ENVIRONMENT AND BIOTECHNOLOGY

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PREFACE

Biotechnology covers a wide spectrum of specialised disciplines which includes not only the latest genetic engineering techniques but also old fermentation process which our forefathers practised thousands of years ago. In recent years there has been tremendous development in this field and it has gained great importance—specially in the eyes of public.

We are grateful to authors for sharing extreme forbearance as it took more than the expected time to bring out this Volume. We thank all the contributors and publishers for their efforts to make this publication successful.

The editors are indebted to Dr. S. Ramchandran, former secretary, Department of Biotechnology, Government of India for his help and guidance in preparation of this volume. Dr. M. V. Nair, Director, NRLC LKO is gratefully acknowledged for providing infrastructure facilities and constant encouragement.

We sincerely hope that readers will be immensly benefited from this book.

SOHAL, H.S.
SRIVASTAVA, A.K.

CONTENTS

1

AQUACULTURE IMPROVEMENT THROUGH BIOTECHNOLOGY

A. Ninawe*

Biotechnological tools now offer immense scope of expansion to aquaculturists by way of incremental output in fisheries with their existing fresh water, marine and brackish-water resources in the different agroclimatic zones of the country. Practically all the sectors, e.g., scientific community, institutes, industry and practitioners stand to reap additional gains from research and development to actual production level. An important co-product of these efforts is better utilization of resources by scientific means, better return on investments, better prospects for direct and indirect employment in rural and coastal areas and increased export surpluses.

Improvement of fish cultivation and production method is of great significance to agronomy. Fishes are the source of high grade protein and essential nutrients. A better and efficient use of aquaculture resources substantially contribute for the wellbeing of the mankind. At present fish production from aquaculture resources is 3.8 Metric Tonnes per annum against the projected need of 13 Metric Tonnes by the end of 2000 A.D.

Greater research strides have been made in the inland fish aquaculture in the last four decades through induced breeding and fish hybridisation, composite culture, nursery management, rearing, stocking and integrated fish farming which have contributed fish production from 600 kg. per hectare

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per annum to the level of 4.6 kg. per hectare per year to achieve optimum productivity from this sector rapid progress in the development of controlled reproduction and associated biotechnologies have opened up new avenues. Possibility of synthesising desired proteins for induced breeding and genetically engineered fish are the alternatives to meet the per capita demand by the turn of the century.

India is one of the richest countries in the world with the vast and varied inland water resources and there is considerable scope for achieving significant increase in food production through inland and coastal aquaculture. Apart from utilising these aquatic bodies for food production, aquaculture appears to be economically more renumerative than the traditional agriculture. Evidently the returns from aquaculture is several times higher than the traditional agriculture. Therefore, investment on biotechnological research and training in aquaculture may not go wrong.

Induced breeding and production of seeds

Several laboratories through out the world are engaged in producing fish seed by multiple spawning as well as prolonging the breeding season. The most commonly used technique of induced breeding for fish seed production has been the treatment of brooder fish with exogenous gonadotropins using pituitary extract. Few workers have demonstrated the release of gonadotropin from fish-pituitary, regulated by hypothalamic gonadotropin releasing harmone (GnRH). The analogoues and superagonist of GnRH has also been tried extensively on different species for prolonging the breeding season, multiple spawning and increased seed production.

Laboratories in India are trying to develop alternate method for induced breeding in major carps and reported different degrees of success in induced breeding of *C. mrigala* through LHRH. They could able to breed Indian major carps for two times a year by GnRH.

Fish genetics and hybridisation

Hybridisation is a rapid route to improve the genetic variety of fish in terms of increased vitality, higher rate of adaptability, accelerated growth and development depending on the phylogenetic relationship of the species to be crossed. Several interspecific and intergeneric hybrids of major carps and those of Indian major carps with exotic carps have artificially produced through hypophysation. Some of the artificially produced hybrids of Indian major carps from the view point of suitability for aquaculture are: Rohu x Catla and Catla x Rohu, Catla x Mrigal and Mrigal x Catla, Calbasu x Catla and Catla x Calbasu. However, among the above hybrids, Rohu x Catla and Catla x Rohu are the most promising in terms of growth, food and feeding habits, viability and higher flesh content. Both are fertile, however, Rohu x Catla hybrid grows faster than Cohu and Catla x Rohu hybrids but not as quick as Catla.

