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GENOMIC DNA ISOLATION AND ELECTROPHORETIC SEPARATION OF PROTEINS FROM TWO CESTODE PARASITES OF THE COUNTRY FOWL

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ABSTRACT

The present study was designed to isolate the chromosomal DNA of the two cestode parasites *R. tetragona* and *R. echinobothrida* which parasitize the common fowl; causing considerable damage to poultry industry. The DNA was isolated from the cestodes with a purity ratio of 2 between *R. tetragona* and *R. echinobothrida* respectively. The DNA samples were analyzed by Agarose gel electrophoresis using λ Hind III as the marker gene. The quantitative analysis revealed that *R. tetragona* had DNA bands exactly at 23Kbps and *R. echinobothrida* had DNA bands above 23Kbps. Qualitative analysis of proteins was performed in different body segments of the parasites and the intestinal tissue of the fowl to establish a host parasite relationship at the molecular level. Mature, Immature and gravid segments of both the parasites were observed for further insight into the parasitic competence of the two cestodes in a common host. The study visualizes the fact that gel electrophoresis is useful in identifying protein patterns and DNA analysis in parasite systems providing a further step in drug designing and vaccine development.

KEYWORDS: *Raillietina tetragona*, *Raillietina echinobothrida*, λ Hind III marker gene, DNA isolation, qualitative protein analysis, vaccine development.

I. INTRODUCTION

The exciting opportunities provided by molecular technology are the possibility of examining genetic variation in cestodes directly and in detail at the DNA level. The most practical method for determining interspecific and intraspecific DNA sequence variation in a cestode is to take sequences from a genomic DNA library and to test its specificity. The genetic

function of the sequence may be unknown, but this is generally unimportant for its taxonomic value (James and Donald, 1989). Microbial genomics will also help pharmaceutical researchers gain a better understanding of how pathogenic microbes cause disease. This information would help to reveal vulnerabilities and identify new drug targets (Spring, 1999). The cestode vaccines are being developed towards practical application. It is possible to achieve a reliable, high level of protection against a complex metazoan parasite using defined DNA isolation and Protein Separation. The high level of efficacy of the vaccines against *Taenia solium* cysticercosis and hydatid disease suggests that they would be effective also if used directly in humans. Bio-technology has underpinned the development of treatments. Over time it has formed the basis of learning about animals and diseases (Lightowlers *et al.,* 2002).

Raillietina tetragona and Raillietina echinobothrida belonging to the family of *Davaineidae* are of common occurrence. According to Southwell (1930) *Raillietina* species are considered quite serious pathogens. Older birds Capillary congestion, cellular infiltration and connective tissue proliferation in intestinal wall and fibrosis are other conditions which have been associated with tapeworm infection (Nath and Pande, 1963). Gel electrophoresis is the standard method used for analyzing and purifying fragments of DNA and proteins. It is also used for separating and analyzing RNAs and oligonucleotides (Sambrook and Russel, 2001). Proteins are linear polymers built from 20 different amino acids using information encoded in genes. Each protein has its own unique amino acid sequence that is specified by the nucleotide sequence of the gene encoding this protein (Dobson, 2000).

Molecular approaches are the most effective and accurate means for the detection of many organisms and for screening of genetic variation among populations (Wongsawad and Wongsawad, 2010). Economic losses due to helminth parasites in sheep throughout the world are considerable (Irfan-ur-Rauf Tak *et al*., 2013). The features of the host – parasite relationships among the taeniid cestodes have formed the basis for vaccine development. Research on the development of vaccines to prevent infection with cestode parasites has brought focus on the taeniid cestodes (Lightowlers *et al*., 2002). Understanding the life cycle and pathogenesis of zoonotic helminths is extremely important because a majority of the infections are acquired from the livestock animals. These animals (including birds) live in close association with the human beings and serve as a reservoir of infections (Biswal *et al*., 2014). Not surprisingly enough in most of these infections the causative agents go unrecorded

due to failure of identification of the parasite. Hookworms are known to be host-specific (Mabvuure *et al*., 2015). The present study was an attempt made to understand the separation of nucleic acids and proteins by electrophoresis in order to identify and evaluate the structural similarity between the two cestode species and also to explore potential wealth of information in host -parasite relationship by protein studies which would form a platform for vaccine development against the cestodes *Raillietina tetragona and Raillietina echinobothrida* and the molecular techniques based on genomics are very useful for epidemiological and diagnostic tools as well as for research on genetic variation of parasitic organisms (Mas-Coma *et al*., 2005). The information presented in this study will help to understand the peculiar biological characters and to design species-specific control tools (Maldonado *et al*., 2017).

