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Identification of Species-Marker Bands in Native and SDS-PAGE Patterns of Soluble Muscle Proteins of Four Species of Genus *Channa* (Channidae: Channiformes) with Evidence of Some Intraspecies Heterogeneity

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Abstract

Soluble proteins of skeletal muscles of four species of genus *Channa* have been analyzed by native and SDS-containing polyacryalmide gel electrophoresis (SDS-PAGE). Native-PAGE patterns of soluble skeletal muscle proteins of all four species are devoid of intraspecies qualitative variations. Sixteen protein bands, each with characteristic electrophoretic mobility, are diagnostic to *Channa gachua* and *C. striatus*, while 10 and 15 bands are to *C. marulius* and *C. punctatus*, respectively. In the same sequence of species, the total number of representative polypeptides in SDS-PAGE patterns is: 18, 14, 13 and 21, respectively. Among species-marker bands, highly acidic proteins are most prominent. Species specific polypeptides have also been identified after resolving soluble muscle proteins by SDS-PAGE. Intraspecies quantitative differences within the individual bands exist in native as well as SDS-PAGE systems; but, contrary to native electrophoretic system, SDS-PAGE patterns of the soluble muscle proteins display a certain degree of numerical heterogeneity also. Most obvious differences are observed in polypeptides with in the M_r range of 26-44 kD, which could be placed under variability-groups of consistency. Identified species-marker bands resolved in either of the electrophoretic systems can also be used for identifying the hybrids and settling taxonomic controversies. In order to facilitate the use of data by other workers, the molecular weights of each band in SDS-PAGE and the relative electrophoretic mobilities of species marker patterns in native gels have been documented.

Introduction

Soluble proteins of muscle sarcoplasm are among the easiest to extract and highly rich reservoir of species specific and biochemical genetic

markers (Tsuyuki et al. 1965, O'Rourke 1974, Ryman and Utter 1987, Buth and Murphy 1999). The repertoire, apart from a few others, embraces the entire range of enzymes and proteins of glycolysis and electron transport released due to rupturing of mitochondria. With the exception of one report (Taniguchi et al. 1982) that described genetic polymorphism in muscle proteins of seabream, the technique of highest resolving power, IEF has also been helpful in comparing various taxa (Basaglia and Marchetti 1990, Basaglia 1992). Application of IEF to a large number of samples is not a cost-effective alternative of routine electrophoretic analysis. In the present study, we have worked out the extent to which native and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) can help discern inter- or intraspecies differences in four species of genus *Channa*. Base line data is provided to characterize these species on the basis of species-marker bands in native and SDS-PAGE patterns. An attempt has also been made to explore the possibility whether polypeptide-specific resolutions by SDS-PAGE can assist in better appreciation of inter and intraspecies comparison of soluble muscle proteins of fish species.

The species of genus *Channa* are important food fishes as well as a substantive component of fresh water and integrative fisheries, apart from representing a distinct adaptive lineage of accessory air-breathing teleosts. Most of the previous efforts in Southeast Asian countries have addressed the problem of species and the variant identification by employing classical morphological criteria. Published biochemical evidence even on electrophoretic markers which could be employed to discriminate freshwater teleosts of the region remains scanty (Hasnain et al. 1973a and b, Hasnain and Siddiqui 1974, Hasnain et al. 1999).

Materials and Methods

Collection of samples and their identification

Live samples of all four species of genus *Channa* Gronov (Teleostei: Channiformes: Channidae) were obtained from a local (Aligarh) fish market where catches from a large area including the nearby districts land. Species identification was carried out according to the key of Srivastava (1980). The four species of genus selected for this study are: *Channa punctatus* Bloch, *Channa marulius* Hamilton, *Channa striatus* Bloch and *Channa gachua* Hamilton. They are well distributed in the areas around Aligarh and other parts of India. During 2000-2001 a total of 91, 45, 67 and 32 samples of both sexes of four channid species in the above order, were analyzed simultaneously on native and SDS-PAGE. Only dorsal white muscles of the anterior-most portion of the trunk just behind the

head were dissected out and traces of colored muscles were carefully removed.

