Fungal Pathogens of Fish Diseases and Nutritional Losses

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Introduction

Human health and nutrition bears direct relationaship with foods, their quality, intake patterns and bioavaility. In this respect fish plays an important role, as fish is well known as an excellent source of food and nutrion. But this important source of nutrition is often contaminated and infected with pathogenic organisms like bacteria, fungi, protozon and viruses. It is observed that the contaminated and infected fish sometime create health hazards in many areas of Bangladesh.

Since effective vaccines are not available, the best approach for controlling fungal diseases is the prevention of fungal infections. But the information and literatures on the diagnostic and identification aspects of fungal diseases and infections of fish are extremely insufficient. At the sametime, the need of rapid fungal identification techniques for fish and fishery products are urgently felt by the fishery extension researchers and the industrialists.

The diagnostic, isolation and identification aspects of bacterial diseases of fish and fishery products has been described in the previous paper.[1]

The purpose of this paper is to describe the major diagnostic and identification procedure used for fungal diseases and infections which will undoubltedly help in controlling diseases and infections in fish and, thus, will help in protecting the nutritional losses of fish and the health hazards of consumers. In most cases of outbreaks of diseases, the rapid identification will expedite information neededd to control fungal infection of fish.

Materials and Methods

Fungal pathogens of fish

Prolific fungal growth may occur after the death of fish. So, sometime, it would be difficult to conclude whether the fungus was a parasitic or suprophytic on necrosed tissue. Consequently histological techniques constitute an important feature of disease diagnosis. Two easily diagnosed fish diseases are lymphocytes and viral erythrocytic necrosis, The lymphocystics infects a wide range of marine and fresh water fish and fish products. These are large charateristic cells with thick hyaline cell walls. Viral crythocytic necrosis infects a number of marine and anadromous fish species and its presence is readily detectable by observing cytoplasmic inclusion in erythrocytes.

Isolation Techniques

In isolation procedure a comparatively wide range of agar containing media are used [2-4]. Details of the isolation

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and identification of the common groups of fungi are shown in Table 1,2 and 3.

Suspected fungal infection make a series of examination which lead to the isolation and selection of specimen. Dead specimen should not be used for diagnostic purposes because of the rapid migration and growth of environmental organisms into the body[5]. Infected tissuses from the deep body of the sample should be examined. The deep-seated tissues are less likely to be contaminated with extraneous organisms, such as bacteria. However, all the samples should be sterilized to avoid contaminations before testing for the fungal infection. A short (1-2 seconds) immersion in ethanol, or washing in starile water are to be completed carefully. Attention should be made so that the sterilent does not pentrate into the tissues and kill all the pathogens. This technique has been used successfully with isolation procedures for *Amphanomyoes astaci* in case of outbreaks of crayfish plaque[6-8]. A small piece of tissue from infected salmonids can be maintained in steriled lake water at 7°C for 7 days and the tissue could be examined regularly for the presence of fungi. Noga and Dykstra (1986)[9] incubated a piece of diseased tissue onto the surface of agar plates, and transferred the newly emerging hyphal tips to fresh media. This method is time consuming and labourious.

In certain circumstances, digestion of samples are important to release the fungi. Gareth Jones (1971)[10] has described a method in which sample of tissuces are digested for 8-10 hr at a temperature o f 35°C in 0.25% (v/v) tryps in and the digested tissue is passed through a fine cloth, centrifuged at 350 g, and washed four times with sterile water. The pellet is resuspended in sterile sea water supplemented with 0.5 mg/ml each of penicilline G and streptomycin sulphate. Propagulas released are used to inoculate in isolation media.

Media

Cultural techniques are required to identify pathogenic infection. This is necessary because different microorganisms sometimes give similar clinical signs (e.g., septicaemia, granulomas, ulcers etc.) and it frequently shows a mixed infection which can be eveluated only by cultural methods.

So, there is a need for antimicrobial agents to be incorporated into the media. Such inhibitors reduce the problem of microbial contamination. Alderman and Polglase (1986)[7] mixed streptomycin (500 µg/ml) and penicillin g (0.5 IU/ml). Where as, Noga and Dykstra (1986)[9] used a combination of penicillin (100 IU/ml). and stretomycin (100 µg/ml) into the media for isolation of fungal pathogens in fish. similarly, therre is a need for sodium chloride upto 5% (w/v) in the media for marine species[11]. Anyway PDA (Peptone destose agar) and SDA (Sabouraude dextrose agar) is used generally in routine diagnostic laboratories for fungi from fresh water fish. A list of recommended isolation media has been included in Table 1 and 2.

