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Original Article

Isolation and Identification of fungi associated with diseased freshwater fishes in Terengganu, Malaysia

Khairulbariyyah Zakaria[1], Sudau Eh Teet[2], Nor Hazwani Hamzah[1], Alia Syafiqah Aznan[1], Mohamed Tajuddin Abdul Manaf[1], Wan Nurhafizah Wan Ibrahim[1], Lee Kok Leong[1], Nurul Aqilah Iberahim[1], Nadirah Musa[1], Laith Abdulrazzak[1], Hassan Mohd Daud[3], Mariam Taib[4], Kishio Hatai[5], Akbar John B[6], Jalal K. C. A.[7], Hassan I. Sheikh[1], and Najiah Musa[1*]

[1] *Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, 21030 Malaysia*

[2] *Department of Agrotechnology and Bio-industry, Politeknik Jeli Kelantan, Jeli, Kelantan, 17600 Malaysia*

[3] *Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, 43400 Malaysia*

[4] *Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, 21030 Malaysia*

[5]*Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, 88400 Malaysia*

[6] *INOCEM Research Station, International Islamic University Malaysia, Bandar Indera Mahkota, Kuantan, Pahang Darul Makmur, 25200 Malaysia*

[7]*Department of Marine Science, Kulliyyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota, Kuantan, Pahang Darul Makmur, 25200 Malaysia*

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Abstract

Fish health problem presents an ongoing challenge for aquaculture as disease outbreaks cause fish mortalities and financial losses. This study investigated the fungal infections in common freshwater ornamental and food fish species in Terengganu, Malaysia. Diseased gold gourami (*Trichopodus trichopterus*), snakeskin gourami (*Trichogaster pectoralis*), angelfish (*Pterophyllum scalare*), African sharptooth catfish (*Clarias gariepinus*), and red hybrid tilapia (*Oreochromis* spp.) with dermal lesions were sampled for fungal isolation. A total of 12 fungal isolates were isolated, and characterized by macro- and micro-morphologies of colonies. Isolates were identified by sequence analysis of ribosomal internal transcribed spacer (ITS) region. Genera *Aspergillus*, *Geotrichum* and *Pestalotiopsis* were more prevalent than *Flavodon*, *Pseudopestalotiopsis*, *Trichoderma* and *Apiotrichum* (*Trichosporon*). This study indicated the advantage of good hatchery settings and management practices in reducing fungal infections.

Keywords: aquaculture, freshwater fishes, fungi, internal transcribed spacer, ITS

*Corresponding author
Email address: najiah@umt.edu.my

1. Introduction

Aquaculture sector serves as an important mean in many countries to compensate the reduced yield of capture fisheries. Yet, significant increases in aquaculture activities also lead to emergence of fish diseases associated with pathogens such as parasites, fungi, bacteria and viruses (Grisez & Tan, 2005).

Diseases in fishes are mostly triggered by poor water quality (Yanong, 2003). Other than parasitic, bacterial and viral diseases, fungal and fungal-like infections in farmed food and ornamental fishes have increased tremendously (Gozlan, Marshall, Lilje, Jessop, Gleason, & Andreou, 2014). Fungal infections are commonly found in temperate fishes with saprolegniasis and *Ichthyosporidium* disease being the most common (Verma, 2008). Fungal infection in fish can be secondary to parasitic, bacterial or viral infections (Yanong, 2003). Most fungi such as those that cause saprolegniasis attack the external tissues of fish, while only few fungi infect the internal organs. *Ichthyosporidium* attacks the internal organs primarily the kidney and liver (Verma, 2008). Fungal infections in farmed food fish also pose potential risk for foodborne illnesses due to the fungi themselves or their byproducts. As such, isolating and identifying these fungi are important not only to understand their etiologic properties but also the sources of infection that are amenable to control while preventing mass mortalities in fish.

According to Van West (2006), the treatment options for fish seriously infected by fungal and fungal-like organisms are currently limited. The available drugs are also neither safe for the fish nor for human consumption of the treated fish. The usage of chemicals such as malachite green and formalin are effective to treat fungal diseases at very low concentrations, however they are hazardous to human, hence, malachite green has been banned worldwide (Robertson, Anderson, Phillips, Secombes, Dieguez-Uribeondo, & van West, 2009; Srivastava, Sinha, & Roy., 2004). Long exposure of fish to hydrogen peroxide and sodium chloride are also able to kill various fungal and fungal-like organisms in mild condition (Iberahim, Trusch, & Van West, 2019; Lilley & Inglis, 1997).