Experimental procedures

The fish were stunned by cerebral blow, pithed and white dorso-lateral muscles of most anterior parts of the body were homogenized in 50 mM Tris-HCl of pH 7.5 at top speed of a mechanical homogenizer (Biospec, U.S.A., model 985-380). Clear supernatant obtained after centrifugation at 10,000 rpm and 4°C were saved and either analyzed fresh or stored at -20°C until electrophoresis could be performed. Modified buffer system as described by Hasnain et al. (1999) was used where separating gel is made in 0.375 M Tris-HCl (pH 8.8), 3 % stacking gel in 0.0125 M Tris-HCl (pH 6.8) and run in 0.0661 M Tris-0.0324 M boric acid (pH 8.3). Tris, acrylamide and linkers were from Sigma Chemical Co. (U.S.A.). Routine staining was carried out with coomassie brilliant blue (CBB) that was from Loba-Chemie (India). The short gels were run in submerged system in BRL Mini-V, 8.10 apparatus, while longer gels were run on a locally fabricated tank.

Protein concentration in the extracts was estimated according to the protocol of Lowry et al. (1951).

Software analysis

Software programme GelPro Analyzer (Media Cybernetics, U.S.A.) was used to estimate molecular weights and for quantitation of the bands in native as well as SDS-PAGE.

Results

Native-PAGE patterns of soluble muscle proteins of selected samples of four channid species are compared in figure 1. Taking presence or absence of comigrating bands as the criteria, software analysis of PAGE patterns reveals that a total of 36 bands are diagnostic to genus *Channa* (Table 1). Out of the above total at the genus level, however, most of the bands are absent in one or the other species, asserting specificity of the number and mobility of various bands at species level. Thus, a maximum of 15, 16, 16 and 10 bands are recorded by the software analysis in *C. punctatus*, *C. gachua*, *C. striatus* and *C. marulius*, respectively (Fig. 1: photographs a, b, c and d). Table 1 documents their relative mobilities for future reference and also demonstrates that relative intensities of even comigrating bands might vary. As shown in table 1, quantitatively the most

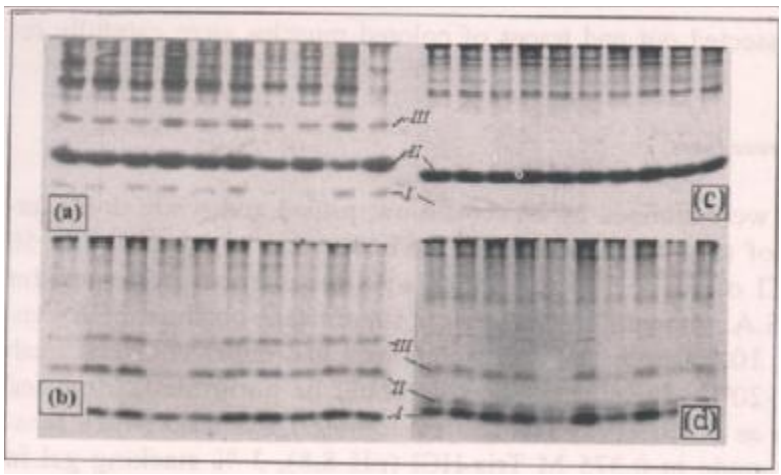

Fig. 1. Native polyacrylamide gel electrophoretic (PAGE) patterns of soluble muscle proteins of four species of genus *Channa*: a, *C. punctatus;* b, *C. marulius;* c, *C. gachua; and* d, *C. striatus*. Highly acidic proteins are marked *I*, *II* and *III*.

Table 1. GelPro analysis of selected lanes of native-PAGE patterns of the soluble muscle proteins from four species of *Channa*, showing constancy in the number along with quantitative variations among protein bands. Amount of protein values are mean±SD of triplicate run under identical conditions

