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# Identification of immunodominant polypeptides of the freshwater fish lice *Argulus siamensis* (Wilson) - preliminary findings

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## ABSTRACT

Successful development of immunoprophylaxis against freshwater fish lice *Argulus siamensis* (Wilson) depends on the proper identification of protective antigens. The present study was undertaken to determine the immunodominant polypeptides of the freshwater fish lice *A. siamensis*, which causes a major disease problem in freshwater aquaculture. Several polypeptide bands ranging from 130.55 to 16.22 kDa were detected in freshwater fish lice homogenates by SDS-PAGE and two polypeptide bands of 75.78 kDa and 79.6 kDa were found as immunodominant polypeptides in western blotting using hyperimmune sera raised in rabbit. This preliminary observation opened up avenues to look into these protein fractions as possible candidate antigens for immunoprophylaxis development against this dreaded ectoparasitic infection in fish.

Keywords: *Argulus siamensis*, Immunodominant polypeptides, Rohu, SDS-PAGE, Western blotting

## Introduction

*Argulus* sp. is a common freshwater ectoparasite belonging to the order Arguloida of the phylum Arthropoda and it causes a disease known as argulosis in fish. This parasite, recognized as a scourge of freshwater fish farming worldwide, is considered as a major economic problem. Rohu (*Labeo rohita*), one of the economically important aquaculture species in India, is the most susceptible fish species to this parasite in aquaculture production (Saurabh, 2009; Saurabh *et al.,* 2010). Several instances of argulosis associated with fish mortality have been reported from culture ponds (Gopalakrishnan, 1964; Singhal *et al.,* 1990; Sheila *et al.,* 2002). Fish suffering from argulosis exhibits behavioural abnormalities including lethargy, irritation and loss of appetite. These ectoparasites puncture the host's skin, inject a cytolytic toxin through its proboscis and feed on blood, besides feeding on mucus and epithelial cells (LaMarre and Cochran, 1992). Feeding sites often become haemorrhagic, ulcerated and provide access to secondary infections by other parasites, fungi and bacteria. Freshwater fish lice *Argulus siamensis* infection has emerged as a significant problem in composite fish culture ponds in India (Mishra, 1991; Saurabh and Sahoo, 2010) and is considered as a notorious pathogen in semi-intensive and intensive aquaculture. Besides direct loss due to mortality, this parasite retards growth, affects behaviour of its host and reduces the market value of fish.

Information on immune responses of host fish to *Argulus* sp. is scanty. Typically the most notable immune response to argulid infections is observed as localised inflammation, appearing as small red spots on the fish's skin (Walker *et al*., 2004). Ruane *et al.* (1995) demonstrated humoral antibody response in rainbow trout (*Oncorhynchus mykiss*) after they were immunized with an antigen extract from *Argulus foliaceus*. Recently, Saurabh and Sahoo (2010) reported non-specific immune responses of the Indian major carp, *L. rohita* naturally infested with different loads of the freshwater fish louse, *A. siamensis.* Modulation of the innate immune response and expression of immune-related genes in *L. rohita* on exposure to *A. siamensis* was also demonstrated by experimental challenge study (Saurabh *et al.,* 2010; 2011). However, no information is available on immunodominant polypeptides of *A. siamensis*, which can act as potential antigens for immunoprophylaxis. The present work was initiated to study the immunodominant polypeptides of *A. siamensis* in order to identify candidate antigen(s) for immunoprophylaxis against this patrasite.

## Materials and methods

### *Argulus siamensis antigen preparation*

Adult specimens of *A. siamensis* were collected from rohu and the whole parasitic extract was prepared by homogenizing 100 numbers of lice in mortar and pestle in 1 ml of phosphate buffered saline (PBS), pH 7.2. The

mixture was centrifuged at 10,000 for 10 min, and the supernatant was collected and stored at -20 °C for further use. Protein concentration of the supernatant was determined by Bradford protein-dye binding assay (Bradford, 1976) and adjusted to a final protein concentration of 1 mg $ml^{-1}$. This antigen was used for immunization of rabbit as well as for SDS-PAGE.

