1979

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Bian, B. Z., Hatai, K., Po, G., & Egusa, S. (1979). Studies on the fungal diseases in crustaceans I. Lagenidum scyllae sp. nov. isolated from cultivated ova and larvae of the mangrove crab (Scylla serrata). SEAFDEC Aquaculture Department Quarterly Research Report, 3(3), 10–13.

http://hdl.handle.net/10862/2353

Studies on the fungal diseases in crustaceans
I. *Lagenidum scyllae* sp. nov. isolated from cultivated ova and larvae of the mangrove crab *(Scylla serrata)*

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In 1978, a highly lethal and fungal disease frequently occurred in ova and larvae of the mangrove crab, *Scylla serrata,* at the wet laboratory of SEAFDEC. A causative organism of the disease is described and compared with the previous members of *Lagenidium* known to be parasitic on the crustacean ova and larvae. For the growth in the liquid culture, the effects of temperature, NaCl concentration and pH are also shown.

In the shaking culture, vegetative thalli appeared as spherical colonies. The fungus on PYGS agar plate inoculated with these colonies appeared whitish and filamentous, and attained a diameter of 2 cm in a week at 25°C. From the morphological observations of these and other cultures in PYGS broth, the following descriptions were made.

Hyphae stout, branched, sparingly septate, infecting the ova or bodies of both zoea and megalopa of the crab, holocarpic. In pure cultures, the hyphae is at first somewhat uniform with a diameter of 7.5-17 μm, generally vacuolate, with numerous minute cytoplasmic oil droplets, then becoming quite irregular and swollen up to 40 μm thick in age, coarsely granular just before spore formation. Vesicle formed at the end of the discharge tube measuring 37-500 x 4-10 μm, 25-72.5 μm in diameter, contains a sphere of protoplasmic material measuring 22.5-65 μm diam. Zoospores monoplanetic, laterally biflagellate, irregular in shape and size, pyriform, globose or elongate, 8-17.5 x 7.5-15 μm, mostly 12.5 x 10 μm, differentiated from the protoplasmic material in the vesicle, discharged simultaneously by rapid deliquescence of the vesicle, or liberated one by one through the opening of the vesicle; collapsed vesicles not persistent. When the discharge failed, zoospores swarming within the discharge tube and vesicle, coming to rest and converted to cystospores which may germinate in situ. Encysted zoospores were spherical, 7.5-15 μm in diam, averaging 10 μm, with walls up to 1.5 μm thick. Sexual reproduction not observed.

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The effects of temperature on growth are summarized in Table 1. The fungus tolerated a wide range of temperature (16 to 42°C) but grew with the optimum at 22.5-31.8°C. For earlier stages of growth, higher temperature seems to be required. The fungus did not grow at 11-13°C, but at least survived beyond 7 days when cultured.

Table 1. Effect of temperature on growth of *Lagenidium scyllae* in PYGS broth

Temperature (C)	Growth-rate at day after incubation*			Survival **
	1	3	7	
9.5	–	–	–	–
11.0	–	–	–	+
13.0	–	–	–	+
14.2	–	–	±	
16.0	–	+	+	
17.4	–	±	++	
19.0	–	+	+++	
20.5	–	+	+++	
22.5	±	++	+++	
30.0	±	++	+++	
31.8	+	++	+++	
33.8	+	++	++	
36.0	++	++	++	
37.6	++	++	++	
39.0	+	++	++	
42.0	±	±	±	
44.0	–	–	–	

* Symbols, – : no growth; + : barely visible growth; +, ++, +++ : increasing amounts of growth from slight to excellent.

** Viability of inoculated spores was checked by re-incubation of the culture after 7 days, at 25 C for more 3 days.

As seen in Table 2, the fungus was also tolerant to relatively high concentrations of NaCl. Its growth occurred over a wide range of NaCl concentrations (0-7% NaCl-PYG broth). Maximum growth was obtained at 2-3% NaCl. Except for a few cases, there was no difference of growth between cultures with 8.0×10^4 spores and those with 8.0×10^3 spores. At higher NaCl concentrations, such as 8 or 9%, larger amounts (8.0×10^4) of the inoculum supported slight growth of the fungus. Smaller amounts of the inoculum had no apparent growth.

The fungus grew well but slowly in the PYG broth. It did not grow in the PYG broth containing 2.5% KCl. The similar growth in KCL-PYG broth was described on *Lagenidium* sp. (Nilson et al., 1976).

Although growth occurred over a wide range of pH (5-11), the greatest yield was obtained at an initial pH of 7 or 8 (Table 3). There was no difference of growth between cultures with 6.8×10^4 spores and those with 6.8×10^3 spores.

Table 2. Effect of NaCl concentration on growth of *Lagenidium scyllae* in PYG broth

Concentration of NaCl*** (%)	Experiment 1*				Experiment 2*			
	Growth-rate at day after incubation			Survival**	Growth-rate at day after incubation			Survival**
	1	3	7		1	3	7	
0	–	+	+		–	+	+	
1	–	+	+		–	+	+	
2	–	+	+++		–	+	+++	
3	–	+	+++		–	+	+++	
4	–	+	++		–	+	++	
5	–	+	++		–	+	++	
6	–	+	+		–	–	+	
7	–	+	+		–	–	+	
8	–	–	±		–	–	–	–
9	–	–	±		–	–	–	–
10	–	–	–	–	–	–	–	–

* Exp. 1: inoculated with 8.0×10^4 spores; Exp. 2: inoculated with 8.0×10^3 spores.

** Viability of inoculated spores was checked by re-incubation that 1 ml of the cultures after 7 days inoculated into 10 ml of newly prepared PYGS broth and then incubated at 25 C for 3 days.

*** Each culture contained very small excess NaCl which was brought from 1 ml of sea water as inoculum.

Table 3. Effect of pH on growth of *Lagenidium scyllae* in PYGS broth

pH	Experiment 1*				Experiment 2*			
	Growth-rate at day after incubation			Survival**	Growth-rate at day after incubation			Survival**
	1	3	7		1	3	7	
4	—	—	—	—	—	—	—	—
5	±	+	++		—	—	++	
6	±	+	++		—	+	++	
7	+	+	+++		±	+	+++	
8	+	++	+++		+	+	++	
9	+	+	++		+	+	++	
10	±	±	++		±	±	++	
11	—	—	+		—	—	+	—
12	—	—	—	—	—	—	—	

* Exp. 1: inoculated with 6.8×10^4 spores; Exp. 2: inoculated with 6.8×10^3 spores.

** Viability of inoculated spores was checked by re-incubation that 1 ml of the cultures after 7 days inoculated into 10 ml of newly prepared PYGS broth and then incubated at 25 C for 3 days.

The fungus could be assigned to a member of the genus *Lagenidium* on the basis of its parasitic, endobiotic and holocarpic thalli and laterally flagellate, monoplanetic zoospores in a vesicle at the end of discharge tube. While the fungus is very similar to *L. callinectes* (Couch, 1942; Bland and Amerson, 1973), there are, however, some differences between the two. In this fungus it needs only 3 h and 15 min for the initiation of spore formation from the transfer of hyphae into sea water; both long and short discharge tubes are found in the fungus; the gelatinous envelope found in *L. callinectes* is not seen and the protoplasmic material nearly fills in the whole inside; and all the spores are simultaneously discharged by the rapid deliquescence of the vesicle or the spores are liberated one by one through the opening of vesicle. From all the other members of *Lagenidium* known to attack animals, this fungus differs in having the non-segmented hyphae. Thus the fungus is proposed as a new species of *Lagenidium, L. scyllae* Bian, Hatai, Po & Egusa.

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