

Polyacrylamide Gel Electrophoresis of Proteins Extracted from *Nematotaenia Dispar* which Isolated from *Varanus griseus* in Saudi Arabia

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Abstract: A large number of mammals are hosts of taeniidae cestodes. *Nematotaenia dispar* was the only worm isolated from the infected gray monitor in Saudi Arabia. The present study was used to examine, the electrophoretic patterns of extracted proteins of *Nematotaenia dispar* isolates from *Varanus griseus* by using SDS-PAGE technique. The whole worm was washed with phosphate buffered saline (PBS, pH 7.4), weighed and homogenized by glass-glass homogenizer in cold physiological saline. Supernatants were examined by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and coomassie blue staining. The SDS-PAGE of the extracted proteins resulted in a total of eleven protein molecules were detected in gel, six bands with apparent molecular weights of 200, 116, 97, 66, 45 and 29 kDa were observed. It is concluded that the present results may give an idea about this worm that we can do antibodies for one of these proteins to be used in the future. In addition, further studies on the functions of these proteins are required.

Key words: *Nematotaenia dispar*, *Varanus griseus*, cestodes, SDS-PAGE, total protein, sulfate-polyacrylamide

INTRODUCTION

A large number of mammals are hosts of taeniidae cestodes (Alyousif *et al.*, 2005). *Nematotaenia dispar* is one of cestoda family (Saeed *et al.*, 2007). This worm was isolated from the reptile fauna which are important group of vertebrate animals in Saudi Arabia (Al-Mohammed, 2009). It has been reported that several species of protozoa can be transmitted through water and cause disease in humans (Gelhaus *et al.*, 2005; Thaumaturgo *et al.*, 2002).

Numerous biochemical and molecular markers are available for the clarification and delineation of different parasites. Electrophoretic analysis of whole cell proteins by one-dimensional protein patterns provides a rough measure of the number and physicochemical properties of gene products (Snider, 1973). Polyacrylamide gel electrophoresis is used to differentiate *Taenia* (Cestoda) by total protein.

The results of this technique revealed that band patterns of different parts of the taeniid strobila were basically identical hence, individual worms were useful for analysis regardless of their state of development when collected (Burse *et al.*, 1980). Electrophoretic techniques allow rapid and accurate identification of organisms. They are especially useful in clarifying relationships at sub specific and population levels when examining individual

genetic variants (Hotchkiss and Kaya, 1984). The protein composition of *Schistosoma mansoni* was analysed by electrophoresis in polyacrylamide slab gels in the presence of sodium dodecyl sulphate (Landa *et al.*, 2010).

The protein composition of *Trichomonas vaginalis* isolates was evaluated using one-dimensional and two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Alderete *et al.*, 1986). This technique is used to analyze trichomonad proteinases (Cabrera-Guzman *et al.*, 2010; Lockwood *et al.*, 1987). This technique is used also to compare the protein expression patterns of worms of different ages (Jamjoom, 2006; Tenenta and Evans, 1997).

As well as enzyme SDS-PAGE electrophoresis has been used in the characterization of many protozoa (Aly *et al.*, 2003; Kordafshari *et al.*, 2010). *Nematotaenia dispar* was recorded for the 1st time as one of the helminthes parasites which infected gray monitor, *Varanus griseus* of both sexes in Saudi Arabia (Al-Mohammed, 2009).

As a result of human population growth, there is now close contact between man, livestock and reptiles. No reports are also available on the protein profiles of *Nematotaenia dispar*. Therefore, the present study would extract proteins from *Nematotaenia dispar* and determine their molecular profiles by SDS-PAGE.