Gene transfer and cloning

Another approach to achieve high yield would be the production of genetically engineered fish. The genetically engineered fish grows faster, consumes less feed and are diseases resistant. The model systems for producing transgenic fish of economic importance has been provided by various laboratories in USA, Canada, France, Japan and China and are actively engaged in transferring different genes in various fish species. In France, the workers are engaged in transferring foreign genes for increased growth rate, disease resistance and fish with high protein content. The recognition of mammalian promoter for metallothionein (MT) to express downstream gene for B_1-galactosidase in Salmon has been studied recently.

The cloning of useful genes from the different organisms is now a routine job in biotechnology. Cloning of fish GnRH gene and introduction of this gene with compatible promoter into the germline by microinjection would generate transgenic lines of major carp which by itself will have multiple spawning, prolonged breeding season under the appropriate induction. Researchers have used GHmRNA from Rainbow Trout pituitaries to synthesise a complementary DNA molecule in *E. coli*. Few of them have cloned Salmon GH in *E. coli* purified growth hormone gene from Tilapia, carp and Chum Salmon. The reports have also appeared regarding antifreeze protein which lowers the freezing point of the blood. This could be an another approach of fish genetic engineering which offers a possibility to make the temperate fish species adaptable to colder habitat.

So far cloning of fish genes has not been initiated seriously in India. Few laboratories engaged in the work of microinjection of genes into the animal germline have endeavoured to introduce hGH gene into Indian major carp embryo for the first time. The technique to introduce foreign gene in fish has already been standardised in some of the laboratories where the initiation has been made to prepare genomic and cDNA Libraries of Indian major carps for is olation of genes such as GH, GnRH and some inducible genes. Generally, the normal major carp takes 8-12 months to attain marketable size, however, the transgenic line of carp will have faster growth rate, better feed conversion ration (FCR) and high flesh bone ratio, which will reduce the production cost considerably.

Induction of polyploidy

During the last few years there has been a burst of research in the production of sterile fish in order to prevent overcrowding and to improve flesh quality. Much interest is being evinced towards the production sterile hybrids of cultivable species by means of inducing triploidy. The triploid hybrids have a great potential in aquaculture as they result in blocking gonadal development and sterility.

Polyploidy in cultivable species is produced using physical or chemical treatments. A remarkable approach for producing sterile hybrids was followed by crossing natural tetraploid common carp with other Indian major carps. They exhibit faster growth rate, possess higher flesh content and show lower seine escapability than the maternal parent. Of the common carp hybrids produced, common Carp x Rohu is the most viable one. Common Carp and Catla is also a promising hybrid in terms of growth and viability.

The success was achieved in producing triploids and tetraploids in *Oreochromis mossambicus*. This broiler fish grows 15 per cent faster than the diploids supermales of *O. mossambicus* have been produced in India by judiciously combining the endocrine sex reversal and selective breeding technique. This technique effectively reduced the time cost of producing YY males from 22 ± 3 to 8 ± 2 months.

Through chromosome manipulation technique it has been possible to produce haploid, triploid, hybrid triploid, supermales and superfemales of *O. mossambicus* for the large-scale commercial production of monosex off-spring. It has also been possible to produce gynogenic and androgenic individuals for the production of pure strains and quality brooders.

Hormonal treatment and vaccine development

Control of sexual maturation in fish to prevent diversion of stomatic growth into the gonadal development by vaccines is another way to improve the productivity of fish. Successful demonstration of mono sexuality and sterility has been done in a variety of fishes through surgical gonadectomy, administration of sex steroids, non-steroids and non-hormonal substitutes, radiation and physical shock treatments and hybridisation, but the most common practice being through the administration of sex steroids such as 17 methyl testosterone and 17-B estradiol or estradiol benzoate in fishes, e.g., *Oreochromis mossambicus* and *Cyprinus carpio*. In India wild spawning of *O. mossambicus* when treated with 30 ug. methyltesterone per gm body weight gave more than 90 per cent of males and also produced monosex males of Tilapia. For obtaining 100 per cent males in Tilapia an artificial feed supplemented with 17-methyl testosterone ensuring a minimum uptake of 1.5 ug. per gm/day should be given to fish. However, 100 percent female Tilapia could be produced by feeding 9-day old fry with de ithytl-stibesterol-supplemented feed. Another hormone miboterone when given to common carp at 75 ppm produced sterile forms. This hormone has been reported to enhance the growth of fish and its flesh showed enhanced protein and lipid contents.