	C. punctatus		***C. gachua***		***C. striatus***		***C. marulius***	
Band #	Amount	Relative mobility	Amount	Relative mobility	Amount	Relative mobility	Amount	Relative mobility
1.	7.98±1.87	0.017	23.68±5.74	0.017	14.29±3.93	0.017	19.98±9.92	0.017
2.	4.85±2.16	0.042	5.19±1.88	0.042	X	X	4.61±1.24	0.042
3.	X	X	X	X	1.94±1.08	0.033	X	X
4.	X	X	X	X	6.87±1.91	0.05	X	X
5.	5.22±1.45	0.067	4.92±0.70	0.067	2.72±0.71	0.067	X	X
6.	X	X	3.27±0.82	0.086	X	X	X	X
7.	9.77±3.69	0.091	X	X	2.37±0.40	0.091	2.35±1.29	0.091
8.	X	X	X	X	X	X	6.38±2.42	0.107
9.	X	X	3.05±1.01	0.108	3.08±1.40	0.108	X	X
10.	6.47±2.47	0.117	1.99±0.92	0.117	X	X	X	X
11.	0.43±1.54	0.15	X	X	3.71±2.53	0.15	X	X
12.	X	X	4.27±1.98	0.166	X	X	X	X
13.	X	X	X	X	X	X	2.2±0.91	0.177
14.	6.09±1.85	0.183	X	X	4.63±1.95	0.183	X	X
15.	2.57±0.88	0.208	X	X	5.29±1.77	0.208	X	X
16.	X	X	4.68±1.13	0.23	5.57±2.80	0.23	X	X
17.	12.55±3.89	0.25	3.79±1.57	0.25	X	X	X	X
18.	X	X	X	X	3.83±3.65	0.26	X	X
19.	4.96±1.02	0.275	X	X	X	X	7.92±2.58	0.275
20.	X	X	X	X	X	X	7.92±2.58	0.277
21.	1.74±0.49	0.3	X	X	7.91±2.94	0.3	X	X
22.	X	X	X	X	8.96±2.5	0.325	X	X
23.	6.73±1.45	0.333	X	X	X	X	X	X
24.	X	X	2.24±0.73	0.342	X	X	X	X
25.	X	X	1.07±0.99	0.383	X	X	5.87±1.67	0.383
26.	9.36±1.52	0.433	X	X	X	X	X	X
27.	X	X	2.4±0.90	0.458	X	X	X	X
28.	X	X	1.48±0.36	0.5	X	X	X	X
29.	X	X	1.10±0.20	0.55	10.3±4.42	0.55	X	X
30.	20.46±4.25	0.6	X	X	X	X	X	X
31.	X	X	38.89±4.09	0.66	X	X	9.82±3.85	0.66
32.	X	X	X	X	4.17±1.1	0.717	X	X
33.	1.77±1.03	0.75	X	X	X	X	X	X
34.	X	X	X	X	X	X	11.41±2.71	0.812
35.	X	X	0.765±0.46	0.867	X	X	X	X
36.	X	X	X	X	20.1±5.96	0.95	20.19±6.38	0.95
Tot. Bands	15		16		16		10	

prominent bands among highly acidic proteins marked as *I, II* and *III*, respectively, are : band # 30 in *C. punctatus*, #31 in *C. gachua*, #34 and #35 in *C. striatus* and #35 in *C. marulius*.

Polypeptide composition of soluble muscle proteins, as determined by SDS-PAGE protocol of Laemmli (1970), is shown in figure 2 and quantitatively compared in table 2. On the basis of software analysis, the

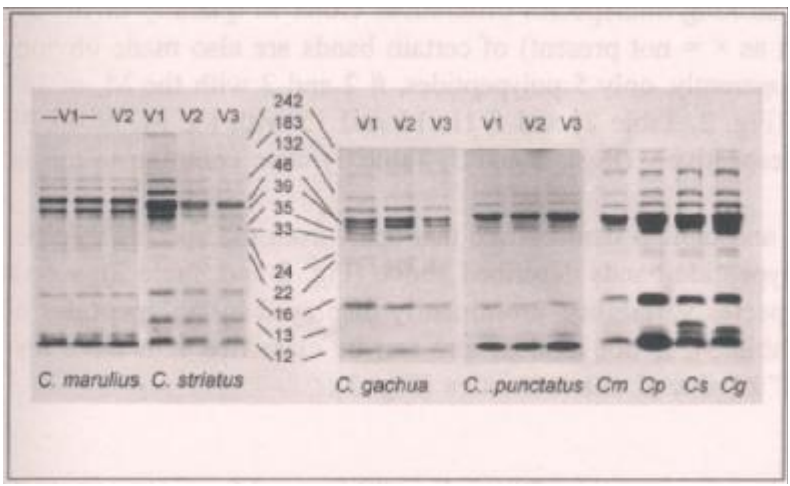

Fig. 2. Selected lanes of three "variability-groups" indicated by V1, V2 and V3, constituted by polypeptides of consistent variability in SDS-PAGE patterns of soluble muscle proteins of four species of genus *Channa*. The last gel on the right shows an aliquot of each species run together in one gel.

