AQUACULTURE ADVANCEMENT IN BIOTECHNOLOGY: REVIEW

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Abstract

Population blast, anthropogenic activities and climate change have caused first class protein like fish to be limited to consumers; the cost of fish flesh proteins are on the increase due to environmental pollution and overfishing has made capture fisheries a nightmare; this gave birth to fish farming/aquaculture and aquaculture advancement in biotechnology. This article reviews aquaculture advancement in genetic engineering/hybridization improvement and biotechnology. Fish farmers should be exposed to primary genetics/hybridization/biotechnology principles; practical skills of hybridization/biotechnology be demonstrated to fisheries and aquaculture undergraduates and farmers. Biotechnology engineering is the way forward for fish protein multiplication, disease resistant fish breeds and high fecundity which farmers need to embrace. A lot of persons have talked down on genetics/biotechnology engineering in fish production but it is a sure way for food security in Africa and the globe at large not forgetting the building of genetics: natural selection in animal husbandry.

Keywords: *Climate Change, Anthropogenic Activities, Aquaculture, Environmental Pollution, Fish Protein.*

1.1 INTRODUCTION

Biotechnology provides powerful tools for the sustainable development of aquaculture, fisheries, as well as the food industry. Increased public demand for seafood and decreasing natural marine habitats have encouraged scientists to study ways that biotechnology can increase the production of marine food products, and making aquaculture as a growing field of animal research. Biotechnology allows scientists to identify and combine traits in fish and shellfish to increase productivity and improve quality. Scientists are investigating genes that will increase production of natural fish growth factors as well as the natural defense compounds marine organisms use to fight microbial infections. Modern biotechnology is already making important contributions and poses significant challenges to aquaculture and fisheries development. It perceives that modern biotechnologies should be used as adjuncts to and not as substitutes for conventional technologies in solving problems, and that their application should be need-driven rather than technology-driven.

The use of modern biotechnology to enhance production of aquatic species holds great potential not only to meet demand but also to improve aquaculture. Genetic modification and biotechnology also holds tremendous potential to improve the quality and quantity of fish reared in aquaculture. There is a growing demand for aquaculture; biotechnology can help to meet this demand. As with all biotech-enhanced foods, aquaculture will be strictly regulated before approved for market. Biotech aquaculture also offers environmental benefits. When appropriately integrated with other technologies for the production of food, agricultural products and services, biotechnology can be of significant assistance in meeting the needs of an expanding and increasingly urbanized population in the next millennium. Successful development and application of biotechnology are possible only when a broad research and knowledge base in the biology, variation, breeding, agronomy, physiology, pathology, biochemistry and genetics of the manipulated organism exists. Benefits offered by the new technologies cannot be fulfilled without a continued commitment to basic research. Biotechnological programmes must be fully integrated into a research background and cannot be taken out of context if they are to succeed. The potential area of biotechnology in aquaculture include the use of synthetic hormones in induced breeding, transgenic fish, gene banking, uniparental and polyploidy population and health management.

1.1.1 Biotechnology In Fish Breeding

Gonadotropin releasing hormone (GnRH) is now the best available biotechnological tool for the induced breeding of fish. GnRH is the key regulator and central initiator of reproductive cascade in all vertebrates (Bhattacharya *et al*., 2002). It is a decapeptide and was first isolated from pig and ship hypothalami with the ability to induce pituitary release of luteinising hormone (LH) and follicle stimulating hormone (FSH) (Schally et al., 1973). Since then only one form of GnRH has been identified in most placental mammals including human beings as the sole neuropeptide causing the release of LH and FSH. However, in non-mammalian species (except guinea pig) twelve GnRH variants have now been structurally elucidated, among them seven or eight different forms have been isolated from fish species (Halder et al., 1991; Sherwood *et al*., 1993; King and Miller, 1995; Jimenez-Linan *et al*., 1997). The most recent GnRH purified and characterized was by Carolsfeld *et al*. (2000) and Robinson *et al*. (2000). Depending on the structural variant and their biological activities, number of chemical analogues have seen prepared and one of them is salmon GnRH analogue profusely used now in fish breeding and marked commercially under the name of Ovaprim throughout the world. The induced breeding of fish is now successfully achieved by development of GnRH technology.

