Indian Journal of Geo-Marine Science
Vol.44(8),August 2015,pp. 1213-1216

Isolation and identification of pathogenic fungi on *Oreochromis aureus*(Steindachner,1864)in the university of Basrah fish ponds

Khalidah S. Al-Niaeem,[1] Fuad Ameen,[2] Ashraf Hatamleh[2] & Marwah Bakri[3]

[1]Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq.
[2]Department of Botany & Microbiology, Faculty of Science, King Saud University, Riyadh 11451, Saudi Arabia.
[3]Department of Microbiology, Dean of Academic Campus for Girls, Jazan University, Saudi Arabia

*[E.Mail: alfouad2004@hotmail.com]

Received 30 December 2014; revised 19 January 2015

Thirty samples of *Oreochromis aureus* were collected from University of Basra fish ponds during the period from February until June 2014.Thirteen fish samples showed fungal infection. A sterile swab was taken from outer surface of body (head, skin, gills, abdomen, caudal fin, dorsal fin and pectoral fin). Potato dextrose agar and glucose yeast agar was used for fungal isolation.In this study six genera were identified and the most common were *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Penicillium* sp., *Brachiomyces* sp. and *Ichthyophonus* sp. Gills and abdomen were the most affected parts of fish. Among the generaobserved*Aspergillus* sp. and *Mucor* sp. were the most prevalent fungi infecting these fishes.

[Keywords: Fungal infection, Fish ponds, *Aspergillus* sp., *Oreochromis aureus*]

Introduction

Fungal diseases are the result of interactions of the pathogens, the fish and the environment. Fish in intensive culture are continuously affected by environmental fluctuations and management practices. All these factors should be considered for fish health control by preventing diseases rather than treatment[1, 2]. Many fungi cause fish diseases such as *Alternaria*sp. and *Poecilia reticulata* in freshwater ornamental fish *Carassius auratus*, *Xiphophorus maculates* and *Poecilia reticulate,*[3]*Aspergillus, Mucor* and *Rhizopus*wasisolated from koi (*Cyprinus carpio*) and shubunkin (*C. auratus*)[4]; *Saprolegnia* sp.from African Catfish (*Clarias gariepinus*)[5]; *Blastomyces* sp.and *Penicillium* sp.from*Catla catla*[6]*, S. diclina* was isolated from eggs of *C. carassius* in Białystok Rivers, Poland[7]; *Achlya* spp.and *Saprolegnia*spp. fromIndian major carps viz. *C. catla, Cirrhinus mrigala* and *Labeo rohita*from Sarangpani Lake[8]*; Ichthyophonus hoferii*from *Mugil capito, M. cephalus,Bighead carp,* and *Oreochromis niloticus* werecollected from 30 farms from different localities atAlexandria, Kafr El-Sheik and El-Behera in Egypt[9]*,Aphanomyces invadans*was isolated from Nile tilapia *O. niloticus* eggs in Thai hatcheries[10].The aim of present study was isolation and identification of fungi present on *O. aureus* in the University of Basrah fish ponds.

Materials & Methods

During the period from February until June 2014, a total of 30 fish samples of *Oreochromis aureus* were collected from University of Basrah fish ponds. Samplingof infected fish was carried out by collecting the fishin polythene bags. These were bought to the laboratory in living condition.Purification of cultures was done by preparing the cultures on Potato Dextrose Agar (PDA) and Glucose Yeast Agar (GYA). To inhibit the bacterial growth 500 µg/ ml each of penicillin and streptomycin was added to PDA and GY agar plates. All the cultures were incubated at temperatures 18±2 °C. Slides were prepared according to (Beakes *et al.*[11]) by taking material from each colony and staining with 0.05% trypanblue in lactophenol. The slides were observed under Digipro-labomed microscope and photographed. The fungi were identified with the help of available fungal identification keys and literature[12].The fish samples were surfaced sterilized with 70% ethanol and rinsed with three changes of sterile distilled water. A 10 g tissue portion of fishwas cut from the abdominal region with a sterile forceps, macerated aseptically in a mortar and mixed in 10 ml of sterile peptone water. From this mixture, further tenfold dilutions were made up to 10^3, and 0.1 milliliter of each dilution was plated in triplicate on potato dextrose agar (PDA) supplemented with streptomycin to

inhibit bacterial growth. Plates were incubated at 28 ± 2◦ C and examined daily for 7 days. The mean number of all fungal colonies appearing in the three plates was taken as the average number of colonies per plate for fish. This was used to estimate the number of colonies per gram of fish sample using a known dilution series.The prevalence of fungi (%) was calculated according to the following equation[13].