Fungi associated with fish such as *Aspergillus* spp. can be isolated using selective methods while identification can be done based on conventional methods such as macro and microscopic features (Wiese, Ohlendorf, Blümel, Schmaljohann, & Imhoff, 2011). However, this technique does not identify the pathogen to species level. Currently, sequence analysis of ribosomal internal transcribed spacer (ITS) region is considered as the most reliable method in fungal identification to species level (Raja, Miller, Pearce, & Oberlies, 2017). This study aimed (1) to isolate fungi from diseased common freshwater fishes in Terengganu, and (2) to identify the associated fungi. It is hoped that this work will provide the basis for more detailed study on fungi associated with fish diseases, and helps provide guidance for control measures to reduce the risk of fish mortalities due to fungal infections.

2. Materials and Methods

2.1 Sample collection

Thirteen live or moribund fish with apparent dermal lesions (hemorrhages, erosion and ulcers) were collected for this study. Gold gourami, a colored variety of three spot gourami *Trichopodus trichopterus* (n=1), snakeskin gourami *Trichogaster pectoralis* (n=1) and angelfish *Pterophyllum scalare* (n=3) were collected from ornamental fish outlets in Kuala Terengganu. African sharptooth catfish *Clarias gariepinus* (n=6) were sampled from commercial fish hatchery at Pengadang Baru, Terengganu. Red hybrid tilapia *Oreochromis* spp (n=1) and African sharptooth catfish (n=1) were sampled from Universiti Malaysia Terengganu (UMT) Hatchery (Kuala Nerus, Terengganu) (Table 1). Fish samples were immediately transported to Fish Disease Laboratory of UMT. The body wet weight (g) and total length (cm) of fishes were measured and recorded. All fishes were physically inspected for general body condition, dermal lesions and clinical signs including swimming behavior. Dermal tissue and the underneath muscle tissue were taken (approximately 1 cm^3), including the leading edge of the lesion and the surrounding tissue (Hatai & Egusa, 1979; Phadee, Kurata, Hatai, Hirono, & Aoki, 2004).

2.2 Isolation and morphological characterization

Each tissue sample was inoculated on the center of sabouraud dextrose agar (SDA) plate incorporated with penicillin G (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}). The plates were incubated at 25 °C for 7 days. Fungal colonies were subcultured on SDA until free from contamination. Pure isolates were stored on SDA slant for preservation. Isolates were characterized by macro- and micro-morphologies of colonies according to Walsh, Hayden, and Larone (2018) and Pitt and Hocking (2009). The macro features included the colony growth, color (surface and reverse), shape, size and gross hyphal structure (Afzal, Shazad, Qamar, & Nisa, 2013; Alsohaili & Bani-Hasan, 2018). The microscopic features included fine hyphae morphology (aseptate or septate), conidia and phialide shapes (Bandh, Kamili, & Ganai, 2012). Mycelial particles were mounted on slides and stained with lactophenol cotton blue stain prior to micro-morphological examinations under light microscope (Thomas, Kuriakose, Kirupashanker, & Maharajan, 1991).

Table 1. Information of diseased fish samples

Fish species	Qty	Origin
Gold gourami *Trichopodus trichopterus*	1	Ornamental fish outlet, Kuala Terengganu
Snakeskin gourami *Trichogaster pectoralis*	1	Ornamental fish outlet, Kuala Terengganu
Angelfish *Pterophyllum scalare*	3	Ornamental fish outlet, Kuala Terengganu
African sharptooth catfish *Clarias gariepinus*	6	Commercial fish hatchery, Pengadang Baru, Terengganu
Red hybrid tilapia *Oreochromis* spp	1	UMT Hatchery, Kuala Nerus, Terengganu
African sharptooth catfish *Clarias gariepinus*	1	UMT Hatchery, Kuala Nerus, Terengganu

2.3 Molecular identification of fungi

2.3.1 Preparation of fungal isolates

Fungal isolates were prepared with delicate aseptic techniques according to Phadee *et al.* (2004). Briefly, fungal isolates were cultured in glucose yeast extract broth (GYEB) (Oxoid, UK) at 25 °C for up to 4 days. Mycelia were harvested when reached 0.5-1.0 cm in diameter, and washed twice with phosphate buffered saline (PBS) and dried on paper towel. Then 20-50 mg of mycelia were transferred to 1.5 mL microcentrifuge tubes and stored at -85 °C until used for DNA extraction.

