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Biotechnological approaches in Fishery Management

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There had been tremendous advances in molecular technology, genomics and biotechnology over the last few decades and the scope of its application in fishery resource management and aquaculture is enormous. The oceans bestow immense resources for research and development and these needs to be conserved for long term sustainability. Fish is the major source of protein for over a billion people, and biotechnology offers a 'give and take management system' whereby biotechnological tools can be used for conservation of ocean resources as well as for sustainable exploitation and utilization of the resources. Responsible fishery management aims to bring about sustainability by adopting an ecosystem approach to fisheries. Biotechnological tools can be effectively utilized to bring about sustainability of the marine ecosystems in many different ways. Again, aquaculture contributes about 41% of the total fish production, (63.6 million tonnes of fish and shellfish annually, FAO, 2012), and is the world's fastest growing food production sector, technological challenge in the aquaculture production and sustainability could also be met through biotechnological innovations.

Marine fish stock assessment

Information about the stock structure of fish populations is very important in devising management strategies which will ensure responsible management of the fishery. Fish stocks are spatially or temporally discrete units which may have different biological characteristics. So if different stocks exist within a particular species, those stocks have to be managed separately. Stocks can also be assessed separately for their sustainability and based on this, sustainable fishing strategies can be devised. If a stock is overexploited, fishing or harvesting can be regulated for that particular stock. There are several methods by which stock structure studies could be carried out. Conventional methods rely on the morphology of fishes and this may be cumbersome and inaccurate. Molecular tools provide a fast and efficient method of stock discrimination.

DNA markers of various types are being used for discerning genetic stock structure of fishes. Mitochondrial genes like cytochrome c oxidase, cytochome b, control region (D-loop), ATPase 6/8 etc have been widely used for stock structure estimates. Mitochondrial genome is circular and double stranded and is maternally inherited. This reduces the effective population size for mitochondrial DNA and this reduced effective population size results in greater genetic differentiation between isolated gene pools which makes it an attractive marker for studying population specificity. It is present in several copies depending on cell type and so a small amount of tissue is needed for its effective amplification. Nuclear DNA markers like Random Amplified Polymorphic DNA and microsatellite and minisatellite DNA have also been successfully used for such studies. Nuclear DNA markers help more accurate delineation of stock structure as it is developed from genomic DNA. RAPD (Random Amplified Polymorphic DNA) markers are dominant markers and the analysis can be completed in a relatively short time. It does not need prior information about the genome of the organism and the protocol is also simple. Since these are dominant markers, the information that can be generated using RAPD markers is very less and this is a serious limitation of the RAPD technique. Microsatellite DNA markers are the most popular for studying genetic stock structure and genetic differentiation. They are repeated DNA sequences having lengths of 1-6 base pairs which are tandemly repeated several times at each locus. Microsatellites are co-dominant in nature and they are inherited in Mendelian fashion. Microsatellites provide information about the effective number of alleles at each locus. Simple sequence repeats or microsatellites have high evolvability which makes them ideal for identifying variations at the level of stocks. Since microsatellite loci are non-coding, the variations are independent of natural selection.

Molecular taxonomy & DNA Barcoding

Documenting the rich biodiversity of tropical seas ensures effective conservation of the natural resources. This information is also very much useful for assessing the community structure and spatio-temporal variations in species distribution. Some of the species may be threatened/endangered which needs to be catalogued accurately. Molecular tools can be used for accurate and effective identification of the species and beyond, including strains, stocks or hybrids.