Table 2. GelPro estimation of Molecular Weights of species-marker polypeptides of soluble muscle proteins of four species of genus *Channa* resolved by SDS-PAGE (Fig. 2). Values are mean ±SD of triplicate run under identical conditions.

Band #	*C. punctatus*	*C. gachua*	*C. striatus*	*C. marulius*
1	X	244±0.443	X	X
2	183±0.332	183±0.254	183±0.254	183±0.216
3	132±0.570	132±0.251	132±0.25	132±0.570
4	X	X	123±0.178	123±0.526
5	84±0.282	X	84±0.192	X
6	X	77±0.514	77±0.222	77±0.222
7	X	X	72±0.086	72±0.400
8	64±0.316	X	64±0.312	64±0.100
9	X	60±0.234	X	X
10*	X	X	X	51±0.396
19*	X	X	X	24.5±0.372
20*	24±0.269	24±0.339	24±0.364	24±0.229
21	X	23±0.387	23±0.387	23±0389
22	22±0.223	22±0.505	22±0.206	X
23	X	17±0.350	17±0.350	X
24	16±0.415	16±0.142	16±0.342	X
25	X	14±0.214	14±0.192	X
26	13±0.173	X	13±0.374	13±0.206
27	12±0.105	12±251	12±0.310	12±0.132
Total Bands	13	16	21	18

*The polypeptides between the M*r* of 26-46 kD (# 11-18) which exhibit heterogeneity are treated separately in Table 3.

number of polypeptides, which comigrate and are either present or absent, reaches a total of 27 (Table 2). This total has been taken as the representative of soluble protein repertoire of the genus *Channa*. As per the analysis of selected lanes of each species (Table 2), individually, 13, 14, 21 and 18 polypeptides in the range of 12-244 kD are diagnostic to *Channa punctatus*, *C. gachua*, *C. striatus* and *C. marulius*, respectively. Some of the striking interspecies differences either in quantity or the absence (shown as × = not present) of certain bands are also made obvious in table 2. Apparently, only 5 polypeptides, # 2 and 3 with the M_r of 183 and 132 kD (Fig. 2, Table 2) and # 11, 14 and 15 with the M_r of 46, 39 and 35 kD, respectively (Figs. 2 and 3, Table 3) were common to all the four species.

Figure 3 and table 3 demonstrate that apart from the species specificity in the polypeptide bands described above (Fig. 2 and Table 2), a consistent intraspecies variability, prominently displayed by polypeptides of 26-46 kD (Table 3), is not random and can be classified into three distinct "groups" of shared characteristics (Fig. 3).

Discussion

Earlier work on soluble muscle proteins of fish species, carried out on most widely employed starch gels electrophoresis has been reviewed

Table 3. Description of the variability groups of heterogeneity among polypeptides of 26-46 kD range revealed by GelPro analysis of SDS-PAGE patterns of soluble muscle proteins of four species of *Channa*. Analysis was done after enlarging selected portions of gel scans (Fig. 3). Values are mean ±SD of triplicate run under identical conditions.

Band #	*C. punctatus*	V1	V2	V3	*C. striatus*	V1	V2	V3
11	46±0.206	p	p	p	46±0.098	p	p	p
12	42±0.82	p	X	X	nil	X	X	X
13	nil	X	X	X	40±0.377	p	p	p
14	39±0.291	p	p	p	39±0.444	p	p	p
15	35±0.521	p	p	X	35±0.346	p	p	p
16	nil	X	X	X	33±0.271	p	p	X
17	nil	X	X	X	nil	X	X	X
18	nil	X	X	X	26±0.408	X	p	p
	C. gachua				***C. marulius***			
11	46±0.098	p	p		46±0.098	p	p	
12	nil	X	X		nil	X	X	
13	nil	X	X		40±0.192	X	p	
14	39±0.399	P	p		39±0.444	p	p	
15	35±0.063	X	p		35±0.18	p	p	
16	33±0.086	p	p		33±0.271	p	p	
7	nil	X	X		28±0.39	p	p	
18	nil	X	X		nil	X	X	

