Aspergillus Diversity Associated with Fungal Diseases on Fish with Molecular Based

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Aspergillus Diversity Associated with Fungal Diseases on Fish with Molecular Based

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Abstract. Fungal diseases are frequently occur in fish culture. The aim of this research was to find out the diversity of *Aspergillus*associated with fungal diseases in catfish and Tilapia based on 16S rDNA gene sequences in central Java Indonesia. The combination between exploratory in the field and experiment, method were applied. In order to find out the *Aspergillus* prevalence, 48 fish were collected from fish pond of Demak, Klaten and Semarang Regency. Based on the clinical sign, 24 moribund fish were chosen for fungus isolation. As a result, 21 fungi isolates (FTD01–FTD05; FTK01-FTK08; FCB01-FCB08) were gained from external wounds of fish with Sabouraud Dextrose Agar (SDA) medium. Based on the Postulate Koch result showed that three isolates (FTD03, FTK07 and FCB01) that were caused 20 – 80% of fish get sick and mortal. On the basis of sequence 16S rDNA analysis, the result showed that FTK01, FTK07, and FCB01 were closely related to*Aspergillus flavus* (100%); *Aspergillus niger* (71%)and *Aspergillus fumigatus* (77%)respectively.

1. Introduction

Catfish has primacy for its fast growth, convenient for culture, and affordable price[1]. In 2010, Central Java Province production on nile tilapia (*Oreochromis niloticus*) reached 11.259 tonnes and on catfish reached 36.394,5 tonnes. Catfish (*Clarias* sp.) was the highest production among the other fish culture production with the highest district producer was Demak while Klaten was the highest producer for nile tilapia. Generally, aquaculture sector production in Central Java increased 31% from 2009 to 2010 [2].

Nile tilapia (*O. niloticus*) and catfish (*Clarias* sp.) culture are threatened by some infectious diseases which causes mortality and economic losses, particularly fungal infection. Fungus could infect fish at any sizes, causing damages on any part of the body. Water currents disperse the fungus so that it can spread rapidly on fish population [3]. Many fungi infected fish in culture as well as in the natural water ecosystem. *Aspergillus* found to be the most occurrence on infected fish. The number of infections may increase due to the imbalance between potential pathogens, the environment, and the

host. Environmental changes and seasonal variation effected the intensity of fungal infection and the occurrence of fungal infection [4].

Research about fungal infection on fish have been carried out in some fishes, basically with fresh water fishes. [5] Some research found that *Aspergillus fumigatus* was the highest fungi isolated from 174 fresh water fishes with incidence of 41,3%. *Aspergillus fumigatus* found on *Channa striatus, Labeo rohita, Mystus seenghala, Cirrhinus mrigala, Macrognathus aculeatus, and Puntius sarana. A. fumigatus* combined with *A. niger* infected *Mystus seenghala.A. fumigatus* and *A.niger* were both pathogenic to *Channa punctatus,* also causing early ulcerative syndrome [6]. *Aspergillus* found infected certain organs both external and internal. On the external organs fungi observed on epidermis of skin.

There are many studies concerning genus *Aspergillus* in aquaculture system [5,7,8]. The present study was commencedto find out the *Aspergillus* diversity associated with fungal diseases in catfish and Tilapia based on 16SrDNA gene sequences in central Java Indonesia.Molecular ccharacterization of fungal using Polymerase Chain Reaction (PCR) should be done to create early warning of fungal disease, including aspergillosis in fish. However, there has been limited research so far regarding the Aspergillus diversity associated with Aspergillosis in fish from freshwater culture-system in Demak, Klaten and Semarang Regency. Therefore, the accuracy of this method for identifying the genus Aspergillus is very important for mitigation and design disease prevention strategy for supporting the fish production.

2. Research Methods

2.1. Sample ofFish

Freshwater pond in that are located at surrounding Demak, Klaten, and Semarang were chosen as sampling locations. Forty eight fish consist of Catfish (*Clarias* sp.) and Nile Tilapia (*Oreochromis niloticus*)in size range 16,6 to 17,2 cm which were presumably infected aspergillosis were collected. The samples were kept in an insulated container and taken to the Aquaculture Laboratory of Fishery and Marine Science Faculty of Diponegoro University for bacterial isolation.

2.2. Fungal Isolation

Twenty onefungal isolates based on morphological differences were obtained external wound usingSDA medium, Based on the morphological performance, colonies were randomly picked and purified by streak plating. Isolationperformedthree replicates to obtain pure isolates, the pure isolates were then storen in SDA medium.

