Achlya spp. แยกได้จากไข่ปลาบึก (*Pangasianodon gigas*, Chevey)

Achlya spp. isolated from eggs of the Mekong Giant Catfish (*Pangasianodon gigas*, Chevey)

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บทคัดย่อ

ไข่ปลาบึก (*Pangasianodon gigas*, Chevey) และตัวอย่างน้ำจากบ่อเพาะฟักไข่ปลาบึกที่สถาบันวิจัยการเพาะเลี้ยงสัตว์น้ำจืด จังหวัดพระนครศรีอยุธยาถูกสุ่มเก็บมา จำนวน 4 ครั้ง ระหว่างเดือนกรกฎาคมถึงกันยายน พ.ศ. 2551 เพื่อทำการแยกและจำแนกเชื้อราน้ำจืด ผลการศึกษาสามารถแยกและจำแนกชนิดเป็นเชื้อรา *Achlya* spp. และผลการศึกษาลักษณะทางชีววิทยาของเส้นใยของเชื้อราที่แยกได้พบว่าอุณหภูมิที่เหมาะสมต่อระยะการเจริญของเส้นใยคือ 30 ^{0}C และผลของโซเดียมคลอไรด์ที่มีต่อการเจริญของเส้นใยคือ15 ส่วนในพัน

ABSTACT

Eggs of Mekong Giant Catfish (*Pangasianodon gigas*, Chevey) and water samples were collected from spawning tanks at Inland Aquaculture Research Institute, Phra Nakhon Si Ayutthaya province, Thailand. The samples were conducted in four times during July - September 2008 for isolation and identification of the fresh water fungi. The results showed that the isolated fungi is *Achlya* spp. Examination of the biological characteristics showed that optimal temperature is 30 ^{0}C and 15 ppt of NaCl is optimal salinity effected on mycerial growth of the isolated.

Keywords: Eggs of Mekong Giant Catfish (*Pangasianodon gigas*, Chevey), *Achlya* spp.

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INTRODUCTION

Fungal infection in fish eggs is usually caused by the fungi, family Saprolegniaceae, including the genera *Saprolegnia* and *Achlya* (Wesley and Wolf,1937; Srivastava and Srivastava, 1977; Srivastava, 1980; Sati, 1985; Padmakumar *et al*., 1985; Czeczuga and Woronowicz, 1993; Czeczuga *et al*., 1995; Kitancharoen and Hatai, 1998). The fungal disease is a problem that occur brood stock husbandry, Occasionally it causes low productivity of fry and low production of fish cultures (Kwanprasert *et al*., 2007). Diseases caused by water fungi occur more often at the eggs stage. The mortality rate dued to fungal infection reaches sometimes 80-100% of incubated eggs (Chukanhom and Hatai, 2004). Mekong Giant Catfish (*Pangasianodon gigas*, Chevey) is the biggest, scaleless fresh water fish in the world. It only naturally found in Mekong River. The fish's meats have a good taste and the price is very expensive. The population of the Mekong Giant Catfish in the Mekong river was decreased rapidly because a lot of adult female were catched in spawning season. Therefore, it was put in endangered species list follow by Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Poulsen *et al*., 2004). In 1983, the Department of Fisheries of Thailand succeed to breed the Mekong Giant Catfish and produced a lot of fly. Moreover in 2001, they have succeed in artificial propagation of Mekong Giant Catfish by first filial generation broodstock (F_1) rearing in earthen ponds at Phayao Inland Fisheries Station (Unakornsawat *et al*., 2001). But the survival rate of fry was very low caused by bacterial contamination and aquatic fungi infected during eggs stages (Purivirojkul *et al*., 2005). The purposes of this research were isolate, identify the fungi genus and study some morphological and biological characteristics of the isolation from eggs of Mekong Giant Cat fish and water collected from hatchery tanks.

