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RESEARCH ARTICLE

ELECTROPHORETIC STUDIES ON THE MUSCLE PROTEINS OF FOUR OCTOPUSES REPRESENTED IN THE TRAWL NET BY-CATCHES OFF VISAKHAPATNAM, EAST COAST OF INDIA

Mohana Rao M[1*]., Yedukondala Rao P[2] and Ramesh Babu K[3]

[1,2,3] Department of Marine Living Resources, College of Science and Technology, Andhra University, Visakhapatnam-530 003

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A B S T R A C T

The comparative electrophoretic patterns of muscle protein in the four octopus species, *Octopus aegina, O. membranaceus*, *O. dollfusi* and *Cistopus indicus* were reported in the present study. The stnadard molecular weights of protein were found to be 232, 212, 140, 116-128, 94, 76, 67, 30, 20, 14, 6.2 and 3.5 KDa corresponds to catalase, myosin, lactate dehydrogenase, β-galactosidase, phoshorelase b, transferrin, albumin, carbonic anhydrase, tripsin inhibitor, α-lactalbumin, aprotinin, insulin B-chain. The molecular weights of protein bands were found to be 232, 126, 67, 20, 14, 6.2, and 3.5 KDa in *O.aegina*; 212, 122, 67, 20, 14, 6.2, 3.5 in *O. membranaceu;* 232, 116, 76, 30, 20, 14, 6.2, 3.5 Kda in *O. dollfusi;* 140, 94, 20, 14, 6.2, and 3.5 KDa in *C. indicus*. The cluster analysis showed that the species *C. indicus* was differing from other three species *O.aegina, O. dollfusi* and *O. membranaceus*. Within the three species of octopuses, *O.aegina* and *O.membranaceus* closely related than *O. dollfusi*.

INTRODUCTION

Octopuses are diverse group of marine organisms and are non-target species incidentally or accidentally caught by shrimp trawling in Visakhapatnam coast. Due to growing demand for octopuses in the international market it is necessity to identify the species at a molecular level, because morphological identification of closely related species rather difficult.

The electrophoresis of protein is an effective technique for generating systematic data from macro molecules (Nevo, 1978; Costante *et al*., 1983; David *et al*., 1983; Johnneesson *et al*., 1989; Vibeke and Kongkiat, 1998; Monolisha *et al*., 2013; George *et al*., 2013). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility (George *et al*., 2013).

Soluble proteins of muscle sarcoplasm are among the easiest to extract and highly a rich reservoir of species specific and biochemical genetic markers (Tsuyuki *et al*., 1965; Ryman and Utter, 1987; Buth and Murphy, 1999; George *et al*. 2013; Monolisha *et al*., 2013).

Thirty eight species of octopuses belonging to the family Octopodidae, Tremactopodidae, Argonautidae abound the Indian seas (Silas, 1985), but only four species (*O. aegina*, *O. membranaceus*, *O. dollfusi* and *C. indicus*) represented in the trawl net by-catches at Visakhapatnam. Among the four species, *O.aegina* and *O.membranaceus* were dominant.

Keeping in view of paucity in molecular taxonomy of octopuses at Visakhapatnam coast, the present study centered to delineating the basic protein zymogram of muscle tissue of four octopuses. It involves the characterization of intra and inter-specific difference among population of *O. aegina*, *O. membranaceus*, *O. dollfusi* and *C. indicus* inhabiting the coastal waters off Visakhapatnam.

MATERIALS AND METHODS

Sample Collection

The octopus samples such as *O. aegina* (300-400mm length and 200-250g weight), *O. membranaceus* (350-400mm length and 200-260g weight), *O. dollfusi* (400-500mm length and 300-475g weight) and *Cistopus indicus* (150-250mm length and 100-150g weight) (plate 1) were collected from trawl net by-catches at Visakhapatnam fishing harbor (Lat.17° 41¹ N, Lon.83° 17¹ E) during October, 2009 to September, 2011.