*Raising of hyperimmune sera*

One healthy New Zealand white rabbit was immunized with *Argulus* antigen to raise hyperimmune serum. Briefly, antigen in PBS was emulsified with Freund's complete adjuvant (FCA) at 1:1 ratio. The animal was injected subcutaneously with 1 ml of the emulsion , which contained approximately 500 mg of lice antigen. Two booster doses were given on 14th and 28th day of immunization with the same dose of lice antigen emulsified in Freund's incomplete adjuvant (FIA). . The rabbit was bled on 42nd day of immunization by puncturing the ear vein and the blood was allowed to clot at room temperature for 30 min and then left at 4 °C overnight. The blood sample was centrifuged at 500 g for 10 min, and the serum collected was aliquoted and preserved at -20 °C for further use.

*Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)*

SDS-PAGE in continuous buffer system was carried out according to the method of Laemmli (1970), in order to determine the number and molecular weight of different polypeptides of *A. siamensis* antigen. Electrophoresis was run on a separating gel of 12% and a stacking gel of 5% acrylamide concentration, in a Bio-Rad mini protean-II electrophoresis cell. Prior to electrophoresis, sample extracts (protein concentration 1 mg $ml^{-1}$) were boiled for 2-3 min in equal volumes of reducing buffer containing 2-mercaptoethanol. A molecular weight marker (Genei, Bangalore) was also run along with the sample at 200 V for approximately 1 h. The gel was then either stained using Coomassie brilliant blue (R-250) or used for western blotting. Molecular weight of the protein bands were determined using AlphaEase®FC Software (Version 5.0.1), Alpha Innotech Corporation, USA using an image analyser.

*Western blotting*

In order to check the specificity of rabbit anti-*Argulus* serum, western blotting was carried out in a mini transblot electrophoretic transfer cell (Bio-Rad, USA) as per the manufacturer's instructions. *Argulus* antigen separated by SDS-PAGE was electrophoretically transferred onto PVDF (Polyvinylidene difluoride) membrane (Hybond-P, Amersham Biosciences, USA) in Tris-glycine buffer, pH 8.3 containing 20% methanol. Electrophoresis was carried out at 100 V for approximately 1 h. After transfer, the PVDF membrane was cut from the middle. One half of PVDF membrane was stained for 2 min in amido black protein staining solution. It was then transferred to destaining solution and shaken for few minutes until the background was clear. The membrane was washed with distilled water and dried on a blotting sheet.

The second half of PVDF membrane was used for immunostaining. It was immediately incubated in blocking agent (5% skimmed milk powder in Trizma buffered saline, TBS) for 3 h at room temperature and the membrane was washed thrice with TBST (TBS with 0.05% Tween 20) at 5 min interval. Then the PVDF membrane was incubated with anti-*Argulus* rabbit serum at 1:1000 dilution for one hour and again washed with TBST. Control rabbit serum used at same dilution with a separate blot was also developed for comparison. Goat anti-rabbit IgG alkaline phosphatase conjugate (Genei, Bangalore) was added at a dilution of 1:1000 and the PVDF membrane was incubated for another 1 h. After washing three times with TBST, the substrate 5-bromo-4 choloro-3 indolylphosphate/nitroblue tetrazolium (BCIP/NBT) (Genei, Bangalore) was added and shaken well until the colour of the band developed. The PVDF membrane was immediately washed with distilled water and dried on a blotting paper.

## Results and discussion

The antibody responses in fish in relation to different parasitic infections were studied by several workers (Reilly and Mulcahy, 1993; Chin *et al.,* 2004; Swennes *et al.,* 2007). Presently, *Argulus* infection is identified as one of the most burning problems in the freshwater aquaculture in India (Saurabh, 2009; Saurabh *et al.,* 2010). Indian major carp, rohu (*L. rohita*), one of the major components of Indian freshwater aquaculture is highly susceptible to this parasite (Saurabh and Sahoo, 2010). To date, no prophylaxis or successful treatment is available to control this ectoparasite. So it was felt necessary to identify the immunodominant polypeptides of the parasite in order to develop immunoprophylaxis against this crustacean ectoparasite.

