Mass mortalities due to bacterial infections were observed while fish were reared in the laboratory. Moribund fish were used for the isolation of the causative bacteria, which was designated as EMS isolate. Being non-clinical and halophilic, together with production of protease enzymes particularly gelatinase activity and Arginine hydrolysis, proved that the EMS isolate can be characterized as *Vibrio aestuarianus*. This forms the first authentic report of *V. aestuarianus* causing ulcerative disease among estuarine fish (*Etroplus maculatus*) in India. The cell free culture filtrate of *V. aestuarianus* exhibited gelatin hydrolysis and casein hydrolysis activities. This activity was found to be temperature dependent and the heat treatment reduced the activity up to 45%.

The peak growth of *V. aestuarianus* was observed at 18 h of culture during which period the total cells were $5.6 \times 10^{11}$ cells/ml, and the viable cell was $1 \times 10^{8}$ CFU/ml. The Generation Time was estimated as 30.15 min.

The survival of experimental fish in response to *V. aestuarianus* cells and its ECP and the LD$_{50}$ value were determined. The LD$_{50}$ for cells was $1.25 \times 10^{4}$ CFU/fish whereas the LD$_{50}$ calculated using probit was $9.8 \times 10^{6}$ cells/fish. After determining the lethal cell density. Further experiments were conducted with the sub lethal ($10^5$ cells) and the lethal ($10^7$) cell densities. The changes in hematological makeup of the fish in response to administration of cells as well as ECP of *V. aestuarianus* were studied. The Hematocrit (Ht) was found to reduce drastically in $10^7$ cells, which was correlated with that of the reduction in the RBC count. Though reduction in Ht was noted in $10^5$ cells, the fluctuation was meager. The Ht of sub lethal dose of ECP (2.03 μg) administered fishes did not show much fluctuation, whereas in the lethal dose of 4.06 μg, the fluctuation was high.

The protein-banding pattern of the lethal ECP was also recorded using SDS-PAGE and silver staining. The major bands were observed with molecular masses ranging between
55 and 30 kDa. The 55-kDa proteins appeared in the 6 h ECP, which gradually increased in concentration towards 72 h. Zymogram studies indicated that the protein of 55-kDa was responsible for the gelatin hydrolysis, which started appearing from the 6th h of bacterial growth onwards.

Histological changes due to *V. aestuarianus* infection were studied using standard histological techniques. In the histological studies, the kidney had more melanomacrophage centers, intact tubules with congestion among the artificially infected fishes. Increased melanomacrophage centers were observed in the spleen. The liver was normal except that some blood cells were noted. The gill showed hyperplasia – coalescence of gill lamellae. Spongiosis and congestion of blood cells were observed among the muscle tissues. Among the infected fishes collected from their natural habitat, the kidney tissues had showed necrotic area, increased melanomacrophage centers with intact tubules and some congestion. The spleen tissue had increased number of melanomacrophage centers. Muscular dystrophy and necrotic areas were seen in the muscle tissue. The other tissues like gill and liver did not exhibit any significant changes than that of the apparently healthy fish.

The efficacy of three seaweeds, two oils, (shark liver extract and cod liver oil) and chitosan (a processed product of prawn exoskeleton), which originate from marine sources were tested against the ulcerative disease of the fish *Etroplus maculatus*. The results of the challenge experiments on fishes fed with *Ulva sp.*, *Gracilaria sp.*, *Sargassum sp.*, and Shark liver extract revealed less protection against *V. aestuarianus*. The gut microflora of fishes fed with *Ulva sp.*, (1.6 x 10⁵ CFU/cm), *Gracilaria sp.*, (6.2 x 10⁵ CFU/cm), and cod liver oil (5.75 x 10⁵ CFU/cm) were decreased dramatically when compared with the control groups (2.6 x 10⁶ CFU/cm). But in the case of *Sargassum sp.*, fed fishes, the difference was insignificant.

The efficiency of the bacterin was evaluated using PRP after challenge. In the bacterin administered fishes, no mortality was noted (within 10 days) while, 80% mortality occurred in the case of control after challenge with *V. aestuarianus*. After 30 days of injection, only 40% mortality was observed in the bacterin-injected group against 100% mortality in control.
The bacterin was evaluated alone and in combination with chitosan. The effect of chitosan and *Vibrio aestuarianus* bacterin alone and in combination in the field conditions were determined by challenge studies. In the control group, all the fish died (100% mortality) after challenges on the 10\textsuperscript{th}, 20\textsuperscript{th} and 30\textsuperscript{th} day of rearing. In the group of fishes injected with chitosan, only 20% mortality was recorded when challenged after 10 days while 80% and 100% respectively was recorded after 20 days and 30 days respectively. The fishes injected with *Vibrio aestuarianus* bacterin showed 20% mortality after 10 days, 60% mortality after 20\textsuperscript{th} and 30\textsuperscript{th} days. But in the case of fishes injected with combination of *Vibrio aestuarianus* bacterin and chitosan exhibited excellent protection against *Vibrio aestuarianus* challenge i.e., 20% mortality after 10 days and 40% mortality after 20\textsuperscript{th} and 30\textsuperscript{th} days. The estimation of total serum protein and total immunoglobulin protein indicated that the injection of marine natural products such as chitosan influence the humoral immune response of the fish. Administration of bacterin and chitosan resulted in higher production of total immunoglobulin (20.2mg/ml) than the other two, and increased antibody titre.