POLICY PAPER 21

Priorities of Research and Human Resource Development in Fisheries Biotechnology

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Background

In India, 16 per cent of the world population has to be sustained on 2.4 per cent of the global land area. Apparently, aquatic resources had to be given high priority for additional food production. Like agriculture, the fisheries sector is also dependent on renewable natural resources. Compared to animal production system, fisheries sector enjoys some advantages. Firstly, fish is the cheapest source of animal protein and a health food. Secondly, high fecundity (up to 1 million eggs) and fast growth rate (growth coefficient often > 1.0) of fishes have no parallel among other animal protein sources, like livestock including poultry. These biological advantages of fishes offer considerable scope for achieving nutritional security in India. Understandably, the projected annual increase in food production target for the next decade is as high as 10 per cent for fish and other aquatic products, whereas it is just 2.5 per cent for cereals.

Asia has been the centre of fishing and aquaculture activities. Among the Asian countries, India ranks second in culture and third in capture fisheries. Fish production has increased by more than 10 times and the contribution of the fisheries to the Indian GDP by more than 3 times during the last five decades; a growth arguably one of the highest within the food production sector.

However, the annual per capita fish availability (8 kg) is less than the world average (12 kg) and the quantity (11 kg) recommended by WHO for nutritional security. India's potential for increasing capture fisheries from the coastal areas is limited. Capture fisheries from rivers is also declining due to water abstraction, sedimentation, habitat destruction and pollution. Hence, India has to depend heavily on aquaculture. At present, the technology exists for rearing only 15 fish species, while as many as 39 and 52 species are profitably cultured by the Chinese and Koreans, respectively. Asian countries suffer an annual loss of more than $ 3,000 million owing to disease problems. Intensive efforts are in place to develop diagnosis. It is also recognised to build trained manpower in this area of fishery research and development.

Fish genetics is a rapidly growing field of science but in India it is still in its infancy. Selective breeding, a proven method for strain improvement, is known to enhance the expression of desired traits by 3 to 5 times; the super strain rohu, 'Jayanthi' is an example. However, it must be indicated that selective breeding may result in transmission of unwanted traits, due to mixing of whole genome of parents. It may also prevent transfer of desired genes from one species to another. On the other hand, transgenesis allows the introduction of novel genes or augmentation of a specific trait by just transferring the tailored copies of gene from the same or other species. In the area of trangenics also, we have just made the beginning and are, thus, far from developing disease-resistant transgenics. Research in genetics and molecular biology of fish is expected to yield probiotics and vaccines to eradicate or contain microbial diseases, which sometimes rapidly wipe out the farmed fishes, even before the disease is diagnosed. Marine plants and animals can be a very important pharmaceutical source to treat dreadful human diseases. Fish is a highly perishable commodity.

Biotechnological research may lead to superior methods of preservation, production of value added products and bioactive compounds, quality control and so on. In order to conserve the valuable germ plasm of Indian fishes, there is need to develop simple and widely practicable methods of sperm preservation and technique for interspecific androgenetic cloning.

In order to discuss aforesaid issues, the National Academy of Agricultural Sciences, organised a workshop. Several aspects of human resource development, molecular markers, genomics and transgenics, aquatic health and management, conservation genetics, and bioactive compounds were covered during the deliberations of this workshop.*

Recommendations

1. Prioritisation of Research in Biotechnology of Fishes

Biotechnology is a fast growing field of science. The number ofpublications appearing on Fish Genetics, Molecular Biology and Biotechnology, especially from USA, Japan, Korea and China are phenomenal. Considering our genetic resources, requirements, expertise and infrastructure, the following areas have been tentatively identified for financial support in the area of research on biotechnology of fishes on a priority basis.