II. MATERIALS AND METHODS

1. Animal procurement (Parasite preparation)

The specimens of tapeworm *Raillietina tetragona* and *Raillietina echinobothrida* were collected alive from the intestine of naturally infected and recently killed country fowls and were rinsed in distilled water to render them free from intestinal contents and kept in separate petridishes for identification. The entire worms were spread out on a glass pane and the length of the worms was measured. The immature, mature and gravid proglottid regions of both the worms were identified and separated and were homogenized with 2ml of distilled water and used for biochemical analysis. In the present study the genomic DNA of two cestode parasites were studied using multisource genomic DNA, mini-prep kit and electrophoretic study by Agarose gel electrophoretic method (Sabry E. Ahmed, 2012).

2. Genomic DNA extraction

Genomic DNAs were isolated on a small scale from 1ml of adult worms using multisource genomic DNA, Mini-Prep Kit, Axgene Biotechnology, U.S.A Cat. No.110420-25, according to manufacture manual. Gene extraction studies do not reveal protein turnover patterns and protein modifications that may be involved in signalling and communication, protein transport and targeting important phenomenon (Savithry Natarajan *et al*., 2014).

3. UV – Spectrophotometer for sample purity

The double beam UV-Spectrophotometer (Shimadzu Corporation) connected to PC UV1650 was used for sample testing and all further operations were done using computer keys. Thus by using UV spectrophotometer the sample DNAs of the cestodes, *Raillietina tetragona* and

Raillietina echinobothrida were assessed for their purity and the ratios of pure preparations of DNA of both the samples were found to be close to 2.0.

4. Agarose gel electrophoresis

Agarose gel Electrophoresis was carried out according to the procedure as outlined by Sambrook and Russel (2001). The DNA analysis of the cestode samples was started by preparing a 0.7% agarose gel to run the samples. TAE was used as the buffer with a concentration of 40mM tris (pH7.6), 20mM acetic acid and 1mM EDTA was used as the marker gene. Bromophenol blue was used as the marker dye at a concentration of 1µl for every 5µl of DNA sample. Ethidium bromide was used as the visualization dye which was mixed in the buffer tank at 0.5mg/ml. The solution was well stirred to disperse ethidium bromide. 20µl of DNA sample were loaded into lane 1 and the marker gene λ Hind III sample was loaded into lane 2. The gel electrophoresis was carried out separately for the two cestode parasites. Current was supplied typically at 100V for 30min. After electrophoresis the gels were observed under a UV lamp wearing a protective gear.

5. Qualitative analysis of protein – (SDS – PAGE)

SDS – PAGE, sodium do decyl sulfate polyacrylamide gel electrophoresis, is a technique widely used in biochemistry, forensics, genetics and molecular biology to separate proteins according to their electrophoretic mobility. The purpose of this method is to separate proteins according to their size. Alkaline SDS – PAGE electrophoresis was performed using 10% gels (SDS – PAGE) was carried out in the slab gel apparatus using electrode buffer with or without SDS in tris - glycine buffer (pH 8.3). Recent advancements in protein separation methods have led to greater use of proteomics to explore and understand mechanisms of resistance and susceptibility of animals to pathogens (Savithry Natarajan *et al*., 2014).

III. RESULTS AND DISCUSSION

A. Agarose gel electrophoresis of DNA of *R. tetragona*

The image of the genomic DNA of *R. tetragona* was revealed in Fig: 1.

- The **lane 1** showed the migration of DNA fragments in 0.7% agarose gel of the cestode parasite *R. tetragona*. The DNA appeared as a single, linear high molecular weight band exactly aligning to the 23 kbps of the Hind III marker DNA.
- The **lane 2** showed the DNA sample that has been degraded and the streak formation was evident RNA appeared as thick orange band at the bottom of the lane because the

cells were broken by SDS treatment to expose the Chromosomal DNA and homogenization of the sample preparation must have broken the DNA into fragments.

- The **lane 3** showed the fragments of Hind III molecular DNA which is a mixture of molecules of known sizes. The Hind III marker DNA is a mixture of 23 kb, 9.5 kb, 6.5 kb, 4.3 kb, 2.3 kb & 2.0 kb.

Fragments of linear DNA of the marker migrated through agarose gel and separation of the components from the marker mixture formed distinct bands in the gel one after the other. The distance a band travels is approximately inversely proportional to the logarithm of the size of the molecule.