1.1.2 Transgenesis

Transgenesis or transgenic may be defined as the introduction of exogenous gene / DNA into host genome resulting in its stable maintenance, transmission and expression. The technology offers an excellent opportunity for modifying or improving the genetic traits of commercially important fishers, mollusks and crustaceans for aquaculture. The idea of producing transgenic animals became popular when Palmitter *et al*. (1982) first produced transgenic mouse by introducing metallothionein human growth hormone fusion gene (mT-hGH) into mouse egg, resulting in dramatic increase in growth. This triggered a series of attempts on gene transfer in economically important animals including fish. The first transgenic fish was produced Zhu *et al*. (1985) in China, who claimed the transient expression and putative transgenic, although they gave no molecular evidence for the integration of the transgene. The technique has now seen successfully applied to a number of fish species. Dramatic growth enhancement has been shown using this technique especially in salmonids (Devlin *et al*., 1994).

Some studies have revealed enhancement of growth in adult salmon to an average of 3-5 times the size of non-transgenic controls, with some individuals, especially during the first few months of growth, reaching as much as 10-30 times the size of the controls (Devlin *et al*., 1994; Hew *et al*., 1995). The introduction of transgenic technique has simultaneously put more emphasis on the need for production of sterile progeny in order to minimize the risk of transgenic stocks mixing in the wild populations. The technical development has expanded the possibilities for producing either sterile fish or those whose reproductive activity can be specifically turned on or off using inducible promoters. This would clearly be of considerable value allowing both optimal growth and controlled reproduction of the transgenic stocks while ensuring that any escaped fish would be unable to breed. An increased resistance of fish to cold temperatures has been another subject of research in fish transgenic for the past several years (Fletcher *et al*., 2001). Coldwater temperatures pose a considerable stressor too many fish species and few are able to survive water temperatures much below 0-1^{0}C. This is often a major problem in aquaculture in cold climates ecosystem. Interestingly, some marine teleosts have high levels (10-25 mg/ml) of serum antifreeze proteins (AFP) or glycoproteins (AFGP) which effectively reduce the freezing temperature by preventing ice-crystal growth. The isolation, characterization and regulation of these antifreeze proteins particularly of the inter flounder *Pleuronectas americanus* has been the subject of

research for a considerable period in Canada. Consequently, the gene encoding the liver AFP from winter flounder was successfully introduced into the genome of Atlantic salmon where it became integrated into the germ line and then passed onto the offspring F3 (3rd filial generation) where it was expressed specifically in the liver (Hew *et al*., 1995). The introduction of AFPs to gold fish also increased their cold tolerance, to temperatures at which all the control fish died-12 hours at 0^0C (Wang *et al*., 1995). Similarly, injection or oral administration of AFP to juvenile milkfish or tilapia species led to an increase in resistance to a 26 to 13^0C, drop in temperature (Wu *et al*., 1998). The development of stocks harbouring this gene would be a major benefit in commercial aquaculture in counties where winter temperatures often border the physiological limits of these species.

The most promising tool for the future of transgenic fish production is undoubtedly in the development of the embryonic stem cell (ESC) technology. There cells are undifferentiated and remain totipotent so they can be manipulated in vitro and subsequently reintroduce into early embryos where they can contribute to the germ line of the host. This would facilitate the genes to be stably introduced or deleted (Melamed *et al*., 2002). Although significant progress has been made in several laboratories around the world, there are numerous problems to be resolved before the successful commercialization of the transgenic brood stock for aquaculture. To realize the full potential of the transgenic fish technology in aquaculture, several important scientific breakthrough are required. They include: (i) more efficient technologies for mass gene transfer (ii) targeted gene transfer technologies such as embryonic stem cell gene transfer (iii) suitable promoters to direct the expression of transgenes at optimal levels during the desired developmental stages (iv) identified genes of desirable traits for aquaculture and other applications (v) information on the physiological, nutritional, immunological and environmental factors that maximize the performance of the transgenic and (vi) safety and environmental impacts of transgenic fish.

1.1.3 Chromosome Engineering

Chromosome sex manipulation techniques to induce polyploidy (triploidy and tetraploidy) and uniparental chromosome inheritance (gynogenesis and androgenesis) have been applied extensively in cultured fish species (Pandian and Koteeswaran, 1998; Lakra and Das, 1998). These techniques are important in the improvement of fish breeding as they provide a rapid approach for gonadal sterilization, sex control improvement of hybrid viability and clonation. Most vertebrates are diploid meaning that they possess two complete chromosome sets in their somatic cells. Polyploidy individuals possess on or more additional chromosome sets, bringing the total to three in triploids, four in tetraploids and so on. Induced triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management. The methods used to induce triploids and other types of chromosome set manipulations in fishes and the applications of these biotechnologies to aquaculture and fisheries management are well described (Purdom, 1983; Chourrout, 1987; Thorgaard, 1983; Pandian and Koteeswan, 1998). Tetraploid breeding lines are of potential benefit to aquaculture, by providing a convenient way to produce large numbers of sterile triploid fish through simple interploidy crosses between tetraploids and diploids (Chourrout *et al*., 1986; Guo *et al*., 1996). Although tetraploidy has been induced in many finfish species, the viability of tetraploids was low in most instances (Rothbard *et al*., 1997).