DNA barcoding using a short sequence of the organism's gene, usually mitochondrial cytochrome c oxidase gene (Hebert et al. 2003) is the most widely practiced method for easy and accurate identification. It often serves as a supporting tool to conventional taxonomy. The Barcode of Life Data System (BoLD) provides an online interface which allows scientists and researchers to work together and share their sequences. Several bar coding projects are being carried out around the world which have already contributed several thousands of barcodes of commercially important fishes. CMFRI has generated partial DNA sequences of cytochrome c oxidase and control region of fin fishes like tunas, sardines, whale sharks, mackerel and shell fishes like oysters, mussels and cuttle fishes. CMFRI has also developed a sex determination technique among marine mammals based on genomic DNA extracted from skin tissues. In addition to accurate identification and molecular taxonomy, DNA barcodes also helps to diagnose the presence of invasive species and their spread in the ecosystem. This will help in efficient quarantine and eradication efforts. Identifying predator – prey interactions by tracking prey species, knowing the presence of pest or pathogen species present in the ecosystem, development of species specific markers and a number of other related research works could be initiated by using DNA barcodes. Species specific markers developed using barcodes can be used as a tool to detect the presence of eggs and larvae of the species. In the mariculture of mussels it is very important to know the timing and location of spat fall. Molecular markers could be effectively used in the forecasting of spat fall, by subjecting a plankton sample from the area of interest to species specific PCR. Species specific identification of larval stages of fishes which contribute to fishery can also be done using DNA based techniques.

Aquaculture biotechnology

Responsible fishery management can only be ensured by supplementing the marine fish production with aquaculture activities. Climate change and overfishing is causing uncertainties in the marine fishery scenario and aqua farming can be one of the viable alternatives. Aquaculture activities also should be sustainable and should not disturb the delicate balance of the ecosystem.

Improving the production performance of aquaculture systems can only be ensured by using superior germplasm. Application of biotechnology is emerging as a novel approach to augment aquaculture production wherein the whole spectrum of aquaculture, right from reproduction, larval rearing, nutrition, bioremediation, health management, to post harvest processing and bioprospecting is benefited. In the early days of carp farming, we have witnessed the control of reproductive cycles through hormone therapy and induced breeding, which revolutionized the Indian carp farming sector. Later on, the nineties witnessed the growth of the Indian shrimp farming industry, which during the last two decades has recorded both successes and failures. Biotechnological innovations and interventions in the areas of induced breeding, larval and growout feeds, DNA-based disease diagnosis and genetic improvement have helped the shrimp farming sector to tide over the adverse phases, thereby taking the industry forward.

Quantitative genetic tools can be effectively used for improving the quality of germplasm. Selective breeding by employing quantitative genetic principles has been employed to produce better brood stock of many commercially important fishes. Knowledge regarding heritability of the trait under consideration, phenotypic and genotypic correlations, heterosis and genotype-environment interactions is essential to plan a proper selective breeding strategy. Improved strains of *Artemia franciscana* have been produced with altered naupliar size in CMFRI using principles of quantitative genetics. Genetic improvement of freshwater carps has already been demonstrated, while similar programmes in brackishwater and marine aquaculture have to gain momentum in India.

Inbreeding and cross breeding strategies have also been used for the improvement of crops and livestock in India. Inbreeding is often applied along with cross breeding to produce superior offspring with hybrid vigor. Many combinations of inbreeding and cross breeding trials have to be conducted for the production of an offspring with hybrid vigor.

Chromosomal engineering is another alternative for the production of superior quality individuals showing higher growth and reproduction. Androgenesis, gynogenesis and ploidy manipulation have been practiced in aquaculture for production of individuals with higher growth and vigor. Androgenesis involves manipulation of reproductive process in such a way that only paternal genetic material is inherited. It will be helpful to produce viable YY supermales in the case of male heterogametic species and it has been successfully applied to produce YY supermales in cyprinids, cichlids and salmonids. Gynogenesis is opposite of androgenesis where only maternal genetic material is inherited into the progenies and thus all female populations of fishes are produced. Androgenesis and gynogenesis is helpful in fishes where males or females alone show superior growth rate and performance.

Ploidy manipulation techniques like induction of triploidy or tetraploidy have also been used mainly in shellfishes for better growth. CMFRI has successfully induced triploidy in edible oyster, *Crassostrea madrasensis*. Triploidy had also been induced in *C. gigas*, *C. virginica*, *Saccostrea glomerata* and *Ostrea edulis*.

Hormonal manipulation of sex and reproduction offer another viable alternative to the production of all male or all female populations. Synthetically produced analogues of hormones are being used for hormonal manipulation. In the classical induced breeding technique for freshwater carps, introduction of hormonal extract containing GnRH (the key regulator and trigger of reproductive cascade in all vertebrates) was done to induce the spawning in carps, and later these were replaced by synthetic hormones such as Ovaprim.