MATERIALS AND METHODS

Nematotaenia dispar were isolated from gray monitor (*Varanus griseus*) in Saudi Arabia (Al-Mohammed, 2009). Protein standards and sodium dodecyl sulfate, polyacrylamide gel were purchased from Amersham Bioscience (Freiburg Germany). Coomassie blue stain and other chemicals were purchased from sigma (St. Louis, MO, USA). A molecular weight marker containing several proteins of known molecular weights was commercially obtained from sigma (St. Louis, MO, USA), containing myosine (200 kDa), dactosidase from *E. coli* (116 kDa), phosphorylase from rabbit muscle (97 kDa), albumin from bovine serum (66 kDa), albumin from chicken egg white (45 kDa) and carbonic anhydrase from bovine erythrocytes (29 kDa).

Homogenate preparation: The whole worm were washed 3 times with sterile Phosphate Buffered Saline (PBS), pH 7.4. Then, they weighed and homogenized by glass-glass homogenizer in 100 μ L of 1 \times SDS gel loading buffer on ice. The homogenates were centrifuged for 30 min at 3000 rpm. The supernatant was drawn and its protein contents were concentrated by adding ammonium sulphate up to 60% (w/v) and left at 4°C for 2 h. The proteins were then collected by centrifugation at 6000 rpm for 10 min. The protein sample was dissolved in 100 mL physiological saline.

Preparing of sodium dodecylsulfate-polyacrylamide gel: The separating gel acrylamide 10% was prepared by mixing the following components (3.3 mL 30% (w/v) acrylamide bis-acrylamide mix, 2.5 mL, 1.5 M Tris-HCl pH 8.8, 0.1 mL 10% (w/v) SDS, 0.1 mL 10% (w/v) ammonium persulphate, 0.004 mL N,N,N',N'-Tetra-methylethylenediamine (TEMED) and poured into the gap between the glass plates (about 4 cm height) followed by 1 mL overlay 0.1% w/v SDS on the surface of separating gel and left vertical at room temperature for 30 min. The overlay was removed and the gel was washed with distilled water. The stacking gel acrylamide 5% was prepared by mixing the following components (2.1 mL H₂O, 0.5 mL 30% (w/v) acrylamide: bis-acrylamide mix, 0.38 mL 1.0 M Tris-HCl pH 6.8, 0.03 mL 10% (w/v) SDS, 0.03 mL 10% (w/v) ammonium persulphate, 0.003 mL TEMED) and added over the separating gel. The plastic comb was inserted into the stacking gel solution taking care to avoid any air bubbles and left for 30 min for polymerisation. The comb carefully removed and the glass plates were assembled to the electrophoresis apparatus containing 800 mL Tris-glycine electrophoresis buffer (25 mM Tris, 250 mM glycine pH 8.3, 0.1% (w/v) SDS).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE):

The protein samples were analyzed by using vertical one-dimensional SDS-polyacrylamide gel electrophoresis according to the method of Laemmli (1970). Protein samples was denaturated and reducing completely before electrophoresis by mixing: 15 μ L 2 \times SDS-gel loading buffer (100 mM Tris-HCl pH 6.8), 4% (w/v) SDS, 0.2% (w/v) bromophenol blue, 20% (v/v) glycerol, 200 μ L MDTT and 15 μ L protein sample (Zimic *et al.*, 2009) and heating the mixture at 95°C in a water bath for 3 min. About 20 μ L of denaturated protein sample were loaded into the gel.

The electrophoresis apparatus was connected to the power supply with 80 V for 2 h. Then the glass plates were removed from the apparatus and the gel transferred into staining solution (0.25% (w/v) coomassie brilliant blue R. 250 in 45% v/v methanol, 10% (v/v) acetic acid) and placed on a slowly rocking platform overnight. The gel was then transferred into destaining solution (30% (v/v) methanol, 10% (v/v) acetic acid) for 4 h on a slowly rocking platform.

The gel was photographed after staining and destaining will be complete. The same conditions were achieved for protein markers at range of 29-200 kDa molecular weights.