The freshly collected octopuses were stored in crushed ice and immediately brought to the laboratory for further analysis. The octopuses were identified morphologically based on standard taxonomic keys (Roper *et al*., 1984; Silas *et al*., 1985). The animals were then dissected and muscle tissue was taken out from both mantle and arms.

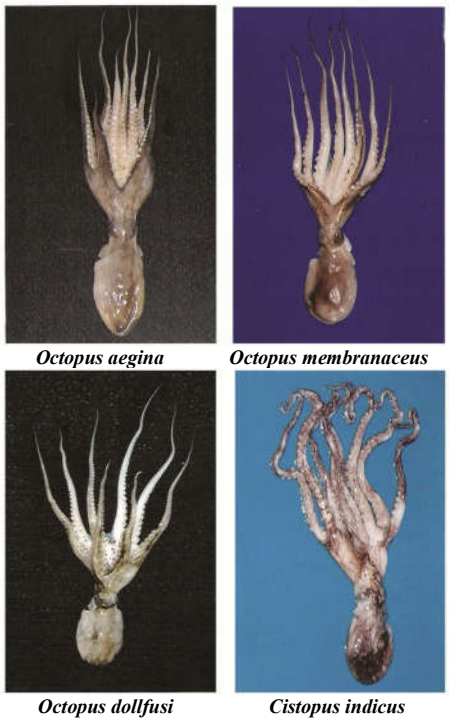

Plate I Octopuses off Visakhapatnam Coast

Extract Preparation

About 1 gm of tissue (both mantle and arm) was homogenized in 2ml tris-HCl (0.5M, 6.8 pH) for 1 minute. Taken 1 ml homogenate into a micro-centrifuge tube and placed on ice. The homogenate was centrifuged at 5000rpm for 15 min. at 4°C. The liquid supernatant was transferred into a second micro-centrifuge tube. Mixed the protein and sample buffer at 4:1 ratio and boiled for 5-10 minutes to denature the proteins to individual peptides and Placed on ice. The obtained supernatant was used for analysis.

Electrophoresis

The SDS-PAGE was performed according to the Hames (1998). Proteins were separated on 12x8 cm dimension and 1 mm thick slab gel. The samples (40µl) were loaded in each well. The instrument was switched on with a current of 22 Amps. The dye after displaced to separating gel, the current was increased to 25 Amps. Electrophoresis was carried for 2 to 3 hours until the die moved down approximately ¾ of the gel. The gel was removed and immersed in a fixative (7% Acetic acid) to guard against diffusion of separated components. Gel was stained in a Commassie brilliant blue staining solution for 2 to 3 hours. Excess stain was removed by washing the gel in a destaining solution.

After destaining the gel was preserved for longer period in 7% Acetic acid. Standard molecular weights ranging from 3.5 to 232 KDa (Bio-Rad) were used to determine the molecular weight of individual proteins. Each fraction obtained has been assigned a qualifying number according to their electrophoretic mobility and protein in the electrophorogram. Molecular weight of an unknown protein was calculated according to Monolisha *et al*., (2013). Cluster analysis was carried out by using SPSS software.

RESULTS AND DISCUSSION

The comparative electrophoretic patterns of tissues of the four octopus species, *O. aegina, O. membranaceus*, *O. dollfusi* and *C. indicus* were depicted in the photomicrograph (Figure 1).

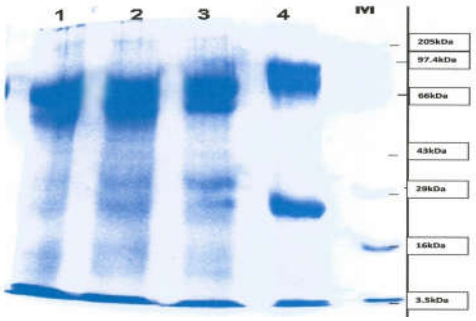

Figure 1 Photomicrograph: A representative of comassie brilliant blue stained SDS-PAGE gel showing protein banding pattern of octopus species.

