PERSPECTIVES IN MARICULTURE

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Biotechnological approach in *in-vitro* pearl production

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ABSTRACT

Explant culture of mantle tissue of the pearl oyster Pinctada fucata *(Gould) was undertaken at the tissue culture laboratory of CMFRI, Tuticorin. Mantle tissues were routinely cultured in Medium 199 and Pf 35 individually and combindly supplemented with 10% foetal calf serum at 28°C, pH 7.8. Explants released numerous epitheloid - like and fibroblast - like cells. Cells were in assorted sizes and formed colonies. Nacreous secretion by the cells was observed on culture plates. The study represents a new tool for cellular approach in in-vitro pearl production.*

Introduction

The Indian pearl oyster *Pinctada fucata* is used for the production of cultured pearls. The pearl production technology was perfected and the techniques involved were standardised. Manipulation of colour and quality of cultured pearls was rather difficult in *in-vivo* culture. Hence an attempt on *in-vitro* pearl production was made

through tissue culture technique. The work on pearl oyster mantle tissue was limited. Bavelander and Martin (1949) organised organ culture of mantle of the marine mollusc *Pinctada radiata* and obtained the deposition of conchiolin crystals typical to those found in normal regenerating shells. Machii and Wada (1989) reported the secretion of organic substance by the cells dissociated from an explant culture of pearl oyster mantle tissue. A similar approach was undertaken at the tissue culture laboratory of the Central Marine Fisheries Research Institute, Tuticorin. The results obtained in the present study are presented here.

Materials and methods

A. *Depuration of oysters:* The pearl oyster *Pinctada fucata* brought from the farm was cleaned thoroughly and placed in U.V. treated running seawater in a fibreglass tank for depuration for 3-7 days. Everyday the oysters and the tank were cleaned to remove the organic waste.

B. *Preparation of tissues :* The depurated oysters were dipped in 70% ethanol for 15 seconds and taken to clean room for further processing. They were cut open by a sterile knife and the mantle tissue was removed. The pallial organs at the free end and the connective tissue at the distal end were cut and removed. The mantle strip thus obtained was cut into pieces of 1 sq.mm.

The mantle pieces were washed six times in 10 ml of sterile seawater (SSW) or in a balanced salt solution (BSS) in petri dishes. The composition of BSS is shown in Table 1.

Table 1. Balanced salt solution for marine mollusc (MM BSS)

Components	MM BSS (g/l)
NaCl	26.22
KCl	1.08
Mg SO_4	3.18
Mg Cl_2	2.20
Ca Cl_2	1.12
Na HCo_3	0.30
Na H2- PO_4	0.044
Glucose	0.30

(After Machii and Wada, 1989)

Table 2. Composition of antibiotics in a sterile seawater

Antibiotics	Quantity
Gentamycin	125 ug/ml
Polymyxin B sulfate	100 ug/ml
Neomycin sulfate	100 ug/ml
Kamamycin sulfate	100 ug/ml
Mycostatin	200 ug/ml
Fungizone	5 ug/ml
Penicillin	200 ug/ml
Streptomycin	200 ug/ml

(After Stephens and Hatrick, 1979)

The mantle pieces were treated in 10% ethanol for 15 seconds and again washed three times in 10 ml of SSW or BSS. They were given treatment in a mixture of antibiotic solution four times each 30 mintues for 2 hours (see Table 2). After this treatment they were washed three times in 10 ml of SSW or BSS. Now the pieces are ready for inoculation.

C. *Explant culture* : The explants were inoculated in petri dishes.

2 ml Medium 199; 2 ml Pf 35; 1 ml Foetal Calf Serum (FCS) and 5 ml of SSW were combined together as medium. No antibiotic was used in the cultures. They were incubated at 28°C, pH 7.8 and the medium was changed once in two days.

Results

At this combination of culture media (Medium 199 - 2ml; Pf 35 - 2 ml ; SSW - 5 ml and FCS - 1 ml) the cells proliferated from the explant in good numbers on day 2 onwards (Fig.1). A mass of epithelial - like and fibroblast - like cells dissociated from the explant (Fig. 2). The epithelial - like cells were spherical in shape and have short pseudopodia. As large

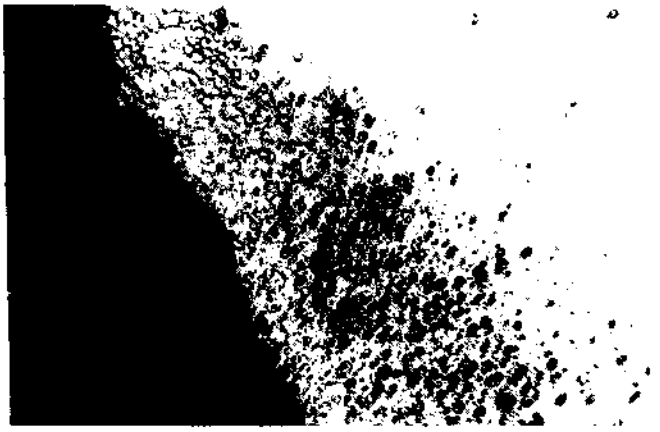

Fig. 1. Cells dissociating from the mantle explant on day 2

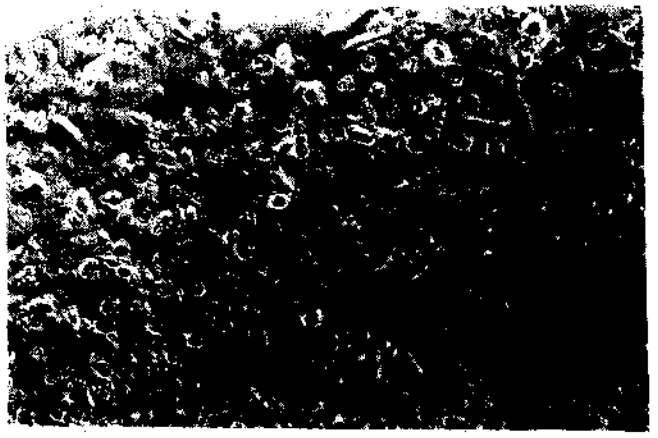

Fig. 2. Epithelial-like and fibroblast-like cells on day 8.

