Genetic Markers in Aquaculture

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Abstract: The aquaculture production throughout the world has reached a plateau in recent years and one of the solutions to this problem can be application of genomic tools in this sector. Genomic tools are already used in research on commercially important fish and shellfish species. Thousands of expressed sequence tags (EST) are now available for some of these species, and the sequencing of complete genomes of tilapia, cod, salmonids, flatfishes, sea bass and Pacific oyster has been proposed. This paper reviews some of the available genomic technologies, especially with special emphasis on genetic markers, their classification and application in aquaculture in Indian context. The potential beneficial application of Marker assisted selection (MAS) were highlighted along with its implications for fisheries management and aquaculture.

Key word: Genetic marker, RAPD, DNA Barcode, Marker Assisted Selection (MAS)

Introduction:

The aquaculture industry has expanded to a great extent in Asia, especially in India, from where aquaculture products are also exported world-wide. However, it is well recognised that fisheries catches have reached a plateau in recent years. Genomics tools combined with the already well-established aquaculture and fisheries management practices can serve as a novel framework for sustainable development in this sector. Genomics is a field of science that deals with the structure, function and evolution of genomes. Genomics often simply implies the use of high throughput DNA or RNA-based methods. Sequencing of genomes facilitates the development of a variety of DNA-based genetic markers that can be used for the management of wild and cultured populations.

Establishment of molecular genetic markers is one of the pre-requisite for stock structure analysis (Hatanaka and Galetti, 2003; Almeida *et al*., 2003). Based on their mode of transmission and evolutionary dynamics, molecular markers can be categorized into i) Protein markers such as general proteins and allozymes and ii) DNA markers such as mitochondrial, DNA, chloroplast DNA and nuclear DNA markers such as RAPDs and VNTRs. All organisms are subject to mutation ultimately leading to genetic variation. For this variation to be useful to geneticists, it must be heritable and discernable to the researcher. It is a gene with a known location or clear phenotypic expression that is detected by analytical methods or an identifiable DNA sequence that facilitates the study of inheritance of a trait or a gene. The markers must either be readily identifiable in the phenotype, (for instance by controlling an easily observable feature) or by being readily detectable by molecular means (for instance, microsatellite marker) (Williamson, 2001; Zaid *et al*., 1999).

Molecular Genetic Markers as Identification Tool for Early Life History Stages:

Genetic markers derived from polymerase chain reaction (PCR) amplification of genomic DNA are an important toolkit for conservation biologists since it helps to identify appropriate fish stock. Early life history stages play crucial role in fish conservation works. Proper identification tool for early life history stages of fish species was of extreme importance for conservation biologists, since, i) Most of the target fish species have a similar breeding ground and monsoon being the breeding season; ii) most of the artificial propagation research efforts and limited culture activity are dependent upon fish stocked from natural collections; iii) extermination of juveniles of numerous non-target fishes due to lack of proper identification, and iv) the differential market potential of various fishes keeping in mind the socio-economic aspect of conservation.

Moreover, the task of routine species identification based on morphological and meristic features has four significant limitations, i) incorrect identification of species due to phenotypic plasticity and genetic variability in the characters involved (Hebert *et al*., 2003); ii) inability to recognize morphologically cryptic taxa (Knowlton 1993; Jarman and Elliott 2000; iii) keys designed for a particular life history stage or gender, might not be effective for the other; iv) misdiagnoses due to lack of expertise and the dwindling pool of taxonomists signal the need for a new approach to taxon recognition (Hebert *et al.,* 2003). Therefore, formulation of precise identification manual for valued fish species through molecular markers is of extreme importance. However, progress is limited by availability of useful genes or identified markers that are linked to important traits. Thus, identification of sufficient numbers of molecular markers is critically important for gene mapping, for marker assisted selection (MAS) and for eventual cloning of

beneficial genes from different fish species (Liu *et al*., 1999). Molecular markers are used extensively for detection of interspecific genetic divergence, establish species specific markers, phylogenetic relationships and resolve taxonomic ambiguities. This provides precise identification of evolutionary significant units important for conservation and management of natural fish population (Lal *et al*., 2006). The markers can detect genetic variation which can be explained within the limits of genetic principles.

