Genetic Diversity Analysis of *Lates calcarifer* (Bloch 1790) in Captive and Wild Populations Using RAPD Markers

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Abstract

*Lates calcarifer* (Bloch 1790) is one of the major economically important cultivable fish species in India. In this study, three populations of *L. calcarifer* was selected to assess the genetic diversity. Of which, two wild (Mudaslodai, Muthupettra) and one captive (Mutukadu) population. The genetic diversity of three populations of this species was studied using Random Amplified Polymorphic DNA (RAPD) markers. Ten random primers were used for the assessment of their genetic diversity and construction of the dendrogram. A total of 589 scorable bands were obtained, 93.12% of them were polymorphic. The Nei’s gene diversity (H) of two wild populations were more (0.0504 ± 0.0670 and 0.0519 ± 0.0953) than the captive population (0.0489 ± 0.0850). The clustering pattern obtained by UPGMA method emphasized the wild populations were clustered in one clade and captive population was deviated into another clade. This study proved that RAPD analysis has the ability to discriminate *L. calcarifer* populations. Further molecular studies, comprising a higher number of molecular tools are still required to precisely evaluate the genetic structure of all seabass populations along the Indian coast.

Keywords: captive and wild populations, genetic diversity, *Lates calcarifer*, polymorphism, RAPD markers

Introduction

*Lates calcarifer* (Bloch 1790) is commonly known as Asian seabass in Asia and barramundi in Australia. This species has been successfully farmed in several Asian countries like Thailand, Singapore, Malaysia, Indonesia, Taiwan, Brunei, Hong Kong, China, Saudi Arabia and Australia (Chou and Lee, 1997; Zhu *et al*., 2006a). Currently, seabass is an increasingly important tropical aquaculture species in Asia-Pacific region and it is inevitable to commencement of various breeding programmes for this species. (Wang *et al*., 2008). Knowledge of genetic variation in seabass is important for the construction of appropriate breeding programs and for a good management of these species. Generally, small effective population size and unmonitored selective breeding programs are the major causes for the loss of genetic diversity in cultured species (Hansen *et al*., 2001).

A number of studies reported that cultured fish stocks showed lower genetic diversity than wild populations (Norris *et al*., 1999). Understanding the genetic diversity of wild populations is needed for setting up a founder population with high allelic and gene diversity, and in turn that is the first step toward successful selection of a hatchery (Norris *et al*., 1999). Regarding seabass, there is limited number of works have been done in genetic variation in India and worldwide. In particular, the study about genetic relationship of Australian populations (*L. calcarifer*) with mitochondrial DNA polymorphism (Chenoweth *et al*., 1998; Doupe *et al*., 1999), and only one broodstock in Singapore which was analyzed with eight micosatellite markers (Yue *et al*., 2002). Availability of more such genetic data could be very useful for stock management, selective breeding programs and sustainable use of wild resources (Kim *et al*., 2004; Liu and Cordes, 2004; Norris *et al*., 1999).

Nowadays, many molecular methods are available for studying various aspects of wild populations, captive broodstocks and interactions between wild and cultured fish stocks (Yue *et al*., 2009). The choice of markers for particular applications is not also straightforward and mostly depends on the experience of the investigators, laboratory facilities and available fund. RAPD is a random amplification of anonymous loci by PCR, has several advantages and has been quite widely employed in fisheries studies. The method is simple, rapid and cheap, only a small amount of DNA is required no need for molecular hybridization and most importantly, no prior knowledge of the genetic make-up of the organism in question is required (Hadrys *et al*., 1992). It is a simple and rapid technique used for population genetic studies (Hardys *et al*., 1992) to determine genetic diversity and to identify useful genetic markers at different taxonomic levels of various marine organisms (Heipel *et al*., 1998; Klinbunga *et al*., 2000; Tassanakajon *et al*., 1998). RAPDs have gained considerable attention particularly in population genetics (Lu and Rank, 1996), species and subspecies identification (Bardaki and Skibinski, 1994), phylogenetics, linkage...
Materials and methods

Sample collection and DNA extraction

Seabass fin clips were collected from three stations namely Mudasalodai, Muthupettai (Wild) and Muttukadu (Captive) in southeast coast of India (Fig. 1). Fifteen samples were collected from each site and the tissue samples were stored in 95% ethanol. Genomic DNA was extracted from stored fin clips by the standard protocol (Sambrook et al., 1989). DNA samples from individuals of each species was diluted to about 25 ng/μl with deionized distilled water and used for PCR amplification.

