A Bacteriological Study of the Natural Flora of Edible Oyster, *Crassostrea madrasensis*

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The total viable bacterial populations in the oysters and the sea water from the edible oyster farm at Tuticorin were in the range of 10^3 to 10^4 per ml and 10^2 to 10^3 per ml respectively. The maximum most probable number of faecal coliform recorded during the one year period of study of both the oysters and seawater were 33 per 100 ml. Pathogenic bacteia (*Salmonella* sp., *Vibrio cholerae,* coagulase positive staphylococci and faecal streptococci were absent in oysters and farm water. Study of 197 (98 taken from oyster liquid and 99 from oyster farm water) randomly isolated cultures indicated that gram negative asporogenus rod-like bacteria of the *Vibrio, Flavobacterium, Achromobacter* and Pseudomonas groups were the dominant flora of the oyster liquid as well as seawater.

Indian coastal waters offer great scope for development of marine aquaculture projects especially for growing oysters and mussels in the sheltered bays and shallow waters. Although immense possibilities exist for mariculture of edible oysters in the east coast of India, oyster farming has not been well established. The reason may be lack of consumer demand. Central Marine Fisheries Research Institute has succeeded in the rack culture of edible oyster, *Crassostrea madrasensis* at Tuticorin. Little information is available on the bacteriology of *Crassostrea madrasensis* and the attempt of the authors to study the natural bacterial flora occurring in the oysters and in the water where they live is presented in this communication.

Material and Methods

Crassostrea madrasensis were grown in racks in a shallow bay area adjacent to the field station of CMFRI, Tuticorin. Fortnightly collections of oysters and farm water were made for a period of one year (July, 1982 to June, 1983). At a time, two samples of oysters and two samples of farm water were collected for bacteriological examination. Oyster flesh and liquid blended with equal weight of sterile phosphate buffer provided the most consistent and high total viable counts (Colwell & Liston, 1961). It was used for plating and for the enumeration of bacterial counts. A sample of 3–5 oysters was used for collecting oyster liquid which was blended with equal amount by weight of the diluent so that 2 ml of oyster liquid contained 1 g oyster meat. Plate counts of the oyster liquid and farm water were determined using tryptone glucose yeast extract agar. The plates were incubated at 37° C (Presnell & Kelly, 1961; APHA, 1976).

Cultures of micro-organisms were obtained by random selection from the count plates of oyster liquid and farm water. They were purified by routine methods and maintained as pure cultures in sea water peptone broth (1% peptone in sea water). The microbiological tests for classification and identification were those described in the Manual of Microbiological Methods (1957).

EC broth with incubation at 44.5° C and eosin-methylene blue agar were used for the enumeration of faecal coliform. For *Salmonella,* brilliant green agar, bismuth sulphite agar and triple sugar iron agar were used. For enumeration of coagulase positive staphylococci, Baird-Parker agar and for faecal streptococci, KF-agar were employed. Alkaline peptone water and thiosulphate citrate bile salts sucrose (TCBS) agar were used for detecting *Vibrio cholerae.*

Pure cultures of all the organisms isolated in this study were streaked on sea water nutrient slants for colonial morphology (Gram stain and motility). Cultures were studied for sensitivity to pencillin discs of 2.5 I.U. pigmentation was observed on sea water agar slants. Other tests and media used were sea water nutrient gelatin for gelatin liquefaction, nitrate broth for nitrate reduction, tryptone broth for indole production, oxides test by Kovacs (1956) method, Hugh & Leifson oxidative and fermentative medium for sugar fermentation (Hugh & Leifson, 1953). The generic classification of the bacterial isolates was done according to a modified scheme of Usio Simidu & Kazuzoshi (1952).

Results and Discussion

The total viable counts of the oyster liquid and the oyster farm water samples were between 10^3 & 10^2 and 10^2 per ml respectively (Fig. 1). The total plate count of the surrounding sea water was consistently lower than the corresponding counts of the oyster of the oyster liquid. This agrees with the findings of Colwell & Liston (1961).

