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

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INTRODUCTION

A large number of mammals are hosts of taeniid 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 delinition 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 taenid strobila were basically identical hence, individual worms were useful for analysis regardless of their state of development when collected (Burney 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 (Hotchkine 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 sulphate-polyacrylamide gel electrophoresia (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 (Jammoun, 2006; Tenenta and Evans, 1997).

As well as enzyme SDS-PAGE electrophoresia 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

**Nematocercia 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 myosin (200 kDa), dalactosidase 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 carboxic anhydride 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 μL of 1×SDS gel loading buffer on ice. The homogenate 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 10000 rpm for 10 min. The protein sample was dissolved in 100 μL 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'-Tetramethylethylenediamine (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 polymerization. The comb carefully removed and the glass plates were assembled to the electrophoresis apparatus containing 300 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 denatured and reducing completely before electrophoresis by mixing: 15 μL 2×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 μL MDTT) and 15 μL protein sample (Zimic et al., 2009) and heating the mixture at 95°C in a water bath for 3 min. About 20 μL of denatured 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 distaining 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 staining solution (30% (w/v) methanol, 10% (v/v) acetic acid) for 4 h on a slowly rocking platform.

The gel was photographed after staining and distaining 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 *Nematocercia dispar* were analyzed by SDS-PAGE technique. SDS-PAGE protein pattern 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 *Nematocercia dispar*. From lane 2, eleven protein bands were observed and their molecular weights were determined related to

![Fig 1: SDS-PAGE electrophogram of proteins isolated from nematocercia dispar](image-url)
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-PAG. 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 (Tendler 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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**REFERENCES**