by O' Rourke (1974). The data, in general, revealed the species-diagnostic nature of electropherograms that also substantiated morphologically known interrelationships (Tsuyuki et al. 1965, 1967, 1968). Similar observations and some information on starch gels electrophoresis has also been published; soluble muscle and a few other tissue proteins of *C. marulius*, air-breathing climbing perch *A. testudinius* and a number of catfishes of different families (Hasnain et al. 1973a and b, Hasnain and Siddiqui 1974). Otherwise, the published evidence on muscle proteins of Indian teleosts from the view point of biochemical systematics or their genetic significance is inadequate. Reports dealing with the native PAGE analysis of soluble muscle proteins of fish species of even temperate regions are either few or lay emphasis on a particular category of well identified proteins (Boback and Slechta 1987, 1988, Focant et al. 1990, 1994, Oberst et al. 1994, Huriaux et al. 1992).

While analyzing soluble muscle proteins of four species of genus *Channa* by PAGE described here, it was observed that for the best and reproducible results the purity of chemicals, particularly the purity of acrylamide and its cross-linker, are highly important. New batches of acrylamide and cross-linker can be tested with appropriate controls (soluble muscle proteins of corresponding species), so that minor adjustments in the concentration of polymer and other running conditions may be made to establish parity with previously obtained results. In modified

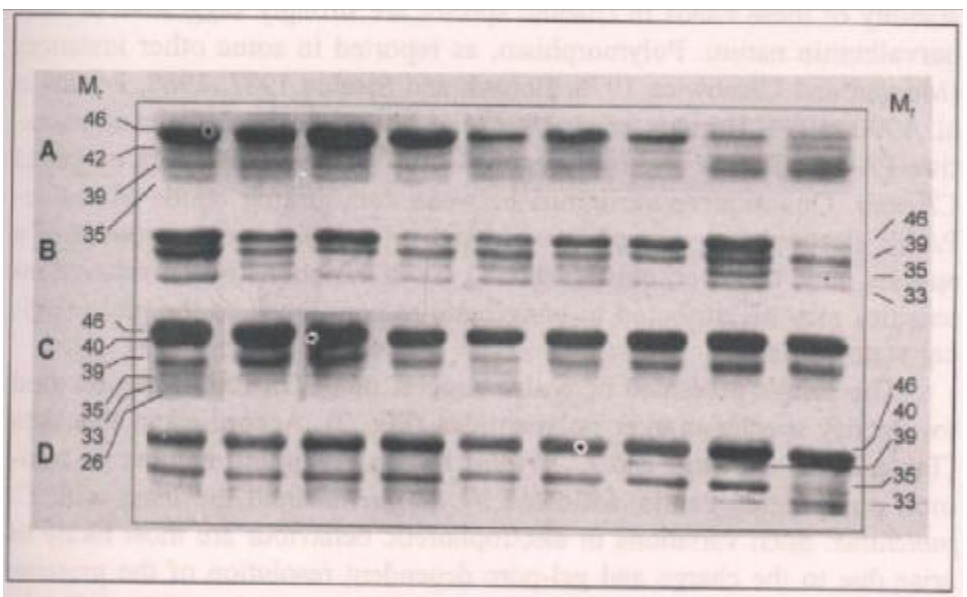

Fig. 3. SDS-PAGE showing most heterogeneous polypeptides of 26-46 kD molecular weights (M_r) within the soluble muscle proteins of : A, *Channa punctatus;* B, *C. gachua;* C, *C. striatus* and D, *C. marulius.* M_r values in kD are shown on either sides.

native system (Pandey and Hasnain 1994) as explained under Materials and Methods as well as in SDS-PAGE (Laemmli 1970), good resolution is obtained following a short pre-run of about 10 minutes with 1 x upper gel buffer in the upper chamber (cathode). This step eliminates trailing, smearing and smiling effects. Samples are subsequently loaded and electrophoresis with the same buffer continued till the tracking dye makes a sharp line in the stacking gel. The upper buffer is then exchanged for the Tris-glycine running buffer.