$$\text{Prevalence (\%)} = \frac{\text{Number of infected fishes}}{\text{Number of examined fishes}} \times 100$$

Results

The species of fungi distributed in 30 samples of *O. aureus* included *Aspergillus* sp.(49% samples), *Alternaria* sp. (25% samples), *Mucor* sp (37% samples), *Penicillium* sp. (27% samples), *Brachiomyces* sp. (30% samples) and *Ichthyophonus* sp. (20% samples). Details of mycofloraisolated from head, skin, gills, abdomen, caudal fin, dorsal fin and pectoral finare shown in (Fig. 1and 2). Gills and abdomenhad higher infection than rest of the organs (Table 1).*Aspergillus* sp. and *Mucor* sp. were the most prevalent fungi infecting these fishes (Table 1) and (Fig. 1).

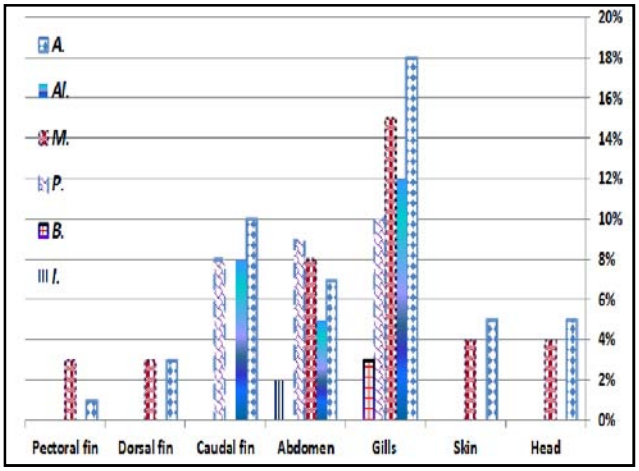

Fig. 1. The prevalence of fungal species: The frequency of isolation (%) from *O. aureus*. *A.: Aspergillus* sp., *Al.: Alternaria* sp., *M.: Mucor* sp., *P.: Penicillium* sp., *B.: Brachiomyces* sp. and *L.: Ichthyophonus* sp.

Table 1−The fungal species isolated from *O. aureus* and fungal colony counts from fish tissue

Fish organ	Fungal species	No. of colonies per gram of fish tissue
Head, Skin, Gills, Abdomen, Caudal fin, Dorsal fin, Pectoral fin	*Aspergillus* sp.	4.1 X 10^3
Gills, Abdomen, Caudal fin	*Alternaria* sp.	2.8 X 10^3
Head, Skin, Gills, Abdomen, Dorsal fin, Pectoral fin	*Mucor* sp.	3.5 X 10^3
Gills, Abdomen, Caudal fin	*Penicillium* sp.	1.3 X 10^3
Gills	*Brachiomyces* sp.	1.4 X 10
Abdomen	*Ichthyophonus* sp.	1.4 X 10

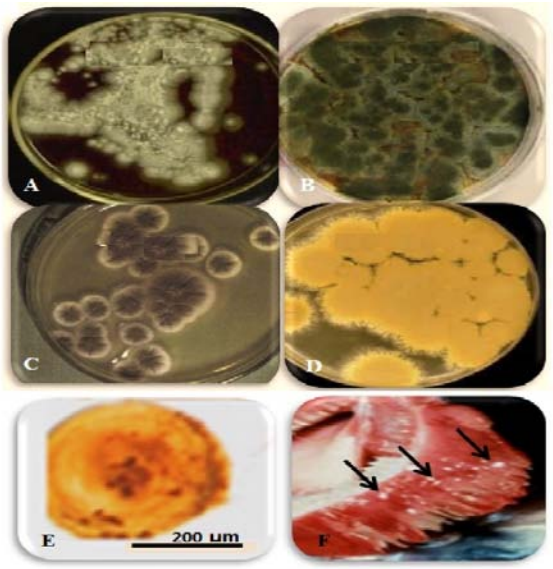

Fig. 2. The fungal species isolated from *O. aureus*: A: *Aspergillus* sp., B: *Alternaria* sp., C: *Mucor* sp., D: *Penicillium* sp., E: *Brachiomyces* sp. and F: *Ichthyophonus* sp.