MATHERIALS AND METHODS

Isolation and identification

Total of 85 eggs of the Mekong Giant Catfish (*P. gigas* Chevey) and 16 water samples were collected from spawning tanks at Inland Aquaculture Research Institute, Phra Nakhon Si Ayutthaya province, Thailand, 4 times during July - September 2008. The specimens were inoculated on glucose - yeast extract agar (GY) (Hatai and Egusa, 1979) at 20 °C. The 500 µg/ml each of ampicillin and streptomycin sulfate were added to the medium to reduce bacterial contamination. A pure culture was made by the single spore isolation (Choi *et al*.1999). For morphological observations, the isolated fungi were inoculated into 50 ml of GY broth and incubated at 20 °C for 3–4 days. The young hyphae in GY broth were washed 5 times with sterilized distilled water (STW). Fungi were identified from the morphological characteristics of zoospore discharged in sterile tap water. The purified fungal isolates were maintained at 20 °C on GY agar and subcultured to fresh GY agar monthly.

Effect of various temperatures on fungal growth

Temperature range and optimum temperature for growth were determined by measuring the radial growth of mycelium of the fungi. The advancing edge of growing colonies were cut by a cork borer (8 mm in diameter) and placed onto center of Petri dishes (100x22 mm) that contained 20 ml of GY agar, the plates were incubated at various temperatures: 10°, 20°, 30°, 37°C. The radial growth of the colony was measured with vernier calipers every day and data analyzed by SPSS program. All experiments were performed in triplicate of each experiment and compared with the other fungi such as *Saprolegnia diclina* NJM 9219 and *Achlya bisexualis* NJM 0611. All of the reference stains of fresh water fungi using in this study supported by Professor Dr. Kishio Hatai, Nippon Veterinary and Life Science University, Tokyo Japan.

Effect of sodium chloride (NaCl) on fungal growth

The actively growing edge of the isolated fungi were cut with a cork borer (8 mm in diameter) and put onto the center of GY agar plates (100x22 mm) containing different concentrations of NaCl (0, 5, 10, 15, 20 and 25 ppt) and incubated at 20°C. The diameter of the colonial growth was measured daily with the vernier caliper for 5 days and data analyzed by SPSS program. Three replicates of each experiment were conducted and compared with *Saprolegnia diclina* NJM 9219 and *Achlya bisexualis* NJM 0611.

RESULTS AND DISCUSSION

Isolation and identification

All of samples including 85 eggs of the Mekong Giant Catfish (*P. gigas* Chevey) and 16 water samples were collected, we isolated and identified 2 isolate fungi in genus *Achlya*. The fungal isolate named E.MCF 2-001 isolated form eggs samples and T.MCF 1-02 isolated from water samples. The morphological characteristics of the fungal isolate following: large white or grey threads of cotton-like mycelia, puffy, whitish and moist colonies on GY agar which reach full plate after 5 days, at 20 ^{0}C. Rigid hyphae penetrated into the agar. Hyphae in GY broth were aseptate, stout and had sharp tips. At the asexual stage, primary zoospores were elongate and discharged from the end of the discharge tube, while spore clusters usually persisted at exit pores in the manner of the achlyoid type (Figure 1A, 1B). Encysted spores were round. Typical zoosporangia of E.MCF 2-001 (isolated from Mekong Giant Catfish eggs) were straight, filiform and occasionally fusiform shape (Figure 1C) and zoosporangia of T.MCF 1-02 (isolated from water samples collected from spawning tanks) were straight, filiform and occasionally fusiform shape too (figure 1D). Zoospore formation occurred within 8-12 hours after mycelia were transferred into STW at 20 ^{0}C. According to the morphological characteristics and mode of zoospores release as described above, both isolates were identified as genus *Achlya* (Johnson 1956).