M= Standard molecular weight markers in (KDa)
L1= *Octopus aegina* L3= *Octopus dollfusi*
L2= *Octopus membranaceus* L4= *Cistopus indicus*

The muscle protein (allozymmes and general proteins) patterns of the octopuses were reproduceble and remarkable variations were recognized in number of bands, relative mobility, and thickness even among the related species of the genus. The muscle protein patterns of four species may be divided into 12 types on the basis of the arrangement, density and position of zones on electrophoresis.The stnadard molecular weights of protein were found to be 232,212,140,116 – 128, 94, 76, 67, 30, 20, 14, 6.2 and 3.5 KDa corresponds to Catalase, Myosin, Lactate dehydrogenase, β-Galactosidase, Phoshorelase b, Transferrin, Albumin, Carbonic anhydrase, Tripsin inhibitor, α-Lactalbumin, Aprotinin, Insulin B chain (Bio-Rad). The molecular weights of protein bands were found to be 232, 126, 67, 20, 14, 6.2, and 3.5 KDa which correspond to Catalase, β-Galactosidase, Albumin, Tripsin inhibitor, α-Lactalbumin, Aprotinin and Insulin B-chain respectively in *O. aegina*. The molecular weights of protein bands were found to be 212, 122, 67, 20, 14, 6.2, 3.5 Kda corresponds to Myosin, β-Galactosidase, Albumin, Tripsin inhibitor, α-lactalbumin, Aprotinin and Insulin B-chain respectively in *O. membranaceus.* The molecular weights of protein bands were found to be 232, 116, 76, 30,20, 14, 6.2, 3.5 Kda corresponds to Catalase, β-Galactosidase, Transferrin, Carbonic anhydrase, Tripsin inhibitor, α-Lactalbumin, Aprotinin and Insulin B-chain respectively in *O. dollfusi.* The molecular weights of protein bands were found to be 140, 94, 20, 14, 6.2, and 3.5 KDa corresponds to Lactate dehydrogenase, Phosphorylase-b, Tripsin inhibitor, α-Lactalbumin, Aprotinin, Insulin B-chain respectively in *C. indicus* (Table 1).

In *O. aegina* Myosin, Lactate dehydrogenase, Phosphorelase-b, Transferrin and Carbonic anhydrase were absent compared to other three species. Catalase, Lactate dehydrogenase, phosphorylase-b, Transferrin and Carbonic anhydrase were absent in *O. membranaceus* compared to other three species. Myosin, Lactate dehydrogenase, Phosphorelase-b and Albumin were absent in *O. dollfusi* compared to other three species. Catalase, Myosin, β-Galctosidase, Transferrin,

Albumin and Carbonic anhydrase were absent in *C. indicus* compared to other three species (Table1).

Table 1 Molecular Weights of Muscle Proteins (Allozymes and General Proteins) in Four Octopuses

S.No.	MW of Protein Standard (Kda)	Name of the Protein	*O. aegina*	*O. mebrana-ceus*	*O. dollfusi*	*C. indicus*
1.	232	Catalase	+	-	+	-
2	212	Myosine	-	+	-	-
3	140	Lactate dehydrogenase	-	-	-	+
4	116 to 128	B-Galactosidase	+	+	+	-
5	94	Phosporilase-b	-	-	-	+
6	76	Transferrin	-	-	+	-
7	67	Albumin	+	+	-	-
8	30	Carbonic anhydrase	-	-	+	-
9	20	Tripsin inhibitor	+	+	+	+
10	14	α-Lactalbumin	+	+	+	+
11	6.2	Aprotinin	+	+	+	+
12	3.5	Insulin B-chain	+	+	+	+

Note: (+) Found; (-) Not found

The cluster analysis (Figure 2) showed that the interrelationship and genetic variation in octopus species. The species *C. indicus* was differing from other three species *O. aegina, O. dollfusi* and *O. membranaceus*. Within the three species of octopuses, *O. aegina* and *O. membranaceus* closely related than *O. dollfusi*.

