Biochemical and molecular characterization of the Cyclophyllidean cestode, *Cotugnia cuneata* (Meggit, 1924), an endoparasite of domestic pigeons, *Columba livia domestica*

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Abstract Analysis of the major biochemical constituents of *Cotugnia cuneata* revealed that the total protein, carbohydrate, glycogen and lipid contents (as percentage of dry weight) were 25.22 ± 0.93 , 32.90 ± 0.30 , 21.33 ± 0.99 and 9.94 ± 0.42 respectively. The results showed that the carbohydrate content was the highest followed by protein and lipid contents respectively. Glycogen content was relatively high which showed that carbohydrate was mainly present in the form of glycogen in these cestodes. The A + T and G + C contents were obtained as 49.82 and 50.18 % respectively. The phylogenetic tree showed that *C. cuneata* branched with its closest cluster comprising of *Raillietina tunetensis*, *Raillietina australis*, *Fuhrmannetta malakartis* and *Raillietina sonini* with 99 % bootstrap support.

Keywords *Cotugnia cuneata* · Biochemical characterization · Gene sequencing and analysis · Phylogenetic tree

Introduction

The Cestodes are a group of endoparasitic helminths which almost exclusively occupy the alimentary canal in preference to other common sites like the bile duct, the gall bladder or the pancreatic duct (Smyth and McManus 2007). The elongated tape-like body of the cestode enables it to live in its tubular habitat (Smyth and McManus 2007).

As an alimentary canal is absent, the worm derives its nutrition from the host's gut across its highly specialized, metabolically active body surface or tegument (Smyth and McManus 2007). Carbohydrates are the most commonly used source of energy in cestodes (Cheng 1986). Lack of protein in the host's diet does not affect the cestodes to a great extent as the lack of carbohydrate does (Cheng 1986). It results in the stunting of growth, decreased egg production and development of morphologically abnormal eggs (Cheng 1986). Glycogen has been reported to be the most important energy reserves in cestodes (Smyth and McManus 2007). The presence of proteoglycans and glycoproteins have been reported from different parts of *Echinococcus granulosus* (Cheng 1986). It has been reported that the protein content of cestodes is relatively lower than that of other invertebrates (Cheng 1986; Smyth and McManus 2007). Study of the amino acid composition of the cestodes shows that they are closely similar to that of hosts only with a few exceptions (Smyth and McManus 2007). Thus, the cestodes obtain their required amino acids by absorption from the host's tissue (Smyth and McManus 2007). The lipid contents of the cestodes are highly variable (Smyth and McManus 2007). Apart from simple lipids, conjugated lipids have also been reported from cestodes (Smyth and McManus 2007). However, there is no evidence which can prove the utilization of fats as stored nutrients (Cheng 1986). The ability to synthesize fatty acids de novo in cestodes has been very much restricted and thus they have to depend largely on the fatty acids of the hosts to fulfill their requirements (Smyth and McManus 2007). Information on nucleic acids and their metabolism in cestodes are limited. However, recent developments in the field of molecular parasitology are providing important and interesting data on molecular characteristics of cestodes that seem to be very helpful for

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their identification, studying the interrelationships between different genera and construction of phylogenetic trees (Eom et al. 2002). Scanty information is available on studies of RNA of cestodes (Smyth and McManus 2007). The phylogeny of Eucestoda has always been a matter of debate and information available are obscure (Hoberg et al. 1997). Some workers have attempted more modern phylogenetic studies on eucestodes using a cladistic approach (Brooks et al. 1991). Use of molecular techniques in the field of taxonomy provides an important aid in the hands of a worker for more accurate interpretations.

However, no such data are available on *C. cuneata* inhabiting the small intestine of domestic pigeon, *Columba livia domestica*. Present work is an attempt to study the biochemical and molecular properties of *C. cuneata*, a cestode parasite of *C. l. domestica*.

Materials and methods

The parasites were obtained from the small intestine of domestic pigeons collected from different parts of Burdwan, West Bengal. The cestodes were washed with 0.85 % normal saline, soaked in a blotting paper and the fresh weight was measured. The collected worms were kept at 50–60 °C for 24 h. and the dry weight was also taken. Entire worms of about the same length were selected and biochemical analyses were performed. The protein, carbohydrate and lipid contents were estimated following the methods of Lowry et al. (1951), Umbreit et al. (1958) and Folch et al. (1957) respectively. Glycogen content was estimated by following the method of Carrol et al. (1956). Phylogenetic affiliation of the *C. cuneata* under study (HQ610177) was done by 28S rRNA gene sequence analysis. Nucleotide BLAST (BLASTN) was done to investigate the most similar sequences from NCBI database. The sequence data was aligned using the ‘ClustalW Submission Form’ and analyzed by ClustalW (Thompson et al. 1994). Evolutionary distances were calculated using the method of Jukes and Cantor (Jukes and Cantor 1969) and the topology was inferred using the ‘neighbor-joining’ method (Saitou and Nei 1987). To assign the taxonomical affiliation of this cestode, phylogenetic tree was prepared following Tamura et al. (2007).

