AQUACULTURE PRODUCTIVITY

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Concepts in Marine Biotechnology and Their Applications for Enhancing Aquaculture Productivity

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Application of biotechnology techniques to agriculture increased the quality and quantity of the final yield. Genetic engineering applied to the production of fish, molluscs and crustaceans although at the rudimentary stage offers promise. Besides, other techniques like tissue culture, chromosomal engineering, cryopreservation of embryos, and production of transgenic organisms also offer immense scope to expand and improve aquaculture operations. Though aspects of marine pollution, microbiology and pharmocology are not directly aquaculture operations, they have also been dealt with in the present paper since many aquaculture operations are inter-linked with such subjects.

Applications in Aquaculture

Applications of biotechnology for enhancing aquaculture productivity needs work along two lines. One is on basic aspects of physiology and genetics of the species of interest. The studies must be oriented towards elucidation of the mechanisms of control of traits like osmoregulation at the biochemical, physiological and genetic level so that it might be possible to manipulate them using biotechnology techniques. The other line of approach has to be on the selection and modification of biotechnology techniques for manipulation of the selected trait.

The traditional genetic technique of selective breeding, inbreeding,

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interstrain crossing, interspecies hybridisation can be selectively combined with more recent techniques like polyploidisation, gynogenesis and monosex culture to increase aquaculture productivity. There is also scope for novel improvements through exploitation of the high fecundity and the flexibility inherent in external fertilisation of aquatic organisms.

The application of biotechnology can lead towards enhancing the growth rate, production of specific bioactive compounds or towards more exotic objectives of producing genetically altered strains of prawn and molluscs cultivable in very low saline or fresh water, reduction in the non-edible portion and producing a strain which can circumvent the marine environment for their breeding cycle.

Some of the biotechnology techniques that can be used in mariculture have been dealt with covering the basic concepts, the current state of the technique, advancements made, potential benefits and the scope of applying these techniques to mariculture in India.

Tissue Culture

Plant tissue culture has been employed for commercial production of ornamental plants and for crop improvement. The widely used approach has been the attempt to manipulate the nitrogen fixation gene from bacteria into plants. In mariculture, tissue culture has a potential application in sea weeds and pearl oyster.

The most widely used approach in sea weeds has been that of screening wild plants for desirable traits such as fast growth (Cheney *et al.*, 1981) or intra, interspecific and intergeneric hybridisation (Sanbonsuga and Neushal, 1978). But these conventional genetic techniques are limited in the extent of genetic improvement that can be attained in a short period of time. Only biotechnology offers scope for producing genetically modified fast growing strains and producing high quality products. This can be achieved by employing such techniques like the protoplast fusion and somatic hybridisation. A major advantage of this technique is that genetic information can be transferred from one species to another without involving sexual reproduction. This technique involves isolation, culture of protoplasts and regenerating into a whole plant. The hybrid cells formed by fusion are further cultured. The selected hybrid cells are regenerated into a whole plant. Cheney (1984) who carried out such studies in mutant *Gracilaria tikvahiae* feels that protoplast is a crucial new tool for genetic improvement of sea weeds. In India, suitable techniques for the culture of *Gracilaria edulis* and *Gelidiella acerosa* have been developed by CMFRI and CSMCRI. Though good production rates have been achieved, the techniques are not economically viable due to high cost of production. If faster growth rate can be induced by techniques like tissue culture, the yield will be economical.

In the pearl oyster, *Pinctada fucata*, a steady supply of good quality mantle tissue is needed for nucleus implantation. The induction of pearl formation and quality of pearls is also dependent on the mantle implant. If

the tissue could be cultured this difficulty could be overcome. Also with more advancement made in tissue culture, it might be possible to grow pearls *in vitro*.

Chromosomal Engineering

The genetic engineering by manipulation of the chromosome set of an organism can be split into techniques on gynogenesis and polyploidy (Purdom, 1983). Gynogenomes are animals derived entirely from maternal chromosomes. In gynogenesis the irradiated spermatozoan induces development, but then degenerates without making a genetic contribution. Gynogenesis provides a method for the rapid production of inbred population for use in cross-breeding programmes. Gynogenesis could also be used to produce all female populations in species in which the female is the homogametic sex. Triploids are animals having three sets of chromosomes which confer sterility in the animal and in few species increased body size. In many fishes like rainbow trout, the secondary sexual characteristics associated with maturity decreases disease resistance and causes deterioration in appearance and flesh quality. This has been avoided by producing all female sterile triploids of rainbow trout. This technique is finding wide commercial application in the UK (Lincoln and Bye, 1984). An increase of 73 per cent in muscle weight in triploid scallops over that of diploid has been demonstrated (Tabarinni, 1984). In India, prawns are a prime candidate species for induction of polyploidy. But the large number of chromosomes in prawns will be an obstacle for the confirmation of polyploidy induction. Triploidy can be attempted in cultivable molluscs since this technique has been extensively applied and also triploid molluscs exhibit increased body size. The potential benefits in *Crassostrea madrasensis* could be a higher growth rate while in *Pinctata fucata* it could be bigger pearls.

Cryopreservation of Embryos

The various aspects of cryopreservation of fish sperm has been dealt with in detail by Scott and Baynes (1980). While there is extensive literature available on cryopreservation of sperm, no published information is available for fish embryos. If such techniques can be evolved they will greatly enhance the scope of production and transport of prawn seed in our country.

Mutagenesis

While most of the mutagenetic work in fishes is of basic interest, such an approach has potential benefits with regard to sea weeds and culture of algae to produce single cell protein. In China, there is commercial production of fast growing sea weed strains developed by induced mutation through X-ray and chemical agents (Tseng, 1981).