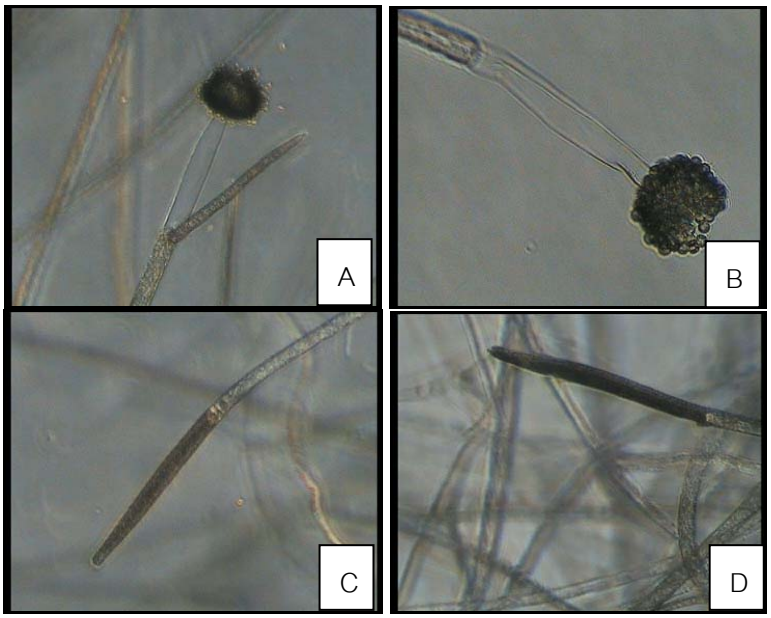

Figure 1 Morphological characteristics of *Achlya* spp. were observed under an inverted microscope at 200x

1A: Mode of zoospores discharged from zoosporangium of the fungal isolate named E.MCF 2-001; 1B: Mode of zoospores discharged from zoosporangium of the fungal isolate named T.MCF 1-02; 1C: Characteristics of many zoospores containing within zoosporangia of the fungal isolate named E.MCF 2-001; 1D: Characteristics of many zoospores containing within zoosporangia of the fungal isolate named T.MCF 1-02

Achlya spp. has been commonly reported in aquatic environment and ccause infected fish and their eggs (Srivastava, 1980; Post, 1983; Kitancharoen *et al*., 1995; Kitancharoen *et al*., 1997; Nejadsattari, 2000; Steciow, 2001; Czeczuga *et al*., 2002).

Effect of temperature on vegetative growth

The effect of temperature on vegetative growth of *Achlya* spp. isolates show in Table 1. *Achlya* spp. grew rapidly in GY agar. The vegetative growth of all isolates grew faster at 30 ^{0}C than 20 ^{0}C. But at 37 ^{0}C only T.MCF 1-02 isolated from water samples was able to grow.

Table 1 Effects of various temperatures on diameter of growth rates (centimeters) of *Achlya spp.* (T.MCF 1-02, E.MCF 2-001) compared with *Saprolegnia diclina* NJM 9219 and *Achlya bisexualis* NJM 0611.