2.3.2 DNA extraction

DNA extraction was conducted following Vandersea, Litaker, Yonnish, Sosa, Landsberg, Pullinger, and Moon-Butzin (2006) using Wizard® Genomic DNA Purification Kit (Promega, USA) according to manufacturer's instructions. Briefly, 40 mg of mycelia were ground to powder in liquid nitrogen using mortar and pestle. The mycelium powders were transferred to 1.5 mL microcentrifuge tubes, and added with 600 µL of lysis solution. The mixture was incubated at 65 °C for 15 min, then added with 3 µL of RNase solution, and incubated at 35° C for 15 min, then left to cool to room temperature for 5 min.

2.3.3 PCR amplification

ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primer pair was used to amplify a fragment encompassing partial 18S rRNA gene sequence, complete sequences of internal transcribed spacer 1, 5.8S rRNA gene, internal transcribed spacer 2, and partial 28S rRNA gene sequence according to White, Bruns, Lee, and Taylor (1990) in a Mastercycler Nexus Gradient Thermal Cycler (Eppendorf, Germany). Amplifications were performed in a final volume of 30 µL containing 1X GoTaq® Green Master Mix (Promega, USA), 0.5 pmol of each primer, 2.0 mM $MgCl_2$ and 1.0 µL of fungal DNA. Amplifications began with an initial denaturation of 2 min at 95 °C, followed by 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 58 °C and 45 s extension at 72 °C, with a final extension of 7 min at 72 °C. Twelve microlitre of each PCR product was electrophoresed in 2.0% agarose gel in parallel with 100-bp DNA ladders (BioLabs, USA) in 1X tris-borate-EDTA (TBE) buffer, and stained with 0.5 µg mL^{-1} ethidium bromide for visualization under UV-transilluminator (Bio-Rad, USA).

2.3.4 Sequencing, sequence alignment and submission

PCR products with a single fragment were purified using GeneMATRIX Basic DNA Purification Kit (EURx, Poland) following the manufacturer's PCR clean-up protocol, whereas PCR products with more than one fragment were subjected to the gel extraction protocol of the kit to extract the most intense fragment. The purified/ extracted products were verified to contain the target fragment by agarose gel analysis prior to sequencing. Dideoxy (Sanger) sequencing was carried out by First BASE Laboratories (Malaysia). The sequences obtained were analysed by nucleotide BLAST at NCBI (http://blast.ncbi.nlm.nih.gov) and deposited to GenBank submission portal (https://submit.ncbi.nlm.nih.gov/subs/genbank/) under Eukaryotic rRNA and rRNA-ITS submissions.

3. Results

3.1 Clinical signs and behaviors

The present work sampled the affected fishes based on the manifestation of dermal lesions. Hemorrhages at the bases of dorsal and caudal fins, and on the ventral part of body, and cotton-wool like growth were the major signs observed, of which the cotton-wool like lesion was most common. The affected fish appeared sluggish. Petechial hemorrhage on operculum, fin erosion and hemorrhagic ulcer were also observed (Figure 1).

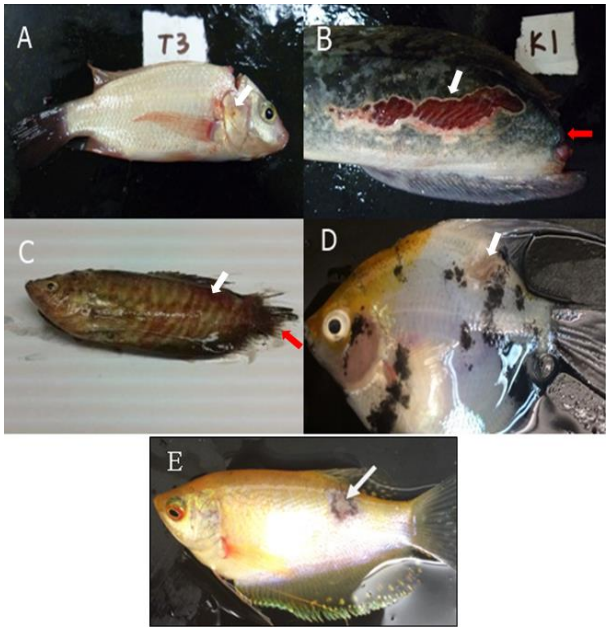

Figure 1. Dermal lesions on affected fishes (A) Petechial hemorrhage on operculum (white arrow) in *Oreochromis* spp. (B) Hemorrhagic ulcers (white arrow) and completely eroded caudal fin (red arrow) in *Clarias gariepinus.* (C) Extensive skin hemorrhages (white arrow) and caudal fin rot (red arrow) in *Trichogaster pectoralis.* (D) Skin erosion (white arrow) in *Pterophyllum scalare.* (E) Skin erosion (white arrow) in *Trichopodus trichopterus*