In teleosts, technique for inducing sterility include exogenous hormone treatment (Hunter and Donaldson, 1983) and triploidy induction (Thorgaard, 1983). The use of hormone treatments, however could be limited by governmental regulation and a lack of consumer acceptance of hormone treated fish products. Triploidy can be induced by exposing eggs to physical or chemical treatment shortly after fertilization to inhibit extrusion of the second polar body (Purdom, 1983; Thorgaard, 1983 and Ihssen *et al*., 1990) triploid fish are expected to be sterile because of the failure of homologous chromosomes to synapse correctly during the first meiotic division.

Methods of triploidy induction in clued exposing fertilized eggs to temperature shock (hot or cold), hydrostatic pressure shock or chemicals such ancolchicines, cytochalasin-B or nitrous oxide. Triploid can also be produced by crossing tetraploids and diploids. Tetraploid induction involves fertilizing eggs with normal sperm and exposing the diploid zygote for physical or chemical treatment to suppress the first mitotic division. Gynogenesis is the process of animal development with exclusive maternal inheritance. The production of gynogenetic individuals is of particular interest to fish breeders because a high level of inbreeding can be induced in single generation. Gynogenesis may also be used to produce all female populations in species with female homogamety and to reveal the sex determination mechanisms in fish. It is convenient to use all female gynogenetic progenies (instead of normal bisexual progenies) for sex inversion experiments. Methodologies combining use of induced gynogenesis with hormonal sex inversion have been developed for several aquaculture species (Gomelsky *et al.*, 2000).

Androgenesis is a method for producing fish in which all the nuclear genetic information originates from male parent (i.e., from the sperm) while the mitochondrial DNA is still maternally derived, which would have commercial application in aquaculture. It can also be used in generating homozygous lines of fish and in the recovery of lost genotypes from the cryopreserved sperms. Androgenetic individuals have been produced in a few species of cyprinids, cichlids and salmonids (Bongers *et al*., 1994).

1.1.4 Biotechnology In Fish Health Management

Disease problem area major constraint for the development of aquaculture. Biotechnological tools such as molecular diagnostic methods, use of vaccines and immunostimulants are gaining popularity for improving the disease resistance in fish and shellfish species world over for viral diseases, avoidance of the pathogen in very important; there is an urgent need for a rapid method for detecting pathogenes. Biotechnological tools such as gene probes and polymerase chain reaction (PCR) are showing great potential in this area. Gene probes and PCR based diagnostic methods have developed

for a number of pathogens affecting fish and shrimp (Karunasagar, 1999). In case of finfish aquaculture, number of vaccine against bacteria and viruses have been developed. Some of these have been conventional vaccines consisting of killed microorganism but new generation of vaccine consisting of protein subunit vaccine genetically engineered organism and DNA vaccine are currently under development. In the vertebrate system, immunization against disease is a common strategy. However, the immune system of shrimp is rather poorly developed, biotechnological tools are helpful for development of molecule, which can stimulate this immune system of shrimp. Recent studies have shown that the nonspecific defense system can be stimulated using, microbial product such as lipopolysacharides, peptidoglycans or glucans (Itami *et al*., 1998). Among the immunostimulants known to be effective in fish glucan and levamisole enhance phagocytic activities and specific antibody responses (Sakai, 1999).

1.1.5 Cryopreservation Of Gametes/Gene Banking

Cryopreservation is a technique, which involve long-term preservation and storage of biological material at a very low temperature usually at -196 ^{0}C, the temperature of liquid nitrogen. It is based on the principle that very low temperature tranquilize or immobilize the physiological and biochemical activities of cell, thereby making it possible to keep them viable for very long period.

The technology of cryopreservation of fish spermatozoa (milt) has been adopted for fish husbandry. The first success in preserving fish sperm at low temperature was reported and fertilizing Herring (*Clupea herengus*) eggs with frozen thawed semen (Blaxter, 1953). The spermatozoa of almost all cultivable fish species has now been cryopreserved (Lakra 1993). Cryopreservation overcomes problems of male maturing before female, allow selective breeding and stock improvement and enables the conservation. It is one of the emerging requirements used by breeders for evolving new strains (Harvey, 1996). Most of the plant varieties that has been produced are based on the gene bank collections. Aquatic gene bank however suffers from the fact that at present it is possible to cryopreserve only the male gametes of finfishes and there in no viable technique for finfish eggs and embryos. However, the recent report on the freezing of shrimp embryos. However, the recent report on the freezing of shrimp's embryos look promising. Therefore, it is essential that gene banking of cultivated and cultivable aquatic species be undertaken expeditiously (Subramoniam and Newton, 1993; Diwan and Kandaswami, 1997).

