



Research Article

ISOLATION AND SCREENING OF PATHOGENIC MICROBES IN POND CULTURED INDIAN MAJOR CARPS

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ABSTRACT

Aquaculture plays a very important role in generating farmer's income. However, the rapid and often uncontrolled development of aquaculture has led to frequency occurrences of infectious diseases that are threatening the sustainability of aquaculture. At the same time, the problem of fish diseases becomes more serious with the development of the intensive breeding and the increase in culture sale and density. Fresh water fishes are an important Protein source for people of many countries. In the present study is to investigate bacterial & fungal infections in three economically important freshwater fishes, such as *Catla catla*, *Labeorohita*, and *Cirrhinus mrigal* were collected from fish culture pond, Thiruvarur District, Tamilnadu. Nearly Three sp of fungi *Aspergillus sp*, *Mucor sp*, and *Trichoderma viride sp*, and two species of bacteria, such as *Aeromonas sp*, and *Vibrio sp*, were isolated.

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INTRODUCTION

Aquaculture is an emerging industrial sector which requires continued research with scientific and technical developments and innovation. The world aquaculture production in 2001 was approximately of 37.9 million tons, which represents around 41% of that obtained from extensive captures for human consumption [FAO, 2003]. Fish is an excellent and relatively cheap source of animal protein that may help to bridge the protein gap because of its nutritional quality. Carp cultured in India has a long history, most of the formed carps are marketed with in the country for domestic consumption in recent years, tillets, flayces and other value-added products of carps has been exported to middle east countries surprisingly, carp production was not reported in the feed and agriculture organization(FAO) fish productions statistics until recently.

Fish farming in various parts of the world has increased many folds in the last decade. As a result, fish culture has now become commercially an important industry worldwide. Diseases in fresh water fishes are a great threat to achieve optimum production and become a limiting factor to economic success of aquaculture. Fungi which are responsible for a microbe of diseases are presented in freshwater.

In aquaculture large scale mortalities of fish occur due to infectious microbial and parasitic diseases caused due to high dense culture (or) by pollution mediated environmental stress. Protecting the fish from diseases can be done by through two ways. One is by strengthening the self, immune power of the organism and the second is through medication. [Stephen Sampath Kumar 2006]

Aquaculture has also been a growing activity for more than 2 decades worldwide and fungal infecting (mycoses) are amongst the common diseases in hatchery and aquaculture systems leading to the demise of fish population resulting in great economic loss [Fisher *et al.*, 2012] Fortunately, in most fungal infection, since only the external tissue of fish and eggs are involved a good antibiotic agent can solve most of the major fungal infection. Fungal infections are mainly caused due to immune suppression. Fungi can attack fishes of all the eggs and it can also present successful hatching when it invades fish eggs. Among numerous aquatic fungi species of oomycetes have special importance because of their effect on fish health.

Bacterial diseases have been reported to the principal liming factor in both with wild and cultured fishes. The most common and frequently encountered bacterial pathogen in India is *Aeromonas hydrophila*. Which causes severe damage to carp production? *Aeromonas hydrophila* is associated with wide range of freshwater fishes. It is an important pathogen in causing stress related diseases in fish with the common symptoms of ulceration, exophthalmia and abdominal distension. The most common approach to treated bacterial diseases is the application of antibiotics.

Fish affected by this classical *Vibriosis* show typical signs of a generalized septicemia with haemorrhage on the base of fins, exophthalmia and corneal opacity. Moribund fish are frequently anorexic with pale gills which reflect a severe anaemia. The *Vibriosis* distribution shows marked seasonal variations in natural reservoirs. During cold months, it is found in the marine sediment, during worn moths. It is found in coastal waters fish and shell fish.

Now a day's microbes are resistance to various antibiotics. The resistant of microbes is due to indiscriminate utilization of commercial antimicrobial medicines supported by many scientists investigation for modern antimicrobial substances from several medicinal plants [Alagesabooopathi and kalaiselvi, 2012]. However, recently use of antibiotics and chemotherapy has been criticized because their use has created problems with resistant bacteria and toxicity both in fish and environment.

REVIEW OF LITERATURE

Toor *et al.*, 1983 reported mortality of *labeorohita* due to fungal infection. Trust *et al.*, 1986 Studied the aeromonas is one of the most important genera of bacterial pathogens, of warm water fishes. Willoughby *et al.*, 1994 Fungi have wide range of infection depending on the management of form and environment. Kapoor *et al.*, 2001 reported that plant based medicine has survived through ages of millions all over the world. *Aeromonas salmonicida* is the causative agent of the fish disease called furunculosis.