(°C)	Isolate	Day						
		0	1	2	3	4	5	6
10	T.MCF1-02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	E.MCF2-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	S. diclina NJM 9219	0.00	9.87±0.13	14.03±0.40	16.50±0.11	19.33±0.33	22.62±0.28	25.10±0.10
	A. bisexualis NJM 0611	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	T.MCF1-02	0.00	11.87±0.20^{a}	25.00±1.15^{b}	36.70±0.42^{b}	49.17±0.79^{b}	61.83±1.42^{b}	75.333±1.76^{b}
	E.MCF2-001	0.00	12.80±0.82^{a}	24.73±1.43^{b}	35.60±0.95^{b}	45.87±1.85^{b}	59.50±1.04^{b}	72.93±1.27^{b}
	S. diclina NJM 9219	0.00	29.57±0.50^{b}	62.27±1.30^{c}	82.33±1.17^{c}	83.50^{c}	83.50^{c}	83.50^{c}
	A. bisexualis NJM 0611	0.00	12.27±0.24^{a}	20.50±0.29^{a}	29.50±0.76^{a}	39.07±0.79^{a}	49.37±1.35^{a}	62.67±1.73^{a}
30	T.MCF1-02	0.00	20.07±0.35^{b}	47.87±0.57^{b}	73.27±1.27^{c}	83.50^{b}	83.50^{a}	83.50^{a}
	E.MCF2-001	0.00	18.32±1.05^{a}	47.53±0.92^{b}	69.33±0.88^{b}	83.50^{b}	83.50^{a}	83.50^{a}
	S. diclina NJM 9219	0.00	44.13±0.53^{c}	81.67±1.83^{c}	83.50^{d}	83.50^{b}	83.50^{a}	83.50^{a}
	A. bisexualis NJM 0611	0.00	18.83±0.44^{a}	39.73±1.13^{a}	57.67±1.85^{a}	69.90±2.97^{a}	81.00±1.44^{a}	83.50^{a}
37	T.MCF1-02	0.00	0.00	10.35±0.65^{a}	30.00±2.00^{a}	56.65±4.35^{a}	81.00±2.50^{a}	83.50^{a}
	E.MCF2-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	S. diclina NJM 9219	0.00	24.27±1.03^{a}	34.97±0.78^{c}	50.83±5.26^{b}	62.23±7.24^{a}	74.47±5.80^{a}	83.50^{a}
	A. bisexualis NJM 0611	0.00	10.00±0.58^{b}	26.40±1.60^{b}	44.57±1.88^{b}	66.20±1.17^{a}	82.33±1.17^{a}	83.50^{a}

Note: different superscripts indicate significant difference at P-value < 0.05.

The results showed that the vegetative growth of the two isolates fungi (T.MCF 1-02 and E.MCF 2-001) grew at 20-35 ^{0}C and 20-30 ^{0}C, respectively. The result related to many *Achlya* spp. that have been reported. Munchan (2003) showed that *Achlya* spp. and *S. diclina* isolates from ornamental fish grew at 5-35 ^{0}C with optimum temperature at 30-35 ^{0}C and 20-35 ^{0}C, respectively. Rakmanee (2004) reported that both *Achlya* spp. and *A. ambisexualis* isolated from African catfish (*Clarias gariepinus*) and common carp (*Cyprinus carpio*) eggs grew at a range of temperature 15-30 ^{0}C with the optimal temperature at 25-30 ^{0}C, while *S. diclina* grew at a range of temperature 10-30 ^{0}C with the optimal temperature at 30 ^{0}C and 25 ^{0}C.

Effect of NaCl on mycelial growth

The effect of NaCl on mycelial growth of all isolates of *Achlya* spp. was shown in Table 2. They grew well on the NaCl free GY agar and they were able to tolerate up to 15 ppt of NaCl. No growth was observed on GY agar containing 20 and 25 ppt of NaCl compared with the other fungi such as *Saprolegnia diclina* NJM 9219 and *Achlya bisexualis* NJM 0611.

Table 2 Effects of various concentration of NaCl on diameters of growth rates (centimeters) of *Achlya spp*. (T.MCF 1-02,E.MCF 2-001) compared with *Saprolegnia diclina* NJM 9219 and *Achlya bisexualis* NJM 0611

NaCl in GY medium	Isolate	Day					
		0	1	2	3	4	5
0 ppt	T.MCF 1-02	0.00	10.17±0.73^a	24.67±1.22^a	43.53±2.66^a	57.85±3.27^a	76.03±2.73^a
	E.MCF 2-001	0.00	12.83±0.44^b	26.33±1.45^a	42.42±1.39^a	58.5±1.22^a	74.1±1.80^a
	A. bisexualis NJM 0611	0.00	14.33±0.93^b	30.42±0.65^b	43.83±1.93^a	64.77±0.39^b	78.03±0.74^a
	S. diclina NJM 2919	0.00	24.50±0.50^c	55.33±1.20^c	80.35±1.15^b	83.50^c	83.50^b
5 ppt	T.MCF 1-02	0.00	10.67±0.33^a	22.67±1.66^b	38.03±1.28^c	52.10±0.95^c	63.60±0.86^c
	E.MCF 2-001	0.00	10.00±0.58^a	21.33±1.45^{ab}	34.17±1.27^b	47.13±1.07^b	59.32±0.68^b
	A. bisexualis NJM 0611	0.00	10.00±0.17^a	19.13±0.71^a	29.80±1.14^a	42.93±1.42^a	53.37±1.67^a
	S. diclina NJM 2919	0.00	25.67±0.88^b	55.6±1.13^c	83.00^d	83.50^d	83.50^d