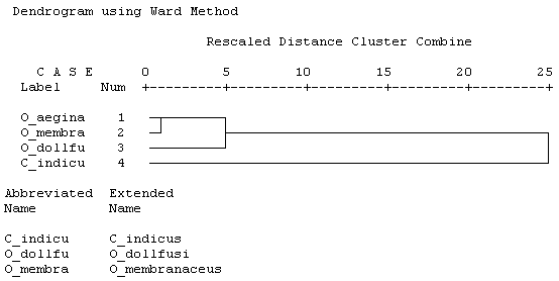

Figure 2 Dendrogram of four octopus species off Visakhapatnam

Rosa *et al*., (2002) reported molecular weights of *O. vulgaris* from Portuguese Coast. The molecular weight standards (Broad Range - Bio Rad) were myosin (200 KDa), galactosidase (116 KDa), bovine serum albumin (66 KDa), ovalbumin (45 KDa) carbonic anhydrase (31 KDa), soyabean tripsin inhibitor (21.5 KDa), lysozyme (14 KDa) and aprotinin (6.5 KDa). In the present study *O. vulgaris* was not analysed but myosin, galactosidase, albumin, carbonic anhydrase, tripsin inhibitor and aprotinin were recognized in four octopuses studied. Monolisha *et al*., (2013) studied the electrophoretic profile of crude powdered sample of octopus species; *O. aegina* and *O. dollfusi* revealed prominent bands from 32.83 KDa to 72.36 KDa and proved that both the species were distinct. Six to eight prominent bands were recognized in the four octopus species in the present study including the bands appeared in earlier study of Monolisha *et al*. The interrelationships of species and genera of different finfish and shellfish were investigated by using values of genetic distance derived from protein similarities and differences (James *et al*., 1982; Matthaeis *et al*., 1983; Gyllensten and Ryman, 1988; Suzuki and Phan, 1990; Hedgecock *et al*., 1993; Alvarez *et al*., 1995; Mohammed *et al*., 2006; Madhu and Rema, 2011; Diyaware *et al*., 2012; George *et al*., 2013). The above observations indicated that the electrophoretic studies were one of the important tools to study the protein pattern to distinguish species diversity and genetic differences in fin fish and shellfish.

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References

Alvarez, E, Zapata, C and Alvarez, G. 1995. Distinct genetic subdivision in sympatric and sibling species of the genus Littorina (Gastropoda: Littorinidae). Heredity, 74: 1-9.

Buth, D.J, Murphy, R.W. 1999. The use of isozyme characters in systematic studies. Biochemical Systematics and Ecology, 27, pp. 117-129.

Constante Ceccarini, Antimo D. Aniello, Marcello, G. Cacace and Paul H, Atkinson. 1983. Purification and characterization of a beta-N-Acetylglucosaminidase from octopus vulgaris. *Eur.J.Biochem*, 132: 469-476.

David, S, Woodruf, Margaret Mulvey, W, Bruce Sauders and Patricia Carpenter, M. 1983.Genetic variation in the cephalopod Nautilus belauensis.Proceedings of the Academy of Natural Sciences of Philadelphia, 135: 147-153.

Diyaware, M.Y, Haruna, A.B, Abubakar, K.A and Omitogun, G.O. 2012. Serum protein electrophoretic characterization of *C.anguillaris, H.bidorsalds* and their hybrids from the north east Nigeria. J.Microbiol-Biotech. Res., 2 (1): 70-77.

George Jesslin, Arumugam Sabaridasan, Ponnu Edwinthangam, Radhakrishanan Palanikani and Ramaiah Soranam. 2013. Electrophoretic studies on the muscle proteins of three species of genus *Puntius* (Osteichthyes-Cyprinidae). *International Journal of Research in Fisheries and Aquaculture*, 3(2): 16-20.