Karatas dugenci and candan, *et al.*, 2003 Bacterial flora of some freshwater fishes in tropical water should that *Aeromonas sp.* Isolated from the skin, gill and intestine of the fish. Karatas Dugenci and candan *et al.*, 2003 reported the bacterial flora of some freshwater fishes in tropical water should that *aeromonas sp* was the most predominant microorganism isolated from the skin, gills and intestine of the fish. Fironzbakhsh *et al.*, 2005 were reported *Aspergillus niger* from common carp. Its eggs and environment. *A.niger* is the cause the internal and external infection of fishes. Jalilpoor *et al.*, 2006 *fusarium sp, saprolegnia sp, penicillium sp,* and *mucor sp,* were reported in the eggs of as pincer percicus. Guojun yin *et al.*, 2008 recently there has been an increased interest in the immune stimulations function of some herbs in aquaculture.

Junaid *et al.*, 2010 were isolated 7 fungal species from stock fish in Nigeria and these included *A.flavus, A.fumigatus, A.Niger, Triphophyton verrucosum, Rhizopus, mucor* and *penicillium sp,* and among these *mucor sp* should the highest occurrence.

Firoz *et al.*, 2011 studied that along this *aspergillus niger* and *neurospora sp* have been isolated first time from the fishes in India. Dhiman *et al.*, 2012 studied the siddha medicine *A.paniculata* used widely to treat fever like cikunguny, swine – flu, typhoid, snake bite and common cold etc.. Das *et al.*, 2012 was isolated *saprolegnia sp,* from farmed raised fingerlings of *L.rohita, C.catla* and *C.mrigal.* Iqbal *et al.*, 2012 infection of *C.catla* and *C.idella* by *saprolegneia sp,* and *achyla sp* was observed.

Das *et al.*, 2012 isolated *saprolegnia sp* from formed raised fingerlings of *labeo rohita, Catla catla* and *Cirrhinus mrigal.* Infection of *L.rohita* and silver carp, *Hypophthalmichthys molitrix* with *aspergillus sp,* and *alternaria sp,* has also been reported. Fisher *et al.*, 2012 reported that more than 600 million people could be feed each year by hunting the spread of fungal diseases in the world's five most important carps. *Aeromonas* are ubiquitous in fresh water, fish and shell fish and also in meats and fresh vegetables. Zafar iqbal and saira saleemi 2013 observed the isolation of pathogenic fungi from a freshwater commercial fish *Catla catla* (Hamilton).

Chandrakala *et al.*, 2013 were reported comparative study on the antivibrio activity of *Vibrio species* isolated from *Penaous monodon, Sillage sihama* against species. Zafar Iqbal *et al.*, 2014 reported the fungal infection in silver carp, *Hypophthalmichthys molitrix* reared in earthen panel. Rashmi kumara and Chandan kumar 2015 reported the fungal infection in some economically important fresh water fishes in Gander River near muzaffarpur region of Bihar.

MATERIALS AND METHODS

Isolation of Bacteria (Aneja, 1994)

The infected Indian major carps were collected from fish culture ponds, Thiruvarur district, Tamilnadu. The scrapped samples were streaked on Aeromonas agar, Thiosulphate Citrate Bile Salt, sucrose agar (TCBS). The plates were incubated at 37°C for 24 to 48 hrs. The colour, colony morphology was observed and the isolates were subjected to series of Biochemical Batteries. The isolates were characterized by compared with Bergey's manual of Determinative Bacteriology (Holt *et al.*, 1994) and a set of keys for Biochemical identification of environmental *vibrio sp* (Alsina and Blanch, 1994),

Isolation of Fungai (Aneja, 1994)

The scrapped sample plated on Potato Dextrose Agar (PDA) (P^H 6.8, Himedia, Mumbai). The plates were incubated at 30°C. After Incubation the fungal isolates were characterized by lactophenol cotton blue method.

RESULTS

The present study was carried out on the isolation of bacteria and isolation of fungi from infected Indian major carps, such as *Catla catla, Labeo rohita, Cirrhinus mrigal.* The finding results are presented in this chapter.

The bacteria isolated from infected fish sample by serial dilution techniques. Two different colonies were observed in *Aeromonas sp & Vibrio sp* are presented in plate I. This study results were compared with Bergey's mamal of classification; the isolated effluent bacterial colonies are confirmed as *Aeromonas sp & Vibrio sp.* The results are present in table 1.

