Research Paper

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Protein Profiles of Selected Commercial Fresh Water Fishes of Nellore, India

Biotechnology

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ABSTRACT

Soluble proteins of muscles of different aquatic fish have been analyzed by SDS- containing polyacryalamide gel electrophoresis (SDS-PAGE). 14 bands are diagnostic to all different types of fish selected with the molecular weight ranging from 250 KD to 10 KD. In comparison, among the selected animals, the high protein content was present in Tilapia mosambica (1180 ug/ml) and low protein content was present in fish species Labeo rohita (580 ug/ml) concluding that the selected aquatic species are rich sources of protein and SDS PAGE can be successfully employed for the separation of muscle proteins in the selected aquatic animals and helps to characterize these species on the basis of species-marker bands in SDS-PAGE patterns.

INTRODUCTION

Fisheries and aquaculture is gaining additional emphasis due to our concern in sustainability, greener solutions, conservation and food security. Detailed studies on physiology, genetics and general biology are therefore in a fish species very much relevant in order to put forward conservation protocols and to propose newer and improved culture practices (Surajit et al., 2011). Fish has been recognized as an excellent food source for human beings for centuries and is preferred as a perfect diet not only due to its excellent taste and high digestibility but also because of unsaturated fatty acids, essential amino acids and minerals for the formation of functional and structural proteins (Kumar, 1992). Even in small quantities, fish can have significant positive impact in improving the quality of dietary protein by complementing the essential amino acids that are often present in low quantities in vegetable based diets (Sargent et al., 1995).

Proteomics is an unbiased, technology driven approach for the comprehensive cataloguing of entire protein complements and represent an ideal analytical tool for the high throughput discovery of protein alterations in health and disease (Hochstrasser et al., 2002) The generation of large data sets on protein expression levels makes proteomics a preeminent hypothesis generating approach in modern biology (Renuse et al., 2011). Currently, research to improve the yield of fish has turned into looking at protein expression profiles or maps using the technique of 2D PAGE (Ian Craig et al., 2005). Proteomic studies of fish can help to identify proteins and enzymes that are responsible for increasing meat yield, the commercially important fish vitamins and unsaturated fatty acids, as well as, treatment of fish diseases. The major theme of this work is to provide protein expression profiles or maps of tropical freshwater fishes common to Nellore region (*Thalapia mosambica, Labeo rohita and Catla catla)* that are seriously lacking in the literature. Nellore district is called the " Aqua capital of India" due to its high production of cultured fish and shrimp. An attempt has also been made to explode the possibility whether polypeptide-spiced resolutions by SDS-PAGE can assist in better application of inter and intra-species comparison of soluble muscle proteins of fish species.

METHODOLOGY:

Sample collection: Selected species of fishes of similar body weight and length (*Catla catla*, *Labeo rohita* and *Thalapia mosambica)* were collected from fish market located at Nellore, A.P. India. All fishes were washed, beheaded, sliced and covered with ice to ensure freshness of the fish tissues. About 25 g of fish muscle tissue was separated for the determination of different tests. The fish muscle tissue was then sliced into smaller pieces and placed in sterile universal bottles and kept at -20^{0}C.

Proximate composition

Moisture: Moisture content in the fish sample was determined by using automatic moisture analyzer (IR 120, Denver, Moisture analyzer). 1 g of the sample (Fish muscle) was subjected to an initial temperature of 100°C and a final temperature of 170°C until a stable weight was achieved. Moisture percentage was obtained from the weight loss due to heating.

Ash: The ash content of the samples was determined by following AOAC method (AOAC. 1995.). 5 g of the fish muscle sample was taken in a previously ignited and weighed silica crucible. It was then transferred to muffle furnace (Phoenix CEM Corporation, USA) and the temperature was raised to 600°C and kept for 6 hours until white ash was obtained. Weight was taken after cooling and the percentage of ash was calculated from the weight difference.

Extraction of total lipids: Total lipids were extracted from muscle tissue according to Folch et al., 1957. The resulting extract of total lipids was stored at 4°Cfor further analysis.

P**rotein extraction**: 1 gram of fresh tissue was grounded to a powder with liquid nitrogen with mortar and pestle. 5 ml of extraction media (0.17 5 M tris HCL), pH 8.8, 5 % SDS, 15 % glycerol 0.3 M DTT) directly add a to mortar and grinding was continued for an additional 30 seconds and homogenate was filtered through muslin cloth, into a 50 ml Falcon tube at room temperature. 4 volumes of ice cold 100 % acetone was added to filter homogenate, mixed by vortexing and placed at -20 ^{0}C for at least 1hour to precipitate proteins. Sample was centrifuged at 5000 rpm for 15 minutes to collect precipitated protein. The pellets washed in 15 to 20 ml of cold 80 % acetone. The final protein precipitate was centrifuged at 5000 rpm for 15 minutes and supernatant was used as protein sample

Protein concentration determination: This was carried out using the method described by Bradford, (1976). The assay was performed in a 96 well plate using Bovine Serum Albumin (BSA) as standard protein. The absorbance was measured at 595 nm.

Sodium Dodecyl Sulphate-Polyacrlyamide Gel Electrophoresis (SDS-page)

SDS-PAGE was performed as described by Laemmli (1970). Ten per cent polyacrlamide gel in a vertical slab gel apparatus (Hoefer) was used.

