ESTERASE PROFILE IN DISTINGUISHING INBREEDS F₁ AND BACKCROSS HYBRIDS IN INDIAN MAJOR CARPS

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Esterase was surveyed in four different tissues namely skeletal muscle, heart, liver and kidney obtained from two Indian major carps viz., rohu (Labeo rohita) and catla (Catla catla) pair crossed F₁ hybrids of catla (♂) x rohu (♀), and back crossed hybrid progenies of (catla x rohu) (♂) x catla (♀) (BC₁C) and of (catla x rohu) (♂) x rohu (♀) (BC₁R). The enzyme was found to be highly polymorphic in different tissues of the inbred as well as in hybrids. Detailed scanning of different activity zones in the zymograms revealed that amidst 15 bands, band numbers 4 and 12 of liver and kidney tissues were found to be common in both the inbreds and hybrids were probably characteristics bands to these two species and their hybrid progenies. Band 4 of rohu and 6/7 of catla were diagnostic bands of pure rohu and catla, respectively, regardless of the nature of the tissue. However, both the bands were present in F₁ hybrid and BC₁C backcross progenies only. Another backcross i.e. BC₁R could not manifest shared bandings of the parents perhaps because of cleavage into or fusion with other sister esterase isomorphs through homo- or hetero-polymerization. Only band 4 was found to be present across all the tissues in BC₁R renders its demarcation difficult from rohu inbreed. However, band 9 and 13 of liver in BC₁R can distinguish it from F₁ and inbreeds. They are likely to be instrumental in distinguishing inbreds from hybrids reliably. The mean pair wise similarity (S) based on esterase profile, between the parents (catla and rohu) and the developed progenies (F₁ hybrids, BC₁C and BC₁R) showed BC₁C was genetically more diverse than the F₁ hybrids and BC₁R for esterase which may be used in carp breeding and improvement programme if it is found to be tightly linked with any characters of economic importance.

INTRODUCTION

Essentiality of an appropriate marker system to distinguish inbreds from hybrids in Indian major carps has long been felt. This is required mainly to pinpoint pure stocks for breeding and culture as well as to study genetics of yield and yield attributing traits for genetic improvement scientifically. Inter-specific hybridization among fishes is more frequent than other vertebrates (Campton and Utter, 1985; Hammer et al., 1991). It is mentionable that congeneric species often produce progenies which remain fertile in subsequent generations (Checassus, 1979; Campton, 1991). Occurrence of natural inter-
generic hybrids was also reported (Elo et al., 1997) earlier. Fertile offspring pose both problems and opportunities to fish farming. Unintentional hybridization of wild and introduced populations or species or genera is a major concern in fishery management (Campton, 1991). On the other hand viable hybrid progenies can be utilized in genetic manipulations of cultured fish (Checassus, 1979; Campton, 1991).

The extensive cultivation of Indian major carps obviously increases the possibility of escape of fish with potential threat to the genetic purity of various wild populations, as available in different geographical regions of the country. This might have far reaching genetic and ecological consequences. Increased anonymous hybridizations may jeopardize the maintenance of pure hatchery brood stocks in cases where wild spawners are needed to establish new stocks or to maintain long-term fish farming.