Table -1 Isolation of Bacteria

S.No	Name of the organisms	Catla catla	Labeo rohita	Cirrhinus mrigal	Colour
1	<i>Aeromonas sp</i>	+	+	+	Green
2	<i>Vibrio sp</i>	-	+	+	Yellow and Green

The study on the infected major carps was examined for fungal infections. Fungi isolated from tissues of fishes were inoculated on agar plates. Four types of fungal colonies appeared on agar plates; Black and green colour colonies plate II. Four fungal genera *Aspergillus Flavus, Aspergillus niger, Trichoderma viride* and *Mucor sp.* The results are present in table - 2.

Table – 2Isolation of Fungi

S.NO	Name of the organisms	Catla catla	Labeo rohita	Cirrhinus mrigal	Colour
1	<i>Aspergillus niger</i>	-	+	+	Black
2	<i>Aspergillus flavus</i>	-	+	+	Green
3	<i>Mucor sp</i>	+	+	-	Black
4	<i>Trichoderma virite</i>	-	-	+	Green

DISCUSSION

In the present study, isolation and identification of bacteria and fungi from infected fish Indian major carps. The investigated results were discussed with previous theoretical values. The high percentage incidence *Aeromonas sp.*, and *Vibrio sp.*, in diseased fishes from various parts of the country strongly suggested the ubiquitous nature of the bacterium. Similar observation were recorded by Liobera and Gacutlan, 1987; shariffe *et al.*, 1988; kumar, 1989; karunasagar *et al.*, 1989; and mukherjee, 1991. The outbreak of a disease, which had more than 75% mortality among Indian major carps, was found mainly due to *A. hydrophila* [lakshman *et al.*, 1989]. Gopalakrishnan (1961) reported many instances of entire population of Indian major carps being wiped out by epidemics of *A. hydrophila*. Besides the usual manifestation as dropsy, ulcerative forms of infection, in fishes are also produced by these organisms. (Mukherjee, 1991 karunasagar *et al.*, 1986).

Vibriosis is characterized by severe anaemia and extensive haemorrhage – especially in the integument surrounding especially in the internal organs of fish including the caeca abdominal fat and kidney. A generalized septicemia with large numbers of bacteria is usually found in the blood of affected fish. Fungi which are responsible for a number of diseases are present in fresh water. Fungal infections are mainly caused due to immune suppression. Fungi can attack fishes of all the ages and it invades fish eggs. Among numerous aquatic fungi species of oomycetes have special importance because of their effect on fish health (West., 2006).

Fungal infection was studied in *C. catla* three fungal genera, *Aspergillus sp.*, *Blastomyces sp.*, and *penicillium sp.*, were isolated from the head, operculum, eye, buccal cavity, abdomen, skin and gills of these fishes. Eye infection may result in blindness and the fungal hyphae may further penetrate into brain and such fishes cannot be treated and these fishes eventually die (Srivastava, 2009). *Aspergillo mycosis* is the disease of African fish tilapia (*oreochromis sp*) and the causative agent of this disease are species; such as *Aspergillus flavus*, *Aspergillus terreus* and *Japonicas* (willough by 1994)

Aspergillus niger from common carp, its eggs and environment (Firouzbakhsh *et al.*, 2005) *Aspergillus niger* is the cause the internal and external infection in fishes. *Fusarium sp.*, *saprolegina sp.*, *penicillium sp.*, and *Mucor sp.*, were reported in the eggs of *Aspencer percicus* (Jalilpoor *et al.*, 2006) *Aspergillus niger* lead to internal and external infection in fish (Firouzbakhsh *et al.*, 2005) Some species of *Aspergillus* produce a group of mycotoxins called aflatoxins. Which is major cause of spoilage of grains and other foods *Aspergillus sp.*, are presume by infectious and spread in fishes through the use of contaminated fish feed (Anderson *et al.*, 2006) (Saleem *et al.*, 2012).

Isolate 7 fungal species from stock fish in Nigeria and these included *A. flavus*, *A. Fumigatus*, *A. niger*, *Trihophytan verrucosum*, *Rhizopus mucor* and *penicillium sp.*, and among these *mucor sp* showed the highest occurrence (Junaid *et al.*, 2010) isolated 11 fungal species from infected eggs of rainbow trout (Shabzhain *et al.*, 2010). Pathogenic fungi are having a pronounced effect on the global biota increasing the

amount of fungal disease on them and this threat is ever increasing. Substantial data to support the idea that fungi pose a greater threat to plant and animal biodiversity relative to other taxonomic classes of pathogens is described and reviewed by (Meyer 1991) fungal disease are second to bacterial infections that cause immenses economic losses in aquaculture (Ramaiah 2006).