Similar to numerous studies carried out on starch gels (O' Rourke 1974), constancy of PAGE patterns of soluble muscle proteins, in terms of the number and relative electrophoretic mobilities in native PAGE, is convincingly diagnostic of each channid species (Fig. 1, Table 1). They are also devoid of such intraspecies differences as displayed by a few nonmarker bands in SDS-PAGE patterns (Fig. 3, Table 3). The documentation of species specific patterns in the present study may also prove useful in identification of hybrids and settling controversies where the occurrence of karyotypically distinct type, *C. punctata* has been documented (Dhar and Chaterjee 1986).

Acidic bands of high intensity in *C. punctatus, C. marulius*, *C. gachua* and *C. striatus*, marked *I-III* in the decreasing order of electrophoretic mobilities (Figs. 1a-d) are: 3, 3, 2 and 3, respectively. The relative electrophoretic mobility of individual bands or the entire set of acidic proteins within a species is diagnostic. Published evidence (Huriaux et al. 1992, Sherwani et al. 2001) and our unpublished data on extreme thermostability of these bands in channid species are strongly suggestive of their parvalbumin nature. Polymorphism, as reported in some other instances (Morgan and Ulanowicz 1976, Boback and Slechta 1987, 1988, Focant et al. 1990, 1994, Huriaux et al. 1992) has not so far been detected in native PAGE patterns in highly acidic soluble muscle proteins of genus *Channa*. Quantitative variations between comigrating bands of native-PAGE electropherograms of several bands of soluble muscle proteins of a species may, however, exist (Table 1). Such variations in the relative intensities may be attributed to nongenetic reasons, such as the physiological state of fish.

The results presented here also suggest that SDS-PAGE can be used to identify species-marker polypeptides (Fig. 2). According to this data (Table 2), *C. marulius* and *C. striatus* have a maximum number of common polypeptide bands, followed by *C. gachua* and the least with *C. punctatus*. Such variations in electrophoretic behaviour are most likely to arise due to the charge and gel-pore dependent resolution of the proteins present in the muscle extracts in native PAGE, which widely differ in tertiary/quaternary structures. In SDS-PAGE, on the contrary, constituent polypeptides of the proteins are resolved according to their molecular

weights (Weber and Osborn 1969). Interspecies patterns of shared bands in native and SDS-PAGE systems, therefore, do not resemble.

SDS-PAGE differs from native-PAGE in revealing the intraspecies heterogeneity among certain polypeptides (bands) of investigated soluble muscle proteins. The observed heterogeneity of high intensity bands, however, displayed consistently variable patterns which facilitated partitioning into definite "variation-groups" (Fig. 3, Table 3). Variation-groups have been specifically constituted on the heterogeneity of high intensity bands within the M_r range of 26-46 kD. Certain minor variations in that range, which could not be satisfactorily reproduced in photographs, have been ignored. Resolution of the bands of M_r of >35-46 kD and <30 kD, however, greatly depends on length of the gels and duration of the pre-run. SDS-PAGE is, therefore, a suitable technique to investigate heterogeneity in soluble muscle protein samples, which in native PAGE systems, appear to be homogenous.

Muscle sarcoplasm embraces more than 50 enzymes of glycolytic pathways alone, many of which are multisubunit complexes. Therefore, identification of subunit structure through allozyme route is not a feasible alternative. Several reports have discussed resolution of non-allozyme protein loci of muscle proteins in native electrophoretic systems as biochemical genetic markers (Tsuyuki et al. 1967, Ryman and Utter 1987, Huriaux et al. 1992, Pandey and Hasnain 1994). Several non-gradient but convenient modifications of SDS-PAGE technique now exist, which may be useful in collecting meaningful data on marker polypeptides and recognize genetic heterogeneity at substructural levels of proteins. The modified protocols have been successfully employed to resolve bands of as low M_r as 1 kD (Schagger and Jagov 1987) or to overcome the main problem of costacking of bands of the same molecular weights (Focant et al. 1990, Martinez et al. 1990).

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