PRODUCTION OF TRANSGENIC ORGANISM

The utilisation of components of genetic machinery of an organism by integrating it with a bacteria for production of specific substances has been generally termed as genetic engineering. The production of human insulin, growth hormone and leucocyte interferon shows the success of genetic engineering techniques. In recent years, this technique has been extended to the production of transgenic animals by transferring foreign genes into fertilised eggs. This transfer has been carried out by direct micro-injection into nuclei or via retroviral vectors. Both these techniques have been demonstrated in a mouse and the germ line transmission of the injected gene has been established in a mouse, *Xenopus* and fruit fly. The production of transgenic culture organisms offers scope for developing genetically altered organisms which can overcome natural barriers that have limited their extensive and mass production. For example, it might even become possible to successfully culture prawns requiring saline water in very low saline or fresh waters.

The experimental introduction of novel genes into fish has been carried out by many workers (Zhu *et al.*, 1985; Ozato *et al.*, 1986; Chourrout *et al.*, 1986; Brem *et al.*, 1988). In the fertilised eggs of goldfish, part of the micro-injected gene consisting of mouse MT-1 promoter and human growth hormone minigene was found to be integrated (Zhu *et al.*, 1985). Ozato *et al.* (1986) produced a transgenic fish (*Oryzias latipes*) by micro-injecting recombinant plasmids containing chicken σ-crystalline gene into germinal vesicles of oocytes. The micro-injection of the human growth hormone gene into the germinal disc and development of 90-day live transgenic tilapia have been reported by Brem *et al.* (1988). The technique of inducing novel genes needs modifications before it can be applied to aquaculture. It needs refining the microinjection technique to a single cell stage of embryo to produce a truly transgenic organism rather than the genetic mosaics most likely now. The use of genes derived from cultured organism itself is an important modification and is dependent on the use of gene libraries constructed for the species of interest.

USE OF RECOMBINANT HORMONES

Incorporation of hormones in feed enhances growth and these have been reviewed by Donaldson *et al.* (1979). With the use of recombinant bovine somatotropin (Gill *et al.*, 1985) it has been found that, in the mariculture of Coho Salmon, a more than double the growth rate has been achieved even when environmental conditions are sub-optimal for growth (Down *et al.*, 1988). These findings demonstrate the potential for using recombinant vertebrate somatotropins in the culture of fish.

Application in aquaculture related fields

MARINE MICROBIOLOGY

Genetic engineering has found its widest application in terrestrial

microbes. Extension of these techniques to marine microbes should be relatively easy than in other organisms. Photosynthetic bacteria (PSB) found in mangroves and estuaries can be used for biotreatment of waste water due to their ability to remove carcinogenic agents and produce antiviral substance. Genetic engineering techniques can be used to develop strains of PSB to degrade compounds, that are not easily broken down. The harvested PSB can be incorporated in fish feed since even at low percentages it enhance fish yield. Also genetic engineering can be used to enhance the effectiveness of sulphur removing bacteria or for the enhanced production of antiviral or antibiotics from marine microbes.

Marine Biofouling

Marine biofouling is highly destructive to vessels and under water and floating structures used for mariculture. The ability of bacteria to find, attach, adhere and elaborate specific primary films are the crucial stages in biofouling. If these factors involved are understood, it is possible to manipulate them by employing biotechnology techniques. Two approaches are being tested to elucidate the molecular basis of fouling (Simon *et al.*, 1984). One is to identify the genes involved in each of this process using recombinant DNA technology. The other is the use of transposon mutagenesis. When a transposon mutant deficient in the expression of adhesion gene is discovered it could be easy for further elucidation of the factors involved in microbial adhesion and then it might be possible to manipulate these factors at a genetic and biochemical level (Simon *et al.*, 1984). This has also implication in aquaculture by enhancing spat settlement of cultivable molluscs.

Pollution

Pollution is one of the major dangers facing the expanding aquaculture industry. Pollution control is a major area where biotechnology can be used. The concept is that most of the organisms do not have the capacity to degrade the toxic elements. But few bacteria have evolved enzyme systems for degrading a specific group of compounds. By genetic engineering it is possible to equip bacteria with such degrading enzymes. By plasmid manipulation, a *Pseudomonas* strain has been genetically altered to have the metabolic capacity to degrade crude oil (Friello *et al.*, 1976). A potential useful technique in this regard is the plasmid assisted molecular breeding (Kellog *et al.*, 1981). This technique involves continuous combined culture of strains containing molecular breeding plasmids with proven degrading genes and wild type having potentially degrading bacterial gene. For combating heavy metal pollution, Vournakis (1984) has suggested cloning of metallothionein gene into species of marine plants and using them as pollution control devices.

Marine Pharmocology

A dramatic example of biotechnology application is that of marine

pharmaceuticals. Extracts from a tunicate belonging to the family *Didemnidae*, inhibit growth of DNA and RNA virus as well as leukemic cells (Rinehart *et al.*, 1981). Marine toxins besides being pharmocological chemicals, also serve as models for development of new synthetic chemicals. The strategy to be undertaken in marine pharmacology would be initial screening of marine organism for bioactive agents. The most useful bioactive compound can be tested and characterised. Then a two pronged strategy could be undertaken. One for evolving techniques for mass culture of the organism and the other for using recombinant DNA technique to identify and clone genes responsible for synthesising the bioactive compound for increasing its production.

Conclusion

Biotechnology technique can be applied for the creation of new and improved strains of culturable species. These would grow faster and larger, cantain more edible fraction, can grow within an expanded range of salinity, are disease resistant, are amenable for mass culture and possess many other improvements. But all these can materialise only if more basic information is available on the physiology, biochemistry and genetics of cultured species and on refining the existing biotechnology techniques.

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