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Variation in the Muscle Tissue Protein of Some Fish Parasitic Isopods

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ABSTRACT

Parasitic attack commercially important fishes are a matter of great concern. Parasites were collected from the Nagapattinam coastal environment, Southeast coast of India. The concentration of protein in the muscles shows variation among the isopods parasites. The muscle proteins from 5 species of isopods parasites was studied, among the species maximum protein content of 18.26% was observed in *Nerocila loveni* and the minimum protein content of 3.44% was noticed in *Catoessa boscii*. After the Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), their clear bands were detected in the gel that represents proteins from five species of isopods. The present study reported for the first time that muscles proteins of isopods were isolated and considered for protein quantification and identification.

Key words: Crustaceans, isopod parasites, muscle, protein, SDS-PAGE

INTRODUCTION

Knowledge of fish diseases and parasites is essential for successful aquaculture, particularly in a country like India with long and highly productive coastal waters. However, the tropical coastal and brackish waters which likely contain many unknown parasites and pathogens, are poorly studied. Parasites play a pivotal role in the biology of fishes and can affect their behavior, health and distribution (Rohde, 1993). Isopods are one of the most diverse orders of crustaceans. Marine isopods are poorly studied animals in many regions. The parasite may be detrimental to the host simply by being there. As a consequent the normal functioning of the host structures can easily be disturbed, especially fins, operculum, mouth and lateral line canal. Pressure atrophy can often be seen in the tissues surrounding the larger isopod parasites (Kabata, 1985). Isopods absorb their nourishment directly from the host's body and they depend upon their hosts for feeding (Pillai, 1958). However, these isopods also possess positive qualities. A nutrient such as protein is needed to fulfil its energy requirement rather than of growth maintenance, is an expensive component in a diet. The reported estimate of protein requirement must be carefully examined because it is dependent on quality (essential amino acid profile) of dietary protein, age or physiological state of crustaceans (D'Abramo and Sheen, 1994). *Nerocila* is a large genus of Cymothoidae including at least 65 species living usually attached on the skin or on the fins of fishes (Bowman, 1978). The *Nerocila*, were collected from the body surface, on the head, on the pectoral fin or from the caudal peduncle of hosts. The *Catoessa boscii* and *Cymothoa eremita* are buccal

parasites. So, the present study was carried out to evaluate and update the protein value of fish parasite in different species of isopods such as *Nerocila phaeopleura*, *Nerocila loveni*, *Nerocila sundaica*, *C. boscii* and *C. eremita* and also to extent which SDS-polyacrylamide gel electrophoresis (SDS-PAGE) used to discern interspecies differences in five species of isopods parasites. Data is provided to characterize these species on the basis of species-marker bands in SDS-PAGE patterns.

MATERIALS AND METHODS

Collection of animal: The parasites were collected from Nagapattinam coastal environments, Southeast coast of India were examined during the years 2011. Species were collected and transported to the plastic cover and washed with fresh water. They were separated into different species. After homogenizing with pestle and mortar, the samples were transferred to centrifuge tubes. The homogenate was centrifuged for 20 min at 4000 xg and the supernatant was collected. Samples were maintained on cool condition at all times to avoid protein denaturation.

Estimation of protein: The amount of protein was measured by spectrometry according to the method of Biuret (Raymont *et al.*, 1964). Biuret reagent was used as a colour reactant and concentration was calculated in response to absorbance at 540 nm in spectrophotometer (Spectro UK-Vis RS).

Determination of molecular weight by using SDS PAGE: SDS-PAGE was used to find out the molecular weight active fractions of the sample. SDS-PAGE was performed in separating gels, according to the method described by Laemmli (1970).

RESULTS

Estimation of protein: Variation in protein substance of the muscles of isopods parasites were shown in Fig. 1. Among the muscles protein from five species of isopods parasites, maximum protein content of 18.26% was recorded in *N. loveni* and the minimum content of 3.44% was noted in *C. boscii* parasite.

SDS-PAGE: The muscle tissue samples were used to estimate the Molecular Weight (MW) of proteins present in it by SDS-PAGE. Different samples were used to determine the molecular weight of proteins in the muscle tissue. The muscle tissue containing proteins were detected. Protein marker with molecular weight in the range of 20 to 50 kDa was used. The bands were detected