3.2 Morphological characterization

Twelve fungal isolates were successfully isolated from infected fishes, and assigned as isolates AY01 to AY12. The morphological features of the fungal isolates were shown in Figure 2 and Figure 3, and were summarized in Table 2. The colonies were consisted of a raised central area and a crusty layer of interwoven hyphae, spores, septate, arthroconidia and conidial heads. These macro- and micro-morphologies are significant source of reference for

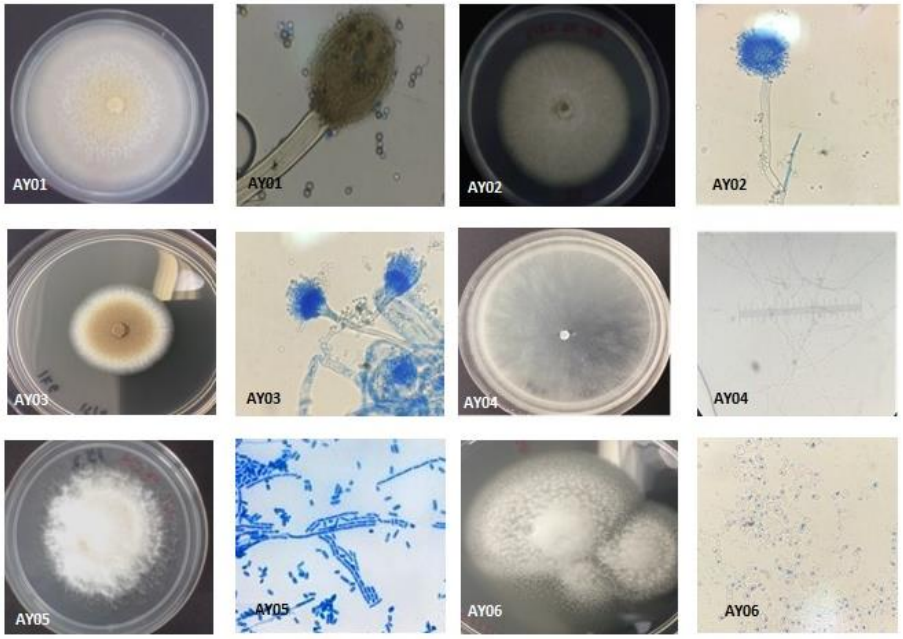

Figure 2. Macro- and micro-morphologies of fungal isolates AY01, AY02, AY03, AY04, AY05 and AY06