To detect interstrain, specific and generic hybridization, morphotyping and metric trait analysis of the progenies were amply used. Isozymes and very recently molecular gene markers like RFLP (Restriction Fragment Length Polymorphism), RAPD (Randomly Amplified Polymorphic DNA), ISA (Inter Simple Sequence Repeat Amplification), SCAR (Sequence Characterized Amplified Regions), AFLP (Amplified Fragment Length Polymorphism), ALP (Amplicon Length Polymorphism) and micro satellite amplification (Wolfus, 1997) are being in use and emerging as more powerful tools. It is mentionable that isozymes are multiple molecular forms of enzymes (Markert and Moller, 1959) with same substrate specificity and different electrophoretic mobility that catalyze same biochemical reaction. They act to endow the organism with greater metabolic flexibility, versatility and precision (Markert, 1959) and have distinct advantages over morphological markers, polymorphic proteins and cellular antigens. Isozymes were used in determining population genetic structure (Shaw, 1965), gene banking maintenance and monitoring of hatchery brood stocks, monitoring and evaluation of stocks of natural habitats as well as in detection of genetic introgression (Ponniah et al., 1994). Electrophoretic pattern of enzymes reveals the overall genetic variation and can be used to examine the genetic relationship between two or more forms. However, in fishes isozyme studies are scanty (Agrell and Kjelberg, 1965; Kossing, 1973; O'Rourke 1974; Fisher et al., 1980; Kirpichnikov 1981; Basaglia 1989), especially in Indian fishes (Krishnaraja and Rege, 1977, 1979; Chaterjee and Dhar, 1985; Sarangi and Mandal, 1996). Keeping all these in backdrop and based on information obtained from our earlier studies (Sarangi and Mandal, 1996) on prevalence of esterase polymorphism in Indian major carp, attempt was made to employ esterase profile in distinguishing inbreed stocks of rohu (Labeo rohita) and catla (Catla catla) from their paired F1 hybrids and backcrosses as well as to study the genetic relationships among them, which are detailed below.
MATERIAL AND METHODS

Fresh tissue samples of skeletal muscles, heart, liver and kidney were obtained from pair crossed F₁ hybrids involving catla (♀) x rohu (♂), back crossed hybrid progenies of (catla x rohu) (♀) x catla (♂) and (catla x rohu) (♀) x rohu (♂) (hereinafter will be referred as BC₁C and BC₁R, respectively and parent rohu and catla were employed for esterase analysis. The F₁ hybrids and backcrossed progenies were developed in the Institute’s farm of Central Agricultural Research Institute, Port Blair (Lat. 11°41’13.04” N., Long 92°43’30.16” E), Andaman, India following standard procedure of induced breeding under controlled indoor hatchery system. The stocks were reared under the same condition of a pond but maintained in isolation by using separate cages. Tissue samples from hybrids (F₁), backcrossed progenies and parents of same age group were collected at 40-45 mm length stage. Individual tissues like skeletal muscle, heart, liver and kidney were dissected out immediately after sacrificing the live specimens. Thirty individual samples in each group were used in preparing tissue lysates. Separated tissues from individual fish specimens were immediately homogenized in small blenders separately in tissue homogenizing buffer containing NADP (10 mg), 2-Mercaptoethanol 100 μl (sigma Cat. No. M7154), Triton X-100 100 μl and dH₂O 100 ml (pH 6.0). Samples crushed in buffer were poured into sterile eppendorf tubes and was spun at 12,000 rpm in a cold centrifuge (Remi, model-C-24) at 4°C for 10 minutes. The supernatants were separated in each case and electrophorased in a 5% native polyacrylamide gel (PAGE) using discontinuous buffer system (Rechardson et al., 1987) in a Bio-Rad electrophoretic system (Mini protein-II; Cat no. 165) at 4°C. On completion of gel run, they were stained through specific histochemical staining technique (Paul et al., 1987). To study details, the gels were scanned on a densitometric scanner (Sharp JX- 330; Pharmacia-Biotech; Cat no. 18-1108-95); relatively density (RD) and area covered by each activity zone of esterase were documented. Bands in different specimens were numbered serially in descending order from the cathodal to anodal and Rf (relative front) values, mean S (similarity) of all tissues and V (s) (variance of similarity) were calculated (Lynch, 1990).

RESULTS AND DISCUSSION

Esterase profile of all the five different groups of inbreeds and hybrids involving catla and rohu either singly or in a cross hybrid combinations are presented in Figs 1a&b.

Across all the tissues (skeletal muscle, heart, liver and kidney) esterase was found to be concentrated in 15 distinct bands. Maximum number of esterase activity was reported to be 10 and were species specific. Through inhibitor sensitivity assay the nature of coding loci was worked out and 3 zones in esterase profile were identified (Chatterjee, 1994). Distribution pattern of esterase in 7 air-breathing fishes was also surveyed (Chatterjee, 1991). Electrophoretic studies on genetics of two species of Indian major
carps and their hybrids were reported earlier (Krishnaraja and Rege, 1977; Krishnaraja and Rege, 1979). In this study inbreed rohu and catla portrayed distinctly different zymograms in respect of all the tissues analyzed. It may be mentioned that esterase are mostly involved in the hydrolysis of esters as acetate, butyrate etc. and are either monomer or dimer and inherit co-dominantly.