Fungal infection was studied in *O. aureus*. Six fungi *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Penicillium* sp., *Brachiomyces* sp. and *Ichthyophonus* sp. were isolated from the head, skin, gills, abdomen, caudal fin, dorsal fin and pectoral fin of these fish samples. *Aspergillus* sp. was the most prevalent fungus infecting all the organs of *O. aureus*, followed by *Mucor* sp. and *Brachiomyces* sp.

Discussion

The infection observed on gills may lead to serious disease condition, and such fishes cannot be treated and these fishes eventually die[4]. Gill infection may interfere with respiratory function of the fish, However, skin and fin infection are considered less serious as compared to gills[6,14]. These fungi may not be considered as non-pathogenic, but they can be better understood as opportunistic fungi[15]as many of them possess virulence factors, which enable them to cause disease, especially under predisposing conditions[16]. Fin infection is considered less pathogenic as such fishes survive but this infection may lead to complete damage of the fins[17, 18]. The single most affected site was gills. The infection on sensitive areas like gills of fish may lead to serious disease conditions[17].

The poor management of fish ponds increases the chances of fungal infection in fishes[19]. This is indicated by isolation of *Aspergillus* sp. from aquarium water[20]. Source of fungal infection may be the consumption of contaminated feed present in the pond. Moreover, the decomposition of this feed may also add to infection[21].There might be certain other conditions in the pond which increasethe possibility of fungal infection including: poor pond management, injured fish or fish having other diseases, or large amounts of decomposing organic matter in pond[10].

In our study,isolation of *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Penicillium* sp., *Brachiomyces* sp. and *Ichthyophonus* sp. from fish samples has given an indication of pond contamination. Source of fungal infection may be the consumption of contaminated feed present in the pond. Moreover, the decomposition of this feed might have also added to infection[22]. There might be certain other conditions in the pond including injured fish or fish having other diseases, or large amounts of decomposing organic matter in pond[23, 24]. 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.

Acknowledgement

This work was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

References

1 Woo PTK, *Fish diseases and disorders*. *Viral. Bacterial and Fungal Infections*, (CABI publishing, London, UK) 2004, pp. 944.

2 Ramaiah, N., A review on fungal disease on algae, marine fishes, shrimps and corals, Indian J. Mar. Sci, 35(2006) 380-387.

3. Haroon, F., Iqbal Z, Pervaiz K, Khalid A, Incidence of fungal infection of freshwater Ornamental fish in Pakistan. Int. J. Agr. Biol. 16 (2014) 411-415.

4. Iqbal, Z. and Sajjad, R., Some pathogenic fungi parasitizing two exotic tropical ornamental fishes.Int. J. Agr. Biol. 15(2013) 595-598.

5. Abolude, D.S., Opabunmi, O.O., and Davies, O.A., Fresh water fungi associated with eggs and broodstock of African Catfish (Clarias Gariepinus, Burchell 1822) in fish hatchery farms, Zaria, Kaduna State, Nigeria. J. Res. Environ. Toxi. 2(2013) 131-135.

6 Iqbal, Z. and Saleemi, S., Isolation of pathogenic fungi from a freshwater commercial fish Catla catla (Hamliton). Sci. Int. (Lahore) 25 (2013) 851-855.

7 Bożena, K., Javier, D.U., and Maria, P.M., Water molds Saprolegnia diclina (FLO) isolated from eggs of Carassius carassius L. in Białystok Rivers, Poland. Afr. J. of Microbiol. Res.7 (2013) 5406-5410.

