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IDENTIFICATION OF MUSCLE PROTEIN PATTERN AND GUT PROTEASE ACTIVITY OF ROHU (*Labeo rohita*), CATLA (*Catla catla*), CLIMBING PERCH (*Anabus testudineus*) AND SPOTTED SNAKEHEAD (*Channa punctatus*) USING SDS-PAGE

Silvia Ferdous, Md. Shamim Ahmed, Zerin Sultana, Md. Monirul Islam and Md. Ayaz Hasan Chisty*

Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna 9208, Bangladesh

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Abstract: The study was carried out to identify muscle protein patterns and gut protease activity of rohu (*Labeo rohita*), catla (*Catla catla*), climbing perch (*Anabas testudineus*) and spotted snakehead (*Channa punctatus*). Twelve percent Polyacryleamide substrate SDS-PAGE was done to visualize the activity of the enzyme after casein hydrolysis and protein pattern of the species. Three alkaline proteases of different molecular weight were observed in rohu, two in catla, five in climbing perch and four in spotted Snakehead from the zymogram. This revealed that the existence of alkaline protein in the alimentary tract of respective species. Fourteen distinct protein bands in rohu, fifteen in catla, sixteen in climbing perch and nineteen in soptted snakehead were visualized from the electrophoretic patterns of fish muscle extract. From the result, it is assumed that heavy molecular weight myosin and low molecular weight actin were prominent in all the experimental species.

Keywords: casein, enzyme, protein patterns, actin, myosin

Introduction

Protein is the most important constituent of the diets that cost higher than any other major nutrient. The fate of dietary protein after ingestion depends on its digestibility. Protein serves as biological catalyst and protective antibodies. The majority of enzymes are proteins. Enzymes are the reaction catalysts of biological system in fishes like all other warm blooded animals. Due to the very high catalytic activity, enzyme increases the rate of reaction million times or even more in biological system (Dixon and Webb, 1979). Enzymes are responsible for digestion of feed ingredients. Enzyme acts as catalyst, transforming feed ingredients into absorbable form (from protein to amino acids). The basic function of the digestive system is to dissolve food by rendering them soluble so that they can be absorbed and utilized in the metabolic process of the fish (Lagler, 1977; Nelson 2005). Fish enzymes are the biological molecule that takes part in important chemical reactions in the fish body that are involved in the digestion and absorption of food in the digestive tract of fish and also involved in tissue maintenance and cell growth Many of these enzymes continue to function after the fish dies, which can reduce its quality by causing tissue breakdown and changes in its flavor and aroma (Champe and Harvey, 2003).

The ability of the fish to utilize ingested nutrients depends on the activities of digestive enzymes present in various locations along the digestive tract. Proteases are the enzymes which

*Corresponding author: <ahc-md@yahoo.com>

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take part in protein digestion. Characterization of digestive proteases in fish species is important for research on nutrition, feeding ecology and biotechnology (Garcia-Carreno 2004). Investigations on protease activities of several marine species have been carried out in order to develop an effective diet for intensive farming of these species through proper understanding of their digestive capabilities towards various feed ingredients. It's very important to understand of the properties, function and optimized conditions for protein hydrolysis of digestive proteases in fish, which will enable and more accurate to measurement of protein digestibility by a particular species (Clark *et al*., 1985). A few researches have been done on the study of digestive enzymes of fresh water fish species. Gel electrophoresis is a major technique for enzyme and protein studies. It has been utilized for the assessment of the composition, molecular mass and classes of proteases present in crude extracts from fish digestive tract. The separation in electrophoresis is based on two characteristics of the molecules being separated electrical net charge and size. A strong detergent which surrounds all parts of the protein chain and causes the protein to unfold into a long string, which will be completely surrounded by the detergent molecules, can be added. The detergent which is usually used to do this is called sodium dodecyle sulphate (SDS). The kind of gel which can be used is called polyacryleamide, because it is a polymer of acryl amide. SDS-PAGE (Sodium Dodecyle Sulphate Polyacryleamide Gel Electrophoresis) is an important technique for identification of protein pattern and enzyme activity in the crude protein and enzyme extract (Laemmli, 1970).