Isolation of *Aspergillus sp* from pond water has given an indication of pond contamination. Hence attention must be paid to carry out good pond and fish health management through the use of good quality inputs such as feed and water, moreover, regular fish health monitoring may also be practiced. Infectious diseases are a major cause of morbidity and mortality worldwide, currently the on going battle against bacteria prevails certainly of evolving resistance, on the other hand advancement in medical field results in more patients being in critical and immune suppressed states, thus creating a perpetual need for new antibiotics. As a result it is the right time discover new antibiotics

References

- Anderson, B and Thrane U. 2006. Food –borne fungi in fruit and cereals and their production of mycotoxins. In ; Hocking A.D.Samson R.A.Pitt J.I and Thrane u.(Eds) Advances in food mycology. *Springer, USA*, pp : 137.
- Aneja, K.R., 1994. Biochemical activities of micro organisms. In: Experiments in microbiology, plant pathology, Tissue culture and mushroom production technology. New age international publishers. (III Ed) pp 245-275.
- Alagesboopathi C ,Kalaiselvi N .2012 .Antimicrobial activity of the root, stem and extracts of *Argemone Mexicana* L. *Int .J.Biosci.* 2 (5): 61 – 68
- Alsina, M and Blanch, A.R, 1994. A set of keys for biochemical identification of environmental *Vibrio Species* *J. Applied. Bacteriol.* 61:431 – 467.
- Chandrakala N , sankar Ganesh P, Prabakaran M , Thangamathi P, Aruljothi R and Bharathidasan R . 2013 . Comparative study on the antivibrio activity of *Vibrio sp* isolated from *Penaesus monodon* ,*Sillago sihama* against species . 42 (7) .
- Dhiman A ,Goyal J ,Sharma K . A , Dhiman S . 2012 . A review on medicinal prospectives of *Andrographis paniculata* Nees JPSI .PP . 1 -4 .
- Das ,S.K.,K,Mumu.,A, Das ,I,Shakuntala R.K Das ,S.V.Ngachan, and S.K.Mjhi.,(2012).studies on the identification and control of a pathogen *saprolegnia* in selected Indian major carp fingerlings at mild altitude. *J.Envior..Biol.*33, 545-549
- Firouzbakhsh, F., E.H.A Ebrahim zadeh Morusavinad A.R Khosravi.2005. Isolation and identification of pathogenic and *saprophytic* Fungi from gill lesion in cultivated cyprinids (*common carp silver carp and grass carp*) *J.Fac.vet.med.univ.Tehran.*, 60(1),15-19.
- Firoz, Mehdi, Hamidreza. 2011. Fresh water fungi isolated from eggs and broodstocks with an emphasis on *saprolegnia* in rainbow trout ferns in west Iran .*Africa J. of microbiology research*.vol.14 (22):3647-3651
- Fisher M.C, Henk D.A,Briygs C.J,Brownstein JS, MadoffLC,*et al.*, 2012 .Emerging fungal threats to animal, plant and ecosystem health, *nature* 484: 186-194