Results and Discussion

Recommended isolation media and properties of fungi as parasites of fish have been studied and completed following the research findings of various workers[12-27].

Greater attention has been mede to fish pathogenic member of the oomycetes, a white 'cottony' growth on fish 23. Recently saccharomyces, a yeast has been identified from rainbow trout from England. Fish attacked by this pathogen displayed oedema in the kidney-an accumulation of ascitic fluid in the peritoneal cavity. Histological study reveals some evidence that there is involvement of yeast in this respect.

The important fungal pathogens of fish with differential characteristics are given below.

1. Candida

Members of the genus candida are characterized by a long vegetative cells which produce multipolar blasto-conidia with hyphae. Arthroconidia, ballistocondia and colony pigmentation are always absent. Fermentation and assimilation tests are also important for the identification of yeasts. *Candida sake* and *C. albicans* are recognized as fish pathogens. Candida sake was first isolated from the gastric *fluid* of a diseased salmona[12]. The yeast grew well in 13.0-32.0^{0}C in 6% Nacl and pH 3-8. *Candida albicans* was isolated from the skin of infected gray mullet (Mugil cephalus)[25], YM agar Teast, malt and agar : 0.3% yeast extract, 0.3% malt extract. 0.5% peptone, 1.0% glucoses, 1.5% agar) and Cornmeal Agar (CMA : 5% cornmeal, 1.5% agar) with tween 80 (1%) are useful for isolation and growth of this pathogen of fish[15].

2. Dermocystidium

Dermocystidium marinum is best known as a pathogen of oystors (Maekin et al, 1950)[28]. Parkins (1976)[29] studied through electrone microscope and found that the zoospores of the organism with a conoid, polar ring, rhoptries and micronemes and concluded the organisms as apicomplexa in protozoa group. Livin (1978)[30] demonstrated that this organisms did not belong to this group and created new genus *Perkinsus manimum*, under a class parkinsus. So, due to lack of detailed studes the taxonomic position of Dermocystidium species from fishes is not clear. The species of this organism is lacking a lid on the spore and is reproted to be a fungi whcih effect the gills and skin of fishes forming visible cysts. It is characterised that the cysts contain many spores with nuclear and special inclusions and reported as parasites of fish. Yeast extract 0.5%, glucose 1.2% and agar media is useful for isolation of this organism.[3]

3. Exophiala

Exophiala pisciphila and *Exophiala salmonis* are considered as fish pathogens. *E. pisciphila* was first reported in catfish by Fijan (1969)[31]. Iwatsu and Udagawa (1984)[32] isolated the fungus *E. salmonis* from retting wood, bamboo and a sandwich containing tuna meat and vegetables . Carmichael (1966)[33] described causative agent of an epidimic cerebral mycetoma of trout and Richards et al (1978)[34] found an

infection in the posterior kidney of Atlantic Salmon in sea water both caused by *E. Salmonis*. In cultured media the Exophiala colonies are restricted, smooth or velvery, dark on mall extract agar. Hyphae are pale, unbranched conidiophores which is mucoid and usually one-celled.

Media containig 1.83% (w/v) potato starch, 60% (w/v) fresh egg mixture, 0.73% (w/v) glycerol, 0.024% malachite green, 0.15% KH2 PO4, 0.015% MgSO4. 7H2 O are important for growth of this fungal pathogen of fish.

4. *Ichthyophonus hofor*

Ichthyophonus disease of fish caused by the fungus *Ichthyophonus intestinalis* and *I. gasterophilum*. The large spore germinates producing long slender branching coenocytic hyphae with thick tips. *Ichthyophonus* produces amoebablasts with thin walls, which rupture liberating mucleate froms and introduce into the intestine of the infected fish. Various spp. of *Ichthyophonus* have been recognized as parasitic to aquatic animal.

This disease producing fungus grow well on SDA (Sabourands, dextrose and agar media) supplemented with beef serum (1%) and glycerine (0.5%) (Neish and Hughes, 1980)[23]. The incubation temparature required for good growth is 10-20°C during 17-30 days.

5. *Fusarium*

Various *Fusarium* sp. are recognised as fish parasites. They produce both micro and macro condida. The macroconidia are several celled, from cylindrical to curved . This parasites are found in gills and scales of aquatic animals.