Gyllensten and Nils Ryman. 1988. Biochemical genetic variation and population structure of fourhorn sculpin (Myoxocephalus quadricornis; Cottidae) in Scandinavia. Hereditas, 108:179-185.

Hames, BD. 1998. Gel Electrophoresis of proteins. A practical approach, 3rd edn, Oxford University Press, Oxford, New York. Pp.345.

Hedgecock, D, Banks, M.A and Mc Goldrick, D.J. 1993. The status of the Kumamoto oyster *crassostrea sikamea* (Amemiya 1928) in U.S. Commercial broad stocks. *Journal of Shell-fish Research*, 12: 215-221.

James B. Shaklee, Clyde S, Tamaru and Robin S. Waples. 1982. Speciation and Evolution of Marine Fishes Studied by Electrophoretic Analysis of Proteins. Pacific Science, 36, No.2.

Johannesson K, Rodstrom EM, Aase H. 1989. Low genetic variation in Scan dinavian populations of *Ostrea edulis* L. – possible causes and implications. J. *Exp. Biol.* Ecol., 128: 177-190.

Madhu, K and Rema Madhu. 2011. Genetic differentiation of black tiger shrimp *penaeus monodon* Fabricicus (Crustacea: Decapoda) in Andaman and Nicobar islands. *Indian J. Fish*., 58 (2): 145-148.

Matthhaeis, E.D, Allegrucci G, Caccone A, Cesaroni D, Coboli sbordoni M and Sbordoni V. 1983. Genetic

differentiation between *Penaeus kerathuras* and *Penaeus japanicus* (Crustacea, Decapoda). Mar. Ecol. Prog. Ser., 12: 191-197.

Monolisha, S, Swathi Elizabeth Mani, Jamila Paterson and J.K. Patterson Edward. 2013. Molecular characterization and Antimicrobial activity of *Octopus aegina* and *Octopus dollfusi* in Gulf of Manner Coast. *IJPSR*., 4(9): 3582-3587.

Nevo, E. 1978. Genetic variation in natural populations : patterns and theory. Theor. Populat. Biol., 13: 121-177.

Roper, CFE, Sweeney MJ., Naven CE. 1984. Cephalopods of the world. Food and Agriculture Organization, Rome, Italy, 3: 277.

Rosa, R., Nunes L and Sousa Reis C. 2002. Seasonal changes in the biochemical composition of *Octopus vulgaris* cuvier, 1797, from three areas of the Portuguese Coast, Bulletin of Marine Science, 71(2): 739-751.

Ryman, N, utter, F, Laikre, L. 1995. Protection of intraspecific biodiversity of exploited fishes. Review of Fish Biology and Fisheries, 5:417-446.

Sarby, S. E1-Serafy; Nassr-Allah H. Abdel-Hamide; Mohammed H. Awwad and Mona S. Azab. 2006. Comparative study on electrophoretic protein pattern characterization of Tilapia species in the River Nile, Egypt. Egypt. *J. Aquat. Biol & Fish*, 10 (2): 147-178.

Silas, E.G. 1985. Cephalopod, bionomics, fisheries and resources of the exclusive economic zone of India. (ED.EG.Silas). CMFRI Bulletin 37: pp.195.

Suzuki, H and Phan. N.V. (1990): Electrophoretic study on intraspecific variations and interspecific relationships of marine catfishes (Siluriformes, Ariidae) of Cananeia (Sao Paulo, Brazil). 1. General Proteins of eye-lens and skeletic muscle. Bolm Inst. Oceanogr, S Paulo., 38(1): 31-42.

Tsuyuki, H, Roberts, E, Vanstone, W.E. 1965. Comparative zone electropherograms of muscle myogens and blood hemoglobins of marine and freshwater vertebrates and their application to biochemical systematics. *Journal of the Fisheries Research Board of Canada*, 22: 203-211.

Vibeke Simonsen and Kongkiat Kittiwattanawong. 1998. Marine mollusks and molecular markers: protein destrophoresis. Phuket Marine Biological Centre Special Publication, 18 (2): 223-236.

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