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Electrophoretic patterns of sarcoplasmic proteins in carangid fishes

BINDHU VERGHESE AND P. JAYASANKAR[1]

Central Marine Fisheries Research Institute, Cochin - 682 014, India

ABSTRACT

The present paper reports muscle myogen patterns of four species of carangids, viz. *Decapterus russelli, D. macrosoma, Selar crumenophthalmus* and *Megalaspis cordyla* using horizontal slab polyacrylamide gel electrophoresis. The number of protein fractions (presumptive loci) in the muscle of *D. russelli, D. macrosoma, S. crumenophthalmus* and *M. cordyla* were found to be 9, 7, 6 and 5, respectively. The R_f value ranged from 21 to 84 in *S. crumenophthalmus*, 2 to 72 in *M. cordyla*, 15 to 89 in *D. macrosoma* and 18 to 96 in *D. russelli*. The difference in number of fractions, their mobility pattern and staining indicated species-specificity. With the limited number of samples used in our study, there has been an indication of intraspecific polymorphism among three species of carangids, being 3.5% in *D. russelli*, 13.5% in *D. macrosoma* and 6.6% in *S. crumenophthalmus*. *M. cordyla* showed no intraspecific polymorphism. The study reveals that protein fractions in carangids can be used as species-specific markers, which could be helpful in resolving disputes in the event of any taxonomic ambiguity.

Introduction

Proteins/enzymes are used as genetic markers which play a significant role in fishery biology and management. It has been established long before that proteins are direct products of gene action (Crick, 1963; Nirenberg *et al.*, 1963; Ochoa, 1963). Gene-controlled proteins form the structural basic source of gentic information at various levels of species organization. Study on protein pattern has become a valuable tool in solving the taxonomic problems.

Electrophoresis of problem has been widely applied for direct study of genetic variation in fish population and identification of genetic stocks of commercially important fishery resources. Electrophoretic techniques has been found to be useful in studying problems involving taxonomic ranks, from relationship between orders to relationship between species. Due to the existence of morphological plasticity among individuals, conventional morphological characters are often found to be deceiving in exact detection of a species (Menon, 1989). This can be overcome by electrophoresis. Genetic markers are superior to artificial markers and tags as they are natural and can be found in all stages of animals (Kapila and Kapila, 1996).

Carangids form the major fishery along the southern states of India (Bal and Rao, 1984). In 1997, 1,37,908 t of

[1] *Author for correspondence.*

carangids were landed which formed about 3.4% of total marine fish landings (CMFRI, 1998). Of the 65 species of carangids found in the western Indian Ocean about 35 species are found in the Indian waters (FAO, 1984). It has been seen that the morphological identification of some species belonging to carangids is difficult. Taxonomic ambiguity was found to exist between species of *Decapterus macrosoma* and *D. macarellus, D. kurroides* and *D. kurroides akaadsi, D. russelli* and *D. macrosoma, D. lajang* and *D. macrosoma* and *Caranx kalla* and *C. para* (FAO, 1984).

Biochemical genetic studies of Indian fishes are scanty and are of preliminary nature, which are mainly the attempts to find out interspecies or species specific protein differences. In common food fishes and prawns, characteristics electrophoretic band pattern was observed which were not altered by storage of fish in ice (Devanesan and Nair, 1971). Based on allozyme patterns, Manezes (1990) reported divergence in three carangids (*Decapterus russelli, Selar crumenophthalmus* and *Selaroides leptolepis*) and opined that the three spccies were clearly divided into two groups. In our work, electrophoresis of general proteins of *Decapterus russelli, D. macrosoma, Selar crumenophthalmus* and *Megalaspis cordyla* was carried out with a view to determining genetic variation within and between the species, to reveal their genetic relationship and to characterize species-specific markers.

Materials and methods

The specimens of *Decapterus russelli, D. macrosoma, Selar crumenophthalmus* and *Megalaspis cordyla* were collected from trawl and gill net landings at Cochin Fisheries Harbour. The vessels generally operate at about 25 km from the coast at 100-120 m depth (9°57'N, 76°14'E). The samples were collected soon after their landings and were transported in crushed ice.