The allelic expression of esterase in skeletal muscles was manifested in rohu by 4 bands (viz., 4, 10, 11 and 12) and in catla by 5 bands (viz., 7, 8, 9, 11 and 12). Presence of bands 4 and 10 separates rohu from catla distinctly (Table 1), whereas catla is having unique diagnostic esterase profile with the presence of band nos. 7, 8 and 9. The F_1 hybrid between catla (♀) x rohu (♂) showed 7 bands viz., 4, 7, 8, 9, 11, 12, and 15 (of which 6 were intense; average RD 1.06, Fig. 1a). Six active esterase zones (4, 7, 8, 10, 11 and 12) were met in BC_1C and 4 bands in BC_1R (viz., 4, 10, 11 and 12) which were highly polymorphic. Profile containing bands namely 4, 7, 8, 9 and 10 in F_1 hybrid, and 4, 7, 8 and 10 in BC_1C (Fig. 1a) made them different from inbreeds. These bands could separate them also from each other. RD of this enzyme in BC_1C was found to be low (0.32, 0.23 and 0.19) for the bands with Rf 0.43, 0.60 and 0.66, respectively in contrast to the high RD of 1.06, 1.06 and 0.04 of F_1 hybrids between catla (♀) x rohu (♂).

Number of esterase bands in heart tissues in parental catla, rohu, F_1 hybrid and back crossed progenies (BC_1C and BC_1R) showed maximum of 15 bands. Bands 4, 10, 11, 12, 14 & 15 in rohu; 7, 8, 9, 12, 14 & 15 in catla; 4, 7, 8, 10, 11, 12, 15 in F_1 hybrid; 1, 2, 3, 4, 5, 7, 10, 11 & 12 in BC_1C, and 4, 10, 11 & 12 in BC_1R were observed (Fig. 1a). Band 4 & 10 of rohu; 7, 8 & 9 of catla; 4, 7, 8, 9 & 10 of F_1 hybrid; 1, 2, 3, 4, 5 & 7 in BC_1C; and 4, 10 & 13 of BC_1R were diagnostic profile (Table 1). Band 4 (Rf 0.43) was found to be common in rohu, F_1 hybrid, BC_1C and BC_1R with varied RD ranging from 0.13-1.06. The characteristics esterase band of catla was observed with Rf. 0.60, 0.64 and 0.70 (band nos. 7, 8 and 9) was also present in F_1 hybrid. In BC_1C diagnostic esterase band was found to be located at Rf 0.6 (band 7) which was characterized with highly polymorphic non-parental band viz., 1, 2, 3 and 5 in heart tissue, while rohu and catla were characterized with the presence of band 4 (RD 1.06) and 7, 8, 9, (RD 0.65-1.06), respectively; the hybrids on the other hand was represented by the expression with peak RD varying from 0.73-1.06. The diagnostic bands displayed parental inheritance in BC_1C and BC_1R which was reflected through the presence of band 4, 7 & 11 and 4, 11 & 12, respectively.

In liver esterase was found (Fig. 1b) to be expressed at 6 active sites in rohu, 7 sites in catla, 6 sites in both F_1 hybrids and BC_1R, and 5 sites in BC_1C. All the bands were anodal, except band 4. Bands 4 and 9 of rohu; 4, 7, 9 & 10 of catla; 4, 7 & 9 of F_1 hybrid; 4, 7 & 10 of BC_1C and 4, 8 & 9 of BC_1R were diagnostic esterase profile separated each groups from another (Table 1). Band 7 (Rf 0.59), a highly active band in catla, was found to be equally expressed in F_1 hybrid and BC_1C albeit stain intensity was found to be
considerably low in BC₁C and was absent in BC₁R. Band 9, another actively expressed band in rohu (RD 1.2), was found to possess low RD activity of 0.37 in catla. The same band was intense in F₁ hybrid, less intense in BC₁R (RD 0.86) and absent in BC₁C. Two another bands (11 & 12) in the anodal end were present in all groups as intense band (average RD 1.45) except with less intensity in catla (RD 0.74).