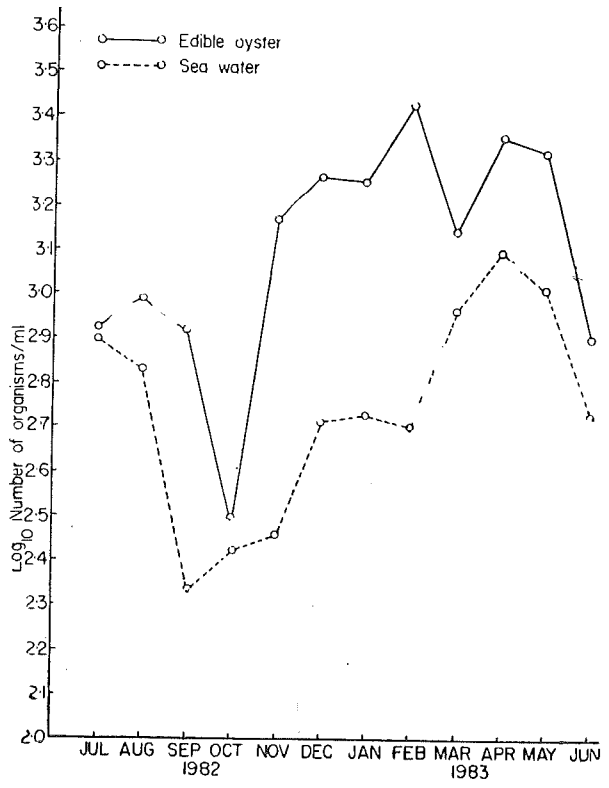

Fig. 1. Total viable counts of oyster liquid and oyster farm water

The MPN of faecal coliform (monthly mean values) of oyster liquid and sea water for the period July 1982 to June 1983 are given in Table 1.

Table 1. *Faecal coliform content of oyster liquid and sea water (MPN/100 ml)*

	Oyster liquid	Sea water
July, 1982	17	16
August	21	18
September	18	33
October	6	13
November	33	12
December	—	9
January, 1983	4	—
February	12	3
March	15	15
April	33	9
May	8	—
June	—	—

The MPN values of the faecal coliform were between 0 and 33 per 100 ml of oyster liquid and sea water. According to New South Wales Pure Food Regulations of Australia (Qadri *et al.*, 1976) and those of Virginia State of U.S.A. (APHA, 1976) a maximum permissible contamination level of faecal coliforms after depuration is MPN 230/100g. The significantly low MPN value of faecal coliform in Tuticorin oysters indicated negligible level of water pollution in the area. The low microbial load (TBC) of the oysters and the surrounding sea water (10^3–10^4 per ml oyster liquid and 10^2–10^3 per ml sea water) and the complete absence of pathogens such as *Salmonella* sp., *Vibrio cholerae* and coagulase positive staphylococci also showed that the oyster grown area was free from pollution. Coliform counts of water were reported to be at maximum under low salinity conditions (Presnel & Kelly, 1961). Low salinity conditions were not observed in the Tuticorin oyster farm.

The generic distribution of 196 organisms isolated from oysters and the surrounding sea water is shown in Table 2.

Table 2. *Generic distribution of bacteria in the cultured Crassostrea madrasensis and surrounding sea water*

	Oyster	% distribution	Sea water	% distribution
Number of isolates	98	100	99	100
Vibrio	31	32	22	23
Flavobacterium	20	21	18	18
Achromobacter	11	11	20	20
Pseudomonas	14	14	14	14
Micrococcus	8	8	6	6
Enterobacteriaceae	7	7	8	8
Aeromonas	4	4	9	9
Corynebacterium	2	2	2	2
Bacillus	1	1	0	0

Gram negative non-spore forming rods constitute 89% of the organisms in oyster fluid and 92% of the organisms in sea water. Vibrio is the dominant genus in both oyster and sea water. *Vibrio, Flavobacterium, Achromobacter* and *Pseudomonas* constitute 78% of the organisms in oyster as against 84% reported for sardine (Karthiayani & Iyer, 1967), 80% reported for mackerel (Surendran & Iyer, 1976) and 74.4% reported for Pacific oyster, *Crassostrea gigas* (Colwell & Liston, 1961). These groups formed 75% in the oyster farm water. Gram positive types represent only 11% and 8% in oyster and sea water respectively. *Micrococcus* is the dominant group among the Gram positive types. Lovelace *et al.* (1968) also reported *Vibrio, Pseudomonas, Achromobacter* and *Cytophaga/Flavobacterium* as the predominant organisms found in the eastern oyster from two natural bars in Chesapeake Bay.

Most of the organisms isolated from oyster fluid were able to ferment sugars either aerobically or anaerobically. Although the natural flora of oysters is almost similar to the flora of marine fish, the major point of difference between the two flora appears to be biochemical. That is, about 60% of the bacterial isolates of Tuticorin oysters were able to ferment glucose anaerobically by Hugh & Laifson (1953) test. This is in agreement with the observation of Colwell & Liston (1961) that about 50% of the organisms isolated by them from oysters fermented glucose anaerobically. This property has a bearing on the spoilage of oysters in that a rapid fall in pH is noticed during early stages of spoilage due to the fermentative activity of these bacteria.

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