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Biological characteristics of an obligate marine strain *Lagenidium thermophilum* isolated from mud crab (*Scylla tranquebarica*) eggs in Sabah, Malaysia

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Abstract

A fungus *Lagenidium thermophilum* was isolated from the eggs and larvae of the mud crab *Scylla tranquebarica* at a hatchery in Sabah, Malaysia. This fungus is responsible for almost 100% of mud crab larval mortality in the hatchery. In this study, the present strain, IPMB 1401, which was isolated from the eggs was identified as an obligatory marine fungus as the strain only grew on PYG agar containing seawater. Varying concentrations of formalin solution were tested to determine the concentration at which hyphal growth of the fungus was inhibited. The findings showed that hyphal growth and zoospore production were inhibited when the colonies were exposed to formalin at 25 ppm for 24 h. Therefore, a bath treatment using 25 ppm formalin could potentially be used to control fungal infection in mud crab larvae aquaculture operations.

Introduction

Mud crab aquaculture is an important economic activity throughout the Asia Pacific region (Keenan, 1999). Mud crabs are commonly found in estuaries, especially estuaries with mangrove forests. In recent years, mud crabs have become increasingly popular in domestic and international markets due to their large size and delicious taste. Consequently, outbreaks of bacterial, viral and fungal infections can have a considerable economic impact on aquaculture operations for this species. Previous studies have shown that, after bacterial pathogens, fungal pathogens are the second most important cause of disease in mud crabs (Karunasagar et al., 2004). The role of marine Oomycetes (lower fungi) as causative agents of egg and larval mortality in mud crabs and some other marine crustaceans has been demonstrated previously (Couch, 1942; Nakamura et al., 1995; Hatai et al., 2000). In the genus *Lagenidium*, *L. callinectes* and *L. thermophilum* have also been isolated from the blue crab *Callinectes sapidus* (Couch, 1942) and the mud crab *Scylla serrata* (Nakamura et al., 1995). This paper describes selected biological characteristics of *L. thermophilum* IPMB 1401, which was isolated from eggs of the mud crab *Scylla tranquebarica* at a hatchery in Sabah, Malaysia (Lee et al., 2016). In addition, the application and effectiveness of formalin for inhibiting the growth of the isolated strain (*in vitro*) were also tested.

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Materials and methods

Effect of concentration of sodium chloride and potassium chloride on fungal growth

PYG agar (0.125% peptone, 0.125% yeast extract, 0.3% glucose, 1.2% agar) containing different concentrations (0, 0.5, 1, 2, 3, 5, 7 and 10%) of NaCl or KCl were prepared. *Lagenidium thermophilum* IPMB 1401 was inoculated onto PYG agar including seawater (PYGS agar) and incubated at 25ºC for 7-15 days to produce a large colony. An agar block of the strain was then excised from the edge of the colony using a No. 2 cork borer (5 mm diameter) and used to inoculate individual PYG agar plates with different concentrations of NaCl and KCl. Fungal growth was examined after incubating the plates at 25ºC for 10 days, and the colony radius was measured at 3, 5, 7 and 10 days.

Effect of salinity on fungal growth

PYGS agar plates with different salinities were made by adding diluted seawater to PYG agar to give salinities of 0, 5, 10, 15, 20 and 30 ‰. An agar block of strain IPMB 1401 was excised from the edge of the parent colony and used to inoculate individual PYGS agar plates at each salinity. The plates were then incubated at 25ºC and fungal growth was determined by measuring the colony diameter at 3, 5, 7 and 10 days, which was recorded as the colony radius in cm.

Effects of formalin on hyphal growth and zoospore production

Different concentrations (6.3, 12.5, 25, 50, 100 and 200 ppm) of formalin solution were prepared using sterile seawater, and sterile seawater without formalin was used as the control. Agar blocks of strain IPMB 1401 were excised from the edge of the parent colony and 10 agar blocks inoculated into each Petri dish containing formalin solutions at different concentrations. After different exposure times (1, 2, 24 and 48 h), two agar blocks of strain IPMB 1401 were removed from each formalin solution and washed with seawater. One agar block was inoculated onto PYGS agar and the other was transferred to fresh sterile seawater and fungal growth was observed after 1, 2, 24 and 48 h and recorded as – (no growth) or + (growth). For the agar blocks in seawater, zoospore production was observed using an inverted microscope Olympus CKX 41and recorded as + (moderate growth), ++ (fast growth) or – (no production).