2.3. PCR Amplification and Sequencing of 16s rRNA Gene Fragments

From thetwenty-one fungal isolates, three isolates were characterised with molecularly approach based on methods previously used by [9] Fungal isolates were extracted from agar plate then suspended in sterile water (Sigma, Germany). The Polymerase Chain Reaction (PCR) was run using Eppendorf Mastercycler (Eppendorf Inc.Germany)with five freezing cycles (-80ºC) and thaw (95ºC).The primers,ITS1 (5'-TCCGTAGGTGAACCTGCGG-3')and ITS4 (5'-TCCTCCGCTTATTGATATGC -3'), were used to amplify nearly complete 16S rDNA gene. Big Dye Terminator V3.1 dyes and automatic DNA sequencer ABI3130 GeneticAnalyzer XL Applied Biosystemsat Macrogen Korea used for sequencing the fungal DNA.DNA sequences of the fungal forward was compared to the BLAST *(Basic Local Alignment Search Tool*) on *National Center for Biotechnology Information, National Institute for Health* database USA to gain the homology[9,10]. Whereas the phylogenetic was constructed with Mega 6 programme [11].

3. Results and Discussion

3.1.Result

3.1.1. Characteristic of the Fungal Isolates

The clinical symptoms of fish infected by fungal diseasefrom fish pond of Demak, Klaten and Semarang Regency were wound and eroded skin with growth of cotton like over the body and dorsal fin(Figure 1).

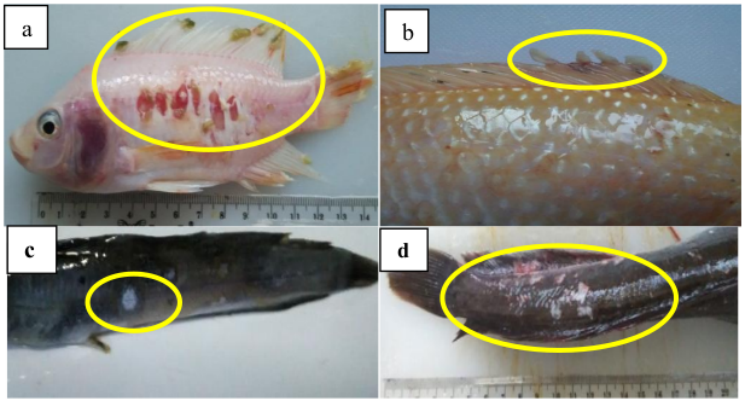

Figure 1. The Clinical Symptoms of Fish Infected by *Aspergillus* from Fish Pond of Demak, Klaten and Semarang RegencyShown by Yellow Circles: (a) Skin lesion with greenness-cotton on dorsal fin (b) White-cotton ondorsal fin (c) growth of cotton like over the body (d) wound and eroded skin

A total of 21 isolates were gained from external wounds of fish were presented in Table 1.

Table 1. Characteristic of Fungal Isolates on Catfish and Nile Tilapia from Demak, Klaten andSemarang

No.	Isolate code	Media	Source	Colony		
				Colour	Texture	Reverse colour
1	FTD01	SDA	External wound	Brown	Powdery	Pale yellow
2	FTD02	SDA	External wound	Yellowish green	Powdery	White
3	FTD03	SDA	External wound	Brown to black	Woolly to powdery	Pale yellow
4	FTD04	SDA	External wound	Yellow	Powdery	White
5	FTD05	SDA	External wound	Blue-greyish	Powdery	Yellow
6	FTK01	SDA	External wound	Blue-greyish	Powdery	Yellow
7	FTK02	SDA	External wound	Brown to black	Woolly to powdery	Pale yellow
8	FTK03	SDA	External wound	Blue-greyish	Powdery	Yellow
9	FTK04	SDA	External wound	Green yellowish	Powdery	White
10	FTK05	SDA	External wound	Yellow	Powdery	White
11	FTK06	SDA	External wound	Brown to black	Powdery	Pale yellow
12	FTK07	SDA	External wound	Brown to black	Powdery	Pale yellow
13	FTK08	SDA	External wound	Brown to black	Powdery	Pale yellow
14	FCB01	SDA	External wound	Blue-greyish	Powdery	Yellow to orange
15	FCB02	SDA	External wound	Blue-greyish	Powdery	Yellow
16	FCB03	SDA	External wound	Brown	Powdery	Yellow
17	FCB04	SDA	External wound	Yellow	Powdery	White
18	FCB05	SDA	External wound	Blue-greyish	Powdery	Pale orange
19	FCB06	SDA	External wound	Yellow	Powdery	White

20	FCB07	SDA	External wound	Blue-greyish	Powdery	Yellow to orange
21	FCB08	SDA	External wound	Blue-greyish	Powdery	Yellow

Based on the morphological character of twenty one isolates, three isolates (FTD03, FTK07 and FDB01)were choosen to further investigation. The morphological characters of three isolateswere presented by Figure 2.