1.1 Molecular Markets

Because of the technical simplicity, protein markers like isozymes and/or allozymes have long been used as 'molecular tags' in identification of species/strain/stock; but this technical simplicity is counterbalanced by the need for fresh tissue. With the advent of PCR technology, molecular markers have become a powerful tool in many areas of fisheries research including identification of species/strain/stock and fishery product(s) of endangered or banned species, evaluation of phylogeny, delineation of stock structure, measuring levels of genetic variation in wild and cultured stocks, conservation genetics, determination of breeding strategies, linkage and gene mapping, marker assisted selection and assessment of gene manipulation techniques. DNA markers include nDNA(RFLP, microsatellite, RAPD, AFLP) and mtDNA (RFLP, PCR). Microsatellites are excellent markers in the study of population genetics whereas, RAPD have been shown to be good markers for pedigree analysis.

1.1.1 Marine Capture Fisheries

An important consideration in the management of exploited fishery resource is the identification of discrete populations or stock units, which are genetically distinct self-maintaining groups. Sex specific markers are useful to assess sex ratio at the earliest embryonic stage and to confirm the genotype of the androgenetic, gynogenetic and hormonally sex reversed progenies. In recent years, aquaculture of the clown fish *Amphiprion* and the food fish *Epinephelus* are targeted. These fishes undergo natural sex

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* *A two-day workshop was organised by the NAAS, at Centre for Cellular and Molecular Biology, Hyderabad, on 25 and 26 August 2003.*

reversal from male to female in the former, and female to male in the latter. There is an urgent need to develop sex specific markers for these fishes.

Sardinella longiceps and *Rastrelliger kanagurta,* which constitute the bulk of Indian exploited marine fisheries must be subjected to an indepth study using protein and microsatellite markers. There is a need to prepare a series of atlas for geographic distribution of these species and description of population size and structure as well as their breeding.

For mariculture, there is a need for developing markers for molecular taxonomy of the genus *Epinephelus* and *Amphiprion.*

There is also a need for developing markers for *Hilsa ilisha.*

Since aquaculture concentrates on a very small range of species and an equally narrow genetic base in these species, deliberate release of stocks for ranching can modify the genetic composition of wild resource. Hence, there is a need to monitor this activity by the concerned agencies.

1.1.2 Freshwater Capture Fisheries and Aquaculture

While genetic background is crucial for success in selective programmes, hardly any programme has been initiated in India for genetic selection of economically important traits such as body growth, disease resistance, fecundity and food conversion rate for even the major carps, which are the mainstay of freshwater fisheries and aquaculture. The vast and diverse Indian aquatic resources are known to provide among the richest genetic resources in the world. Yet, there is no recorded information describing various strains of major carps, which occur in different agro-climatic zones of India. Japanese have made a series of atlases for the distribution of selected fish species characterised by different ploidy status and there are only a couple of records on the occurrence of haploids and tetraploids in India. To promote aquaculture, Chinese have made a large scale annual production of over 100 million triploid crucian carp seedlings by crossing tetraploid and diploid Japanese crucian carp. Considering these deficiencies, the following programmes are suggested:

- Identification and preservation *of* different strains *of rohu* and *catla, Penaeus indicus* and *Macrobrachium rosenbergii.*
- Development *of* reference and/or resource family lines, genetically distinctive populations/strains of Indian major carps and identified penaeid and palaemonid species by crossing and rearing under controlled conditions.
- Isolation and characterisation of polymorphic DNA markers for rohu, tiger and giant prawns.
- Construction of genetic linkage map containing at least 500 markers each for rohu and catla.
- Development of markers, products and morphologically indistinguishable progenies/larval stages of exotic/banned fishes.

1.2 Genomics

Marker assisted selection can accelerate the selective breeding process and such markers can also be used for genome mapping. In this context, a commercially important foodfish, *Labeo rohita* and a shellfish, *Macrobrachium rosenbergii* are recommended for genome mapping through RFLP, RAPD, AFLP and microsatellite markers.

