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Prospects of Genetic Transformation Techniques in Culture of Marine Molluscs in India

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ABSTRACT

Overfishing is posing a major problem that has dangerously reduced fish and shellfish stocks. People are, therefore, looking at aquaculture to not only increase the fishery production but to improve the declining stocks. Among all the cultivable species of fish and shellfishes, molluscs such as oysters, mussels and clams form a sizable group that can be cultured successfully along the coast of India. Though the molluscs sustain regular and very productive fisheries in our waters, only a few of the mussels, clams and oysters are now generally eaten. But molluscan diversity has shown a declining trend due to the commercial exploitation, pollution and environment hazards that cause death of molluscs and to a lesser magnitude, the professional shell collection from wild. There is an urgent need to conserve the depleting stocks and to improve the quality of these bivalves. It is necessary, therefore, to develop efficient culture methods to increase the production of clams, oysters and mussels to meet high worldwide demand for these organisms. Genetic manipulation in culture promotes faster growth and maturation, increased disease resistance and triploidy. Some of the techniques are discussed in the current paper.

INTRODUCTION

All over the world, overfishing is posing a major problem that has dangerously reduced the fish and shellfish stocks. Some of the fish and shellfish stocks are so low that many of them are declared as endangered. People are, therefore, looking at aquaculture to not only increase the fishery production but to improve the declining stocks. Today, high-density aquaculture of many freshwater as well as brackish water fishes is practised in many countries. Traditional aquaculture encompasses low input technologies to propagate, harvest and market fish, algae, crustaceans and molluscs. Among all these candidate species of fish and shellfishes, molluscs (bivalves) such as oysters, mussels and clams form a sizable group that can be cultured successfully along the coast of India. Tools of biotechnology are being used today to improve and increase the yield and quality of various bivalves.

The molluscs are soft-bodied, heterogeneous group of animals with great diversity. Out of the 80,000 to 100,000 species of molluscs recorded from various parts of the world, from India a total of 3271 numbers of molluscs are known to occur belonging to 220 families and 591 genera, of which 1900 are gastropods, 1100 are bivalves, 210 are cephalopods, 41 are polyplacophores and 20 are scaphopods. Oysters, mussels, clams, pearl oysters and chank are the important molluscs, which are exploited as sustenance fishery. Many gastropods and bivalves are also used for edible purpose, source of lime, as decorative shells or for industrial purpose. Though, the molluscs sustain regular and very productive fisheries in our waters, only a few mussels, clams and oysters are now generally eaten. Many of the bivalves have a good market demand in local as well as in foreign markets, but they are still considered as poor man's food. The molluscan diversity has shown a declining trend due to the commercial exploitation, pollution and environment hazards that cause death of molluscs

and to a lesser magnitude, the professional shell collection from wild. Indiscriminate fishing from natural bed may lead to depletion of stock of most of the molluscan resources. This has been particularly noticed during the study of Kalbadevi estuary of Ratnagiri (Mohite 2007). The disturbance, caused by dredging and handpicking by the local fishermen, has affected the growth and survival of bivalves such as *Paphia malabarica* and *Meretrix casta* in this estuary. Similar situation exits in Ashtamudi estuary. The last 15 years of commercial exploitation of undersized clams of *P. malabarica* led to depletion of stock in the recent years (Appukuttan 1996). In the same way the other bivalves (Pearl oysters) and gastropods (Chank, Turbo, Trochus, Babylonia spp.) are also being extensively and indiscriminately fished in different parts of our country and also in Andaman and Nicobar Islands. Due to this, the molluscan resources are facing a lot of constraints such as genetic erosion and resource management. New and multidisciplinary approach, especially through biotechnology, will greatly help in solving these problems and bring about the much needed sustainable 'blue revolution' in the country.