Horizontal slab polyacrylamide gel electrophoresis was done in the present work. Modifications were made with respect to gel concentration, tissue ratio, homogenising media and time of homogenization from the standard methodology. Initially muscle, liver and eye-lens were dissected out from the samples for analysis. However, subsequently only muscle was used because it was giving more number of bands. Extreme care was taken to avoid red muscle while preparing tissue samples. Approximately 1 g of tissue was taken from each fish and stored at –20°C prior to homogenization. Protein extraction was done using double distilled water and 0.2M sucrose medium at different ratios of the sample and homogenising media (W/V) (1:1, 1:2 and 2:1) and 2:1 was found to be ideal. Homogenization was done usig a manual glass homogenizer as well as mechanical homogenizer (Remi). The homogenised sample was then transferred to eppendorf tubes and centrifuged at 4°C and 10,000 rpm. Duration of centrifugation was varied at 15 min, 30 min and 1 h for standardization and best results were obtained at 30 min of centrifugation. The supernatant obtained was then transferred to another eppendorf and kept at –20°C for later use. Table 1 shows the three different gel composition used for standardization. Best result was obtained with 7.5% gel. While different tissues, such as eye-lens liver and muscle were tried, best results were

obtained in electrophoretic patterns of muscle protein.

The separating gel was stained for 2.5 h in 0.25% coomassie brilliant blue (Huriaux and Focant,1977) and destained in a mixture of 150 ml methanol, 70 ml acetic acid and 780 ml double distilled water. The band patterns were observed in a transilluminator over visible light and zymogram of each gel was recorded carefully on a graph sheet for species/genera wise comparison and for scoring intraspecific variation; samples were always run in the same gel and under identical condition. In the zymogram bands were serially numbered, with the fastest band among all the species getting the first number and the slowest, the last. The most commonly occurring protein profile of each species was considered as the base picture of that species and any variation to this pattern was separately recorded to study intraspecific polymorphism, if any. The relative frequency of each fraction was estimated using the formula:

$$\text{Relative frequency } (R_f\%) = \frac{\text{Distance travelled by the fraction} \times 100}{\text{Total distance travelled by the marker dye}}$$

Results and discussion

The number of sarcoplasmic protein fractions of four carangids varied from 5 to 9 (Fig. 1). Maximum number of bands (9) were found in *Decapterus russelli* (presumptive loci=9) and minimum number was in *Megalaspis cordyla* (presumptive loci=5). *Selar crumenophthalmus* exhibited 6 bands (presumptive loci=6), while *Decapterus macrosoma* showed 7 bands (presumptive loci=7). The relative frequency (R_f) value ranged between 2 and 96 (Table 2). The staining was most intense for the fractions 5, 6, 8, 10 and 11 for *D. russelli;* 10 for

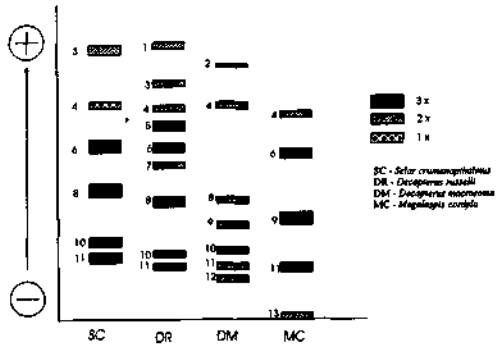

Fig. 1. Zymogram of general proteins in muscle tissue of carangids showing interspecific/ intergeneric difference.

TABLE 1. *Gel composition used in electrophoretic analysis of carangids*

Gel composites	7.0% Separating gel (50 ml)	7.0% stacking gel (25 ml *	7.5% Separating gel (50 ml)	7.5% stacking (25 ml	8% Separating gel (50 ml)	8% stacking gel (25 ml
30% acrylamide bis-acrylamide	11.67	2.91	12.5	3.13	13.33	6.67
Tris buffer (pH 8.9)	6.25	-	6.25	-	6.25	-
Tris buffer (pH 6.8)	-	6.25	-	6.25	-	6.25
Double distilled water	7.08	15.85	6.25	15.62	5.62	12.08
10% APS	400*+	100*	400*+	100*	400*+	100*
Temed	-	25	-	25	-	25

* micro litres. + 0.16% of APS in 25 ml double distilled water.

D. macrosoma; 6, 9 and 11 for *M. cordyla* and 6, 8, 10 and 11 for *S. crumenophthalmus.*

Clear cut interspecific variations were observed in the protein profile of *D. russelli* and *D. macrosoma* (Fig. 2). In *D. macrosoma,* the total number of bands were 7, while 9 bands were observed in *D. russelli.* The protein banding pattern of the two species showed much variation particularly, with regard to the bands in the middle region. In *D. russelli,* the middle region is occupied by intensely staining bands 5, 6 and 7; while in *D. macrosoma,* the entire region appeared without any

The banding pattern and staining intensity differed widely between *S. crumenophthalmus* and *D. macrosoma* (Fig. 1). Among these species, only one fraction (band number 10) showed same staining intensity and only 4 bands, viz. 4, 8, 10 and 11 showed common R_f value (Table 2). The protein profile of *M. cordyla* was distinct with a prominent additional band at cathodal end (band number 13) which was absent in all other species (Fig. 4). Totally there were 5 fractions of which the fastest (band number 4) and the slowest (band number 11) showed the same R_f value and staining intensity with 3 other species. Bands 4, 6 and 11 of *M. cordyla* showed similar R_f value and staining