As discussed earlier, the sustainability of aquaculture depends on the costing of the feed. The success of fish culture of valuable fish species viz. rohu (*L. rohita*), catla (*C. catla*), climbing perch (*A. testudineus*) and spotted snakehead (*C. punctatus*) depends on suitable low cost feed development. As protein is the most costly nutrient in diets and the enzymes that are involved in protein digestion might vary from species to species, it is important to get the muscular protein pattern and the proteases present along with the digestive tract of the species to help selecting the feed ingredients as well as to maintain the protein levels in feed. Therefore, the present study was designed to identify gut protease activity and muscular protein pattern of different commercially valuable species.

Materials and Methods

To observe the muscular protein pattern and the gut protease activity of the valuable fish species, different procedures and protocols were followed:

Sample collection: Four different commercial valuable fish species were selected as follows: (Table 1). Live fish (viz. rohu, catla, climbing perch and spotted snakehead) were purchased from local market (Gollamari bazaar) and kept in aquaria (climbing perch and spotted snakehead) and ponds (ruhu and catla) until needed. Upon the arrival of the fish, they were acclimatized by commercial feed (protein level 40%) in the aquaria and in the ponds. Later they were sacrificed for muscle protein and gut enzyme collection, according to Garcia-Carreno *et al.,* (1994).

Table 1. List of the species

Common name	Local name	Scientific name
Rohu	Rui	*Labeo rohita*
Catla	Catla	*Catla catla*
Climbing perch	Koi	*Anabas testudineus*
Spotted snakehead	Taki	*Channa punctatus*

Preparation of enzyme extract: Live fish were caught from the aquaria and pond and sacrificed by dissection to collect the guts of the species. The guts of the same species were pooled together, kept at chilled condition (≤4°C) and weighed using electronic balance (Electric balance, Model-AND GF-300, Japan) and homogenized in a Potter Thomas tissue grinder with a Teflon pestle at cool

temperature (≤4°C) by keeping the tissue grinder on to ice and diluted with cool distilled water (4°C) at a ratio of 1:10 W/V. The homogenate were poured into 1.5ml microfuge tubes (previously kept into ice and marked) and immediately centrifuged at 12000×g for 15 minutes at 4°C in a refrigerated centrifuge (Hettich Zentrifugen, model- D 78532, Mikro 22 R, Germany). The upper lipid layer of the supernatant, after centrifuging, was discarded. The aqueous supernatant was collected in previously cooled microfuge tubes, frozen and stored at -20°C until use (Chisty, 2005).

Preparation of protein extract: Live fish were sacrificed by dissection to collect the muscle just beneath the 1st and or 2nd dorsal fin, but above the lateral line of the respective species. The muscle of the same species were pooled together, kept at chilled condition (≤4°C) and weighted. Later, the muscle was homogenized in a Potter Thomas tissue grinder with a Teflon pestle at cool temperature (≤4°C) by keeping the tissue grinder on to ice and diluted with cool distilled water (4°C) at a ratio of 1:10 w/v. The homogenate were poured into 1.5ml microfuge tube and centrifuged at 12000×g for 15-20 minutes in a centrifuge (Eppendorf, model-5415D, Germany). The aqueous supernatant was taken in previously cooled another microfuge tube, after centrifuging. Immediately after collection of the muscle protein extract of electrophoresis was done to identify the protein pattern according to Bollag and Edelstein (2000).

Sample preparation for enzyme activity : Ten µl crude enzyme extract and 10µl sample buffer was taken, vortexed (Rocking on rotary sucker, Type 16700 Mixer, Thermoclyne) for 30 second, after vortex, the sample was ready for electrophoresis. The sample buffer was prepared according to Bollag and Edelstein (2000).

Sample preparation for protein pattern: Ten µl crude protein extract and 10µl sample buffer was taken. It was then vortexed for 30 second and heated 3-5 min at 95°C temperature in water bath (Grant instruments Ltd., Model- Hearts SG86PZ, England). Again it was vortexed and centrifuge at 12000×g for 15-20 min in a centrifuge (Eppendorf, Model- 5415 D, Germany) and was taken in a previously cool microfuge tube and was ready for electrophoresis.

