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Isolation and culture in artificial media of *Lagenidium* from *Penaeus monodon* larvae

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Fungal infection of *Penaeus monodon* larvae is a problem in hatchery operations. The fungus, which attacks the nauplius to postlarval stages and causes up to 100% mortality, has been tentatively identified as belonging to the genus *Lagenidium.* This pathogenic organism has recently been isolated and cultured.

P. monodon larvae infected with the fungi were collected from culture tanks. Bacteria and other organisms adhering to the larvae were reduced by serial washing with sterile seawater. One or two infected larvae were placed in different agar media and the others were placed in test tubes of broths. The media were supplemented with streptomycin and penicillin to prevent bacterial growth. These were then incubated at 23 to 35°C and examined daily for signs of hyphal growth.

Growth of the hyphae became evident after 48 hr in Sabouraud dextrose broth, tryptose broth, Sabouraud broth and Sabouraud agar. The hyphae are irregularly branched, non-septate or sparingly septate, 1.9 to 5 microns wide, granular and filled with numerous oil globules. The colonies in the Sabouraud agar appeared whitish and filamentous. Growth in brain heart infusion broth was slower, becoming evident only after 96 hr. The fungus which grew in this medium had irregular, broader hyphae measuring 15 to 30 microns in width. Very slow growth was observed in other agar media, specifically in Sabouraud maltose agar, triple sugar iron medium, and dextrose agar. Growth of the colonies was observed only after 11 days incubation. The colonies were oval, small, averaging only about 2.9 x 4.7 mm in size compact, convex, and creamy in color. The hyphae were irregular and much wider than those grown in broth, ranging from 12.5 to 45 microns in width.

Attempts to induce sporulation were done by transferring the fungus from each culture medium to sterile seawater.

The sporulating fungi, as observed from infected larvae, were similar to the *Lagenidium* species observed by Lightner and Fontaine (1975) in the larvae of white shrimp *Penaeus setiferus,* by Bland and Amerson (1973) in eggs of the blue crab, and by Johnson and Banner (1963) in barnacles. The hyphae of a sporulating fungus measured 2.5 to 6.25 microns wide and the discharge vesicles were 14.4 to 25 microns in diameter, from which 14 to 32 spores, 3.75 to 6.25 microns wide and 5 to 6.25 microns long, were released. Despite the differences in the sizes of the vegetative and reproductive structures, the appearance of these structures and sporulation are similar to those of *Lagenidium callinectes* as described by Bland and Amerson (1973). *L. callinectes* heavily infects the eggs of the blue crab *Callinectes sapidus* Rathbun and could be transmitted to eggs of the oyster crab *Pinnotheres ostreum* and the mud crab *Neopanope texana* (Rogers-Talbert, 1948). The fungus of the same genus is apparently a very active pathogen also in larvae of the lobster *Homarus americanus,* causing large mortalities in the hatchery as observed by Nilson et al. (1976a, 1976b) and Fisher et al. (1976). As in the *P. monodon* larvae, *Lagenidium* in lobster also replaces the tissues of the larvae and produces extramatrical germ tubes. Terminal

discharge vesicles then develop from which zoospores are later released into the water, thus infecting the other larvae (Schuur et al., 1976). The fungus has a wide range of hosts, among which is the alga *Ectocarpus* (Fuller et al., 1964). Like the *Lagenidium* isolated from *Ectocarpus,* the sporulating fungus produces a discharge vesicle which is formed when the cytoplasm of the adjacent hyphae follows into the extramatrical tube. The formation of this vesicle with the sporogenic cytoplasm appearing amorphous inside occurs in only 5 to 10 min. Spore formation then proceeds for 10 to 20 min after which the spores may be seen moving slowly inside the vesicle. The spores then become active and are released 10 to 15 min later. The vesicle, however, seems to disappear immediately after the discharge, which is typical of *Lagenidium chthamalophilum* isolated by Johnson from the eggs of the barnacle *Chthamalus fragilis* (Johnson and Banner, 1960; Fuller et al., 1964). The fungus in the present study has an extramatrical, sparingly septate hyphal system and short discharge tubes which are also characteristic of *L. callinectes.* As far as the mentioned characters are concerned, the authors believe that the isolates are similar to *L. callinectes.* Sporulation of the isolates, however, is still to be observed and until then, they cannot be definitely assigned to *Lagenidium callinectes.*

Contamination of the cultures by other fungi has been more rampant in the agar media despite the antibiotic supplements. Cultures are being maintained in broth media at 15 to 16°C.

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