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Bacteria from seawater used in *Penaeus monodon* larval cultures

By

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Bacteria cause a number of diseases in shrimps and other crustaceans. Known to bring about varying degrees of mortality in many stages in the development of crustaceans of the species *Vibrio* such as *V. panulirus* on *P. japonicus* (Kusuda and Watada, 1969), *V. alginolyticus* and *V. anguillarum* on *P. setiferus* and *P. aztecus* (Lewis, 1973) and *P. californensis* (Lightner and Lewis, 1973); *V. parahaemolyticus* on *P. setiferus* (Vanderzant et al., 1971). *Aerococcus viridans,* formerly known as *Gaffkya homari,* is another, having been found to cause 'gaffkemia' in lobsters (Cornick and Stewart, 1968; Stewart et al., 1969). Many relatively less known forms of shell rot are known to be due to concerted action of different genera including *Benekea, Vibrio* and *Pseudomonas,* through their chitinoclastic activity (Cook and Lofton, 1973).

A few of the symptoms of shrimp diseases are recognizable in larvae reared at the SEAFDEC hatchery in Tigbauan, Iloilo. Mortality in *P. monodon* larvae has at times been suspected of being bacterial in etiology. We believe that linkage of certain bacterial groups to such mortality would be very difficult to prove owing to many uncontrollable parameters when a challenge on a population of larvae is made.

As a takeoff point for further studies, a survey of potentially pathogenic bacteria in water used in the culture of larvae was made for a period of 13 months (November 1975 to December 1976).

Seawater samples were collected in 250-mL Erlenmeyer flasks from 200-ton spawning tanks containing *P. monodon* nauplii. These were brought to the microbiology laboratory for dilution and plating.

Water samples of 1-mL were drawn aseptically from the flasks and diluted in a series (10^1 to 10^5). The 10^2 – 10^5 homogenates were then pipetted out, delivered at the rate of 1 mL per Petri plate in triplicate, and overlaid with Bachmann's agar. The plates were incubated at 27°C for 96 hours and the resulting colonies tabulated. Colonies from the 10^3 plates were stabbed onto Sulfide-Indole-Motility agar (SIM) for maintenance.

Identification of each isolate was facilitated by the use of modifications in the morphological and biochemical protocols of Shewan et al. (1959) and Kazanas (1966).

Table 1 shows the ranges of the total initial bacterial counts in 25 samples of water used in the rearing of *P. monodon* nauplii as recovered on Bachmann's agar. The mean was 1.48×10^4 cells/mL. The results point to the wide daily variation in the total bacterial counts in the water and reflect the efficiency of the filtration system used in the hatchery.

Table 1. Ranges and frequency of total bacterial counts of water used in the culture of *P. monodon* at nauplii stage, based on 25 platings

Total plate counts (cells/mL)	Frequency
$1.0 - 5.0 \times 10^2$	2
$5.1 - 9.9 \times 10^2$	5
$1.0 - 5.0 \times 10^3$	7
$5.1 - 9.9 \times 10^3$	1
$1.0 - 5.0 \times 10^4$	8
$5.1 - 9.0 \times 10^5$	2

It is perhaps not the total bacterial count, but the kind of bacteria present (as is the case of drinking water) that is of interest to the aquaculturist. No hatchery has so far come up with a standard bacterial load of the water used for its daily operations.

The different genera isolated and their relative abundance based on 124 strains identified are presented in Table 2. Of this number, 98 (or 79%) were Gram-positive. This is indicative of the limited efficiency of Bachmann's medium in recovering Gram-positive bacteria.

Table 2. Bacterial profile of water used in the culture of *P. monodon* at nauplii stages, based on 24 isolates.

Genera	Number of isolates	Percentage
A. Gram-positive isolates		
Micrococcus	42	33.3
Staphylococcus	16	12.3
Planococcus	8	6.2
Kurthia	8	6.2
Bacillus	1	1.0
Microbacterium	1	1.0
Mycobacterium	1	1.0
Unidentified 1/	21	16.1
B. Gram-negative isolates		
Acinetobacter	8	6.2
Moraxella	8	6.2
Flavobacterium	7	5.4
Alcaligenes	5	3.8
Branhamella	2	1.5
Enterobacteriaceae	1	1.0

1/14 resembling *Peptostreptococcus*; 7 *Pediococcus*

Enumeration of bacteria from marine sources using varied media has always shown more Gram-positive than Gram-negative strains (Colwell and Liston 1960; Shewan et al, 1960; Kazanas 1966; Adams et al, 1964; Liston, 1958, Lee and Harrison, 1968). The only exception is the case of pond-reared shrimps (Vanderzant et al, 1971). This suggests that there are plenty of materials of terrigenous origin.

As far as the present isolation and identification shows, the culture water used over the period was relatively free of potentially pathogenic bacteria such as *Vibrio, Aeromonas, Aerococcus* and *Pseudomonas.* Whether these were present but were not detected in routine isolation remains to be proven by the use of other plating media.

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