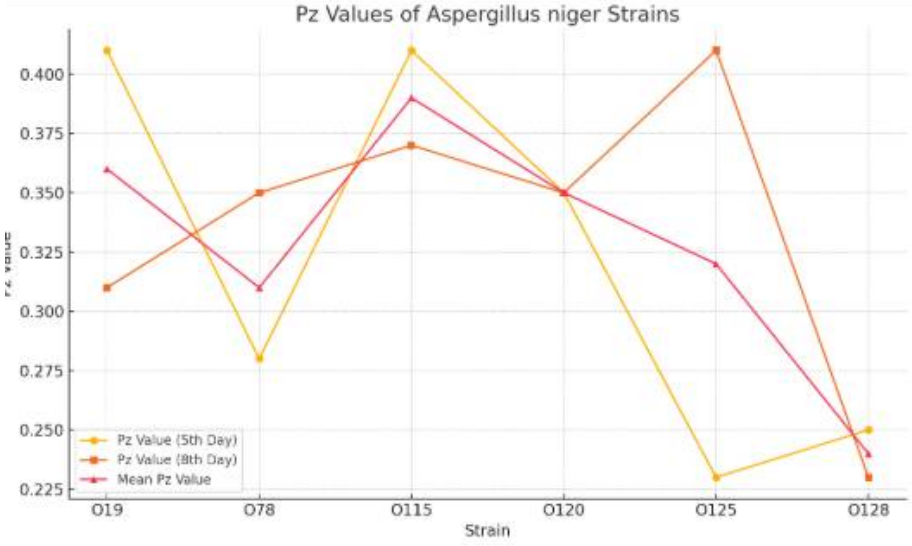

Fig 1: Graph showing the Pz values of different ***Aspergillus niger*** strains on the 5th day, 8th day, and their mean

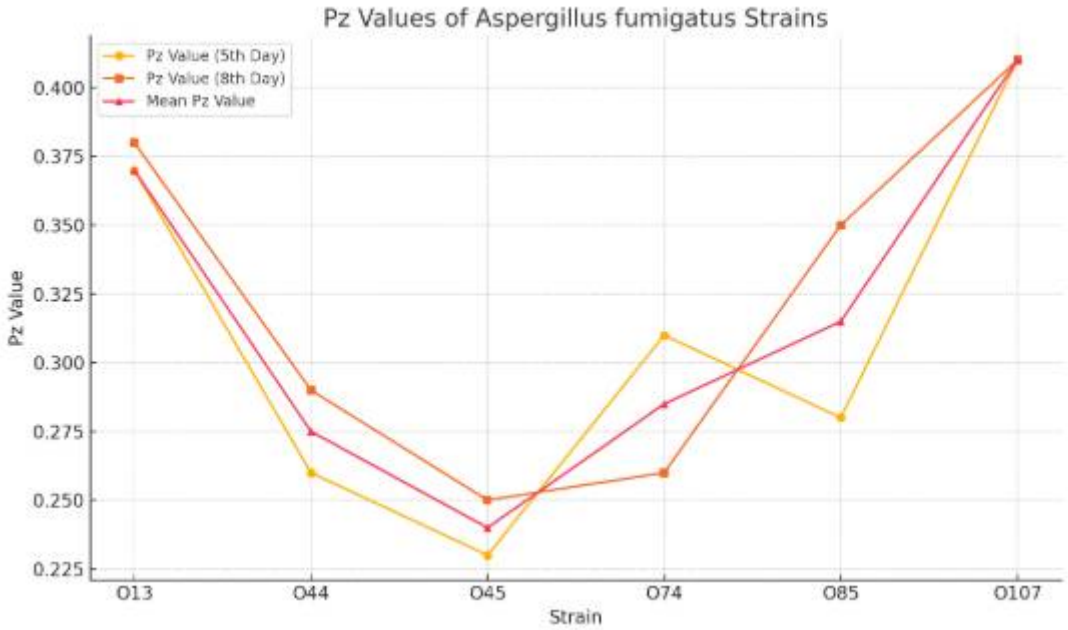

Fig 2: Graph showing the Pz values of different ***Aspergillus fumigatus*** strains on the 5th day, 8th day, and their mean.

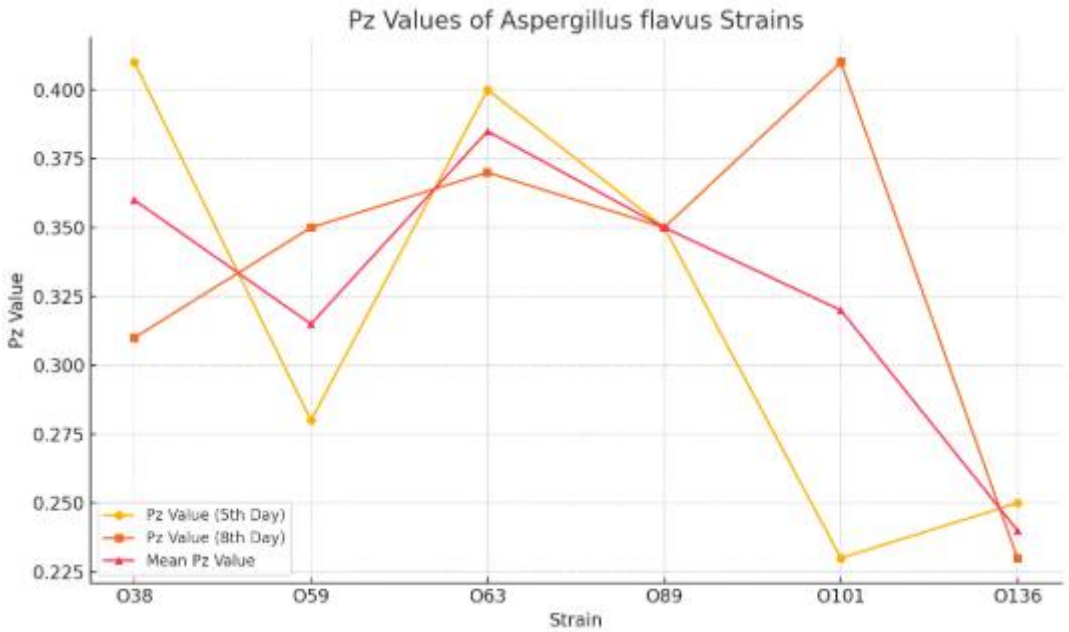

Fig 2: Pz values of different ***Aspergillus flavus*** strains on the 5th and 8th day, along with their mean values.

CONCLUSION

Fungi that contribute to the pathogenicity and virulence of infected fish have successfully adapted to the aquatic environment. The present study was conducted to characterise extracellular enzyme production of fungi solated from catla catla. *Aspergillus niger, Aspergillus fumigatus* and *Aspergillus flavus* was successfully isolated. The test strain of fungi that was screened for this study turned out to be a productive source of enzymes that break down proteins and polysaccharides. The production of the extracellular phospholipase enzyme by *Aspergillus*-like species demonstrated the isolate's virulence and potential to spread infection.

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