Kidney tissue displayed almost similar esterase pattern (Fig. 1b) like liver in all the groups except absence of anodal bands, namely 14 and 15 in the former tissues. Profiles were characterized with the presence of bands viz., 4, 8, 9, 11 & 12 in rohu; 6, 9, 10, 11 & 12 in catla; 4, 6, 8, 11 & 12 in F₁ hybrid; 4, 6, 11 & 12 in BC₁C; and 4, 6, 11 & 12 in BC₁R. Band nos 4, 8 & 9 in rohu; 6, 9 & 10 in catla; 4, 6 & 9 in F₁ hybrid; 4, 6 & 8 in BC₁C and 4 in BC₁R (Table 1) were characteristic profile which distinguished each entries from another.

Table 1. Diagnostic esterase profile (band and band combinations) indistinguishing inbreed catla and rohu from F₁ hybrids and backcross in different tissues

<table>
<thead>
<tr>
<th>Species tissue</th>
<th>Liver</th>
<th>Kidney</th>
<th>Skeletal muscle</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rohu</td>
<td>4, 9</td>
<td>4, 8, 9</td>
<td>4, 10</td>
<td>4, 10</td>
</tr>
<tr>
<td>Catla</td>
<td>4, 7, 9, 10</td>
<td>6, 9, 10</td>
<td>7, 8, 9</td>
<td>7, 8, 9</td>
</tr>
<tr>
<td>F₁ hybrid</td>
<td>4, 7, 9</td>
<td>4, 6, 9</td>
<td>4, 7, 8, 9, 10</td>
<td>4, 7, 8, 9, 10</td>
</tr>
<tr>
<td>BC₁C</td>
<td>4, 7, 10</td>
<td>4, 6, 8</td>
<td>4, 7, 8, 10</td>
<td>1, 2, 3, 4, 5, 7</td>
</tr>
<tr>
<td>BC₁R</td>
<td>4, 8, 9</td>
<td>4</td>
<td>4, 10, 13</td>
<td>4, 10, 13</td>
</tr>
</tbody>
</table>
The peak of RD of band 4, 6 and 9 was low. Band 4 was present in all the groups except catla, while band 6 was present in catla, F1 hybrid and BC1C only. Considering all the electropherograms of inbreeds and hybrids specific shared banding patterns are highlighted in Table 2. Bands 4, 11 & 12 for rohu; 6/7*, 9, 11 & 12 for catla; 4, 6/7, 9, 11& 12 for F1 hybrid; 4, 6/7 & 11 for BC1C; and 4, 11, 12 & 13 for BC1R would be instrumental in separating inbreeds hybrids and backcross progenies. We found that bands 4 and 12 of liver and kidney tissues were found in all the groups analyzed. Band 4 of rohu and 6/7 of catla were diagnostic generic bands regardless of tissue nature. Seemingly they may be used as marker in monitoring inheritance pattern of different esterase isozymes as well to distinguish different groups of fishes.

The pair-wise similarity (SAB) for esterase loci (Table 3) indicated the genetic relationships of inbreeds and hybrids. Tissue specific expression of esterase was also observed at genetic level. With respect to liver the F1 hybrid was found to be close to catla (S=0.87) and rohu (S=0.77). The backcross BC1C showed greater divergence from parental catla and rohu (S=0.61 and 0.62) and was relatively closer to hybrid (S=0.57). However, the S values of BC1C from both the parents were almost equal (S=0.61 to catla and 0.62 to rohu) which is indicative of its nearly similar genetic divergence from both the parents. Mean similarity is an upward biased estimation of the mean homozygosity of population (Lynch, 1990). Comparatively low level of homozygosity as observed from mean similarity values and higher degree of variance of BQC indicates the manifestation of heterosis which may be exploited in breeding programmes for selection of elite fishes. Kidney tissues represents a stable esterase pattern across Fi hybrid, BC1C and inbreeds with a very high genetic similarity (S=0.80) indicating the possession of similar charges by different isomorphs of esterase for migration.