- Goplakrishnan.V. 1961 .observation on infections dropsy of Indian carps and its experimental induction.*J.Sci.Ind.Res.*,20(C):357-358
- Gvojun yin ,Laszlo Ardo ,zsigmond Jeney pao Xu ,Galina Jeney .2008 .Chines herbs (*Lonicera japonica* and *ganoderma lucidum*) .Enhance the non-specific Immune response of Tilapia.VI 269-282.
- Holt, J.G., Kreig,N.R., Sneath, P.H.A., Stantey, J.T and Williams, S.T., 1994. Bergey's manual determinative Bacteriology (IXEd). Pp 259-279.
- Iqbal,Z.,Asghar.,Mand Rubaba.2012 a . *Saprolegniasis* in two commercially important carps.pak.*J.Zoal*,vol.(44)jeikh and 2: 515-520.
- Jalilpoor.J.,S.A.Mosoules and M.Masoumzoden.2006 . fungal flora in acipensersicus eggs with particular emphasis on *saprolegniapa* (oomycetes)and mortlity during mass incubation at the sahid Behesti hatchery.*J.App.Icthyol*.22(suppl.1),265-268.
- Junaid ,S.A.Olarubofin ,Fand olabode ,A.O.2010 . Mycotic contamination of stock fish sold in Jos ,*Nigeria*. *J.Yeast and fungal Res*,1:136-141.
- Karunasagar,I.,P.K.M.M.Ali.G.Jeyasekaran and I.karunasagar ,1986 . Ulcerative from of *Aeromonas hydrophila* infection of *Catla catla*.*curr.sci.*,55:1194-1195
- Karunassagar,I.,G.M.Rosalind,I.karunasagar and k.Gopal Rao ,1989 . *Aeromonas hydrophila* septicemia of Indian major carps in some commercial fish farms of west Godavari dist. *Andra Pradesh curr.sci*,58:1044-1045
- Kumar,D.,1989 . Epizootic ultertive syndrome out-break in india.summer instant fish Dis. and Health Man.in freshwater Aqua .syst ,5-24 June CIFA ,Bhubaneswar.
- Kapoor L.D ,2001 . Hand book of Ayurvedic medicinal plants .crc press florida 416.
- Karatas Digeenci,S,candan ,2003 . A isolation of *Aeromonas* strains from the intestinal floa of atlantic (*salmon salmosalar* L.1758).Turk *J.vet.Anim.sci.*,(27):1071-1075.
- Lakshmanan.M.,Sunder,K.Lipton,A.P.1989. Isolation and characterization of *Aeromonas hydrophila* in Indian major carp, *laberorohita* (zan).*curr.sci.*,55:1080-1081.
- Liobrera, A.T and R.O.Gacuttan, 1987. *Aeromonas hydrophila* associated with ulcerative disease. Epizootics in Lagunade Bay, philippnes. *Aquacult.*, 67:273-278.
- Meyer F. P .1991.Aquaculture disease and health management. *J Anim sci*(69):4201-4208.
- Mukherjee, S.C,1991 . Epizootic ulcerative syndrome in india.Training course in fish Dis, and contrl.CIFA, Bhubaneswar, P:22-27
- Ramaiah N, 2006. A review on fungal disease of algae, marine fishes,shrimps and corals, *Ind J.mar sci* (35): 380-387.
- Rashmi kumara and DR.Chandan kumar, 2015 .Fugal infection in some economically important fresh water fishes in Gandank river near muzaffarpur region of BiHar.vol(5).
- Shariff,K.D.,J.L.Torres,L.A.Theem and M.N.Shamsudin ,1988 . EVS threatens aquaculture industry .Research News ,UPM,20PP.
- Stephen sampath kumar,J.Ananthraja.k. 2006 .Herabal health care in Aquaculture the Indian experience.
- Srivastava,R.C. 2009. Fish mycopathology.Today and tomorrow's publishers New Dehli, India. pp106.
- Shahbazain, N.Ebrahimzaden, M.Soltani, M., Khosravi, A.R.Mirzagai, S and I.sharifpour, I. 2010. Fungal contamination in rainbow trout eggs in Kermanshah province propagation with emphasis on *saprolegniaceae* Iranian *J.Fish sci*,9:151-160.
- Saleem ,M.J.A.Hassan,A.V Nisa and T.A.Qasir, 2012 . Occurance of aflatoxin in mazine seed under different conditions. *Int.J.Agric.Biol*.14:473-476.
- Toor,S.H.,Sehgal.H.and Sehdev,R.S.1983 . A Case study of acute fish diseases in tanks loaded with high level of organic manures.*Aquaculture*.35:277-282
- Trust ,T.J. 1986. Pathogenesis of infections diseases of fish.*Ann.Rev.Microbial*.40:479-502
- Von Sonntag,C 1987. The chemical basis of radiation biology.Taylor and francis Ltd.,London,United kingdom.
- Willoughby,L.G.1994. Fungi and fish disease. pisces press,stirling.UK.pp57.
- West P.V, 2006. *Saprolegnia paracitica*,an oomycete pathogen with a fishy appetite : New challenges for an old problem,mycologist.
- Zafar Iqbal and saira saleemi., 2013 . Isolation of pathogenic fungi from a fresh water commercial fish ,*Catla* 25(4),851-855.
- Zafar Iqbal, Uzma Najam and saira saleemi. 2014 .Fungal infection in *Silver carp*, *Hypophthalmichthys molitrix* reared in earthen pond .26(1), 261-266.