RESULTS

Extracted proteins from a whole *Nematotaenia dispar* were analyzed by SDS-PAGE technique. SDS-PAGE protein patterns was shown in Fig. 1. After staining with coomassie brilliant blue R. 250, some of the protein spots were stained more heavily as shown in the Fig. 1. In Fig. 1, lane 2 represents the protein bands extracted from the whole worm *Nematotaenia dispar*. From lane 2, eleven protein bands were observed and their molecular weights were determined related to

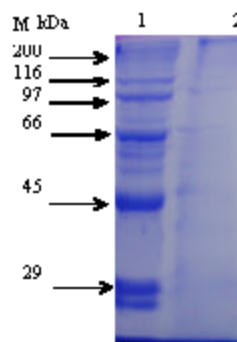


Fig. 1: SDS-PAGE electrogram of proteins isolated from *nematotaenia dispar*; Lane 1: Protein markers (M.Wt 30,000-200,000 kDa); Lane 2: Protein patterns of *Nematotaenia dispar*

protein markers. The prominent polypeptide bands were 200, 116, 97, 66, 45 and 29 kDa. The other bands of 11 polypeptides were with faint color and unknown molecular weights.

DISCUSSION

Electrophoretic protein banding pattern of an organism can be used to elucidate reliable biochemical genetic markers of this organism. It can also provide information about structural genes and their regulatory systems which control the biosynthetic pathways of that protein banding pattern (Abdelsalam *et al.*, 1992). In addition, electrophoretic techniques for identification and classification are widely utilized and are particularly useful for organisms that are difficult to distinguish by other means (Hotchkiss and Kaya, 1984). Soluble proteins of *Nematotaenia dispar* were analyzed by SDS-PAGE. In the present study, 11 bands with the molecular weights in the range of 29-200 kDa were observed in identifying protein profile extracted from *Nematotaenia dispar*. The results are in compatible with the previous (Abdelsalam *et al.*, 1992). Many spots on our gels remained to be identified (Fig. 1).

The observed changes in protein banding patterns in the present study could be reasonably interpreted to be the result of gene mutation. This conclusion is in accordance with Abdelsalam *et al.* (1992). However, other researchers as Ashour *et al.* (1995) traced such changes back to the induction of chromosomal abnormalities such as bridges breaks, laggards and micronuclei which can lead to loss of some of the genetic material. Therefore, some electrophoretic bands disappeared due to the deletion of their corresponding bands. Disappearance of some bands could also be explained on the basis of a mutational event at the regulatory genes which are suppressed at transcription level.

Meanwhile, the appearance of new bands could be explained on the basis of a mutational event at the regulatory system of unexpressed gene(s) that activate them (Kordafshari *et al.*, 2010). In other words, several factors may be considered as primary determinants of the number of bands observed on a gel including the number of coding genes, their allelic states (homozygous or heterozygous) and the quaternary of the protein products (El-Hady, 2010).

Cross reaction among helminths has been extensively described for decades (Hillyer, 1995). Thus, it has observed that Sm14, the 1st fatty acid-binding protein homologue derived from *Schistosoma mansoni* adult worm extract, induces immune cross-protection against infection by *F. hepatica* in Swiss outbred mice

(Tandler *et al.*, 1996). It has been reported that Sm14 was the 1st fatty acid-binding protein homologue identified in helminthes (Thaumaturgo *et al.*, 2002). Therefore, it is better to investigate the identification between the worm of the present study (*Nematotaenia dispar*) and different helminthes that infected humans. This test is important to protect, the humans against *Nematotaenia dispar* infection in the future.

CONCLUSION

The present study is the 1st description of the protein patterns of *Nematotaenia dispar*. Therefore, it is better to identify and know the amino acid sequences of each protein bands isolated from *Nematotaenia dispar*. In addition, we would investigate the cross reaction of *Nematotaenia dispar* with different helminthes particularly human parasites. In the future, Sm14 was the 1st FABP homologue identified in helminthes. Thereafter, members of the same family were identified in several helminthes species.

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