Results

Effects of concentration of NaCl and KCl on fungal growth

Strain IPMB 1401 did not show any growth on PYG agar containing NaCl (Table 1) or KCl (Table 2). Growth of the strain was only observed on PYGS agar, which was used as the control.

Effect of salinity on fungal growth

Strain IPMB 1401 grew on PYGS agar at salinities of 15, 20 and 30ppt (Table 3). The fungus did not grow at salinities of less than 10 ppt.

Effect of formalin on hyphal growth and zoospore production?

Hyphal growth of the fungal isolate was inhibited after 24 h exposure to formalin at 25, 50 and 100 ppm (Table 4). The fungal isolate did not produce zoospores after exposure to formalin at 25 ppm and above concentrations.

Discussion

Five fungal genera (*Lagenidium, Haliphthoros, Halocrusticida, Halioticida* and *Atkinsiella*) have been reviewed by Hatai (2012) and reported

Table 1. The effect of NaCl concentration on hyphal growth of the strain IPMB 1401.

NaCl (%)	Days after incubation on PYG agar			
	3	**5**	**7**	**10**
0	-*	-	-	-
0.5	-	-	-	-
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
5	-	-	-	-
7	-	-	-	-
10	-	-	-	-
PYGS agar	0.84**	1.55	2.17	3.21

* no growth
**colony radius in cm

Table 2. The effect of KCl concentration on hyphal growth of the strain IPMB 1401.

KCl (%)	Days after incubation on PYG agar			
	3	**5**	**7**	**10**
0	-	-	-	-
0.5	-*	-	-	-
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
5	-	-	-	-
7	-	-	-	-
10	-*	-	-	-
Control	0.76**	2.0	2.55	3.89

*no growth
**colony radius in cm

Table 3. The effect of salinity on hyphal growth of colony radius of the strain IPMB 1401 after incubation for 10 days on PYGS agar with different salinity.

Salinity ppt	Days after incubation			
	3	5	7	10
0	-*	-	-	-
5	-	-	-	-
10	-	-	-	-
15	1.68**	2.39	3.43	4.77
20	1.60	2.41	3.63	5.17
PYGS agar	1.96	2.92	4.16	5.76

*no growth
** colony radius in cm

Table 4. Effect of formalin on hyphal growth and zoospores production of the strain IPMB 1401.

Concentration of formalin, ppm	Time of exposure (h)							
	1		2		24		48	
	hyphal growth	zoospores production	hyphal growth	zoospores production	hyphal growth	zoospores production	hyphal growth	zoospores production
0	+	+++	+	+++	+	+++	+	+++
6.3	+	+++	+	+++	+	++	+	++
12.5	+	++	+	++	+	++	+	++
25	+	++	+	++	-	-	-	-
50	+	+	+	+	-	-	-	-
100	+	+	+	+	-	-	-	-

Symbols, -: no growth; +, ++, +++: increasing number of zoospores from slight to excellent.

to affect marine crustaceans (Nakamura et al., 1995; Leano 2002). Among the members of the genus *Lagenidium*, *L. callinectes* was first reported from eggs of the blue crab *Callinectes sapidus* (Couch, 1942). *Lagenidium thermophilum* was subsequently reported as a new species isolated from eggs and larvae of the mud crab *Scylla serrata* by Nakamura et al. (1995). To date, *L. thermophilum* has been reported twice, first from eggs and larvae of the mud crab *S. serrata* (Nakamura et al., 1995), and then in the black tiger shrimp *Penaeus monodon* (Muraosa et al., 2006).

Morphological characteristics of the present strain IPMB 1401 revealed that it produced zoospores in a similar manner to *L. thermophilum*. However, in *L. thermophilum*, the vesicle separates from the discharge tube before the zoospores are released. Conversely, in *L. callinectes*, the vesicle does not separate from the discharge tube when the zoospores are released (Hatai, 2012). This characteristic of zoospore release is the main distinguishing feature between *L. callinectes* and *L. thermophilum*. Strain IPMB 1401 also differed from *L. thermophilum* NJM 9338 (Nakamura et al., 1955) and NJM 0031 (Muraosa *et al.*, 2006) in its mineral salt requirements. Since *L. thermophilum* NJM 9338 and NJM 0031 were both able to grow on PYG agar containing 0% (w/v) NaCl and KCl, it is suggested that both strains are not exclusively marine. However, strain IPMB 1401 was clearly shown to be an obligatory marine fungus as it was only able to grow on PYGS agar containing seawater. Further, strain IPMB 1401 was not able grow on PYG agar containing seawater at salinities below 10 ppt. Indeed, the optimal salinities of this fungus are similar to that of the environment and of the hatcheries where these crabs are cultivated.