Sodium Dodecyle Sulfate Polyaccrylamide Gel Electrophoresis: SDS-PAGE was done to identify the enzyme zymogram and protein pattern. Bio-Rad Mini -Gel Apparatus (Bio-Rad, Mini-PROTEIN 3 cell) was used for this purpose. In brief, Twelve percent polyaccrylamide was used to prepare the linear slab gel at a dimension of 10 cm × 8 cm × 0.75 mm. In brief, the gel was prepared by pouring the separating gel in the glass slab. Stacking gel was poured onto separating gel, just after the polymerization of the separating gel. Comb (0.75 mm thickness and with 10 teeth) was inserted on it. Electrophoretic buffer was poured properly according to Bollag and Edelstein, (2000) and 10 µl sample solution was loaded onto each well. The tub was placed in a bowl containing ice to maintain the temperature below 10°C. For running the gel, 120V constant power supply was ensured (Bio-Rad, Power pack 1000). Electrophoresis continued until the dye front migrated about to 1 cm above the bottom of the gel (around 1 hr) then Spacer (0.75 mm) was removed carefully by inserting the spacer in one corner between the plates and the gel plates were apart gently. To obtain the enzyme zymogram, the gel was soaked in 2% caesine solution (w/v) by using caesine and Tris HCl buffer, (pH 7.8) and kept at 4°C for 30 min that helped to absorb casein into the gel. Later, keeping the gel into the same solution; it was incubated for 1.5 hrs at room temperature. To obtain the protein zymogram, soaking in caesine solution was omitted. Now the gel was subjected for staining and distaining.

Gel staining: The gel was picked up and transferred to a small container, containing a small amount of coomassie gel staining solution by using coomassie Blue R-250, methanol, distilled water and glacial acetic acid and the gel, keeping in to the staining solution, was agitated gently for 1-2 hrs. After staining of the gel, it was taken out from the solution, washed a few times with distilled water and was subjected to destaining.

Destaining of gel : The gel was soaked in destaining solution and kept over night with a gentle stirring. In brief the destaining solution was prepared by addition of methanol, distilled water and glacial acetic acid. Finally the gel was taken out from the solution, washed with distilled water and preserved for the zymogram.

Results

Emzyme: Ten µl of prepared gut enzyme extract and sample buffer of different fish species was loaded onto each well to obtain zymogram. The existence of different alkaline protease in gut of all species was observed (Fig. 1). At least three alkaline proteases of different molecular weight were observed in rohu (*L. rohita,* Lane: 1 and 2), two in catla (*C. catla*, Lane: 3 and 4), five in climbing perch (*A. testudineus*, Lane: 5 and 6) and four in spotted snakehead (*C. punctatus*, Lane: 7) (Fig. 1).

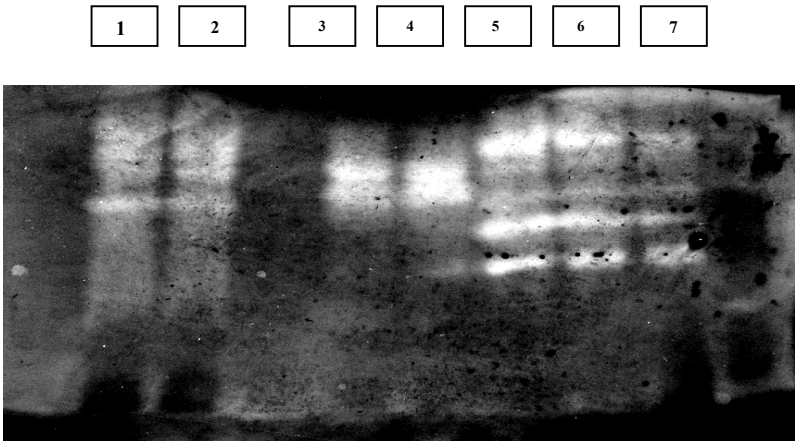

Fig. 1. Zymography patterns of protease present in the digestive tract of four different fish species (Lane: 1 & 2 for rohu; 3 & 4 for catla; 5 & 6 for climbing perch and 7 for spotted snake head)

Protein: Muscular protein pattern of different fish species were visualized after electrophoresis. Fourteen distinct bands in rohu (*L. rohita*) were visualized (Lane: 1 and 2) from the electrophoretic patterns of fish muscle extract. And that of fifteen in catla (*C. catla,* Lane: 3 and 4), sixteen in climbing perch (*A. testudineus*, Lane: 5 and 6) and nineteen in spotted snakehead (*C. punctatus*, Lane 7 and 8) were visualized from SDS-PAGE (Fig. 2).