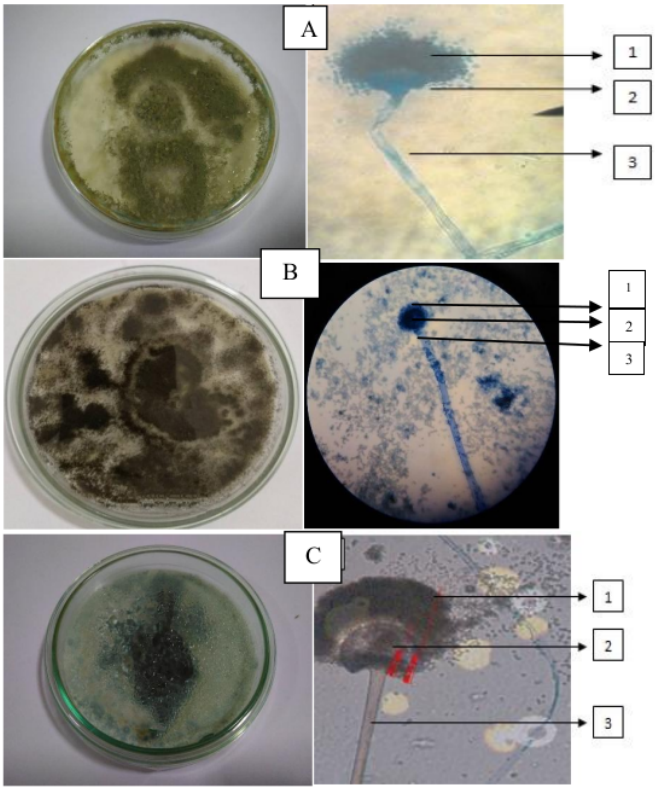

Figure 2. Morphology Character of Fungal Isolat with Code of FTD03,FTK07 andFDB01(1) Konidia (2) Vesikel (3) Konidiofor(400X magnification)

3.1.2. Postulate Koch

Postulate Koch test results also showed that three isolates (FTD03, FTK07 and FDB01) were causing sick range of 20 – 80 % and mortality. Therefore, these isolates (FTD03, FTK07, FDB01) were positively confirmed as causative agents associated with fungal diseases in catfish from Demak, Klaten and Semarang Regency.

3.1.3. PCR Analysis

Based on the sequencing analysis indicated that all isolates (FTD03, FTK07 and FCB01) are the members of *Aspergillus* as shownby Table 2.

Table 2. Molecular Identification of Three*Aspergillus*Associate with Fish from Fish Pond of Demak, Klaten and Semarang Regency

No.	Isolates	Closely Relative	Homology (%)	Acc. Number
1.	FTK01	*Aspergillus flavus*	96	HQ645490.1
2.	FTK07	*Aspergillus niger*	97	MF422165.1
3.	FCB01	*Aspergillus fumigatus*	96	KX664390.1

On the basis of 16S DNA sequence analysis, the result shows that the *Aspergillus* associatedwith Aspergillosis in fish from Fish Pond of Demak, Klaten and Semarang Regencywere closely related to *Aspergillus flavus* (FTD03); *Aspergillus niger*(FTK07)and *Aspergillus fumigatus*(FDB01) with homology range between 96–97%. The phylogenetic of three *Aspergillus* was seen in Figure 3.

