Development of technology in *in-vitro* pearl production in India

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The technology for production of cultured pearls from the pearl oyster *Pinctada fucata* was first developed in India in 1973 [1]. Techniques related to the technology were standardized [2]. The need for further improvement was greatly felt and hence a programme of advanced research has been taken up on pearl oyster mantle tissue culture. Earlier studies in this field had reported deposition of conchiolin and crystals in an organ culture of mantle of marine pearl oyster *Pinctada radiata* in 1949[3]; secretion of organic substance from an explant culture of mantle tissue of pearl oyster *Pinctada fucata* in 1974 [4]; and deposition of trefoil-shaped material in an explant culture of pearl oyster mantle in 1989 [5]. The advances in the present investigation are a stepping stone for further research in *in-vitro* pearl production in India. The details of the study are presented here as a first report.

Mantle tissue fragments of the pearl oyster *Pinctada fucata* were used for explant cultures. Prior to excision of mantle tissue, the pearl oysters were depurated for three days in U.V. irradiated running seawater. The depurated oysters were wiped externally with 70% ethyl alcohol. The oysters were opened carefully by severing adductor muscle and the mantle tissue was removed aseptically. The strip was processed by trimming pallial organs at the free end of mantle and connective tissue at the lower portion. The middle region of the mantle was retained and washed three times in sterile seawater (SSW) or in artificially prepared balanced salt solution (BSS). It was then treated in SSW containing 1000 µg/ml streptomycin and 2000 IU/ml penicillin. The strip was again washed two times in SSW and cut into small fragments of about 2 mm square under aseptic condition. Three fragments were inoculated as explants in each T25 flask. 3 ml of Medium 199 with nutrient salt solutions and 10% foetal calf serum was added to each flask.

Organ cultures were organized in sterile petri dishes. In each petri dish a sterile shell bead nucleus of 4 mm diameter was placed over a glass ring to avoid rolling of the bead and a mantle piece was kept over it in such a way that the outer face of mantle touched the shell bead. Medium 199 was added only up to lower portion of mantle and the upper phase was kept free from the medium. Medium exchange was done every 4 days. The cultures were maintained at 28°C and pH 7.4.

Epithelial-like spherical cells emerged from the explant in large numbers on day 1. Cells having short pseudopodia formed a cell sheet around the explant (Fig.1). The spherical cells were of two types - granulocyte and agranulocyte.

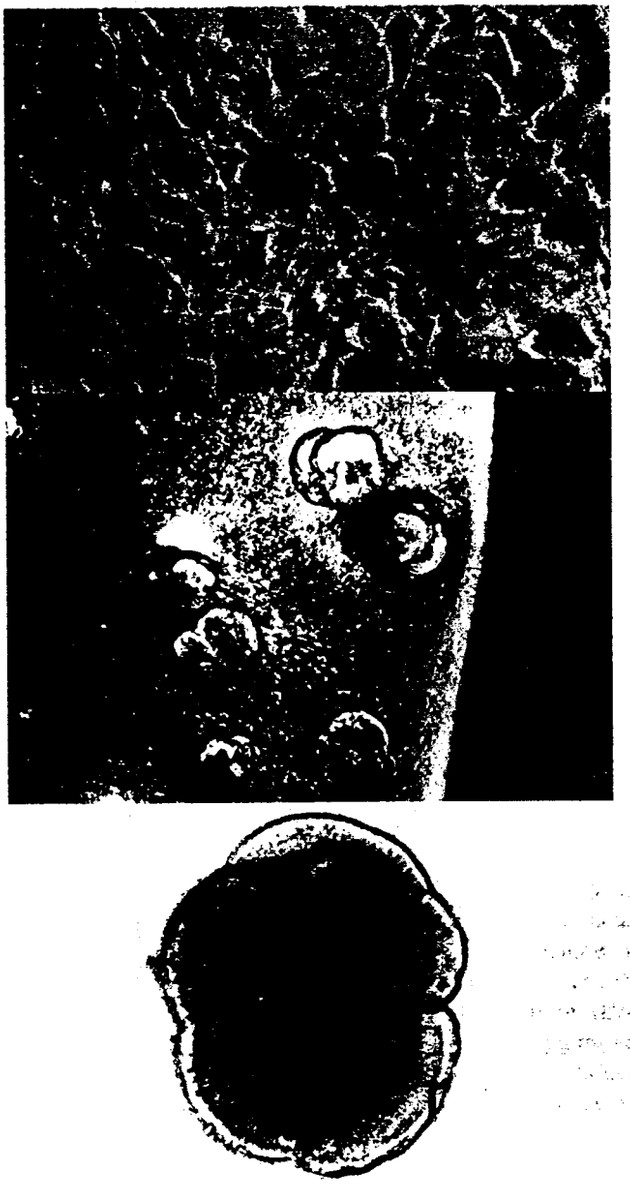

Fig. 1 (top). Formation of cell sheet with pseudopodia in an explant culture of mantle tissue of pearl oyster *Pinctada fucata*.
Fig.2 (centre). Crystals formed in an explant culture of pearl oyster mantle tissue.
Fig.3 (bottom). Secretion of alveolar material in an explant culture of pearl oyster mantle tissue.

These cells were the dominant type on day 4. Smaller epithelial-like cells and spindle shaped fibroblast-like cells appeared subsequently at the distal end of the cell sheet on day 8. The number of cells increased on day 13 and occupied the entire surface of culture flask consisting of variety of cells. Meanwhile the explant changed its colour from creamy to dark brown. Secretion of organic material occurred on the surface of the explant. When the cultures continued, deposition of crystals

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museum so preoccupied him that his stay in Cambridge was reduced to five weeks teaching each year, and, from his former pupil's collections, he selected only two *Opuntia* species from Galapagos and the Keeling (Cocos) Isles flora for study. Thanks to Anne Stow's expertise as scientific librarian at Cambridge, this biography has brought forward much previously unpublished material. The book includes genealogical tables, a chronology, brief biographies of persons mentioned, and lists of eponymous taxa, local botanical records and Henslow's works. The authors are to be congratulated on producing a book that is a thoroughly enjoyable read, and it will be a useful addition to any library's collection of Darwiniana.

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started at the periphery of the explant (Fig.2). According to Machii (1989) the organic substance is a kind of pearl formed *in-vitro*. The shape of the crystals resembled the crystals formed *in-vivo*. On day 20, an alveolar structure formed in the culture flask. It showed no birefrigency (Fig.3). Cells liberated from the mantle tissue in organ culture spread over the shell bead and started depositing crystals. Crystals varied in shape in different trials and species. Addition of crystals converts the shell bead into an *in-vitro* pearl.

The authors are grateful to Dr. (Prof) Mohan Joseph Modayll, Director, CNFFRI, Cochin and to Dr. K.K.Appukuttan, Head, Molluscan Fisheries Division, Cochin for their interest. We sincerely thank the Department of Biotechnology, New Delhi for financial help in establishing the marine invertebrate tissue culture laboratory at Tuticorin and the National Agriculture Technology Project (ICAR, New Delhi) for providing funds to continue the research work.

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