Aldermann and Polglase (1986)[14] stated that PDA (Potato extract, 2.0% dextrose and 1.0% agar) is important media for the identification of Fusarium species. The best method for observation of above characteristics of Fusarium is to grow them in slide culture and the growth rate is maintained on PSA (Potato, sucrose and agar) media at 25°C for 4-6 days (Hatai et al, 1986)[15].

6. *Phoma*

Hatai et al, (1986)[15] described a fungus with characteristics of genus phoma, isolated from diseased ayu fry *(Plecoglossus altirelts)*. They are also isolated from hatchery reared coho salmon, chinok salmon and rainbow trout (Ross et al, 1975[16] and Sparks et al, 1979)[35]. This type of fungus was also isolated from the air bladder of infected fish. A pure cuture of fungus was obtained from diseased fish. Colonies appeared on GY Agar medium at 21-22°C for 7-14 days.

The mycelial mat was whitish to greyish with varius shades of green colour. Mycelia were composed of slender, smooth, septate hyphae of 3-6 μm wide. The fungi belongs to the genus phoma are compared in plate cultures on oatmeal agar (OA) and malt agar (MA) because it stimulates the production of pycnidiospores and pycnidia.

7. *Saprolegnia*

Saprolegnia dicilina type I is the main pathogen of salmonids whereas type II is found in perch. This fungus produces non-septate, multinucleated, variably branched, transparent hyphae of 32-40 μm in diameter. Two types of zoospores may be produced by this organism which

can swim away from the zoosporangium before encystment. The primary cyst usually releases a secondary zoospore whcih is bean-shaped biflagellate and capable of active swimming. This encysts form secondary cysts with bundles of long hooked hairs which then germinate and produce mycelia[24]. *S. achlya* does not produce free-swimming primary zoospores. They produce encyst at the mouth of the sporangium, where they form a holow ball[25].

8. Ochroconis

Ochroconis was classified in the genus Scolecbasidium[36]. Colonies are brown to olivaceous in colour. Gonidiogenous cells are scattred, arising from undifferentated hyphae or intergrated in short lateral branches, elongate to cylindrical, sometime slightly swollen. Gonidia are one to four-celled, pigmented, smooth walled, ellipsoidal to cylindrical, broadly rounded at the tip, narrowed abruptly at the base and usually provided with a distincet hilum. *O. tshawytshae* and *O. Humicola* have been reported as fish pathogens. The fungus, however, was isolated from kidney mycoses of chinook salmon[37]. Although a fungal infection due to *O. tshawytschae* and not been reported since 1946. *O. humicola*, a previously undescribed fungal pathogen of fish was isolated from salmon by Ross or Leek (1975)[16]. The kidney was the most affected organ in natural infections. Ajellow et al (1977) also reported this pathogen in rainbow trout. The colonies of *O. tshawaytshae* on malt agar (MA) attain a diameter of about 15mm in 10 days, appearing slightly domes, velvety, brown to olive, Hyphae are 1.5-2.5 µm wide. Conidiopharos are slightly differentiated, *O. humicoloas* colonies grew on maize meal agar (MMA) attain a diameter of about 7mm in 10 days. Hyphae are subhyaline to pale olivaceous, being 1.5-2.5 µm wide. Conidiophores are slightly differentiated, cylindrical and upto about 100 µm long, 2-35 µm wide with thin septa.

9. Phialophora

Phialophora colonies grow on SDA media with 25mm diameter after incubation at 22°C for 2 months. An aerial mycelium does not develop[17]. The hyphae of Phialophora attains a thin walled smooth septate, cylindrical hyphae of 1-3 µm in width. Conidal are hyaline, aseptate, thin walled and smooth.

10. Haliphthoros

Two species of *Hliphthoros have* been described s parasites of rainbow trout. *H. milfordensis* is filamentius, branched, non-septate, 10-25 µm in diameter, occasionally developing thick sellings. The protoplasm is clear or slightly granular or foamy. They are transofrmed into sporangia with irregular to partly coiled or straight tubes. Zoospores are globose to pyriform, variable in sized 8 µm long, 4-8 µm wide [38]. *Philippinensis* is found in salmon and trout26. Hyphae are strongs, branched, irregular, non-septate. In pure culture, the hyphae are homotrichous, at first some what uniform, 10-37 µm in diameter. Zoospores of the primary type are biflagellate, elongate. formed within the sporangium, cleared by vacules, liberated through the opening of the

sporangium, poorly swimming and usually encyst after a period of mobility. Encysted spores are sperical, 5-7.5 µm on diameter.