8 Rekha, C., Pinky, K., and Shivani, S., Pathogenicity of some species of Achlya and Saprolegnia on Indian Major carps viz. Catla catla, Cirrihinus mrigala and Labeo rohita. J. Environ. Sci. Comp. Engi. Tech. 1(2012) 422-428.

9 Shawer, R., Safinaz, G., Saleh, W., Soliman, M.K., Khalil, R., and Mona, S.Z., Some studies on fish deformity in freshwater fish in Egypt. Life Sci. 8 (2011) 415-422.

10 Prasatporn, B., Pithai, K., Chutima, H., Kanit, C., Daishi, F., and Kishio, H., (2009) Effects of Thai Herbs on the control of fungal infection in Tilapia eggs and the toxicity to the eggs. Aquaculture Sci. 57 (2009) 475-482.

11 Beakes, G.W., Wood, S.E., and Burr, A.W., Features, which characterize Saprolegnia, isolates from salmonid fish lesions. A review. In: Salmon Saprolegniasis (Mueller GJed.). Report to Bonneville Power Administration, Portland. (1994) 33-66.

12 Willoughby, L.G., Fungi and fish diseases. (Pisces Press, Stirling, UK) 1994.pp. 57.

13 Margolis, L., Esch, G.W., Holmes, J.C., Kuris, A.M., and Schad, G.A., The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). J. Parasitol.68 (1982) 131-133.

14 Fadaeifard, F., Raissy, M., Bahrami, H., Rahimi, E., and Najafipoor, A., Freshwater fungi isolated from eggs and broodstocks with an emphasis on Saprolegnia in rainbow trout farms in West Iran. *Afr. J. Microbiol. Res*. 4 (2011) 3047–3651.

15 Refai, M., Attia, S., Salem, R.M., and El-Dahsham, E.M., Studies on the pathology of *Aspergillus fumigatus*, *A. flavus* and *A. niger* isolated from chicken and their environment. *Egyptian J. Comp. Path. Clinic. Path*. 17 (2004) 193–205.

16 Refai, M., Laila, K., Amany, M., and Shimaa, El-S.M.S., The assessment of Mycotic settlement of freshwater fishes in Egypt. *The Journal of American Science*. 6 (2010) 595–602.

17 Iqbal, Z., Asghar, M. and Rubaba, M., Saprolegniasis in two commercially important carps. *Pak. J. Zool*. 44 (2012a) 515–520.
18 Iqbal, Z., Sheikh, U., and Rubaba, M., Fungal infections in some economically important freshwater fishes. *Pak. Vet. J*. 32 (2012b) 422–426.
19 Eli, A., Briyai, O.F. and Abowei, J.F.N., A Review of some fungi infection in African fish Saprolegniasis, Dermal Mycoses; Branchiomyces infections, Systemic Mycoses and Dermocystidium. *Asian J. Med. Sci*. 3 (2011) 198-205.
20 Prabhu, P., and Balasubramnian, U., Analysis of physiochemical parameters and fungal population in various tissues of *Catla catla*. *Adv. Appl. Sci. Res*.3 (2012) 2103-2107.
21 Panchai, K., Hanjavanit, C., and Kiatancharoen, N., Characteristic of Achlya bisexualis isolated from eggs of Nille Tilapia (Oreochromis niloticus Linn.). *KKU Res. J.* 12(2007) 195- 202.
22 Chukanhom, K., Study on fungal diseases of fresh water fishes in South East Asia. *Bull. Nippon. Vet.Ani. Sci. Uni.*54 (2005) 63-65.
23 Robert Durborow, M., David Wise, J., and Jeffery Terhune, S., Saprolegniasis (Winter Fungus) and Branchiomycosis of commercially cultured channel Catfish. *Southern SARC Publ*. (2003) No. 4700.
24 Firooz, F., Mehdi, R., Hamidreza, B., Ebrahim, R., and Ahmad, N., Freshwater fungi isolated from eggs and brood stocks with an emphasis on Saprolegnia in rainbow trout farms in west Iran. *Afri. J. Microbiol. Res*. 4 (2001) 3647-3651.