Polymerase Chain Reaction

Ten commercially available decamer random primers (An1-An10) (Tab. 1) from Chromous Biotech Pvt Ltd (Bangalore, India) were used for this study. The amplification reaction was carried out in a 25 μl reaction volume containing 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 2.5 mM MgCl₂, 0.001% gelatin, 100 μM each of dATP, dCTP, dGTP, and dTTP, 0.2 μM of each primer, 1 U of Taq DNA polymerase (Bangalore Genei, India) and 25 ng of genomic DNA. RAPD-PCR was performed in a thermocycler (Lark, India) for 40 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 35°C for 30

Tab. 1. The details of the RAPD primer used in this study

| Sl. No | Primer name | Sequence      | Ta (°C) |
|--------|-------------|---------------|---------|
| 1      | An1         | GATGACCGCC    | 35      |
| 2      | An2         | GCCACCATTC    | 35      |
| 3      | An3         | GGTGGCGCCTT   | 35      |
| 4      | An4         | GTGCAGGTCT    | 35      |
| 5      | An5         | GGCATGACCCT   | 35      |
| 6      | An6         | GTGCCCGTCA    | 35      |
| 7      | An7         | TCCCCACGCAG   | 35      |
| 8      | An8         | TGACCGGACA    | 35      |
| 9      | An9         | TGCGCTCCAT    | 35      |
| 10     | An10        | TGGTGCTGGTC   | 35      |

seconds, and extension at 72°C for 60 seconds. The final extension was carried out at the same temperature for 5 minutes. The resulting products were electrophoretically analyzed through 1.5% agarose gels, stained with ethidium bromide, and visualized using a UV transilluminator. Subsequently the gel was photographed using a gel documentation system (Lark, India).

Data analysis

Sizes of RAPD bands were determined by comparison with a 100 bp ladder and genetic similarity/distance between the three species was estimated using PopGene software (Version 1.31, Yeh et al., 1999). Nei and Li’s (1979) genetic similarity (GS) among the three populations was computed and converted by PopGene into genetic distance (GD), according to Hillis and Mortiz’s (1990) formula, GD = 1-GS. The GS reflects the proportion of the bands shared between individuals and values range from (1) when present, to (0) when absent. Phylogenetic relationship based on genetic distance values generated from RAPD data among three populations were made and dendrogram was plotted, using UPGMA method (Sneath and Sokal, 1973).
Results and discussion

Totally, 589 bands were scored by using 10 random primers in three seabass populations. Among these three populations, Muthupettai showed high number of bands (294) than the counterpart wild population, Mudasalodai (150), whereas, the captive population (Muttukadu) showed 145 bands. Number and size of the fragments produced by ten RAPD primers and molecular weight of the bands in three populations of *L. calcarifer* was given in Tab. 2. Although there was some variation observed between individuals within population but most of the bands were less variable among individuals in a population.

The genetic distance between Mudasalodai (wild) and Muttukadu (captive) was higher (0.0097) than Muthupettai (0.0063), the wild population (Tab. 3). The genetic distance between Muttukadu and Muthupettai was 0.0077. The genetic identity was more (0.9937) between Mudasalodai and Muthupettai (0.9923), the captive population. The overall observed and expected polymorphic loci of three populations of *L. calcarifer* are given in Tab. 4. The genetic diversity (H) of two wild populations were more (0.0504 ± 0.0670 and 0.0519 ± 0.0953) than the captive population (0.0489 ± 0.0850). The UPGMA tree (Fig. 2) grouped the three populations into separate clusters emphasizing the distinct population status of Mudasalodai, Muthupettai and Muttukadu. Among these three populations, Mudasalodai and Muthupettai (wild) were closely related than Muttukadu (captive).

This is the first report to assess genetic variation in both wild and captive *L. calcarifer* populations of India. This present study revealed 589 polymorphic loci, indicating high level of polymorphism in three populations and supporting the suitability of RAPD markers for effectively discriminating different populations. Also this study compared the genetic variation between wild stocks and hatchery bred individuals, which might assist in formulating good management practices for increased aquaculture production.