The high worldwide demand for clams, oysters, mussels and other shellfishes requires an efficient culture method to increase their production. Genetic manipulation in culture promotes faster growth and maturation, increased disease resistance, and triploidy (Barnum 1998). Normal diploid oysters loose their flavours during their reproductive phase due to formation of massive amount of reproductive tissue. Triploid Pacific oysters were obtained in culture by treating the eggs with cytochalasin B, an inhibitor of normal cell division. The eggs with double number of chromosomes were then fertilized with the normal sperm, forming a zygote with three sets of chromosomes. These sterile oysters were found to retain their flavours, as they do not form the reproductive organs. These oysters also grew larger and faster than the diploid oysters and could be harvested earlier. Triploid oysters (Pacific northwest oyster hatchery) thus cultured, form almost 50% of the total oyster production in United States. To avoid the use of cytochalasin B, trials are going on to produce triploid oysters by mating tetraploid oysters with normal diploid ones.

Successful genetic transformation requires reproducible and reliable methods for the transfection, i.e., the physical transfer of genetic material, the expression, i.e., the functionality of the transfected sequences, and the integration, i.e., the possibility to guide and control the insertion of the transgene in germinal cells to obtain stable expression and to transmit the new trait to the next generation or to create a bivalve cell line (Cadoret 1997).

GENETIC TRANSFORMATION TECHNIQUES

Sustainable aquaculture depends on the continuous breeding of cultured stocks of bivalve molluscs. Application of genetics and biotechnological tools to aquaculture in the fields can be used for the development and maturation cycle, gamete production and storage, sex and ploidy manipulation, transgenesis, selective breeding and marker-assisted selection. The initial interest in stock improvement that triggered the genetic studies in fishes has been shifted to chromosome manipulation in commercially important bivalves. Four major techniques of genetic transformation have been applied in the bivalves.

1. Electroporation: Variations in the electric field have been used to create polyploidy oyster embryos (Cadoret 1992). The equipment used for this technique is relatively small scale, simple and inexpensive. In similar experiment, first transgenic bivalve, *Mulinia lateralis* was produced (Lu 1996), but this technique is unreliable and standardization of the results is also difficult.

Instantaneous perturbation of cell membranes by sudden changes of electrical fields has widely been used to introduce foreign genes into animal cells. Exposing eukaryotic cells to a brief but high voltage electric field can cause local areas of reversible membrane breakdown, allowing exchange of molecules through the transient pores in the membrane. Cells can be transformed to different phenotypes by exposing them to a high voltage DC electrical impulse in the presence of transgenes (Neumann & Forster 1982). This method has been exploited successfully for the introduction of cloned DNA molecules into different kinds of cells for permanent transformation or for transient expression of gene products.

Although electroporation has been used to produce transgenic animals successfully, the biophysical mechanisms by which DNA uptake is induced and the structural characteristic of the pores are not well known. In general, electroporation instrumentation consists of three main components: the pulse generator for creating and delivering the electric field, the electroporation chamber in which cells are suspended between two electrodes, and an oscilloscope for monitoring the wave form and amplitude of the generated pulse. The pulse generator produces the electrical field and allows controlled discharge through the electroporation chamber. The pulses produced by electroporation generators are most commonly exponentially decaying or square waves. The voltage applied to the electroporation chamber is the most critical parameter for successful gene transfer. The electric field generated across two fixed flat plate electrodes is governed by Ohm's law, V = IR. Thus, the resistance (or conductivity) of the medium in which cells are suspended has major effects on the current generated.

2. Lipofection: Due to the difficulty in production of molluscan cell lines, primary cultures from oyster heart cells have been used in the experiments of lipofection mediated DNA transfer techniques. Several parameters such as culture medium, the culture temperature, the cell culture time before transfection and the DNA/liposome ratio (Boulo et al. 1996, Boulo 1997) were considered. The medium containing seawater, which is rich in polyvalent anions such as phosphates, was seen to strongly influence the transfection efficiency.

Lipofection was shown to be 50 to 100 times more efficient than the calcium phosphate method in some vertebrates and insect cell. Transfection experiments using calcium phosphate using bivalve cells have shown poor results (Ellis 1991).