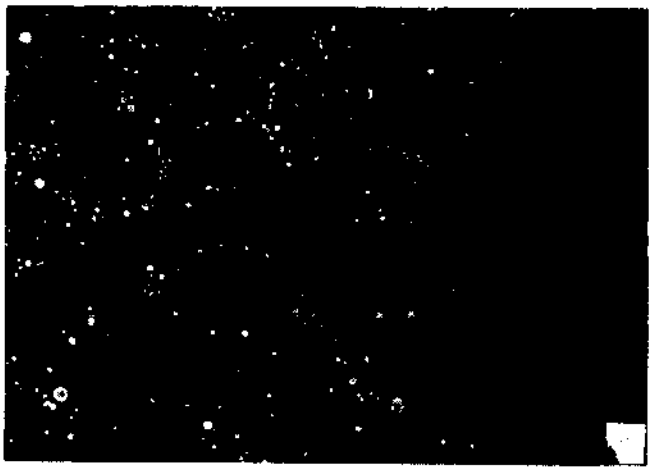

Fig. 3. Formation of colony by the mantle cells on day 15.

Fig. 4. Secretion of crystals by the cells in *in-vitro* culture.

number of cells were emerging, the cells at the distal end had moved away from the explant on day 4. On day 8 a mixture of spindle shaped, elongated, string like and spherical cells were seen. Meanwhile the explant was found to have undergone a noticeable change from a flesh

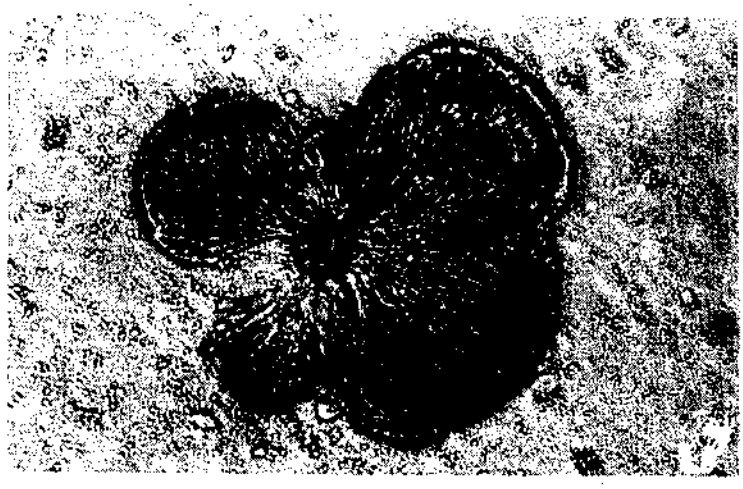

Fig. 5. Secretion of alveolar material on day 20.

colour to a dark brown colour on day 10. The number of cells had increased since day 13 and the colony formation had occurred on day 15 onwards (Fig.3). The colony of cells secreted an organic substance (Fig.4) and deposited on the culture plates. Prolonged culture of these cells resulted in trefoil - like crystals in the colony. The crystals were in different sizes. Apart from these crystals there were alveolar materials secreted by the colony of cells on day 20 (Fig.5). The alveolar materials showed no birefrigency. They were subjected to acid testing and found to be calcareous matter. On day 14-15 the explant became fragile as large number of cells had dissociated from it.

Discussion

Although research on *in-vitro* culture of marine invertebrates was undertaken, establishment of cell lines from these animals was rather limited. However some studies were carried out on the culture of mantle tissue from pearl oysters. Bevelander and Martin (1949) could demonstrate the deposition of conchiolin and formation of crystals through an organ culture of mantle from the marine mollusc *Pinctada radiata.*

The nature of conchiolin and crystals was typical to those found in normal and regenerating shells. Subsequently Machii (1974) reported that the secretion of organic substance through an organ culture of mantle from the pearl oyster *Pinctada fucata* was nothing but calcium crystals. Later the secretion of calcium crystals was also reported from the cell line culture of pearl oyster larvae (Machii, 1985) and from an explant culture of pearl oyster mantle tissue of *P.fucata* (Machii and Wada, 1989). A similar result was obtained in the present study on the explant culture of mantle tissue of *Pinctada fucata*. In our study large number of cells had dissociated from the explant and a cell sheet formed on day 4. It was identical to the study by Machii and Wada, 1989 where the formation of cell sheet took place between day 3 and 7. On day 12 the explant underwent considerable change in colour and since then the secretion of organic substance occurred. It was quite similar to our study where the change of colour and the secretion of organic substance occurred on day 10. The deposition of alveolar material was obtained on day 30 in both the studies. The high content of calcium in the crystal as reported by Machii and Wada (1989), indicated that it must be a prism, a kind of pearl formed *in vitro*.

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