Type of Molecular Genetic Marker Available:

Genetic markers are classified into two categories: type I and type II. Type I are markers associated with genes of known function, while type II markers are associated with anonymous genetic segments. According to this classification, allozyme, EST, barcodes of species based on Cytochrome c oxidase I (COI) and most RFLP markers are type I markers where as RAPD, AFLP, Microsatellite markers are examples of type II markers. The significance of type I are increasing day by day in studies of genetic linkage, comparative genomics, genome evolution, candidate gene identification and enhanced communication among laboratories. These serve as bridge for comparison and transfer of genomic information from a map-rich species (like human, mouse, zebra fish) into a relatively map poor species (such as most of our indigenous fish species). Curently, large insert bacterial artificial chromosome (BAC) libraries are available for several fish species, like channel cat fish(Quiniou et al., 2003), Tilapia (Katagiri et al., 2001), Atlantic salmon (http://bacpac.chori.org/salmon214.htm) and rainbow trou (Thorgaard et al., 2002). Type II markers also have proven its importance in species, strain and hybrid identification, in breeding studies (Yoon and Kim, 2001; Holsinger *et al*., 2002), management for sustainable yield and preservation of genetic diversity (Dinesh *et al*., 1993; Garcia and Benzie, 1995; Tassanakajon *et al*., 1997, 1998), population genetics and as markers linked to QTL.

Application of Type I and II markers in Indian Perspective:

The significance of molecular markers was not fully appreciated in the early stages of aquaculture genetics in India. However, molecular genetic studies are gaining momentum today. In this discussion, emphasis was given on application of RAPD and Barcodes as an example of type II and type I marker respectively in Indian aquaculture sector. The RAPD markers have been successfully used in genetic diversity studies in the past for several species by many (Govindaraju and Jayasankar 2004; Das *et al*., 2005). National Bureau of Fish Genetic Research (NBFGR) has analyzed population structure of *Lactarius lactarius* from east and west coast of India using RAPD, allozyme and TRUSS analysis. Species specific mitochondrial ARFLP and allozyme pattern for *Clarius batrachus,* exotic *C. gariepinus* and *C. macrocephalus* has also been developed by NBFGR. Barman *et al*. (2003) used RAPD assay as a source of genetic markers to generate species-specific RAPD profiles for four species of Indian carp and to estimate genetic variation among them. Jayasankar and Dharmalingam (1997) studied potential application of RAPD and RAHM markers in genome analysis of scombroid fishes with 35 arbitrary primers. Jayasankar *et al*. (2004) combined Truss morphometry, protein polymorphism and RAPD analysis, i.e. one phenotypic and two genotypic methods to determine stock relationships among Indian Mackrel off east and west coast of India.

RAPD was employed to discriminate different populations of commercially important anadromous *Tenulosa ilisha* by Dahle *et al*. (1998), Shifat *et al*., 2003 and Brahmane *et al*. (2006). RAPD fingerprinting of ornamental aquarium fish *Badis badis* and *Dario dario* has been deviced by Brahmane *et al*. (2006) with the help of seven arbitrary oligodecamer primers to calculate inter specific as well as intraspecific genetic similarity. Genetic divergence in two featherback fishes (*Chitala chitala* and *Notopterus notopterus*) has been studied by Lal *et al*., (2006) by allozyme and RAPD loci to determine species specific markers and genetic relatedness. They have identified 20 and 31 species specific RAPD fragments of *Chitala chitala* and *Notopterus notopterus* respectively and indicated a large genetic divergence between the two species. Species specific bands in five Indian sciaenid species through eight random primers have been generated by RAPD (Lakra *et al*., 2007). Phylogenetic relationship between eight commercially important indigenous fishes were analysed using RAPD markers by Sengupta and Homechaudhuri, 2012.