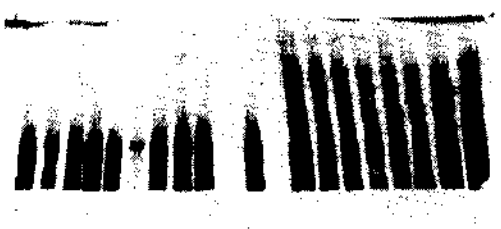

Fig. 2. Sarcoplasmic protein pattern in *D. macrosoma* and *D. russelli.*

Fig. 3. Sarcoplasmic protein pattern in *D. russelli* and *S. crumenophthalmus.*

bands. Of all the bands, only bands 4, 8, 10 and 11 alone showed similar R_f values in both species (Table 2). In *S. crumenophthalmus,* the protein fractions corresponding to bands 1, 5 and 7 of *D. russelli* were absent (Fig. 3); this was the only striking difference between the two genera. Five out of 6 bands of *S. crumenophthalmus* showed similar staining intensity with 5 out of 9 bands of *D. russelli.* In *S. crumenophthalmus* and *D. russelli,* the R_f values were similar for bands 3, 4, 6 and 11 (Table 2). Overall, both the genera appeared to exhibit similar protein banding patterns.

TABLE 2. *R_f values of the four species of carangids*

Fraction No.	*Decapterus russelli*	*Decapterus macrosoma*	*Selar crumenophthalmus*	*Megalaspis cordyla*
1	96	-	-	-
2	-	89	-	-
3	83	-	84	-
4	74	75	75	72
5	68	-	-	-
6	61	-	61	58
7	55	-	-	-
8	42	42	45	-
9	-	33	-	35
10	23	25	26	-
11	18	18	21	18
12	-	15	-	-
13	-	-	-	2

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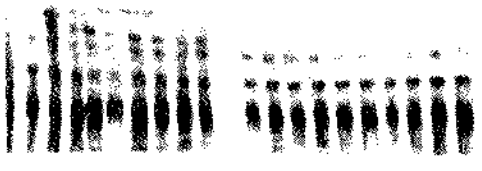

Fig. 3. Sarcoplasmic protein pattern in *D. russelli* and *S. crumenophthalmus*.

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11	18	18	21	18
12	-	15	-	-
13	-	-	-	2

pattern of corresponding bands of *S. crumenophthalmus* (Table 2). The intensely staining band 9 of *M. cordyla* showed similar R_f value with a less intense fraction of *D. macrosoma*.

The comparative picture of the four species provided clear out species specific banding patterns (Fig. 4). Species specific bands for *D. russelli, D. macrosoma, M. cordyla* and *S. crumenophthalmus* were, respectively 5, 12, 13 and 8. Close similarity of protein fractions was seen between *S. crumenophthalmus* and *D. russelli* with regard to the position and staining reaction of protein fractions rather than the pattern found in *D. macrosoma* and *D. russelli* the individulas of the same genus. Three out of 5 bands (fractions 4, 6 and 11) of *M. cordyla* were also found to be closely similar with the same fractions of *S. crumenophthalmus*.

The variation in the number of bands was comparatively less within species in *Decapterus russelli* (Fig. 5). Only one out of 30 samples exhibited an additional band between the first and third bands, with staining intensity 2x and R_f value 87 (Table 2). The indicative polymorphism in *D. russelli* was found to be 3.5%. In the 30 samples of *D. macrosoma* studied, all except four

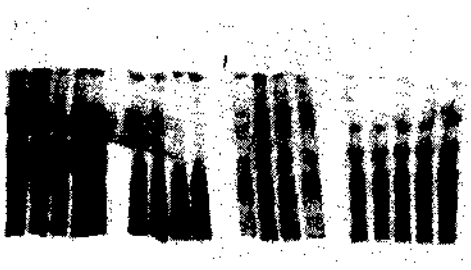

Fig. 4. Sarcoplasmic protein patterns in *M. cordyla, D. macrosoma, D. russelli* and *S. crumenophthalmus*.