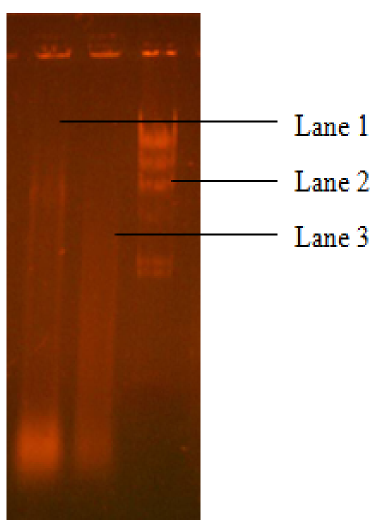

Fig: 1 Agarose gel electrophoresis of DNA *R. tetragona*

1. The lane 1 showed the migration of DNA fragments in 0.7% agarose gel of the cestode parasite *R. tetragona.*
2. The lane 2 showed the DNA sample that has been degraded and the streak formation was evident RNA appeared as thick orange band at the bottom of the lane
3. The lane 3 showed the fragments of Hind III molecular DNA which is a mixture of molecules of known sizes

B. Agarose gel electrophoresis of DNA of *R. echinobothrida*

The image of the genomic DNA of *R. echinobothrida* was revealed in Fig: 2.

- The **lane 1** showed the migration of DNA Fragment in 0.7% agarose gel of the cestode parasite *R. echinobothrida.* The DNA appeared as a single thick intense orange colored band moving close to 23 kb of the Hind III marker DNA.
- The **lane 2** showed the Hind III marker components of known sizes of molecular weight. DNA fraction of *R. tetragona* can be observed to give a faint linear orange colored band and DNA fraction of *R. echinobothrida* gave a bright intense orange colored band.

Hence the color differences are helpful in identifying small proportions of the substance in the DNA mixture. The DNA fraction of *R. echinobothrida moved* in close association with 23 kb fraction of Hind III marker. The DNA of *R. tetragona* has high molecular weight and it is

found to contain approximately 23 kilobase pairs. DNA fraction moved a bit faster than the DNA fraction of *R. echinobothrida,* it moved to almost the same distance as that of *R. echinobothrida*, thus presenting the difficulty in qualitative analysis of the sample (Fig: 2).

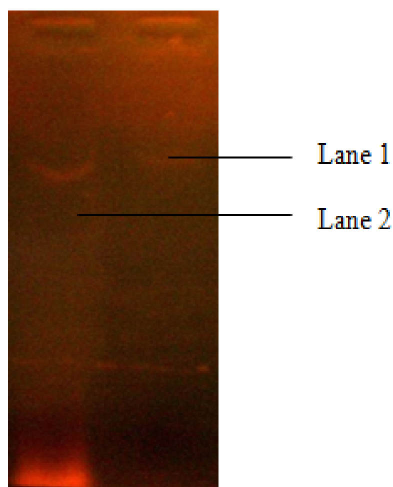

Fig: 2 Agarose gel electrophoresis of DNA *R. echinobothrida*

1. The lane 1 showed the migration of DNA Fragment in 0.7 % agarose gel of the cestode parasite *R. echinobothrida*. The DNA appeared as a single thick intense orange colored band moving close to 23 kb of the Hind III marker DNA.
2. The lane 2 showed the Hind III marker components of known sizes of molecular weight. DNA fraction of *R. tetragona* can be observed to give a faint linear orange colored band and DNA fraction of *R. echinobothrida* gave a bright intense orange colored band.

C. Electrophoretic studies of proteins of *R. tetragona*

The electropherogram of protein fractions of the whole worm and the strobilar regions of *R. tetragona* was revealed along with the host tissue (fowl) (Fig: 3).

- The **lane 1** represented the protein fractions of the intestinal tissue of the host (fowl) which fractionated into nineteen bands.
- The **lane 2** represented the protein fractions of the full worm of the parasite *R. tetragona* which fractionated into sixteen bands intense bands were seen beyond and at 95 *kDa* and 40 *kDa*. Individual faint bands were seen between 66-47 *kDa* and below 40 *kDa*.
- The **lane 3** represented the marker protein ranging from 95 *kDa* to 20 *kDa* (95 *kDa*, 66 *kDa*, 47 *kDa*, 35 *kDa* and 25 *kDa*).
- The **lane 4** revealed the protein fractions of the immature region of the parasite, *R. tetragona* which fractionated into seventeen bands. Intense bands were observed at 95 *kDa* and at 40 *kDa* approximately. Two intense bands were observed beyond 95 *kDa.* Many faint bands were seen individually between 95 *kDa* and below 40 *kDa* approximately.
- The **lane 5** represented the protein fractions of the mature region of the parasite, *R. tetragona* which fractionated into fourteen bands. Most of the bands in this region are found to be similar to the immature region of the parasite.