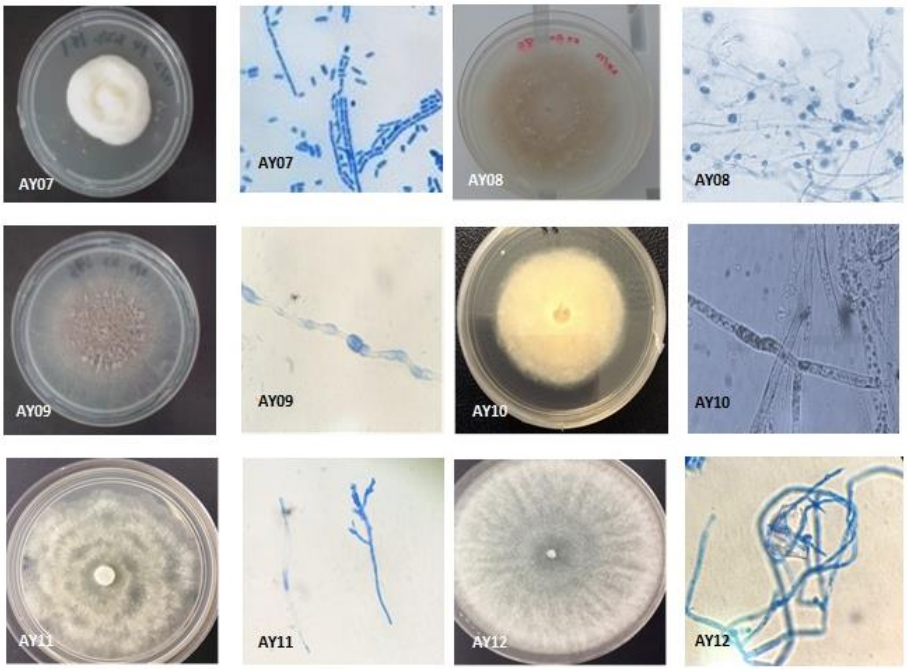

Figure 3. Macro- and micro-morphologies of fungal isolates AY07, AY08, AY09, AY10, AY11 and AY12

comparative research of fungi (Shah *et al.*, 2019), and morphogenetic studies in fungal taxonomy (Ullah *et al.*, 2018).

3.3 Molecular identification

ITS PCR amplified a single fragment in the range between 500-600 bp from all isolates except AY01, which resulted in an intense fragment between 700-800 bp, and an additional faint fragment between 600-700 bp (Figures 4 and 5). Nucleotide BLAST analysis of the fragment sequences showed high similarity with *Aspergillus niger* (AY01), *Aspergillus versicolor* (AY02), *Aspergillus terreus* (AY03), *Flavodon flavus* (AY04), *Geotrichum candidum* (AY05, AY06 and AY07), *Pestalotiopsis microspora* (AY08), *Pestalotiopsis photiniae* (AY09), *Pseudopestalotiopsis theae* (AY10), *Trichoderma asperellum* (AY11), *Apiotrichum* (*Trichosporon*) *montevideense* (AY12), respectively. The

Table 2. Macro- and micro-morphologies of fungal isolates

Isolate no.	Morphology	
	Macro	Micro
AY01	White-yellow mycelium with a dense felt of conidiophores	Brown-black with biseriate phiallides
AY02	White mycelium with red and yellow basement	Coenocytic hyphae with biseriate conidial head
AY03	White-green mycelium with a dense felt of conidiophores	Coenocytic hyphae with uniseriate conidial head
AY04	Thin surface of mycelium with suede-like surface	Septate hyphae
AY05	White thick surface of mycelium with suede-like surface	Septate and double layer hyphae
AY06	White thick surface of mycelium with suede-like surface	Septate and double layer hyphae
AY07	White thick mycelium surface with suede-like surface	Compartment fragmentation of hyphae
AY08	White-yellow thin surface of mycelium	Cylindrical spores with septate hyphae
AY09	White-red and thin mycelium with suede-like surface	Septate hyphae
AY10	White-yellow, thick mycelium	Septate hyphae
AY11	White, thin suede-like mycelium	Pseudohypahe
AY12	White, thick mycelium	Septate hyphae

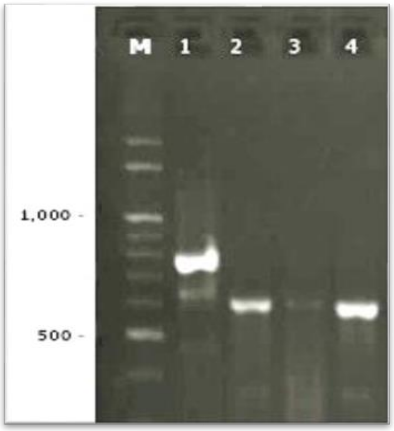

Figure 4. Gel electrophoresis of PCR products from isolate 1 (AY01), isolate 2 (AY02), isolate 3 (AY03) and isolate 4 (AY04) in parallel with 100 bp DNA ladder

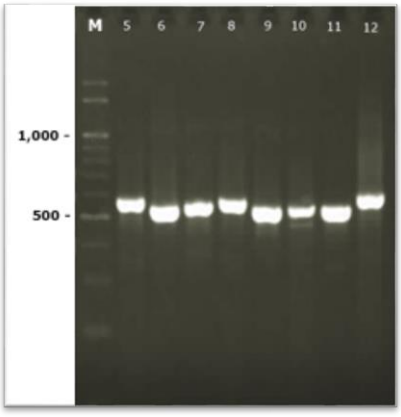

Figure 5. Gel electrophoresis of PCR products from isolate 5 (AY05), isolate 6 (AY06), isolate 7 (AY07), isolate 8 (AY08), isolate 9 (AY09), isolate 10 (AY10), isolate 11 (AY11) and isolate 12 (AY12) in parallel with 100 bp DNA ladder

DNA sequence deposit accession numbers and the GenBank reference accession numbers are shown in Table 3.