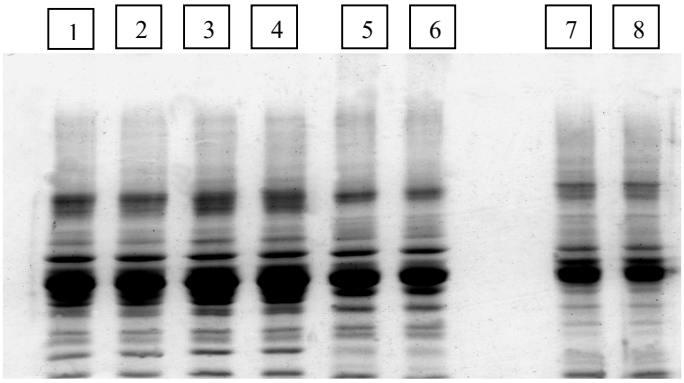

Fig. 2. Gel electrophoretic patterns of muscle extracts of four different fish species (Lane: 1 & 2 for rohu; 3 & 4 for catla; 5 & 6 for climbing perch and 7 & 8 for spotted snake head)

Discussion

This study was carried out to identify the existence of alkaline proteases present in the gut of rohu, catla, climbing perch and spotted snakehead. Four active zones of protease in spotted snakehead (*C. punctatus*) were visualized from the zymogram. Five active zones of protease in climbing perch *(A. testudineus*) were visualized from the zymogram. Two active zones of protease in catla (*C. catla*) and three active zones of protease in rohu (*L. rohita*) were visualized from the zymogram. Hidalgo *et al*., (1998) conducted experiment on a comparative study of the proteolytic and amylase activities in six species (rainbow trout, gilthead sea bream, european eel, common carp and gold fish) of fish with different nutritional habits. The ratio of total amylase: total proteolytic activity was higher in omnivorous fish species, the carp having the greatest value, whereas in trout this ratio was lower than one. The present study is also supported by the findings of Hidalgo *et al.,* (1998) as comparatively lower number of proteolytic activity/ active bands were obtained in Indian major carps (rohu and catla) than the carnivore species, spotted snakehead and climbing perch. Lemos *et al.,* (1999) reported that shrimp have trypsin between 17.7 and 22kDa. Gimenez *et al.,* (2001) conducted experiment on digestive proteases of red shrimp (*Pleoticus muellen*) with different moulting stages and ten active bands distributed from 17.4 to 66kDa were detected in all moulting stages from Zymograms of proteases activity were determined.

The presence of metal protease (36.1-39.8kDa), trypsin (73.3-76.5kDa), non trypsin or chymotrypsin serine proteases (58.7-61.4kDa) and chymotrypsin (19.2-21.8kDa) were detected in the intestine of discus (Garcia-Carreno, 2002). Four or more enzymes are available in the digestive extract having the molecular mass of 65.3kDa, 44.9kDa, 38.8kDa and 26.6kDa climbing perch (*A. testudineus*) has been reported by Manjuara (2006). The present study is also in support of the findings of Manjuara (2006). Several protein bands from the electrophoresis were visualized in the muscle of rohu, catla, climbing perch and spotted snakehead. Probably heavy chain myosin with high molecular weight and actin with comparatively lower molecular weight were prominent in all species. Also other protein bands were visualized with comparatively thin bands. Molecular weight of myosin and actin has been reported to be 230 kDa and 42 kDa respectively (Nelson, 1982). it is support by the statement of Chang *et al.,* (2002) they investigated the molecular evolution by analyzing fish proteins and found myosin heavy chains in glavoc, pirka, catfish, trout and tuna, myosin light chains in glavoc, pirka and fish sticks, actin in glavoc, prika, catfish, trout and tuna. Most of the samples contain both myosin and actin.

Conclusion

The result of this study as three alkaline proteases of different molecular weight were observed in rohu (*L. rohita*), two in catla (*C. catla*), five in climbing perch (*A. testudineus*) and four in spotted snakehead (*C. punctatus*). Muscular protein pattern of different fish species was visualized after electrophoresis. Fourteen distinct bands in rohu were visualized from the electrophoretic patterns of fish muscle extract. And that of fifteen in catla, sixteen in climbing perch and nineteen in spotted snakehead fish ware visualized from SDS-PAGE. So, the findings of the research would be useful tools for to identify gut protease activity and muscular protein pattern of different experimental species.

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