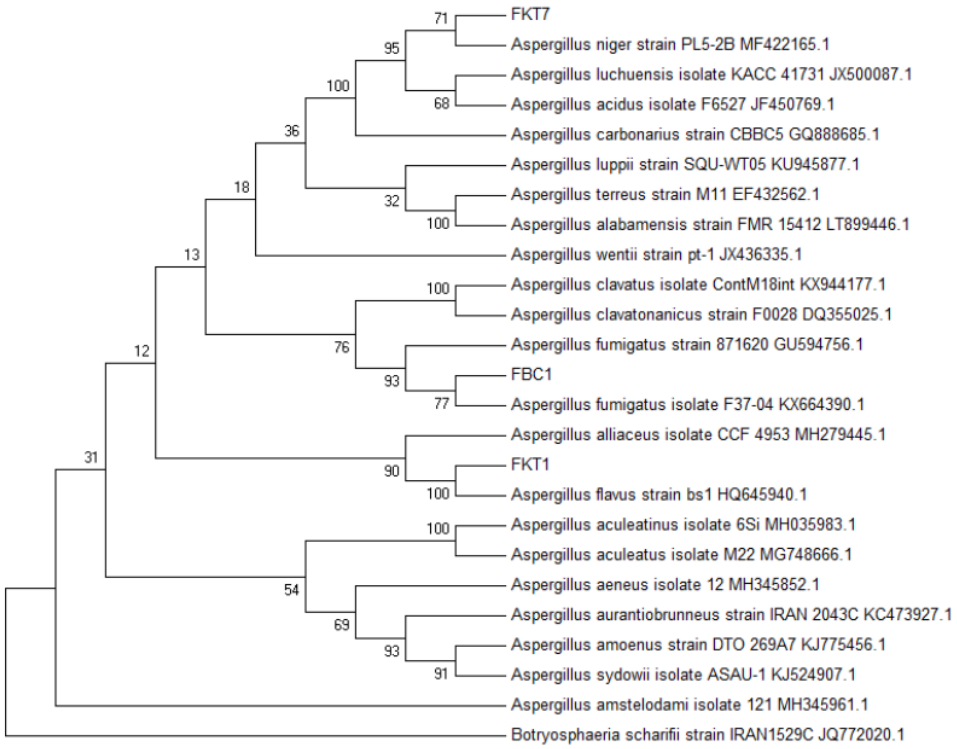

Figure 3. Phylogenetic of The *Aspergillus*Associated withAspergillosis in Fishfrom Fish Pond ofDemak, Klaten and Semarang Regency

3.2. Discussion

Fungi infected and moribund fishes both catfish (*Clarias gariepinus*) and nile tilapia (*Oreochromis niloticus*) from fish pond of Demak, Klaten and Semarang Regency were taken and isolated. The fishes showed clinical symptoms that were wounded, lesions, ulcer, fin rot and cotton-like grow on skin and wound. The clinical sign appeared on fungal infected fish were common with [6] had found.

The diversity of *Aspergillus* related to Aspergillosis in fish fish pond of Demak, Klaten and Semarang Regency, using molecular approach obtained three *Aspergillus* strains namely *Aspergillus flavus* (FTK01); *Aspergillus niger*(FTK07)and *Aspergillus fumigatus*(FCB01) respectively. *A. fumigatus* and *A. niger* discovered infected murrel fishes (*Channa punctatus*) [6], the infection showing mycelia growth on body surface and in some fishes wound and lessions appeared on gills, fins, and skin. While *A. flavus* foundto be the one of the most occurrence fungi on *Clarias gariepinus* isolated from fish and water from dams and farms [12].

A. fumigatus infected fishes, both catfish (*Clarias* sp.) and nile tilapia (*Oreochromis niloticus*) and causing the fish moribund. *A. fumigatus* known as the causal of invasive aspergillosis, the most pathogenic fungi among the genus, infecting human and animal [13]. *A. fumigatus* ubiquitous in the environment for it has ability to defend itself from in any type of environment. It defends itself by abundant efflux pump and producing potent secondary metabolites.*A. fumigatus* has 16 identified different secondary metabolites, such as gliotoxin [14].

A. niger found infected fish from fish pond of Demak, Klaten and Semarang Regency. *A. niger* as a member of *Aspergillus*, is easy to find in environment. So that *A. niger* could infected various kind of fish such as *Mystus seenghala* and *Puntius ticto*[5].*A. niger* also found on gold fish (*Carrasius auratus* L.) [15].

A. flavus was found from collected fish from fish pond of Demak, Klaten, and Semarang Regency. *A. flavus* is a widely spread fungi, it can be found on soil, air, and can easily attach to something. Not only can be found infected catfish (*Clarias* sp.) and nile tilapia (*Oreochromis niloticus*), *A. flavus* can be found on other fishes such as*Channa punctatus*[3]. On the other hand, *A. flavus* isnot always found infected organism, but it can found on organism without causing infection.*A. flavus* found on apparently healthy and apparently infected fishes, *Oreochromis* and *Clarias gariepinus*[16]. It shows that *A. flavus* can cause natural infections and yet considered as a normal mycoflora.

The present research revealed that *Aspergillus* found infected catfish (*Clarias* sp.) and nile tilapia (*O. niloticus*) from fish ponds of Demak, Klaten and Semarang Regency considered as *A. fumigatus, A. niger,* and *A. flavus*. They can cause infection and mortalities [6], infected other species such as *Channa striatus, Labeo rohita, Mystus seenghala, Cirrhinus mrigala, Macrognathus aculeatus, and Puntius sarana* [5].

Conclusion

On the basis of 16S DNA sequence analysis, the result shows that the *Aspergillus* associatedwith Aspergillosis in fish from Fish Pond of Demak, Klaten and Semarang Regencywere closely related to *Aspergillus flavus* (FTD03); *Aspergillus niger*(FTK07)and *Aspergillus fumigatus*(FDB01).

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