1.3 Transgenesis

Although selective breeding is an excellent method for strain improvement of crops and farm animals, it suffers from drawbacks such as: trans-species barrier, mixing of whole genomes resulting in transmission of unwanted traits and requirement of relatively longer time. Since transgenesis allows introduction of novel genes or augmentation of a specific trait by transferring tailored copies of the gene from the same or different species, it has become a powerful tool to bio-engineer an organism to provide products and services. Fish has been regarded as the best vertebrate model system because of its oviparity and fecundity. However, non-availability of piscine vectors has been a handicap. At Madurai Kamaraj University, in order to construct transformation vectors for the indigenous fishes, growth hormone genes of rohu, *Labea rohita,* catfish*, Heteropneustes fassilis* were isolated, cloned and sequenced; their fidelity was confirmed in prokaryotic and eukaryotic systems. A vector was constructed with grass carp b-actin promoter driving the expression of r-GH. The sperm electroporation technique was standardised to ensure 25 per cent hatchling survival and 37 per cent presumptive transgenics without suffering any deformity. Genomic integration was confirmed in 15 per cent of the tested individuals (Ti) belonging to family lines 2 and 3; another 25 per cent of the juveniles (Te) were also proved transgenic but with the transgene persisting extrachromosomally for longer than 1 to 2 years. Further like drought resistant gene, cloning and sequencing of salinity tolerant gene may also prove a boon to aquaculture. Development of salinity tolerant transgenic fish and shrimp may be a long-term programme but may not be an insurmountable task.

Considering the above constraints and accomplishments, the following programmes are recommended:

- Follow-up research on transgene of growth hormone gene transmission on rohu need to be pursued further.

- Isolation, cloning, sequencing and construction of vector for genes responsible for aestivation and dormancy and their introduction into animals and plants of desirable species.

- Isolation, cloning, sequencing and construction of vector for genes responsible for salinity tolerance and their introduction into eggs to generate a salinity-resistant fish or shrimp.

Existing transgenic technology, like, microinjection, electroporation and lipofection are successful in isolated cases. Establishing methods of gonadal and embryonic stem cell isolation and manipulation for transgenesis in fishes would offer new methodology and far-reaching implications. Blastula stage cells can be transplanted successfully into another blastula by microinjection. It has been shown that donor cells colonise in the gonads of fishes like zebrafish, trout and medaka.

- Transgenesis through gonadal stem cells is to be explored.

1.4 Aquatic Medicine and Health Management

Bacterial, fungal, protozoan and viral infectious diseases are widespread among the natural fish populations. However, the fishes raised with intensive aquaculture systems are more vulnerable to diseases. Frequent outbreaks of diseases, especially those caused by viruses in recent years, are recognised as the major constraint to aquaculture. Effective control and treatment of the diseases require access to diagnostic tests that are rapid, reliable and highly sensitive. Conventional methods of bacterial identification are time-consuming and not amenable to mass scale screening. Molecular genetic tools facilitate mass screening and classification of the microbial pathogens. Highly discriminatory molecular typing methodologies allow rapid and sensitive differentiation within even a single serotype. For example, there are over 200 serotypes of *Vibrio cholerae,* but only serotypes 01 and 139 cause cholera. Following recommendations are made in the area of aquatic medicine and health management:

- It is strongly recommended that a Network Project on microbial Diseases of Fishes and Shellfishes is established.

- The proposed project may focus on two most important diseases *viz*., white spot syndrome disease of shrimp and white muscle disease of freshwater prawn. In addition, participating institutions may take up research on diseases that are locally important in the areas like disease surveillance and reporting, indigenous kits for rapid identification of microbial pathogens, vaccines and immunostimulants for improving host-defence, novel probiotics and bioremediations, cell lines from host species, disease-resistant broodstock through marker-assisted selection, aquaculture health products for the presence of exotic pathogens, exploratory research on exotic pathogens in molluscs and other aquatic species, DNA probes and monoclonal antibodies as bio-sensors to determine fish quality, techniques for rapid identification of pathogens in aquaculture systems and chemical pollutants by biofluorescence and bioluminescence in aquaculture systems.