Table 2. Species specific shared band profile (common in skeletal muscle heart liver and kidney)

<table>
<thead>
<tr>
<th>Species</th>
<th>Unique profile with specific bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rohu</td>
<td>4.11.12</td>
</tr>
<tr>
<td>Catla</td>
<td>6/7.*9.12</td>
</tr>
<tr>
<td>F1 hybrid</td>
<td>4.6/7.9.11.12</td>
</tr>
<tr>
<td>BC1C</td>
<td>4, 6/7, 11</td>
</tr>
<tr>
<td>BC1R</td>
<td>4, 11, 12, 13</td>
</tr>
</tbody>
</table>

* Band 6 is present (Rf 0.59) in kidney; whereas band 7 is present in other all tissues (Rf 0.61)

Esterase profile of skeletal muscle showed maximum similarity between the F1 hybrid with catla (S=0.83), and BC1C and rohu (S=0.80). This indicates the presence of similar coding sequences for muscle protein esterase among the entries or similar post-translational changes might have occurred to produce similar esterase. The esterase
pattern of heart tissue showed more closeness of F₁ hybrid with catla (S=0.71) and rohu (S=0.61). On the contrary, the BC₁C was found to be divergent from rohu (S=0.53) and F₁ hybrid (S=0.55). This may be indicative of the extent of inheritance of parental characters as well as tissue specific inheritance of esterase in these groups of fishes.

Table 3. Mean S and V (S) over four different tissues (skeletal muscle, heart liver and kidney) in catla, rohu their F₁ hybrids and backcrosses

<table>
<thead>
<tr>
<th>Species</th>
<th>Catla</th>
<th>Rohu</th>
<th>F₁</th>
<th>BC₁C</th>
<th>BC₁R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rohu</td>
<td>0.55</td>
<td>0.80</td>
<td>0.59</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.037)*</td>
<td>(0.018)</td>
<td>(0.030)</td>
<td>(0.042)</td>
<td></td>
</tr>
<tr>
<td>Catla</td>
<td>0.67</td>
<td>(0.029)</td>
<td>0.63</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>F₁ hybrid</td>
<td>0.69</td>
<td>(0.024)</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC₁C</td>
<td>0.61</td>
<td>(0.035)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC₁R</td>
<td>0.67</td>
<td>(0.032)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean pair wise similarity (S) (Lynch, 1990) and variance (V(S)) are presented in Table 3. Genetic similarity between the parents (catla and rohu) and the developed progenies (F₁ hybrid, BC₁C and BC₁R) showed maximum value between BC₁R and rohu (0.84) followed by F₁ hybrid and catla (0.80); hybrid and BC₁C (0.69); hybrid and rohu (0.67); and hybrid and BC₁R (0.64). Variance [V(S)] (17) of similarities among the fishes showed that BC₁C was genetically more diverse than the other two varieties viz., F₁ hybrid and BC₁C.

We conclude from the foregoing studies that esterase profile might be used in identifying inbreds rohu and catla from their F₁ hybrids and backcross progenies. Catla poses a distinctly different esterase profile from rohu in which bands 4 and 6/7 of catla were diagnostic bands of pure rohu and catla, respectively. Similarly, bands 9 and 13 of liver in BC₁R can separate from F₁ and inbreeds. A shared band profile of catla and rohu manifested in F₁ hybrid. Presence of diagnostic band profile containing 4, 6/7 and 11 clearly distinguishes BC₁C from the parental inbreeds, F₁ and BC₁R. Thus, pedigree of natural occurring anonymous strains/ hybrids may be detected with the aid of esterase analysis. In an optimistic view, we are of opinion that esterase profile may be used in tagging useful characters in fish breeding and selection programmes (Vuorinen, 1988; Rymen, 1983; Elo et al., 1997) if appropriate mapping populations are developed.
REFERENCES


INTRODUCTION

Carps culture

The hatcheries of the State. These include rohita, mrigal (Ctenopharyngodon idella), and catla. All these species are used extensively bred in the State. The catla is the main species, and it has led to the production of the breedstock, which is imported. The small scale fish farms use the breedstock of these fish species for their domestic requirements. In small scale fish farms, closely related is to the accumulation of the interval and smaller accumulation of the procedure analyses.