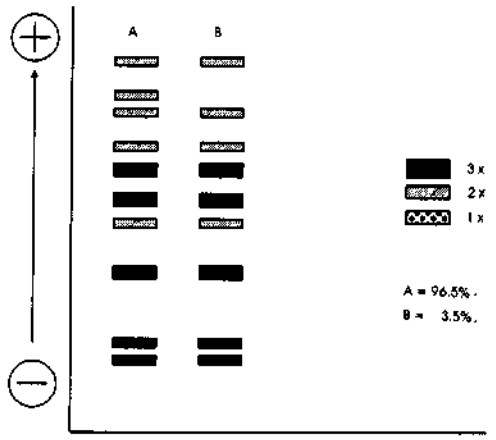

Fig. 5. Zymogram showing intraspecific polymorphism in sarcoplasmic protein pattern of *D. russelli*.

showed similar protein fraction (Fig. 6). Out of these four individuals, three exhibited a double banding pattern of intensity 2x and R_f values of 54 and 58 was found between bands 4 and 8 in the middle zone (Table 2). The fourth specimen showed a single band in the place of double band which showed an intensity of 2x and shared the same R_f value 58 of the faster band of the former variant. In *D. macrosoma* the indicative polymorphism was 13.5%. In *S. crumenophthalmus*, all the samples showed similar banding patterns except in two individuals (Fig. 7), in which there was an additional protein fraction towards cathodal end with intensity 2x and R_f values 17 (Table 2). The indicative polymorphism was found to be 6.6%. All the thirty samples of *M. cordyla* showed similar banding patterns (Fig. 1).

Protein differences between species is specific for individuals representing a group. This could elucidate taxonomic problems in the case of disputed species (Smith *et al.*, 1979). In electrophoretic technique, closely related species share many electrophoretic alleles but also

differ at some gene loci at which they are fixed for different alleles (Smith *et al.*, 1990). If two different species have same number of electrophoretic fractions, further close comparison of relative mobility of one or more bands could reveal well defind species-specific differences. Species-specificity of a protein should mean primarily the elctrophoretic mobility differences of one or more bands. In the present study, all the four species exhibited species-specific bands which can be used as diagnostic genetic markers for species identification. Further, banding pattern and mobility were also species-specific. The electrophoretic banding patterns of *D. russelli*, *D. macrosoma*, *S. crumenophthalmus* and *M. cordyla* confirms their status as valid species which have undergone speciation long back indicating that the four species are wide apart in their origin.

The individuals of one species may differ from another if they have distant origin. It is not necessary that morphologically identical species have similar

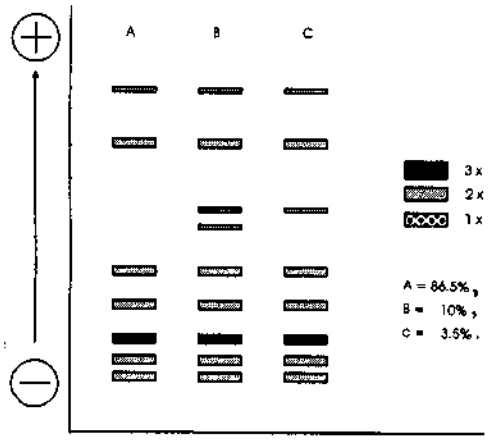

Fig. 6. Zymogram showing intraspecific polymorphism in sarcoplasmic protein pattern of *D. macrosoma*.

A = 86.5%, B = 10%, C = 3.5%

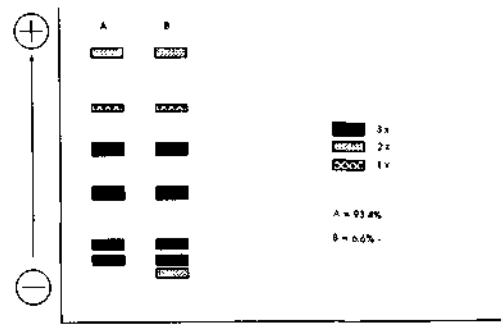

Fig. 7. Zymogram showing intraspecific polymorphism in sarcoplasmic protein pattern of *S. crumenophthalmus*.

A = 93.4%, B = 6.6%

protein banding; this characteristic of the protein banding is used to resolve problems of taxonomic ambiguity and other related disputes (Ponniah, 1988). The dissimilarity in protein banding pattern between *D. russelli* and *D. macrosoma* in the present work shows that the species are wide apart from the biochemical point of view eventhough morphologically they are almost identical. On the otherhand, *D. russelli* and *S. crumenophthalmus* exhibit a close biochemical relationship, despite the fact that they belong to two genera. *M. cordyla* showed similarity to *S. crumenophthalmus* in protein banding pattern. This leads to a possibility that *D. russelli*, *S. crumenophthalmus* and *M. cordyla* are closely related or have a common origin. However, to confirm this, further work has to be done using isozymes, mt DNA and nuclear DNA.

Molecular genetic markers which reveal intraspecific polymorphisms are valuable tools to determine heterozygosity which determine the potential of a population. In our present short term

study, *D. russelli* was showing minimum intraspecific polymorphism, while *D. macrosoma* exhibited maximum intraspecific polymorphism. To reach a proper conclusion, an in-depth study is needed using more number of samples from the same population.

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