This is a preliminary attempt to identify an immunodominant polypeptide present in *A. siamensis*, among several polypeptides bands with molecular weight ranging from 130.55 to 16.22 kDa detected in freshwater lice antigen under SDS-PAGE. The major polypeptide bands detected in lice antigen were 97.25, 88.16, 79.60, 75.78, 63.05, 60.25, 54.86, 43.99, 31.33 and 16.22 kDa respectively (Fig. 1). Raune *et al.* (1995) observed polypeptide bands in the range from 100 kDA to 15 kDa of for *Argulus foliaceus* in SDS-PAGE.

In the present study, western blotting did not clearly identify components of molecular weight greater than 100 kDa in *A. siamensis.* Two prominent bands of 75.78 and 79.6 kDa besides few faint smaller bands were noticed

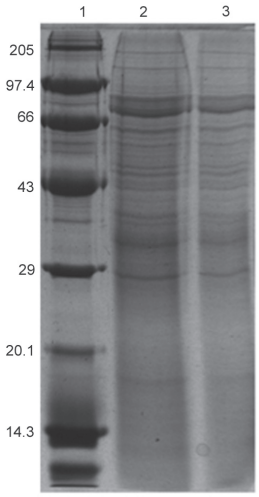


Fig. 1. SDS-PAGE analysis of *A. siamensis* antigens in 12% polyacrylamide gel. Lane 1: Standard molecular weight protein marker; Lane 2 and 3: *Argulus* antigen

(Fig. 2). No reactivity was detected with control serum. This may reflect the absence of such components or more likely, their quantity and immunogenicity, relative to components of lesser molecular weight. Reilly and Mulcahy (1993) reported that 110, 116 and 200 kDa polypeptides were the major antigenic components in sea lice, *Lepeophtheirus salmonis*, a similar louse affecting marine fish. On the other hand, Raune *et al.* (1995) reported 82 to 15 kDa polypeptides of *A. foliaceus* probed with rabbit anti-*A. foliaceus* serum in western blotting. In the present study, the 79.6 kDa and 75.78 kDa polypeptides were the most immunodominant antigens detected on western blot

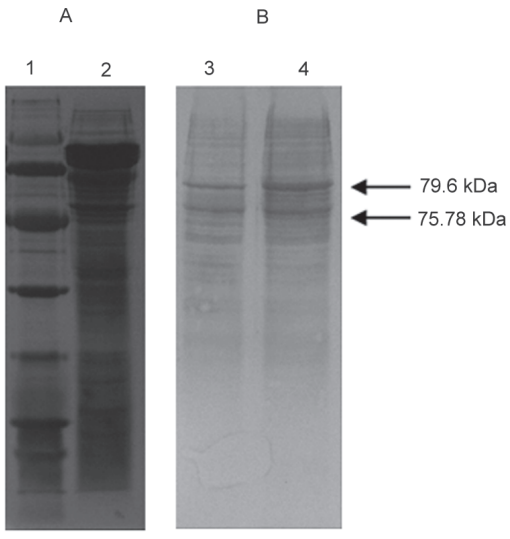


Fig. 2. Reaction of *A. siamensis* antigens with rabbit *anti-A. siamensis* hyperimmune serum in Western blotting. Strip A, Amido black staining (Lane 1: Standard molecular weight protein marker; Lane 2: *Argulus* antigen); Strip B, Immunostaining (Lane 3 and 4: *Argulus* antigen)

analysis. These antigens are potential targets for immunoprophylaxis development against argulosis. The successful immunoprophylaxis against the freshwater lice *A. siamensis* depends on the proper identification of protective polypeptide (s). The present study has opened up avenues to look into few protein fractions which could be considered as candidate antigens for immunoprophylaxis development. Further work is required to investigate the protective response of immunodominant polypeptides in rohu and other Indian major carps against this crustacean parasite, as it may help to control this dreaded ectoparasite, which is causing huge economic losses to freshwater aquaculture sector in India.

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