Cryopreservation of gametes and embryos in the cold environment is another biotechnological intervention for improving aquaculture processes. Cryopreservation ensures year round supply of fish gametes which can be efficiently utilized for controlled reproduction. This will avoid increased dependence on wild collected seeds. Some of the important candidate species of fishes like seabass and grouper are sequential hermaphrodites (seabass-protandrous; groupers-protogynous) and so it is difficult to get males or females from the wild for controlled hatchery production. In fishes, sperm cryopreservation is widely practiced whereas cryopreservation of ova and embryos is yet to be perfected due to some inherent disadvantages of fish eggs and embryo.

Genetic engineering tools can also be employed for the production of superior genetic stock. Transgenic fishes can be produced using genetic engineering methods for the production of fishes with faster growth, disease resistance and improved environmental tolerance. The gene of interest like antifreeze protein genes, growth hormone genes or fluorescent protein genes can be incorporated into the genome of the desired species of fish which will get expressed in the progeny. In India, preliminary success has been reported in developing gene transfer technology in zebra fish, medaka and Indian catfish. Genetically modified zebra fish (Glofish) which glow in the aquarium, modified to produce fluorescent pigments red, green and yellow has been very popular and is widely used as a household aquarium pet.

Marker assisted selection (MAS)

Marker assisted selection (MAS) is a selection process by which prospective breeders are chosen based on genotypes using molecular markers. Diverse types of molecular markers like allozymes, mitochondrial DNA, RFLP, RAPD, AFLP, microsatellite, SNPs and ESTs are being used for marker assisted selection programmes. Identification of genetic relatedness, genetic diversity, pedigree determination, molecular tagging, tracking family and population lines and strain identification are the potential application of markers. It also envisages identifying markers linked to quantitative trait loci or QTL.

Feed biotechnology and nutrition

Feed biotechnology and nutrition of cultured fishes is one of the most essential aspects of any aquaculture venture and fruitful biotechnological interventions can be made in nutritional research so as to improve the performance of candidate fishes. Enzymes can be incorporated into feeds so as to increase the availability of nutrients in formulated feeds. These enzymes should be able to withstand variations in physico-chemical parameters like increased temperature conditions and they also should have a long shelf life. Phytase is one such enzyme which can be incorporated into feeds which will help in breaking down indigestible phytic acid in plant based nutrient sources and thus it will help in the release of digestible phosphorous.

Incorporation of probiotic bacteria into formulated feeds is another application of feed biotechnology to improve the disease resistance in cultured fishes. Probiotics are live microorganisms which when incorporated into diets confer some kind of a health benefit on the host. It is based on the principle of competitive exclusion where they exclude pathogenic bacteria competitively. These bacteria also release enzymes which will help in the digestion of food. The common organisms in probiotic products are *Aspergillus oryzae*, *Lactobacillus acidophilus*, *L. bulgaricus*, *L. planetarium*, *Bifidobacterium bifidium* and *Saccharomyces cerevisiae*.

Prebiotics are feed for probiotic organisms and they are resistant to attack by endogenous enzymes and consequently they allow the proliferation of gut microflora. Prebiotics can withstand high pelletizing temperatures in the feed and have a long shelf life. Supplementing dietary amino acids using genetically enhanced microorganisms have also been tried to increase the quality of feed. Essential amino acids like lysine and methionine have also been incorporated into feed as such to improve feed quality. Recent innovations in feed biotechnology include incorporation of nucleotides into feed as feed additives and thereby increasing the expression of some desired traits like growth or disease resistance. Nutrigenomics involves the application of functional genomics principles into nutrition research whereby the influence of nutrients on an organism can be studied at molecular levels.

Fish Health

Sustainability and economic viability of aquaculture ventures could only be ensured if adequate health management measures are taken at the right time. Disease diagnosis using conventional methods is time consuming and its specificity, sensitivity and speed is very limited. Disease diagnosis using biotechnological tools is accurate and efficient and valuable decisions could be taken at an appropriate time for devising optimal management strategies. Finfish and shellfish aquaculture is affected by a number of viral, bacterial and fungal diseases. White spot viral disease of the shrimp *P. monodon* has been a major threat to shrimp aquaculture and research institutions like CMFRI and CIBA have developed kits for the early detection of white spot virus which was a boon to aquafarmers.