- The **lane 6** revealed the protein fractions of the gravid strobilar regions of the parasite, *R. tetragona* which fractionated into sixteen bands. Intense bands were seen beyond 95 *kDa*, 50 *kDa* and at 40 *kDa* approximately.

Many individual bands were found to be similar to the above two regions. The intensity and the nature of the bands in the three regions of the parasite were not different from each other. The gravid region showed intense protein fractions compared to the immature and mature regions of the parasite. The numbers of low molecular weight protein fractions were found to be more in the gravid region below 25 *kDa*.

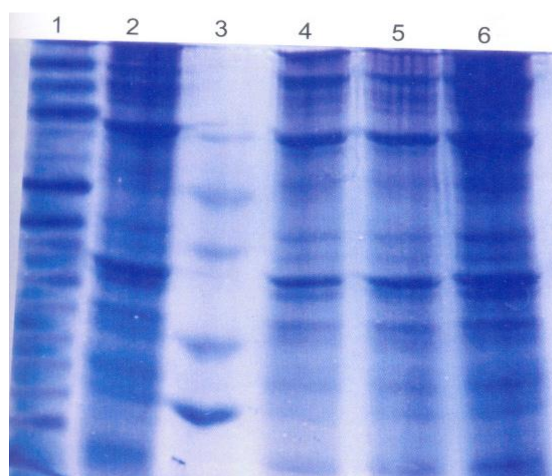

Fig. 3 The electropherogram of protein fractions of *R. tetragona.*

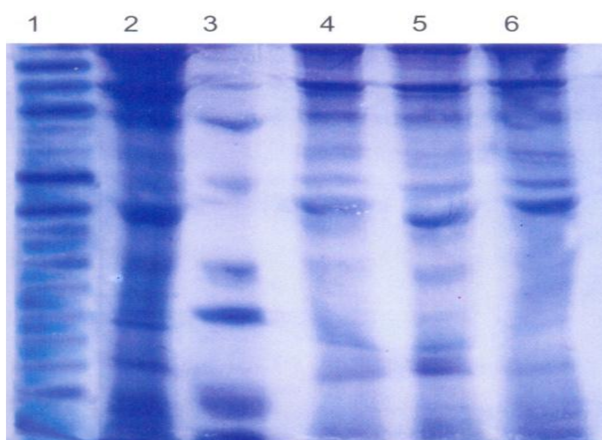

Fig. 4 The electropherogram of *R. tetragona* of *R. echinobothrida.*

D. Electrophoretic studies of proteins of *R. echinobothrida*

The electropherogram of protein fractions of the whole worm and the strobilar regions of *R. echinobothrida* along with the host tissue (fowl) were revealed (Fig: 4).

- The **lane 1** represented the protein fractions of the intestinal tissue of the host (fowl) which fractionated into nineteen bands.
- The **lane 2** represented the protein fractions of the full worm of the parasite *R. echinobothrida* which fractionated into fifteen bands. Intense bands were seen beyond 95 *kDa* and at 95 *kDa*, 66 *kDa*, 40 *kDa*, and at 35 *kDa*. Intense bands were also found between 25-20 *kDa*.
- The **lane 3** represented the marker proteins ranging from 95 *kDa* to 20 *kDa* (95 *kDa*, 66 *kDa*, 47 *kDa*, 35 *kDa*, 25 *kDa* and 20 *kDa*).
- The **lane 4** revealed the protein fractions of the immature region of the parasite, *R. echinobothrida* which fractionated into sixteen bands. Intense bands were seen beyond 95 *kDa*, and at 95 *kDa*, 66 *kDa* and at 40 *kDa*. Many individual faint bands were seen between 100 *kDa* to 90 *kDa*, between 90 *kDa* to 40 *kDa* and below 40 *kDa*.
- The **lane 5** represented the protein fractions of the mature region of the parasite, *R. echinobothrida*, which fractionated into thirteen bands. Most of the bands found in this region were similar to the bands found in the immature region. The protein fractions between 25-20 *kDa* were thick and dark compared to the immature region of the parasite.
- The **lane 6** revealed the protein fractions of the gravid strobilar regions of the parasite, *R. echinobothrida* which fractionated into fifteen bands some of which were similar to the immature and mature regions of the parasite. The grand region showed more number of low molecular weight proteins than the immature and mature region of the parasite.