2.1 BIOTECHNOLOGY ADVANCEMENTS IN AQUACULTURE

2.1.1 Nutrition

Indian aquaculture industry lags behind in the science of nutrition. A good approach would be to improve the quality and utilization of various agricultural and industrial by-products for efficiency. Various foodstuffs having potential values as fish feed ingredients have not been nutritionally utilized as they contain toxic or anti-nutritional factors. The need of biotechnology is to develop new varieties of foodstuff with totally free or low content of such intoxicants.

Other developments of improved feeds are:

- Developments of food additives, such as anabolic steroids and thyroid hormones, have resulted in increased growth of salmonid fishes.
- Development of new artificial diets, such as micro-encapsulations for filter-feeders (oysters and mussels).
- Improved culture of single-celled feed organi-sms (algae, yeast, bacteria).

2.1.2 Feeding Stimuli And Chemical Signal

To obtain the full sequence of feeding behaviour from initial recognition of feeding "strike" to ingestion of food particles, it is necessary to identify the feeding stimuli or chemical signal. It is thus required to genetically design or engineer the bacterial strain with characteristics of having better cellulose degradation capacity.

2.1.3 Reduce The In-Pond Chemical Oxygen Demand

Microbial processing of livestock manure through anaerobic digestion in biogas plants reduces the in-pond COD. It also improves the fertilization quality of the inputs, Biotechnology has attained great significance in aquaculture development by increasing the nitrogen fixation levels in ponds through bio-fertilization with blue-green algae (*Azolla* spp.) and green-manuring and through genetic manipulation of bacteria to improve their nitrogen fixing capacity. This has definitely increased fish production.

2.1.4 Administration Of Mammalian Hormones

Mammalian gonadotropic hormones have been used extensively for spawning of carps, tilapias and catfishes. Hormonal manipulation of sex is being practiced to control unwanted reproduction in prolific breeders such as common carp and tilapias. Administration of mammalian growth hormones through feed or injection has enhanced fish growth. Manipulated mono-sex population improves nutritive value of fish flesh tissue and growth.

2.1.5 Cryopreservation

Cryopreservation refers to preservation of gametes and embryo generally at below freezing temperature for ready availability of genetic material for breeding and research purposes. Cryopreservation of milt has been successfully developed for several fishes. It is essential since the males of some fish species mature earlier than the females and there would be death of oozing males when fully mature female fishes would be available. Gametes are most likely preserved in liquid nitrogen at a very temperatures. Cryopreservation of zygotes and embryos have facilitated large scale fish seed production programme.

2.1.6 Use Of Allomones And Pheromones

In large scale induced breeding, use of allomones and pheromones is of great significance. Under biotechnological programme it is necessary to study the role of pheromones in alarm and social behaviour, species and sex recognition, sex behaviour and territorial and space recognition.

2.1.7 Genetic Manipulation/Engineering

Change in genetic makeup of any organism by application of improved biotechniques is referred to as genetic manipulation or engineering. Fishes, generally, can tolerate a wide range of genetic manipulation than terrestrial farm animals.

2.1.7.1 Chromosomal Manipulation

Through chromosome manipulation, fish stock can be improved for breeding and culture by way of gynogenesis, androgenesis and polyploidy.

i. Gynogenesis (All Maternal Inheritance): - Gynogenesis in fishes can be induced by stimulating parthenogenetic development of fish eggs by artificially inactivated spermatozoa. Through X-ray or UN-rays, the genetic contents (DNA) in spermatozoa can be destroyed. With such spermatozoon fertilization would occur without any contribution from them. The diploid parthenogenetic individuals would give rise to offspring that would be females having maternal inheritance only, e.g. Grass carp, Salmon, Rainbow trout, etc.
ii. Androgenesis (All Paternal Inheritance): - Similarly with X-rays or UN-rays, the genome of ova can be destroyed and fertilized with normal spermatozoon would result in homogametic males having paternal inheritance only, e.g. Common carp.
iii. Polyploidy: - Polyploidy (individuals having extra sets of chromosomes) can be achieved by subjecting fertilized eggs to heat, cold, pressure or chemical shocks. Polyploidy produces:

- Sterile individuals
- Enhanced growth and survival
- Improved quality of flesh in some fishes
- More viable and ideal for producing new hybrids.