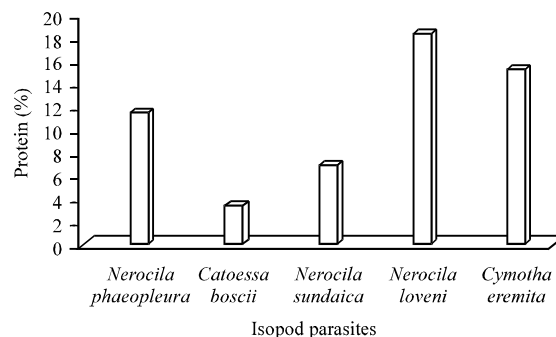


Fig. 1: Comparison of protein content in the isopod parasites

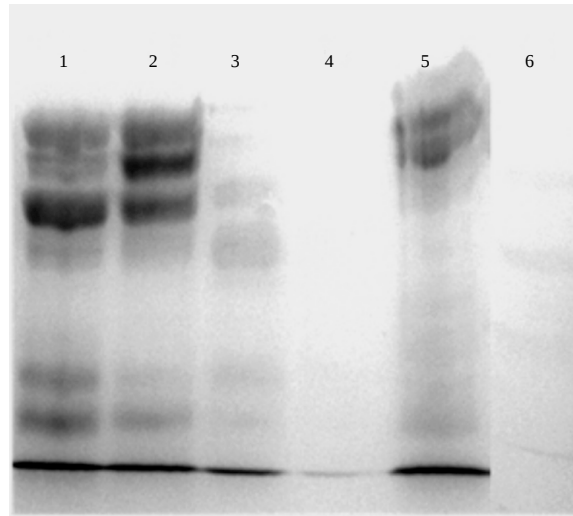


Fig. 2: SDS-PAGE gel showing variation in molecular weight of muscle protein in different isopod parasites. Lane 1: *N. loveni*, Lane 2: *N. sundaiica*, Lane 3: *C. eremita*, Lane 4: *C. boscii*, Lane 5: *N. phaeopleura*, Lane 6: Marker

Table 1: Molecular weight of muscle protein in isopod parasites

Molecular weight (kDa)					
<i>N. loveni</i>	<i>N. sundaiica</i>	<i>C. eremita</i>	<i>C. boscii</i>	<i>N. phaeopleura</i>	Marker
54	53	50	18	56	50
47	45	41	-	50	35
39	39	33	-	34	25
32	32	22	-	27	20
23	22	20	-	23	-
20	20	-	-	19	-
19	19	-	-	-	-

-: Not detected bands

in the gel that represents proteins. SDS-PAGE was shown to have protein patterns of parasites samples (Fig. 2). Seven bands with molecular weight of 54, 47, 39, 32, 23, 20 and 19 KDa were found in *N. loveni*. *N. sundaiica* also seven bands were detected with molecular weight of 53, 45, 39, 32, 22, 20 and 19 KDa. *C. eremita* had five bands with molecular weight of 50, 41, 33, 22 and 20 kDa. *C. boscii* had only one bands were detected with molecular weight of 18 kDa. *N. phaeopleura* showed six bands with MW of 56, 50, 34, 27, 23 and 19 kDa. Only few bands were detected and they were basically similar for all species except *C. boscii* (Table 1).

DISCUSSION

Information about these five isopod species is important because each infects and reduces the productivity of commercially important species of fishes. Proteins were, in proportion, the main body component and also the most stable. Parasites were found to have protein, maximum content of protein in the muscles of parasites *N. loveni* (18.26%) and *C. eremita* (15.24%) followed by *N. phaeopleura* (11.51%), *N. sundaiica* (6.83%) and the lowest content of (3.44%) was reported in

C. boscii. Among the five parasites body surface parasites of *Nerocila* genus, *N. loveni* show maximum value in both estimation of protein and molecular weight. In the buccal parasites *C. eremita* shows Maximum value in protein estimation and molecular weight. *N. phaeopleura*, *N. loveni* and *N. sundaica* had maximum bands were detected in compared with *C. boscii* and *C. eremita*. Most of the researchers have concentrated on the quantification, identification and isolation of protein from edible crustaceans like crabs, shrimps and lobsters. Isolation of protein has been earlier reported in the hemolymph of crustaceans in the brachyuran crabs (Rameshkumar *et al.*, 2009). The present study was observed percentage protein in the muscles of parasites. The protein content of *P. pelagicus* and *P. sanguinolentus* was 0.47-15.91% and 12.81-13.6% (Radhakrishnan and Natarajan, 1979). Reported on the protein value in blue crab was 17.17% (Anonymous, 1999). In general, the average body proteins of the crab were directly related to the levels of protein in the diet (Khan, 1992). Similar pattern of protein changes was reported by Alava and Lim (1983) in *P. monodan* and in *M. malcolmsonii* by Soundarapandian and Ananthan (2008). The results of the present study indicate that the concentration of protein in the muscles showed wide inter specific variation among the parasites. In the present investigation samples showed that protein level was subjected to SDS-PAGE to estimate the molecular weight. After electrophoresis band were detected in the gel which represented proteins of molecular weight. Further, the identification of protein pattern of the host fishes could give sufficient knowledge about the feeding, energy requirement and developmental needs of isopods which might pave way for devising a strategy to reduce the infection. To date, although progress has been achieved in this respect, knowledge on the protein requirements of isopod parasites was still too insufficient to provide reference for effective diet levels. In addition experiments presented in the study represent a preliminary analysis to compare the information obtained by electrophoretic methods, namely SDS-PAGE. This method allowed to separate proteins and to determine their molecular weights.

CONCLUSION

The results of the present study indicated that muscle tissue protein content shows wide inter specific variation among the fish parasitic isopods. No study has been previously dealt with a trial to estimate the protein content in isopods. They were considered to be least important but the present study has initiated a new baseline in parasitological. The study means more as the parasites acquire energy from the host in the form of proteins.

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