Formalin is widely used in aquaculture as a disinfectant and for inhibiting the growth of bacteria, fungi and parasites (Herwig, 1979; Mohamed et al., 2000). Hyphal growth and zoospore production in strain IPMB 1401 were both inhibited after exposure to 25 ppm formalin for 24 h. This formalin concentration falls within the range recommended by Hamasaki and Hatai (1993) for preventing Lagenidiales infection in the swimming crab *Portunus trituberculatus* and the mud crab *S. serrata*. Bath treatment with 25 ppm formalin was found to be effective for inhibiting infection by the fungal pathogen, *Haliphthoros* sp., while remaining non-toxic to newly hatched larvae of the mud crab *S. serrata* (Kaji et al., 1991).

References

Couch JN (1942). A new fungus on crab eggs. *Journal of the Elisha Mitchell Scientific Society* **58**, 158-162.

Hamasaki K and Hatai K (1993). Prevention of fungal infection in the eggs and larvae of the swimming crab *Portunus trituberculatus* and the mud crab *Scylla serrata* by bath treatment with formalin. *Nippon Suisan Gakkaishi* **59**(6), 1067-1072.

Hatai K, Roza D and Nakayama T (2000). Identification of lower fungi isolated from larvae of mangrove crab, *Scylla serrata* in Indonesia. *Mycoscience* **41**, 565-572.

Hatai K (2012). Disease of fish and shellfish caused by marine fungi in "Biology of Marine Fungi", pp 16-52. Springer.

Herwig N (1979). Handbook of drugs and chemicals used in treatment of fish diseases. Charles C. Thomas, Springfield, IL.

Kaji S, Kanematsu M, Tezuka N, Fushimi H and Hatai K (1991). Effects of formalin bath for *Haliphthoros* infection on ova and larvae of the mangrove crab *Scylla serrata*. *Nippon*

Suisan Gakkaishi **57**(1), 51-55.

Karunasagar I, Karunasagar I and Umesha RK (2004). Microbial diseases in shrimp aquaculture. *In Marine Microbiology: Facetes and Opportunies*, edited by N, Ramaiah (National Institute of Oceanography, Goa, India), 165-186.

Keenan CP (1999). Aquaculture of the mud crab, genus *Scylla* – past, present and future. In Keenan, C.P. and Blackshaw, A. Mud Crab Aquaculture and Biology. Proceedings of the international scientific forum held in Darwin, Australia 21-24 April 1997. *ACIAR Proceedings* **78**, 216p.

Leano EM (2002). *Haliphthoros spp*. from spawned eggs of captive mud crab, *Scylla serrata*, broodstocks. *Fungal Diversity* **9**, 93-103.

Lee YN, Hatai K and Kurata O (2016). First report of *Lagenidium thermophilum* isolated from eggs and larvae of mud crab (*Scylla tranquebarica*) in Sabah, Malaysia. *Bulletin of the European Association of Fish Pathologists* **36**(3), 107-113.

Mohamed, S, Nagaraj G, Chua FHC and Wang YG (2000). The use of chemicals in aquaculture in Malaysia and Singapore. In: J. R. Arthur, C. R. Lavilla-Pitogo and R. P. Subasinghe (Eds.) Use of chemicals in aquaculture in Asia: *Proceedings of the Meeting of the Use of Chemicals in Aquaculture in Asia 20-22 May 1996* Tigbauan, Iloilo, Philippines, 127-140.

Muraosa Y, Lawhavinit O and Hatai K (2006). *Lagenidium thermophilium* isolated from egg sand larvae of black tiger shrimp *Penaeus monodon* in Thailand. *Fish Pathology* **41**(1), 35-40.

Nakamura K, Nakamura M, Hatai K and Zafran (1995). *Lagenidium* infection in eggs and larvae of mangrove crab (*Scylla serrata*) produced in Indonesia. *Mycoscience* **36**, 399-404.