2.1.7.2 Gene Transfer/Transgenesis

Transgenesis results in formation of "Transgennic fish". The techniques involved is through micro-injection and electroporation, genes are introduced into one-celled embryo or oocyte. The potentialities of transgenesis are:

- Tolerance to physical factors, e.g. tolerance to cold using antifreeze gene
- Accelerate growth by using growth hormone genes
- Efficient use of food by fishes through manipulation of biochemical pathways
- Increase disease resistance by using specific disease resistance genes (e.g. T-cell receptor, immunoglobulin, lymphokines, etc.).
- Behavioural modification (maturation, repro-duction, sex control, etc.) by regulation of endocrine function.

2.1.7.3 Application Of Cell Culture Biotechnology To Marine Macro-Algae

Seaweeds can be easily genetically engineered. In many maritime countries, tons of seaweeds are produced through mariculture, which are used as human food (green seaweeds) and for commercial production of phyco-colloids (agar, algins, carrageenas, etc.) for industrial purposes.

2.1.8 Hormonal Manipulation For Sex Control

Sex hormones (steroids) are used to produce monosex for culture of tilapia in particular (e.g. GIFT). Tilapia being a prolific breeder, creates problem in pond culture as it results in overcrowding with small size fishes and thus, in decreased production. Therefore, monosex culture controls reproduction and also helps in increased production. To obtain only males by sex reversal method, the early fry of tilapia are fed with androgen 17-a methyl testosterone for induction of sex reversal from genetic females to phenotypic males. Similarly, in species where females are bigger and grow faster than males, only females can be produced for monosex culture by feeding fry with estrogen 17-b-Estradiol benzoate or diethyl stibestrol.

2.1.9 Fish Disease Management

Fish diseases can be controlled through development of biotechnologically produced vaccines. Vaccines against vibriosis and furunculosis have been developed and are available in the market. However, they are for limited fish species. There is an increased demand to use biotechnology for developing vaccines against bacterial, viral, fungal and other diseases affecting several commercially important culturable fish species.

2.1.10. Current Breakthrough and Essentials for Future Research

Technological advancement has led to freshwater and marine pearl culture product development such as chitin, chitosan, etc. Further studies for even more efficient and large scale production technologies is essential. Attention should be drawn towards genetic improvement of fish stock through selection and genetic engineering, ex-situ conservation methodologies, gene banking both with live and gamete level approaches, which would subsequently lead to future research. Social impact of fisheries and aquaculture development has to be studied and the need is to develop parameters

to undertake Social Impact Assessment (SIA). Thus, for the development of biotechnology, it is urgently required to upgrade laboratory facilities and attract young blood for research and training. It should be noted that water resources in the country are not exclusively available for aquaculture as there is excessive pressure on their use from several other sectors. Therefore, any further growth in aquaculture for quantum jump would have to be vertical and technology based. The transfer of technology from the laboratory to the field has had its own causalities.
Today, only about 30% of the proven technology available in various fields of aquaculture has been manifested in the field. There is thus, urgent need to narrow the gap between the results of research and those in the farmers' field.

3.1 CONCLUSION

Biotechnological research and development are growing at a very fast rate. The biotechnology has assumed greatest importance in recent years in the development of fisheries, aquaculture, agriculture and human health. The science of biotechnology has endowed us with new tools and tremendous power to create novel genes and genotypes of plants, animals and fish. The application of biotechnology in the fisheries and aquaculture sector is a relatively recent practice. Nevertheless, it is a promising area to enhance fish production for fast growing, immune and fish harvest. The increased application of biotechnological tools can certainly revolutionize our fish farming besides its role in biodiversity conservation and management. This review briefly consider the current progress and thrust areas in the transgenesis, chromosomal engineering, use of synthetic hormones in fish breeding, biotechnology in health management and gene banking as climate change threatens aquatic fish biodiversity and the ecosystem. Biotechnology came about through observance of animals' and plants' best performing breeds/cultivars and natural selection was firstly applied by the farmers then technology was engineered on outstanding traits of species.

3.1.1 RECOMMENDATIONS

Climate change and overexploitation of aquatic resources have informed biotechnology engineers to branch gap of wild fish recruitment and harvest; most fish species grow faster and bigger in their single (mono) strand (all male or all female) production in natural selection.
Fish farmers should be exposed to primary genetic/hybridization/biotechnology principles. Practical skills of hybridization/biotechnology be demonstrated to fisheries and aquaculture undergraduates and farmers. Biotechnology engineering is the way forward for fish protein multiplication, disease resistant breeds and high fecundity which farmers need to embrace.
A lot of persons have talked down on genetics/biotechnology engineering in fish production but it a sure way for food security in Africa and the globe at large.

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