Administration of DNA vaccines is another kind of biotechnological intervention that can be made in disease prevention and management. DNA vaccines are made from the DNA of the infectious organism and this can be introduced into the host DNA so that it gets expressed.

Pathogenic bacterial infections can be treated using phage therapy wherein lytic bacteriophages are used. A phage virus is used to infect and kill the bacteria and the phage should not interact with the surrounding tissue or with other harmless bacteria. Since the virus replicates quickly, a single, small dose is sufficient. Research related to the use of lytic bacteriophages for the treatment of *Vibrio* related infections are underway at Mangalore Fisheries College, National centre for aquatic animal health (NCAAH), Cochin and CIBA, Chennai.

RNA interference (RNAi) is a kind of RNA-guided regulation of gene expression in eukaryotic cells. Short chains of double stranded ribonucleic acid (dsRNA) present in the cell will interfere with the expression of genes having sequences complementary to this dsRNA. RNA interference is a kind of post transcriptional silencing of genes where dsRNA binds to specific mRNA and induce degradation of the homologous endogenous transcript, which causes reduction or loss of gene activity. RNAi is a promising tool for the management of viral diseases.

Genetically modified organisms with altered genetic material can be produced using recombinant DNA technology. Research efforts are being carried out to produce transgenic fishes with disease resistance.

Marine Bioprospecting

Marine bio prospecting is an emerging area where new sources of chemical compounds, genes, micro-organisms and other valuable products are explored from the sea. Biotechnological tools can be used in a sustainable way for making use of these biological resources and it also can be applied for the socio-economic development of the local communities. Marine organisms are potential sources of pharamaceuticals, enzymes, cryoprotectants, cosmaceuticals, and neutraceuticals. Novel drugs can also be extracted from marine organisms which may offer a substitute to antibiotics. Anti-inflammatory agents and anti-cancer agents can also be extracted from the secondary metabolites of marine bacteria and invertebrates.

Contributions of CMFRI to marine biotechnological research

Application of biotechnology in fisheries and aquaculture in India is in its developmental phase, and CMFRI has been the pioneer in many areas of marine biotechnological applications. Biotechnological innovations have left its footprint in many of the recent success by CMFRI - in the breeding of Cobia and silver pompano, hatchery rearing of clown fishes, development of the 'Varna' range of formulated feeds for ornamentals and in the development and commercialization of nutraceuticals from green mussel and seaweeds. Various other areas related to aquaculture such as value added products, novel processing methods and food safety are also benefitted by novel biotechnological methods.

The major contributions of CMFRI include;

- Selective breeding of native *Artemia* strains and production of a strain with altered naupliar size
- Production of triploid oysters
- Use of molecular taxonomical tools for stock structure studies of Indian oil sardine, Indian mackerel, threadfin breams, Bombay duck, groupers, clown fishes and sergeant major.

- DNA bar coding of several species of marine mammals, tunas, cuttlefishes, bombay duck and shrimps
- Technology for *in-vitro* pearl production
- Development of molecular diagnostic tools for white spot virus and *monodon* baculo virus (MBV)
- Production of low cost feeds for crabs and ornamental fishes
- Use of enzymes as feed additives
- Development of probiotics
- Neutraceuticals from Green mussel and Green algae

Conclusion

India is endowed with a coast line of about 7500 kms, an Exclusive Economic Zone (EEZ) of about 2 million sq. kms and vast brackishwater water resources of about 1.9 million ha, offering great opportunities to harness these aquatic resources using the science of aquaculture biotechnology. Biotechnology being a multidisciplinary system, requires the integration of scientific advances in biology, chemical sciences, material sciences and engineering. Aquaculture Biotechnology is an emerging and evolving area where marine resources are utilized for the development of novel applications, products and quality foods for the benefit of mankind. In India, aquaculture biotechnology is in its initial phase of development, and a concerted effort involving players from both Government and Private sectors under an umbrella or network of Aquaculture Biotechnology could bringout meaningful outcomes in the areas of food production, value added products, creation of employment and providing alternative livelihood.

Suggested readings

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