3. Microinjection: Microinjection of cloned DNA fragments into pronuclei of fertilized mammalian eggs has been demonstrated to be the most successful method for producing transgenic animals. This technique of transfection can be used in mussel or oyster embryos. But the larval development is fast in this case and the zygote becomes d-shaped veliger within a day. Transfection should be done when it is still unicellular to avoid mosaicism. The "window" for effective transfection is available only for an hour. During this available time, about 100 eggs can be microinjected by a skilled manipulator. The smaller size (about 80 μm) of these eggs also proves to be a problem as the equipment suitable to microinject eggs is heavier. Thirdly, the dense yolk prevents the direct entry of DNA into the nucleus, as it is possible in other cases. Considering these aspects, a technique for microinjecting eggs and embryos of the oyster *Crassostrea gigas* and the mussel *Mytilus edulis* was developed (Cadoret 1997). In these experimental trials, approximately 40% of microinjected embryos survived. This technique was used to microinject beta-galactosidase, for which specific detection techniques were developed. A reporter construct (CMV-beta) based on a promoter of cytomegalovirus linked to the beta-galactosidase-encoding gene was then microinjected, and the expression level of this construct was monitored. The technique can be suitable in terms of its application to the

manipulation of bivalve molluscs in pathology and genetics. Cytoplasmic injection can also be tried as it was shown in case of sea urchin (Hough-Evans 1988).

4. Particle bombardment: This is a technique of bombarding living material with DNA coated with gold or tungsten particles (Sanford et al. 1987). The particles or the microcarrier accelerate due to the rupture of the Kapton disk at a fixed pressure. This technique is much suitable for mass transfection. This can be made use of in case of the bivalves as a large number of eggs are produced by them. Secondly, this method helps in delivering the DNA right into the nuclei. Out of the two methods i.e., dry biolistic and aqueous biolistic (Mialhe & Miller 1994), the latter was successfully used (Cadoret 1997) to transfect oyster and mussel eggs. Transient expressions of various heterologous and homologous expression vectors were obtained. In the same experiment, suitability of tungsten as microcarrier in particle bombardment on the whole excised tissue of oyster was tested. The results were similar to that of particle bombardment with gold on embryos. This proves the suitability of this technique for transfecting three different stages of oyster.

CONCLUSION

Over the past decade, a revolutionary technique was developed that allows the introduction of a defined fragment of cloned DNA into germ lines of an animal. Once foreign DNA is integrated into a host genome, the DNA now called transgene, can be stably transmitted into progeny from generation to generation. Since the first transgenic animals (mice) were produced in 1980, the scientific community has become enthused with the idea that this technology will bring us several steps closer to understanding the actions of genes in intact organisms. Of fundamental importance has been the observation that transgenes are often expressed, that is, they are functional, and that this expression is subject to correct tissue-specific, developmental, and physiological regulation. It is, therefore, possible to analyse the role and regulation of specific cloned genes within the whole organisms. Of all the technical achievements that have advanced the biological sciences, few have opened up such possibilities as transgenic methodologies. The ability to change selectively the genetic makeup of a multicellular organism and thereby permanently alter the activity of particular proteins has important bearing on all areas of biological investigation.

Commercial application of bivalve genetics is a process of development and transfer of genetically improved stocks to industry through a team effort (Wada 1999). The goal of genetics in bivalve aquaculture is bringing about the sustainable breeding of particular species for better productivity in a sound sustainable aquaculture environment (Wada 1999). The prospects of farming transgenic bivalves though appear distant and with many blockages, but a priority can be given to the research areas of production of disease resistant and fast growing bivalves of commercial importance. Transgenesis indeed appears to be a very appealing strategy to achieve the goal of sustainable aquaculture of bivalve molluscs. But the controversial issues and the ethical aspects in these regards need to be evaluated. From the ecological point of view, the application of such a strategy will depend on the availability of the sterile transgenic bivalves to prevent the spread of genetically modified organisms (GMOs) in the natural environment. Hence, programmes to understand sex determination in bivalves should be undertaken. On a holistic approach the production of sterile transgenic bivalves could help in protecting the ecological equilibrium by limiting the overcrowding in natural rearing beds by overstocking. The techniques of gene manipulation is being applied in the commercial cultures of common carp, catfish, coho salmon, tilapia, etc. but the application of this technique in the bivalve culture in India needs a proper research approach and understanding.

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