During this investigation, the RAPD profile showed 93.12% polymorphic loci among the three populations. As compared to previous studies, 75% of polymorphic loci were reported in three *Catla catla* populations (Islam *et al.*, 2005). Barman *et al.* (2003) reported 45% of polymorphic loci in four Indian major carp population using RAPD primers. Islam and Alam (2004) observed 46.5% of polymorphic loci in four different *Labeo rohita* population. In the present study, relatively high level of genetic polymorphism was observed due to the small sampling size.

While considering the Nei’s gene diversity (H), it was high (0.0504 ± 0.0670 and 0.0519 ± 0.0953) in wild populations compared to the captive population (0.0489 ± 0.0850). This result also coincides with previous study revealed by microsatellite marker, reported that cultured...
Tab. 4. Overall observed number of alleles (Na), Effective number of alleles (Ne), gene diversity (H), Shannon's Information index (I), Number of polymorphic loci (Np) and Percentage of polymorphic loci (Pp) in three populations of Lates calcarifer

| Populations     | Na        | Ne        | H          | I          | Np | Pp (%) |
|-----------------|-----------|-----------|------------|------------|----|--------|
| Muthuppetai (wild) | 1.5008 ± 0.5004 | 1.0594 ± 0.0915 | 0.0504 ± 0.0670 | 0.1009 ± 0.1191 | 331 | 50.08  |
| Mudasalodai (wild) | 1.2481 ± 0.4322 | 1.0680 ± 0.1343 | 0.0519 ± 0.0953 | 0.0898 ± 0.1604 | 164 | 20.01  |
| Muttukadu (captive) | 1.3041 ± 0.4604 | 1.0621 ± 0.1212 | 0.0489 ± 0.0850 | 0.0890 ± 0.1452 | 201 | 23.03  |
| Overall         | 2.0000 ± 0.0000 | 1.0599 ± 0.0536 | 0.0546 ± 0.0390 | 0.1239 ± 0.0624 | -  | -      |

Fish stocks of L. calcarifer showed lower genetic diversity than wild populations. Frost et al. (2006) illustrate that hatchery management practices have the potential to significantly impact on the retention of genetic diversity in this species. Other results also reveal high levels of polymorphism in Atlantic salmon using microsatellite markers in which farmed salmon showed less genetic variability than wild salmon in terms of allelic diversity but not necessarily in terms of overall heterozygosity (Norris et al., 1999). This reduction may be the effect of small effective population size, low genetic variation of founder populations, and low reproductive success of some founders. Nei’s genetic identities in wild populations were high (0.9937) as compared with captive (0.9903 between Muttukadu and Mudasalodai; 0.9923 between Muttukadu and Muthuppetai). Nei’s genetic distance was also low (0.0063) between wild populations compared to distance between wild and captive populations (0.0077 between Muttukadu and Muthuppetai; 0.0097 between Muttukadu and Mudasalodai). This result coincides with the previous study in which farmed population were most similar when compared to wild populations of Atlantic salmon, Salmo salar (Norris et al., 1999). Xu et al. (2001) also observed same type of result in the black tiger shrimp, Penaeus monodon, where the genetic diversity was more in Philippines wild populations than captive population and the genetic distance was also more between the wild populations than the captive one.

The clustering pattern obtained by UPGMA method wherein wild populations were clustered in one clade and captive population was separated in another clade. The similar clustering pattern was obtained with other RAPD profiling analysis study on three wild populations of Catla carp, Catla catla and one hatchery reared population of Bangladesh which form separate clusters for wild and hatchery reared carps (Khan et al., 2009). In Brycon opalinus, the Brazilian freshwater fish species, Hilsdorf et al. (2002) showed the genetic diversity was more than the captive populations using mitochondrial markers. The dendrogram analyses of microsatellite studies on Brycon opalinus showed a regional structure grouping individuals of closer regions (Barroso et al., 2005). They also reported that all six wild populations and one captive population was deviated significantly to HWE showing deficit of heterozygote and they suggested that it was due to anthropogenic habitat deterioration and fragmentation.

Conclusions

Despite some limitations, the RAPD analysis can be used effectively for initial assessment of genetic variation among fish species, particularly like economically important cultivable fishes. The present study represents a first step towards the generation of RAPD markers for population identification and assessment of genetic diversity of captive and wild populations of seabass. Further molecular studies, comprising a higher number of molecular tools are still required to precisely evaluate the genetic structure of seabass along the Indian coast.

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