Hormone technology in crustaceans has not been well developed. The molting hormone ecdysone can be used effectively to abbreviate molting in larval stage of the shrimp in hatchery production.

Immunological methods to neutralise the gonads stimulating hormones may be another way to increase the stomatic growth. The field of fish immunology is almost untouched in India. However information is available which mainly involves the identification of important diseases.

Cryopreservation of gametes

As fish farming expands and harvesting of wild stocks become more intense, the technique for gametes storage is needed to facilitate artificial reproduction and to preserve the desirable gene pool. Considerable progress has been made in the cryopreservation of different species of salmonid spermatozoa. It has shown a lot of promise in tropical, freshwater and salt water species with potential, for aquaculture of silver carp, *Mugil cephalus*, Indian carp, grass carp and milk fish.

Very little information is available regarding ova and embryo cryopreservation. Gamete cryopreservation is still in the infant stage, however crab gamete cryopreservation and cryopreservation of prawn embryos are under developing stage.

Nutritional aspects and cost-efficient feed formulation

The basic promise in fish culture is to maximise growth at minimum cost with an end-product that is of high nutritive value and aesthetically acceptable to the consumer. The successful cost efficient fish culture depends on the proper feeding habits and nutritional requirements of the available species. Growth studies on the different species of fish has been done throughout the world. The studies has been directed at the various aspects of nutrition such as evaluation of low cost raw materials for aquaculture feed, bioenergetics, digestibility, protein, lipid and carbohydrate contents and nucleic acid concentrations in estimating growth rates. These studies will emphasis the need for improving the growth rate and health status of fish.

In India, reports are available on the quality of diets and their development and demonstrated the protein, fat and carbohydrate requirements of carps. The studies have been made to find out the effects of amino acid balancing on growth and diet utilisation and amino acid profile of conventional feed mixture, poultry litter and micro-encapsulation of diet for carp fry spawning. The various laboratories are engaged in finding out the cost efficient nutritionally balanced diets for fish and prawn aquaculture.

Supplementation of natural food organisms

The most interesting feature of fish and prawn are the different food requirements during the different stages of their life cycle. These varying food habits in different life stages pose certain problems to culturists who for successful culture should provide the correct type of food in right quantity. The natural food of larvae from mysis stage onwards consist mainly of zooplankton including rotifers, copepods and copepodites, very small worms and larval stages of various aquatic invertebrates. *Artemia nauplii* are considered to be the best food for prawn larvae and the pelagic post larvae. Several algae have been identified for their beneficial applications in aquaculture for biological nitrogen fixation and high yielding nutritive protein fish feed.

Fish pathology

Intensification of aquaculture practices involving high density stocking, supplementary feeding and fertilisation programme has brought in problems of fish and shell-fish disease outbreak in ponds and tanks in the recent years. Parasitic diseases in fishes in warm water ponds of Asia and Far-East has also reviewed but very little information is available on the biology of this parasites and their pathological effects on the host. Bacterial and fungal diseases have also been encountered in our fish culture system. The major bacterial diseases are the tail and fin rot, ulcer diseases, columnaris disease, bacterial haemorrhagic septicemia, dropsy and eye disease. The most common fungal diseases are caused by the fungus *Saprolegnia* species and *Brachiomyces* species. Recently, a severe fish disease outbreak known as "Ulcerative syndrome" has been encountered in some of our bordering states affecting fishes in all types of water bodies. Mass mortality of the brood fish of Indian major carps due to *Argulus* infection has been reported and successfully controlled by a combine treatment using melathion and potassium permaganate.

As environmental problems are among the significant causes of disease, it is essential to routinely monitor and record the various environmental factors including biota. Proper management and feeding programmes can go a long way in maintaining good environment. Recently, many breeders have suggested polyculture of shrimp and fish in the same pond as the basis of a balanced ecological system.