4. Discussion

Fish mortalities due to fungal infections occur secondary to factors such as stress, mechanical injuries, poor water quality or culture conditions (Yanong, 2003). *Aspergillus*, *Flavodon*, *Geotrichum*, *Pestalotiopsis*, *Pseudopestalotiopsis*, *Trichoderma* and *Apiotrichum (Trichosporon*) are members of Ascomycota, also known as sac fungi. Ascomycota have long been known to be able to turn from non-pathogenic to pathogenic (Klein & Tebbets, 2007). Sac fungi are known to produce spores in a distinctive type of microscopic sporangium called ascus. Other examples of sac fungi are yeasts, morels, truffles and *Penicillium* (Chiu & Fink, 2002). In this study, infectious fungi were isolated from fishes maintained in ornamental fish outlets, commercial fish hatchery and university hatchery. The fungal infections seemed more prevalent in the commercial hatchery which practices high-density fry culture in canvas tanks, and the ornamental fish outlets compared with the less intensively operated university hatchery that also practices stricter hygiene measure. The ornamental fish outlets in the present study use public utility water (piped water) as water source without additional end-user water disinfection such as UV radiation and ozonization. In view of the reported presence of a high variety of fungi in tap water (Babič *et al.*, 2017), this type of rather simple aquarium setup is deemed more prone to contamination by opportunistic fungi in water.

Trichopodus trichopterus fish is resistant to wide range of pH, temperature and dissolved oxygen (Rodrigues-Filho, Gurgel-Lourenço, & Sánchez-Botero, 2018). *T. pectoralis* is also a gourami species that can be easily maintained in a wide range of water parameters *Trichogaster pectoralis* is a fish species that can be easily maintained in a wide range of water parameters (Cole, Tamaru, Bailey, & Brown, 2000). In this study, *T. trichopterus* and *T. pectoralis* samples were infected with fungi identified to be *A. niger* and *A. versicolor*, respectively. A recent study (Malgundkar, Pawase, Dey, Tibile, & Shelke, 2019) reported that this species is susceptible to vitamin C deficiency which could lead to a variety of conditions such as anorexia, anemia hemorrhages and infections. *Aspergillus* spp. are part of the normal mycoflora of fish capable of causing serious infections in unhealthy fish (Haroon, Iqbal, Pervaiz, & Khalid, 2014; Iqbal & Saleemi, 2013). *Aspergillus* mycotoxins are also

Table 3. Nucleotide BLAST analysis of fungal isolates

Isolate no.	Deposit accession	No. of nucleotide	Host fish	Origin	GenBank reference accession	Percentage identity (%)
AY01	MT771988	578	*Trichopodus trichopterus*	Ornamental fish outlet, Kuala Terengganu	*Aspergillus niger* (MH237648.1)	99.83
AY02	MT772031	543	*Trichogaster pectoralis*		*Aspergillus versicolor* (KY509550.1)	99.63
AY03	MT772080	578	*Clarias gariepinus*	Commercial fish hatchery, Pengadang Baru, Terengganu	*Aspergillus terreus* (KU743892.1)	99.48
AY04	MT772081	602	*Pterophyllum scalare*	Ornamental fish outlet, Kuala Terengganu	*Flavodon flavus* (KM277995.1)	99.33
AY05	MT772084	402	*Pterophyllum scalare*		*Geotrichum candidum* (KF112070.1)	100.00
AY06	MT772092	348	*Pterophyllum scalare*		*Geotrichum candidum* (MK713424.1)	99.40
AY07	MT772096	350	*Clarias gariepinus*	Commercial fish hatchery, Pengadang Baru, Terengganu	*Geotrichum candidum* (KX218269.1)	98.52
AY08	MT772094	581	*Clarias gariepinus*		*Pestalotiopsis microspora* (KM438013.1)	100.00
AY09	MT772095	527	*Clarias gariepinus*		*Pestalotiopsis photiniae* (AY682945.1)	99.62
AY10	MT772148	539	*Clarias gariepinus*		*Pseudopestalotiopsis theae* (KR709041.1)	98.87
AY11	MT772146	474	*Oreochromis* spp	UMT Hatchery, Kuala Nerus, Terengganu	*Trichoderma asperellum* (MN176379.1)	99.58
AY12	MT772147	513	*Clarias gariepinus*		*Apiotrichum* (*Trichosporon*) *montevideense* (KP132872.1)	99.60

