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TECHNIQUES FOR QUALITY PEARL PRODUCTION

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Introduction

The success of pearl production adopting culture techniques depend on the quality of pearls rather than the quantity produced. It is quiet evident that the cost of production would go up in future and hence pearl quality alone can meet their challenges to make profits in this industry. There is long way to go to meet the challenges through application of advanced biotechnological tools for quality pearl production. The techniques related to pearl production being a biological process, priority for production of quality pearls can be achieved mainly by improvement in the quality of mother oyster stock and also by manipulation of environmental characters and through tissue culture.

Quality improvement of mother oyster stock

At global level the pearl culture mainly depend on the wild stock for pearl production and when the pearl oyster population in the natural beds shows a sign of depletion, the techniques of spat collection from the wild have been adopted. During this period the genetic qualities of wild stocks are not ascertained. It is feared that the limited stock may be degenerating in terms of pearl quality. Hence there is an urgency to identify the genetic characteristics of wild stock and to build up the selected stock capable of producing desirable quality of pearls, resistant to diseases, adaptability to environmental stress, high growth potential. In Japan such studies were already taken up in *P. fucata.* Through such studies Wada (1975, 1984, and 1986) demonstrated that pearls without yellow pigments could be produced. Hatchery technology has been developed in several countries for the commercially important species *Pinctada maxima*, *P.margaritifera* and *P.fucata*. A selective breeding programme can easily be carried out to develop a particular strait of character to be inherited. In India selective breeding programme has already been initiated in *P. fucata* to improve the colour and quality of pearls and also to increase the shell cavity to accommodate large sized nucleus. These characters have been found to inherit in *P. fucata.*

In India the consecutive use of the succeeding generations as parent stock resulted in the poor growth of the successive generations hence it was suggested to out breed those stocks. The inbreeding of these stocks resulted in the production of higher percentage of yellow nacre, and more thickness of nacre was also observed. Hence from the natural stocks a breeding stock is selected based on the desired characters and the next generations produced by assortative mating of the selected group. Genetic profiling of proteins from the selected oysters of wild stock as well as the newly produced generations reveals the heritability of the characters. By repeating the selection and genetic marking of proteins of the heritable characters we can produce a better stock of oysters, which produce better quality pearls

Pearl culture environment

The quality of pearl is influenced by the environmental factors. Basic studies on the pearl culture grounds have been made in Japan on this aspect. The outcome of the study facilitated the establishment of farms at suitable sites and shifting of culture grounds for improvement of quality of pearls. Availability and quality of phytoplankton, which form the food item of pearl oysters, influence pearl quality (Matsui, 1960) via its role of energy supply. The type of phytoplankton available at different seasons at different sites has been studied thoroughly. Studies revealed that phytoplankton's influence the colour of pearls and areas were identified and the pearl bearing oysters were shifted to the site before pearl harvest in order to get a final coating of desirable colour. This is otherwise called 'make-up' culture. Similarly the amount of trace elements influences the colour of pearls. The level of these trace elements at different pearl culture grounds has been studied thoroughly in Japan. The locality having optimal level of trace elements was chosen for rearing nucleated oysters. At Tuticorin, preliminary trials indicated that it is possible to manipulate the colour of the nacre and thence the pearl in captivity. The medium lethal concentrations (LC 50) of Copper, Cobalt and Ferric were administered through feed and initial results show the differentiation in colour of new nacre secreted with bluish nacres by Cobalt below LC50 concentrations.

Influence of depth on quality pearl production

Apart from the environmental factors such as temperature, salinity, pH, turbidity, detritus, plankton, trace elements, nutrient salts, the physical factors such as the depth, light intensity, topography of area, wind velocity, tidal effect and wave action play a composite role in pearl quality. Among the characters; depth plays prominent role in pearl quality and the temperature varies with the depth. Culture practices of oysters are decided on the optimal level of temperature. For example, in summer the surface temperature in the Ago Bay, Japan was around 28 to 30°C and hence the nets were lowered to a depth of about 7 m where the temperature was around 23 to 26 ° C. During spring and autumn the surface water temperature was around 23 to 25° C and therefore the nets are kept at 2 to 3 m depth (Alagarswami, 1970).

Dharmaraj (2002) studied the influence of depth on pearl quality. The results indicated that the pearl growth was 12 % more at 1 m depth than in 3 m depth. In view of this, the cages containing nucleated oysters in Indonesia are kept at the seabed having a depth of 10-20 m in order to get good quality pearls (Tun and Winanto, 1988). When the cages are suspended at 5-8 m depth during dry season they are subjected to strong sunlight, which induced nacre-secreting cells to produce calcite crystals resulting poor quality pearls. The lustre and colour are better at higher depths although the rate of nacre deposition is slow.