Statistical analysis: Data presented as mean ± standard error of the mean (SEM), and significant differences between the means were determined by using SPSS (version 16).

RESULTS AND DISCUSSION

This experiment was performed to study the percentage of moisture, ash content, total lipids and protein content in different fresh water fish of Nellore AP, India. The data recorded through physical and chemical analyses was subjected to a statistical analysis which showed significant variations in total lipids and proteins content in the muscle of these three fresh water fishes. The proximate composition of the selected fishes shows that the differences in all the contents analyzed and shown in the Table 1 and Fig 1. From all fish species, the high protein content was

found to be present in *Thalapia mosambica* i.e 1180µg/ml and low protein content was present in *Labio rohitha* i.e. 580 µg/ml.

Table 1: Proximate Composition (%) of different selected freshwater fishes

Nutrients	Thalapia mosambica	Labeo rohita	Catla catla
Moisture*	73.49±5.9	75.46 ±6.0	77.5 ±6.5
Lipid**	7.90±0.63	2.9 ±0.21	1.2±0.08
Ash**	3.74±0.28	2.20 ±0.17	2.7 ±0.22

***=Non-Significant, **= Significant**

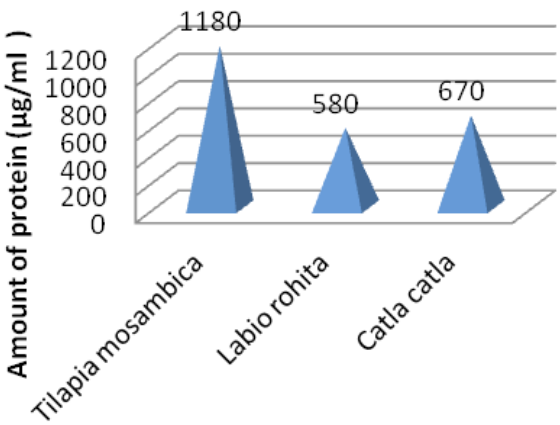

Fig 1: Amount of protein present in selected fresh water fish muscle

The banding patterns on gel reveal information about the protein composition of muscle from fish samples. PAGE patterns of soluble muscle proteins of selected samples of different fish are compared in Fig 2. Taking presence and absence of co- migrating bands as the criteria in analysis of PAGE patterns reveals that a lot of 14 bands are diagnostic to all different types of fish selected with the molecular weight ranging 250 KD to 1 0 K D.

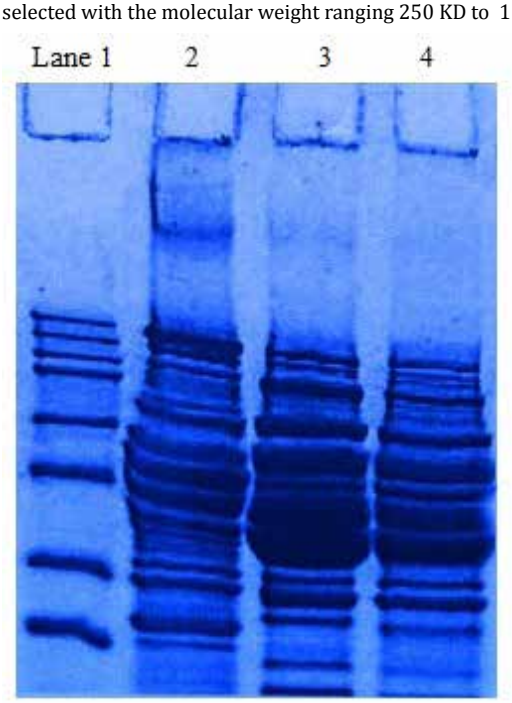

Fig 2: SDS-PAGE patterns of soluble muscle proteins of selected fish (Lane1:Protein marker, Lane 2: *Thalapia mosambica* ,Lane 3: *Labeo rohita* , Lane 4: *Catla catla*)

The useful determination that can be made from a protein gel is relative protein abundance. A thick, broad, darkly stained band indicates an abundance of molecules in that band, compared to a faint or narrow band. Base line data is to characterize these species on the basis of species-marker bands in SDS-PAGE patterns. Different proteins with in a sample are present in different quantities and the same protein may be present at different levels in different samples. Since created by closely related organisms share similar DNA sequences, the proteins encoded by their DNA should be very similar as well, and similar protein compositions should be reflected in banding patterns (Choi et al., 1986). These banding patterns will be very useful for the studies of protein patterns under different diet and environmental conditions in order to improve all aspects of the fish industry as well as generating new knowledge (Jitender Kumar et al., 2012). Variations in an organism's protein may reflect physiological adaptations to an ecological niche and environment but they originate as chance DNA mutations (Javed et al., 1992). By keeping the importance of protein profiling in assessing various climatic and diseased conditions as it is highly useful in exploiting health status of animals (Guha et al., 2013).

CONCLUSION:

In the present study, we have investigated the extent to which SDS-PAGE can help discern inter-or intra species differences in selected fish. The results showed that all the fishes have similar protein profiles, where each protein band consisted of identical proteins. Furthermore, the relative intensity of protein bands of all the fishes analyzed is also similar. These maps will be very useful for the studies of protein maps under different diet and environmental conditions in order to improve all aspects of the fish industry as well as generating new knowledge.

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