Table 2 (continuted)

NaCl in GY medium	Isolate	Day					
		0	1	2	3	4	5
10 ppt	E.MCF2-001	0.00	8.17±0.17[a]	12.67±0.33[a]	16.42±2.55[a]	23.93±1.09[ab]	32.30±1.91[b]
	A. bisexualis						
	NJM 0611	0.00	8.00[a]	9.87±1.04[a]	13.43±0.83[a]	18.52±1.04[a]	22.78±1.21[a]
	S. diclina						
	NJM 2919	0.00	21.93±0.99[b]	40.30±4.00[b]	59.67±3.84[b]	76.87±4.45[c]	83.50[c]
15 ppt	T.MCF1-02	0.00	0.00	0.00	0.00	8.33±0.33[a]	10.00[a]
	E.MCF2-001	0.00	0.00	8.00[a]	9.80±0.20[a]	12.02±0.56[b]	13.47±0.86[b]
	A. bisexualis						
	NJM 0611	0.00	0.00	7.67±0.17[a]	9.20±0.32[a]	10.67±0.17[b]	12.23±0.33[ab]
	S. diclina						
	NJM 2919	0.00	18.13±0.70	34.73±0.96[b]	50.53±1.11[b]	62.13±1.16[c]	75.17±0.93[c]
20 ppt	T.MCF1-02	0.00	0.00	0.00	0.00	0.00	0.00
	E.MCF2-001	0.00	0.00	0.00	0.00	0.00	0.00
	A. bisexualis						
	NJM 0611	0.00	0.00	0.00	0.00	0.00	0.00
	S. diclina						
	NJM 2919	0.00	10.88±0.27	22.57±0.7	33.47±1.17	44.87±1.66	54.6±2.08
25 ppt	T.MCF1-02	0.00	0.00	0.00	0.00	0.00	0.00
	E.MCF2-001	0.00	0.00	0.00	0.00	0.00	0.00
	A. bisexualis						
	NJM 0611	0.00	0.00	0.00	0.00	0.00	0.00
	S. diclina						
	NJM 2919	0.00	0.00	15.87±0.29	26.10±0.78	34.73±1.12	39.97±1.32

Note: different superscripts indicate significant difference at P-value < 0.05.

The result showed that the isolates of *Achlya* spp. grew up to 15 ppt of NaCl and no vegetative growth at 20 and 25 ppt of NaCl. Chukanhom and Hatai (2004) showed that *Achlya klebsiana* was isolated from eggs of common carp (*Cyprinus carpio*) grew up to 10 ppt of NaCl and no vegetative growth at 20, 30 and 40 ppt of NaCl. Kwanprasert *et al.*(2007) reported that *Achlya* spp. isolated from eggs of Nile Tilapia (*Oreochromis niloticus* linn.) showed maximal growth in GY agar without NaCl and could grow up to 15 ppt of NaCl, while *S. diclina* BKKU 0506 grew well in 0-15 ppt of NaCl and was tolerated up to 20 ppt of NaCl.

CONCLUSION

The results of isolation and identification showed the isolated fungi is *Achlya* spp. including the fungal isolate named E.MCF 2-001 isolated from Mekong Giant Catfish eggs and the fungal isolate named T.MCF 1-02 isolated from water samples collected from spawning tanks were not growth at 10 ^{0}C. The optimum temperature for vegetative growth was 30 ^{0}C. The E.MCF 2-001 isolated from Mekong Giant Catfish eggs had no growth at 37 ^{0}C but the T.MCF 1-02 isolated from water samples could grow buy slowly. The both isolates were tolerated up to 15 ppt NaCl.

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