Fig: 3 represented the protein fractions of the different regions of *R. tetragona* and Fig: 4 represented the protein fractions of the different regions of *R. echinobothrida.* The number of protein fractions of *R. tetragona* was found to be more than in *R. echinobothrida*. Qualitative analysis of DNA by agarose gel electrophoresis using different markers of Hind III suggests that DNA is present in both the samples analyzed. The intensity and thickness of the bands obtained in the two parasitic cestode species are comparable to the quantity of the DNA content of the respective species. The results of the present study on the two cestodes *R. tetragona* and *R. echinobothrida* confirms to the observation made by the above results also suggest that the parasite acquires its immunity by deriving immune proteins from the host as well as by synthesizing its own immunoproteins has been reported by (Hammerberg *et al*., (1980) and Joshua *et al*., (1989). These results reveal that the *Davaineidae* parasites, *R. tetragona* and *R. echinobothrida* shows similarities to other species belonging to the order *cyclophyllidea* in the protein profile. The chemical analysis found in *Echinococcus*

granulosus shows the presence of an appreciable amount of proteins in the membranes and stated that mucopolysaccharides constitute the principle structural material (Kilejian and Schwabe, 1971).

Similar results on total proteins were observed in *M. benedini* by Vinayakam (1980), Jailani (1998) in *R. tetragona*, Vijayalakshmi (2001) in *A. lahorea* and Radha (2003) in *R. echinobothrida*. The occurrence of soluble proteins in different strobilar regions of the parasite implies its role in metabolic activities and growth. The insoluble proteins of the parasite especially in the parasitic regions suggest that they may be involved in the membrane's structural organization (Smyth, 1969). The protein components of both soluble and insoluble moiety may have different functional significance such as the synthesis of collagenous and elastic substances and mucopolysaccharides etc. (Vijayalakshmi, 2001). Moreover the soluble fraction of protein may be to maintain the internal osmotic pressure so as to withstand the unnatural changes in the surrounding host environment (Radha, 2003).

E. Qualitative analysis of DNA of both parasites

Qualitative analysis of DNA by agarose gel electrophoresis using different markers of Hind III suggests that DNA is present in both the samples were analyzed. The intensity and thickness of the bands obtained in two cestode parasites are comparable to the quantity of the DNA content of the respective species. It is inferred from the qualitative studies that DNA of the cestode *R. echinobothrida* has the maximum number of the nucleotide base pairs than the DNA of *R. tetragona*. The occurrence of this predominant DNA band in *R. echinobothrida* accounts for the maximum intensity of the thick orange colored band by agarose gel electrophoresis. With this knowledge of observing the color developed and their relative mobilities, it was inferred that the intensity of orange color was great in *R. echinobothrida* and less in *R. tetragona*. Hence the DNA content of *R. echinobothrida* was found to be heavier than *R. tetragona*. The above results also suggest that the parasite acquires its immunity by deriving immune proteins from the host as well as by synthesizing its own immunoproteins has been reported by (Hammerberg *et al*., (1980) and Joshua *et al*., (1989). The results of protein biochemistry and DNA studies of the cestode parasites have a great importance in chemotherapy and vaccine production. Recombinant oncosphere antigens have been found to be remarkably effective when used as vaccines. Comparison of the structural features of these proteins and then associated genes suggest common features between antigens (Lightowlers *et al*., 2002). Identification of structural conservation of genes

encoding antigenic proteins across several taeniid species suggest that the encoded proteins play important roles in host infection and parasitic survival.

IV. CONCLUSION

The results of the protein fractions in the immature region (which include the scolex and immature region) of *R. tetragona* and *R. echinobothrida* evidenced that the concentration was very high and the number of protein fractions were more when compared to mature and gravid proglottides. Both the parasites live inside the host intestine and share the common habitat with regard to nutrients uptake and the environment is quite conducive for the co-existence of the two species. The results of similar protein fractions found in both the parasites confirm their sharing of protein resource. However the results clearly demonstrate the usefulness of gel electrophoresis in identifying protein pattern and DNA in the parasite systems and proven that this technique could be a valuable tool in taxonomic and phylogenetic studies of helminth parasites.

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