reported to come into contact with cultured fish through contaminated feed, especially in feed pellets stored for prolonged period of time and/or in moist conditions (Hashem, 2011; Iqbal & Saleemi, 2013). More recently *Aspergillus* sp. was found in contaminated trout pellet feed (Haroon *et al.*, 2014). *Aspergillus niger* is by far the most abundant species causing numerous internal and external infections in fishes (Iqbal & Saleemi, 2013). *Aspergillus versicolor* has been previously isolated from diseased *C. gariepinus*, *Tilapia zilli* and *Liza ramada* (Thinlip mullet), and found to produce sterigmatocystin (Hashem, 2011).

Angelfish *Pterophyllum scalare* are naturally found in South American countries such as Venezuela, Colombia and Brazil (Gallani, Sebastião, Valladão, Boaratti, & Pilarski, 2016; Winemiller, López-Fernández, Taphorn, Nico, & Duque, 2008), and are imported, cultured and sold as ornamental fish (Adel, Saeedi, Safari, Azizi, & Adel, 2013). In Malaysia, at least 22 strains of *P. scalare* are commonly traded in the market (Bibi-Koshy, Oyyan, & Sekaran, 2008). The fungal isolates from diseased *P. scalare* were identified as *Flavodon flavus* (AY04) and *Geotrichum candidum (*AY05 and AY06), respectively. *Flavodon flavus* has been previously isolated from seagrass (Raghukumar, Mohandass, Kamat, & Shailaja, 2004) and mangroves in Thailand (Anke & Schüffler, 2018). *Geotrichum candidum* is part of the normal mycoflora of the digestive system in humans and many other mammals. They can also be found in the air, water and soil (Pottier, Gente, Vernoux, & Guéguen, 2008). These pathogens could have come into contact with *P. scalare* via contaminated water. Considering that angelfish are not hardy fish, ornamental fish outlets and hobbyists should look into possible circumvention including storing feed is in cool dry condition, and use within shelf-life, as well as further treatment of piped water by in-line processes such as UV radiation and ozonization to get rid of possible infectious contaminants. Besides, prophylactic in-tank water treatment with methylene blue after water change also helps prevent fungal infections in fishes.

In the present study, *Clarias gariepinus* cultured in canvas tanks suffered a number of mortalities possibly due to infections at time of sampling. *Aspergillus terreus* isolated from the diseased *C. gariepinus* is a saprotrophic soil fungus (Park, Jun, Han, Hong, & Yu, 2017). The occurrence of *A. terreus* was possibly associated with the use of groundwater for fish culture without appropriate treatment. This finding was in line with a previous study where *A. terreus* showed a maximum frequency of 58% in the earthen pond ecosystem in Dumaratarai Talab in the south of Raipur city, India (Saju & Tiwari, 2011). *A. terreus* was also previously isolated from bony bream *Crenidens crenidens*, and was found to produce patulin, a highly toxic mycotoxin (Hashem, 2011). *Geotrichum candidum* isolate from diseased *C. gariepinus* is an anamorph to *Galactomyces* (Kolecka *et al.*, 2013). *G. candidum* is widely distributed in the nature, and can be found in the air, water and soil (Pottier *et al.*, 2008). Other *C. gariepinus* samples were infected with *Pestalotiopsis microspora* (AY08), *Pestalotiopsis photiniae* (AY09) and *Pseudopestalotiopsis theae* (AY10). *Pseudopestalotiopsis* is relatively new genus that was established in 2014 by Maharachchikumbura research group and two new species of *Pseudopestalotiopsis* (*Ps. myanmarina* and *Ps. vietnamensis*) have been described in 2017 (Nozawa *et al.*, 2017). Though *Pestalotiopsis* are plant pathogens (Chen, Hu, Luo, Zhu, & Zhou, 2013), many *Pestalotiopsis* species have been isolated from polluted water (Maharachchikumbura, Guo, Chukeatirote, Bahkali, & Hyde, 2011; Maharachchikumbura,

Hyde, Groenewald, Xu, & Crous, 2014). Another fungus isolated from diseased *C. gariepinus* in the present study was *Apiotrichum* (*Trichosporon*) *montevideense*. This genus is also widely distributed in the nature, inhabiting soil, salt and freshwater. *A. montevideense* was previously reported to be pathogenic to humans (Colombo, Padovan, & Chaves, 2011), while *T. jirovecii* has been recently reported to infect red swamp crayfish (*Procambarus clarkii*) (Abdallah, Mahmoud, & Abdel-Rahim, 2018).