Summary

Fish are often contaminated and spoiled by various pathogenic microoraganisms like bacteria, fungus and viruses. This paper has described the major fungal diseases and provided informations about sampling, isolation, culture media and identification techniques. Many of these methods could be used in accurate determination of fish diseases to help developing system usable for monitoring the health of fish population with minimum time, effort and equipments.

Table 1 : List of recommended isolation media for fungal parasites of fish [3, 12, 34]

Fungal species	Recommended isolation media
Candida	Bacts Mycobiotic agar : (1% w/v Bacto-Soytone, 1% (w/v) (Bacto-doxtrose, 1.5% (v/v) Bacto-agar, 9.04% (w/v) agar).9.04% (w/v) actidione, 0.005% (w/v) chloromycetin); agar (1% w/v soypeptone, 1.05% (w/v) glucose 0.005% (w/v) cholramphemicol, 1.5% (w/v) agen
Dermocystidium	YG agar and FME agar : (0.5% yeast, 1.2% glucose and fish meat extract 1.5% (w/v) agar).
Exophiala	PDA : 1.8% (w/v) potato staech, 60.0% (w/v) fresh egg mixture, 0.70% (w/v) glycerol, 0.22% (w/v) asperagine, 0.024% (w/v) malachite green, 0.15% (w/v) KH_2PO_2, 0.015% (w/v) MgSo4, 7H20, 0.037% (w/v) $Mg_3(C_5H_5O_7)_2$, H_20.
Aphanomyces	RGY agar : (o.1% (w/v) yeasr extract, 0.5% (w/v) glucose. 1.2% (w/v) agar) propared in river water incubated at 25°C.
	0.3% (w/v) Difco Bacto-peptone, 0.6% (w/v) glucose, basal medium2.
Ichthyophonus	Sabouraud dextrose medium supplemented with 1% (v/v) beef serum. SDA containing 0.5% (v/v) glycerine and 1.0% (v/v) beef serum.

Table 2 : List of recommended isolation media for fungal parasites of fish[3, 6, 7, 5, 22, 23].

Fungal species	Recommended isolation media
Ichthyophonus	Hagem's fungus medium (modified) : (1.8% (w/v) mutrient agar, 1.0% (w/v) glucose, 0.5% (w/v) malt extract, 0.05% (w/v) MgSo, $7H_2O$, 0.05% (w/v) NH_4Cl, 0.05% (w/v) KH_2P_4 0.0005% (w/v) thiamine. Hydrochloride, preparod in deionized water).
Fusarium	Bacto Mycobiotic agar : (1% (w/v) Bachto-soytine, 1% (w/v) Bacto-dextrose, 1.5% (w/v) Vacto-agar, 0.04% (w/v) actidione ; Mycosal agar : (1% (w/v) soypoptone, 1.05% (w/v) glucose, o.04% (w/v) cycloheximide, 1.5% (w/v) agar).RGY agar : (yeast, glucose, river water).
Phoma herbarum	Sabouraud's dextrose agar (SDA)
Phoma fimeti	Potato dextrose agar (PDA)
Phoma Sp.	GY agar (Glucose Yeast); Oatment agar : (OA : 6.0% (w/v) eatmeal, 1.25% (w/v) agar).
Saprolognia	PYG (1.2% (w/v) agar, 0.1% (w/v) glucose, 1% (w/v) gelatin hydrolysate, 0.001% (w/v) liver extract, 0.001% (w/v) yeast extract) prepared in sea water. After cooling (50.°C) 0.5g Streptomucin sulphate and 0.5g penicillin G. Peptone-glucose yeast extract agar (PYGEA): 0.5% (w/v) mycological peptone, 0.1% (w/v) glucese, 0.25% (w/v) yeast extract, 1.0% (w/v) agar, prepared in sea water or 2.0% (w/v) Nacl.

Table 3 : List of recommended isolation media for fungal parasites of fish[13, 15-18, 24, 34]

Fungal species	Recommended isolation media
Ochroconis Sp.	Sabouraud's dextrose agar, potato dextrose agar and cereal agar at 25°C.
Phialophara Sp.	Potato carrot agar : (2.0% (w/v) potato extract, 2.0% (w/v) carrot extract, 1.5% (w/v) agar).
Haliphthoros	Nutrient agar in 90% sea water, 0.1% (w/v)
mitofordensis.	gelatin hydrolysate, dried overnight at 37°C
sirolpidium	and spreaded with 0.2 ml aliquots of an infected larval culture together with 200 IU of crystalline penicillin G and 0.5 mg of streptomycin sulphate.

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