1.5 Conservation Genetics

Due to habitat destruction, overexploitation and chemical and biological pollutants, several species of fishes are designated at the global level as endangered/threatened. As a major agricultural country, India uses annually 18 mt of NPK fertilisers, 0.55 mt pesticides, 0.125 mt synthetic detergents; at least 25 per cent of these manmade chemicals ultimately enter the sea through rivers. Large scale earth moving and clearance of forests have also led to an unprecedented sedimentation rates of major Indian rivers. Consequently, siltation rate of reservoirs is also many orders higher than that estimated at the time of construction. Hence, there is an urgent need for researches in conservation genetics. This aspect could be explored on the following lines:

1.5.1 Sperm Preservation

Strategies adopted for *in situ* conservation requiring legislation for the protection of habitats and germ plasm are of regional importance. Conversely, *ex situ* strategies like cloning and cryopreservation of germ plasm, involving scientific and technical development, have global significance. The National Bureau of Fish Genetic Resources, Lucknow has a sperm bank facility for only about a dozen fish species: *Catla catla, Labeo rohita, L. dussumieri, Cirrhina mrigala, C. carpio, O. mykiss, Salmo trutta, Tor putitora, T. khudree, Tenualosa ilisha* and *Horabagrus brachysoma* and protocols for other commercially important species need to be developed.

- Explorative research on protocols for year round production of adequate seedlings of (i) endangered species, (ii) eggs of commercially important *Epinephelus tauvina* and sperm of the bycatch E. *merra* and (iii) migratory species like the eel using eggs of suitable species.

1.5.2 Development and Preservation of Cell Line

Barring very few exceptions, development of fish cell lines in India is highly restrictive. Keeping that in front, there is an urgent need to develop and preserve cell lines for culturably important penaids, *Penaeus monodon, P indicus* and *Macrobrachium rosenbergii* and to diagnose viral diseases, and to screen and certify imported brooders and seedlings, and exportable shrimp commodities.

1.6 Inventory of Bioactive Compounds

Aquatic organisms can play an increasingly important role in biotechnology and medicine. They are rich sources of novel and structurally divergent compounds. Some of these bioactive compounds could serve as models for new drugs, antifouling compounds, morphogens, signal substances, pheromones, neurotransmitters and antifertility compounds. Hence, genetic control of the biosynthetic pathways of selected bioactive compounds, needs to be worked out. Studies are also warranted to determine whether the genes for biosynthesis can be sequenced and cloned for practical pharmaceutical applications. Thus, large quantities of the compound can be made without disturbing the organisms, from which they are isolated. Application of DNA technology may thus offer exciting opportunities for advancing science and technology associated with bioactive products from the aquatic environment. Following suggestions are made:

- Preparation of an inventory of anti-viral and anti-bacterial compounds with potential application in aquaculture.

- Isolation, purification and evaluation of heparin from nudibranchs and algae development of fermentation technology for production of enzymes and enrichment of aquafeeds using mangrove microbes.

- Development of enzymes from specified sources with higher activity.

2. Human Resource Development

The programmes of trading and research are weak and hardly match with those seen in some well established institutes. Hence, it is recommended that an adequately funded Centre for Excellence in Fisheries Biotechnology is established with specific mandates to (i) offer post-graduate course in Fisheries Biotechnology to train annually a dozen students, initially to meet the Indian requirement but later to benefit the Third World countries in Asia and Africa, (ii) organise advanced level (three months) mid-summer training in fishery biotechnology at the identified units to a dozen graduate students, selected through competitive examination, who will work on cloning, sequencing and gene expression, as well as cell and tissue culture and (iii) generate manpower with competence for DNA microarray technology, 2-D gel electrophoresis, sequencing and characterisation of genome and protein. In order to make most efficient use of existing manpower and infrastructure, it was recommended to encourage multidisciplinary programmes of research and development by promoting alliances among conventional universities, national laboratories, fisheries institutes and fisheries colleges.