On the other hand, DNA based approaches to solve taxonomic ambiguities through DNA barcoding is an emerging global standard today. As a consequence, it will make the Linnaean taxonomic system more accessible, with benefits to Aquaculture practitioners ecologists, conservationists, and the diversity of agencies charged with the control of pests, invasive species, and food safety. However, very little has been done in India for generation of barcodes of our valued fish fauna. A major national programme on DNA barcoding of fish and marine life was initiated in India by the scientists of National Bureau of Fish genetic Resources (NBFGR) during 2006. 115 marine fish species covering Carangids, Clupeids, Scombrids, Groupers, Sciaenids, Silverbellies, Mullids, Polynemids and Silurids representing 79 Genera and 37 Families from the Indian Ocean have been barcoded for the first time using cytochrome c oxidase I gene (COI) of the mtDNA. Many indigenous freshwater fish species were also barcoded by the scientists of NBFGR and a few by Sengupta and Homechaudhuri, 2013 .

Application of Marker assisted selection (MAS) in Fisheries sector:

Marker assisted selection (MAS) refers to a selection procedure which is improved using information from genetic markers. Allelic variation in genetic markers can be linked to the variation in traits of economic interest, and thus the marker provides DNA level information on the inheritance of the traits. The practical use of markers in selection can be roughly divided into three classes: 1) removing genetic disorders, 2) marker breeding value-selection, and 3) genomic selection. Recessive genetic disorders, determined by a simple Mendelian one-locus method can be effectively removed from a population using a gene test done on a small tissues sample. Individuals carrying a deleterious allele are culled, and no computationally demanding selection tools are needed. Such tests are in practical use in terrestrial farm animals (e.g. Sironen et al. 2006). Marker breeding values of individuals can be estimated by combining information on phenotypes and a single or several QTL segregating within a pedigreed population (Fernando and Grossman 1989). Genomic selection refers to selection directed on allelic variation identified across the whole genome. The use of SNP analysis is the most promising method for such whole-genome analysis. Using current technology, variation in tens of thousands of SNP can be simultaneously estimated. For this method to be effective however, Hayes et al. (2006a) suggested that 10–20 QTL need to be found for each trait and up to 30 000 SNP may be needed to obtain a dense enough marker map. Thousands of putative SNP have been detected in Atlantic salmon (Hayes *et al*. 2007). Sauvage *et al*. (2007) reported a very high level of DNA polymorphism in the Pacific oyster (i.e. one SNP every 60 bp in coding regions and one every 40 bp in non-coding regions). However, there is hardly any data available for utilisation of MAS in Indian aquaculture sector.

Conclusion:

At the end of this length discussion, it can be concluded that the major chunk of the work on identification and analysis of fish molecular markers in Indian scenario were performed by the scientists of NBFGR and some other scientists. However, there is paucity of described molecular markers and MAS in case of Indian fishes. Therefore, the study pinpoints the requirement of developing new molecular markers and crosscheck existing ones with whatever data available with an aim to the following:

1. The implementation of genomic approaches should be encouraged in the fields of fisheries and aquaculture by supporting the development of genomic resources, such as BAC libraries, fine scale linkage maps, EST and barcode databases and expression profiling.
2. Studies of local adaptations in the wild and hatchery populations should incorporate genomic approaches to further understand the footprints of selection at a genome wide level.
3. The potential of molecular marker assisted selection (MAS) and the domestication process in aquaculture species should be further explored, benefiting from the development of new genomic resources and computational and analytical tools.
4. SNP data provide attractive possibilities for genomic selection. Genomic methods for SNP data analysis need further development.
5. Alternative strategies of using genomic information in broodstock selection need to be assessed, and their cost effectiveness needs to be evaluated in Indian perspective.

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