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NOTE

BACTERIAL BLACK SPOT DISEASE OF SHRIMP *(M. MONOCEROS)* IN BANGLADESH

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ABSTRACT

The black spot disease which causes extensive mortality of shrimp. *Metapenaeus monoceros* in Bangladesh has been studied from January to December 1981. The disease was caused by a Pathogenic bacteria, *Pseudomonas* sp. The sensitivity of this bacterium to four antibiotics was tested and tetracycline was found to be most effective in controlling this disease in shrimps.

Key-words : Black spot disease, *Metapenaeus monoceros*, *Pseudomonas*, Bangladesh.

Black spot disease in *Metapenaeus monoceros* is characterised by brown to black spot on the external carapace or cuticle. With the advancement of the disease, considerable erosion and destruction of the cuticle takes place. This disease causes mortality both in the hatchery and culture ponds.

The effects of black spot disease on individual shrimp is apparently a break down of cuticular protection, thus causing loss of hemolymph and invasion by internally destructive pathogens. Black spot disease in penaeids is fairly common, at least in early manifestation (Couch, 1978).

The role of pathogenic bacteria causing disease in penaeid shrimp has been established in recent years. Cook and Lofton (1973) reported isolation of three genera of bacteria, namely, *Beneckea*, *Vibrio* and *Pseudomonas* from penaeid shrimps infested by black spot disease. This disease has also been reported from many other decapod crustaceans by Rosen (1970).

In Bangladesh no report has been made till date regarding black spot disease in shrimps which causes heavy mortality in culture ponds.

The present work has been undertaken to find out the role of bacteria in the formation of black spots in shrimps and the sensitivity of the pathogen towards some antibiotics.

Pieces of black spotted muscles and shells were taken from the infected area of diseased shrimps and rubbed on the surface of the seawater nutrient agar plates by a sterilized forceps and incubated for 48 hours at room temperature (28 ± 2 °C).

The colonies developed on the plates were removed, checked for their purity and maintained on seawater nutrient agar slants.

Final selection of the strains was made by observing pathogenicity of the strains as follows: Cells of 24-48 hours old cultures of each strain was harvested in sterile saline water (1.0% NaCl) and diluted to a cell density that allowed 70% light transmission at 540 μm and 0.05 ml was administered directly into the experimental shrimps.

Juvenile shrimps collected from local bait camp, Chittagong were used in experiments to ascertain the pathogenicities of the strains.

Ten animals were inoculated intramuscularly in the abdominal segment with 0.05 ml of cell suspension of each strain using 1.0 ml sterile syringe. Ten control shrimps were also injected with only 0.05 ml sterile saline (1.0% NaCl) water in the same location.

Inoculated and control shrimps were maintained in separate glass aquarium tanks at room temperature (28-29 °C) by providing continuous and

Table I. Characterization of the pathogenic strain.

Cell morphology	:	Non sporing, short rods (0.3 to 0.5μ× 1.3 to 2.2μ)
Gram reaction	:	—
Acid fast stain	:	—
Mortality	:	+
Agar colonies	:	Small, round, convex translucent (1 mm), filiform to spreading.
Seawater broth	:	Pellicle and sediment formed.
Catalase	:	+
Oxygen relationship	:	Facultative anaerobic
Indol formation	:	—
Nitrate reduction	:	+
Gelatin hydrolysis	:	+
H_2S production	:	+
Urease	:	—
Protease	:	+
Amylase	:	—
Methyl red test	:	—
V.P. test	:	+
Growth with NaCl (1%)		
0	:	++
2.0	:	+++
4.0	:	+++
6.0	:	++
8.0	:	++
10.0	:	Trace
Fermentation test	:	Acid and no gas from starch, Sucrose, Arabinose, Glucose, Xylose, Glycerol and Fructose.
Identification	:	*Pseudomonas* sp.

occasional aeration for 5 days. Proper food was supplied and shrimps were checked four times daily for clinical signs of the disease and mortality. Dead shrimps were removed as and when necessary.

Sensitivity of the pathogenic strain to four antibiotics, viz., oxy-tetracycline, chloramphenical, tetracycline and amphicellin at concentrations of 10, 20, 30 and 40 ppm were tested as follows:

A young culture of pathogen was streaked crosswise (X) on the surface of shrimp extract seawater nutrient agar in petriplates. A sterile 'Porous clay cylinder' was placed at the point where two streaks crossed each other. Five drops of antibiotic solution were poured inside the cyclinder. The procedure was repeated three times for each concentration in different plates. After 48 hours of incubation at 37 °C the zone of inhibition formed, was recorded.

From the pathogenicity test it was found that among the 9 strains isolated from the diseased shrimps only strain No. 8 was found to be pathogenic. The isolated pathogenic strain which caused the black spot disease was identified as a member of the genus *Pseudomonas* (Table 1). Cook and Lofton (1973) had also isolated *Pseudomonas* and *Vibrio* from penaeid shrimps suffering from this .

The pathogen was resistant to 10 and 20 ppm, while it was slightly sensitive to 30 and 40 ppm of both oxy-tetracycline and chloramphenicol. In the case of tetracyline and amphicilline it was slightly sensitive to 10 ppm concentration but highly sensitive to 30 and 40 ppm.

REFERENCES

Cook. D.W. and Lofton. S.R., 1973. Chitinoclastic bacteria associated with shell disease in penaeus shrimp and blue crab *(Callinectes sapidus)*. *Journal of Wildlife Diseases*, **9**: 154–159.

Couch, J.A., 1978. Diseases, parasites and toxic responses of commercial penaeid shrimps of the Gulf of Maxico and South Atlantic coasts of North America. *Fisheries Bulletin*, **76**: 1–45.

Rosen, B., 1970. Shell disease of aquatic crustaceans. In: *A Symposium on Diseases of Fish and Shell Fishes*, edited by S.F. Sneiszko, American Fisheries Society, Special Publication, **5**: 409–415.