There is not much useful information available on the proper treatment of shrimp diseases. In some cases, treatment is taken too late to have a beneficial effect and drugs and disinfectants may even have a negative effect on the fish or shrimp or pond environment and just be wasted. For treatment to be successful, it is important to diagnose the disease as early as possible and treat the specific disease problem using an efficacious method.

Environmental monitoring and protection

Aquaculture production is mainly dependent on the availability of the water quality which forms the major component of environment, influences the life of aquatic organisms. It is, therefore, necessary to protect environment from polluting canals and sea by avoiding discharge of pond sediments and effluents during pond preparation.

In intensive and semi-intensive farming system the capacity of the environment can be increased through the adoption of technology, for example, pond construction, water depth, irrigation system, number of pumps and paddle wheels are technological inputs which will interact with soil and water qualities in determining the capacity of the pond. Raising the depth of water, number of pumps and paddle wheels; the carrying capacity of the pond can be improved.

An increase in the stocking rate will bring about an increase in dissolved organic matter in the water as precipitate on the pond bottom as a result of left over fish and shrimp feed faeces. This will utilise the growth and phytoplankton due to the process of eutrophication. The load of organic matter can be reduced though water exchange and a self purifying capacity process, whereby microbes present in the pond will disintegrate into the minerals and gaseous compounds. Sometimes such compounds will be produced in much concentration which will be dangerous to the health of the animals. It is, therefore, very essential to take proper precaution to maintain water quality in the pond eco-system for the better aquaculture practices.

To sum up the above biotechnological aspects for increasing aquaculture productivity it may be necessary to consider promises of marine biotechnology for developing countries where fish and shell-fish forms a major source of protein rich food and foreign exchange earnings as well. The tropical country like India possesses abundant marine and estuarine natural resources and, therefore, the thrust should be given to explore the possibilities in marine biotechnology since easy access and availability of marine life which permits direct utilisation of new and or unique products through genetic engineering and ensures continuous production in the laboratory, eliminating the fluctuations caused by weather conditions.

Application of genetic engineering and marine biotechnology promotes cloning of genes which control production of larval attractants and inducers for metamorphosis and growth. Recombinent DNA probes and templates can also be used to analyse and control life cycle processes of marine invertebrates.

Advances in applied molecular biology and genetic engineering can be applied to aquaculture in the control of microbially mediated diseases like *Vibrio* waterspread among fish. Production of vaccine strains employing genetic engineering for excision of virulence factors as has been in *Vibrio cholerae* and other agents of human diseases, should be equally effective for controlling *Vibrio* diseases of fish and shell-fishes.

Biotechnology thus offers a means for obtaining recalcitrant species in culture which represent excellent opportunities for gene selection, manipulation and amplification. Aquaculture productivity can advance significantly through the production of vaccines, employing both hybridoma technology and genetic engineering.

A directed search for biologically active natural products in the marine environment, as new sources of industrial chemicals, with the help of extensive screening for such products. Cloned genes effective for producing desired compounds in a non-marine industrially adopted vector offers rich sources of new products. The need for their collection, culture and screening of marine organisms from which bioactive compounds can be isolated and characterised as the first step before genetic engineering could be employed.

The recent development in the aquaculture biotechnology have been very spectacular and opened up possibilities of exponential growth through the major components of genetics, biotechnology, disease control, feed formulation and commercially viable systems. The Indian scene is very encouraging and we should attempt to bring about a blue revolution in aquaculture through creation of trained manpower and infrastructure.

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Safety and Regulatory Arrangements in Biotechnology

K. Narayanaswami*

Introduction

The advent of recombinant DNA (rDNA) technology has heralded new opportunities for beneficial applications in agriculture, animal and human health, industry and environment. Distinctly different species could be recombined by purposeful and directed manipulation at the molecular level towards improvements of plants or animals or to develop microorganisms and products for specific purposes.

While recombinant technique has potential for multifarious beneficial applications it has also raised concerns about possible.

(i) Unknown hazards from bridging natural species barrier and

(ii) the uncertain consequences of the novel organisms may have on environmental and public health.

To encourage development of safe biotechnology in all sectors, the RDAC drew attention to the state of art and the approach to safety followed among nations through guidelines and regulations while developing one for India. As a result the rDNA safety guidelines have been brought out by the DBT in January 1990 giving adequate safeguards in research, production and application.

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