The pearls from 1 m depth were of ivory in colour having poor lustre whereas the pearls from 5 m depth were of light yellow in colour with good lustre. Knowing the influence

of depth on pearl quality, the pearl farms in Australia are located at 33 m depth and in Japan at 10 m depth (Alagarswami, 1970).

Improvement in surgical techniques

The pearl quality could be improved by treating the graft tissue with appropriate stains, which are capable of activating the nacre secreting cells and by taking utmost care in the surgery procedures. The application of stains like azumin and eosin was to improve the quality of pearl, percentage pearl production and survival of operated oysters. When the implantation is made with graft tissue treated with azumin, the oysters showed high survival when compared to eosin or filtered seawater treated ones. The treatment with stains (azumin and eosin) improved the pearl production in *Pinctada fucata*.

Several other surgical techniques were also employed on he improvement of pearl quality, retention of nucleus, prevention of contamination, enhancement of pearl production, reduction of oyster mortality, improvement in wound healing process. One of such techniques in surgical improvement was the use of tetracycline hydrochloride (2 % TC-HCL), 0.4 % succinated antherocollagen and polyethylene glycol 6000 coating on the nucleus. The survival rate of oysters treated with this antibiotic was higher (86.7 %) than that of the control group (63.3 %) treated with uncoated nuclei. The nuclei retention rate was also high in the treated group.

Norton *et al* (1976) and Aquilina (1999) carried out a research programme aiming at improving the production of gem quality pearls in *P.margaritifera*. Treatments incorporating modern surgical techniques were applied to seeding operations. Use of prophylene phenoxetol for narcotization of oysters and the use of cyanocrylate adhesives to fuse incision site yielded poor results. But efforts are being undertaken using modern techniques.

The Pearl Developing Group (PDG) carried out improvement of quality of pearls with PDG alpha antibiotic coating on nucleus. PDG is able to offer three distinct advantages to pearl farmer for better price, better quality and better science.

Biotechnological aspects are undertaken in Japan in which the nuclei are coated with antibiotic powder. It is inevitable that insertion of nucleus after causes infection in oysters. It resulted either in the rejection of nucleus or the death of oysters. Therefore the nucleus coated with antibiotic powder was to improve the retention rate of nucleus from 70 to 80 % as against 50 to 60 % with uncoated nuclei.

Studies on bio – mineralization of nacre

Elaborate work on mineralisation and spectral characteristics of pearls was undertaken (Wada, 1972, 1983). The outer epithelium of the mantle of bivalve molluscs secretes extrapallial fluid that crystallizes in to aragonite crystals. The fluid contains carbonate and other inorganic ions (Wada, 1972). The organic matrix and the growth of nacreous layer are formed alternatively. The nature of crystals either aragonite or calcite and their size are related to the secretary activity of the outer mantle epithelial cells. The

activity differs in species, in environment and in physiological condition of animal (Wada, 1972). A thorough knowledge on these aspects would help in controlling the quality of pearl. These studies may have to be taken up in future for improved quality pearl production.

Tissue culture techniques

Culture of mantle tissue of pearl producing molluscs has been undertaken in recent years. Machii (1974) cultured mantle tissue of *P. fucata* and reported the types of cells proliferated from the tissue and the secretion of organic substance in *in-vitro*. The latest breakthrough obtained in the culture of mantle tissue of *P. fucata* and the abalone *Haliotis varia* is a milestone in tissue culture research. It created the possibilities of not only the production of pearls in large numbers but also different coloured pearls.

In the context of the dwindling quality pearl production initiated mantle tissue culture of the Indian pearl oyster, *Pinctada fucata* and the progastropod abalone *Haliotes varia* was organized in the laboratory. In the explant tissue culture, the cells proliferated and migrated away from the explant and multiplied *in-vitro*. This resulted in the formation of a cell sheet. The round cells develop pseudopodia that later covered the entire surface of culture plates and formed an organic matrix.

In an organ culture, the mantle tissue of a pearl oyster/ a kept in nutrient rich medium resulted in the formation of nacreous layer with organic matrix and a pearl sac within 3 months after organization of cultures. The basic technology developed through tissue culture method can totally eliminates the dependence on natural environment for pearl production. It provides scope for manipulation of the technique to produce pearls of the desired quality.

By organizing explant cultures of pearl producing molluscs, the epithelial cells capable of producing aragonite crystals may be collected and stored in cell bank. The cells can be used at any time for the production quality pearls in *in-vitro*. The cells in suspension would form the pearl sac that would secrete nacre to form a pearl. Isolation and the type of epithelial cells that would secrete the aragonite crystals, which form the top quality pearls, can be done.