C. gariepinus is a very hardy fish that can tolerate extreme environmental circumstances (Abraham, Mallick, & Paul, 2018). All the fungi isolated from diseased *C. gariepinus* in the present study are potentially pathogenic, and are commonly found in soil. The presence of these fungi might be associated with the use of groundwater for fish culture without prior disinfection. *C. gariepinus* has been previously reported to be susceptible to fungal infections during spawning period (Melaku, Lakew, Alemayehu, Wubie, & Chane, 2017). Thus, the use of adequately disinfected water is warranted during this period.

UMT Hatchery is a university teaching hatchery relatively better equipped compared with the ornamental fish outlets and commercial hatchery in terms of water quality control and biosecurity. Of the hundreds of red hybrid tilapia inspected during sampling, only one fish was found with apparent dermal lesion (petechial hemorrhage on operculum), and from where AY11 was isolated and identified as *T. asperellum*. *Trichoderma* are highly competitive soil fungi widely distributed in the tropical habitat (Schuster & Schmoll, 2010). However, *T. asperellum* is considered a rare and low-pathogenic fungus in fish, thus finding it in the present study was unexpected. In a toxicity test on juvenile rainbow trout (*Oncorhynchus mykiss*), *T. asperellum* resulted in two mortalities in tanks at 1.5×10^9 and 2.4×10^{10} CFU L^{-1}. Despite being low-pathogenic, *T. asperellum* can cause high turbidity, bubbling and coloration in tank water, which in turn leads to adverse effects on water quality (Environmental Protection Agency, 2010). Considering that only one out of hundreds of tilapia was infected, the occurrence was considered just an outlier.

4.1 Treatment and control of fungal infection in fishes

Potassium permanganate, formalin and povidone-iodine are commonly used for treatments of fungal infections. According to Patel, Patel, Bariya, Pata, and Ghodasara (2018), NaOH (10-25 g L^{-1} for 10-20 min), $KMnO_4$ (1 g in 100 L of water for 30-90 min) or $CuSO_4$ (5-10 g in 100 L water for 10-30 min) bath can be used to treat infected fishes. However, over dosage of chemical treatments may cause tissue damage in fish, and predispose them to recurring infections. Environmental management is important for adequate resolution of chronic fungal diseases. Good management practice will make the environmental circumstances less favorable for growth of fungi. Prevention is better than treatment against fungal infection in fishes. Great care must be taken to avert moving the disease from one area to another. Formalin and copper sulphate ($CuSO_4$) can be applied to help stop mortalities; however, all tanks, raceways and aquaria must be sanitized and dried. Ponds should be dried and treated with quicklime (calcium oxide) and copper sulphate (2-3 kg/ha). An extended bath in Acriflavine neutral or forma-green for seven days also helps reduce fungal infection. Dead fish should be buried. Good water quality and circulation, dodging of crowd to curtail injury (particularly during spawning) and good nutrition can help prevent fungal infection. Ponds with dirt or gravel bottoms need months of drying to totally eliminate the fungi. Complete disinfection of tanks or aquaria and raceways is encouraged (Patel *et al.*, 2018). Besides, good attention should be given to feed management especially feed storage. Feed should be properly stored for no longer than 6 months in dry and well-ventilated areas to prevent, or at least to minimize growth of fungal contaminants (Leaño, 2001).

5. Conclusions

The present study isolated *A. niger*, *A. versicolor*, *A. terreus*, *F. flavus*, *G. candidum*, *P. microspora*, *P. photiniae*, *Ps. theae*, *T. asperellum* and *A. montevideense* from diseased fishes. The genera *Aspergillus*, *Geotrichum*, *Pestalotiopsis*, *Trichoderma* and *Apiotrichum* (*Trichosporon*) are known to produce toxic metabolites that can cause diseases in plants, animals and humans, hence potential opportunistic pathogens to fish. The advantage of using advanced hatchery and maintenance system has been clearly shown in this study. Continuous monitoring of water quality parameters, feed storage condition and fish health condition are also crucial.

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