Results

Total Protein, Carbohydrate, Glycogen and Lipid contents (as percentage of dry weight) of *C. cuneata* were 25.22 ± 0.93 , 32.90 ± 0.30 , 21.33 ± 0.99 and 9.94 ± 0.42 respectively (Fig. 1) [Values have been expressed as mean of five observations $\pm$ S.E.]. The major biochemical

Fig. 1 Total Protein, Carbohydrate, Glycogen and Lipid contents of *C. cuneata* affecting domestic pigeons

Fig. 2 Mol% of the nucleotides of *C. cuneata*

constituents followed the order, Carbohydrate > Protein > Lipid. The A + T and G + C contents were obtained as 49.82 and 50.18 % respectively. The mol% of the nucleotides obtained has been shown in Fig. 2. The phylogenetic tree has been shown in Fig. 3.

Discussion

A lot of work has been done on the biochemical composition of cestodes. Some recent studies include those on *Lytocestus* sp. (parasitizing the intestine of *Clarias batrachus*) whose protein, lipid and glycogen contents were estimated at 11.2 mg/g wet weight of the tissue, 22.5 mg/g and 12.61 mg/100 ml of solution respectively (Jawale et al. 2011); protein, glycogen and lipid contents of *Senga* sp. (from *Mastacembelus armatus*) have been reported as 14 mg/gm wt. of tissue, 14.4 mg/100 ml solution and 25.07 mg/gm respectively (Fartade et al. 2011); protein, glycogen and lipid contents of *Circumoncobothrium* sp. (from *Mastacembelus armatus*) have been reported to be 13.44 mg/gm wt. of tissue, 13.5 mg/100 ml soln. and 24.8 mg/gm respectively (Fartade et al. 2011); protein, glycogen and lipid contents of *Moniezia* sp.

Fig. 3 Neighbour-joining tree constructed based on partial 28S rRNA genes sequences of *C. cuneata* along with other 28S rRNA genes of cestodes retrieved from NCBI database. Numbers at nodes indicate percent bootstrap values above 80 supported by 1,000 replicates

(from *Capra hircus*) have been reported to be 13.10 mg/gm wet wt. of the tissue, 14.12 mg/100 ml solution and 12.58 mg/gm respectively (Humbe et al. 2011); protein, glycogen and lipid contents of *Stilesia* sp. (from *Ovis bharal*) have been reported to be 13.90 mg/gm. wet wt. of the tissue, 16.60 mg/100 ml of sol and 12.64 mg/gm respectively (Humbe et al. 2011). From the above studies the workers have inferred that the proportion of lipids was higher as compared to that of protein and glycogen contents in cestodes which is quite contrary to earlier studies in this field. However, the results of the present investigation (Fig. 1) shows that carbohydrate is the major organic constituent of this species and supports earlier works. The role of carbohydrates in the life processes of parasitic worms is of immense importance (Smyth and McManus 2007). The parasites living in the anaerobic and semiaerobic habitat obtain their energy mainly through fermentation of carbohydrates because under anaerobic conditions, carbohydrates act as better substrates for energy

production in comparison to that of protein or fat (Von Brand 1950). The body covering or the tegument of the cestodes consists mainly of mucopolysaccharides often complexed with proteins to form mucoproteins or glycoproteins (Smyth and McManus 2007). Thus, the glycocalyx accounts for a substantial portion of the carbohydrate content of the species. The present study reveals that total glycogen accounts to about more than 50 % of the total carbohydrate (Fig. 1) in the species. Thus, carbohydrate occurs mainly in the form of polysaccharide, glycogen. Previous data shows that the total glycogen content varies from 6 to 48 % of the dry weight of cestodes (Smyth and McManus 2007). The present study shows the glycogen content to lie in that range. Protein is the most essential component of all living organisms. Cestodes are known to synthesize polypeptides rapidly (Barrett 1981) owing to their high turnover rates of their tegument and high rates of egg-production (Harris 1983). The protein content of *Cotugnia digonophora* (from *Gallus gallus domesticus*) was

reported to be 5.77 mg/gm wet weight of the tissue (Nanware et al. 2012), while that of *Davainea shindei* have been reported to be 13.20 mg/g wet weight of the tissue (Jadhav et al. 2008). The total protein content of cestodes usually lies between 20 and 40 % of the dry weight that is comparatively lower than that of other invertebrates (Smyth and McManus 2007). The protein content obtained in the present investigation (Fig. 1) shows similar results. Structural proteins account largely for the estimated protein content besides cellular protein. These structural proteins mainly include the glycoproteins of the cestode tegument (Smyth and McManus 2007). Other structural proteins include sulphur-rich keratin present in the hooks and sclerotin present in the eggs found in the gravid proglottids (Johri and Smyth 1956). Collagen has also been reported to be an important structural protein of cestodes (Smyth and McManus 2007). Lipids also form an integral part of a tissue and exhibit a variety of cellular functions (Smyth and McManus 2007). The lipid content of *Raillietina echinobothrida* (from domestic fowl) has been reported to be 4.05 % of wet weight (Mondal et al. 2009). Earlier works show that the lipid content of cestodes varies between 7.5 and 37.3 % (Smyth and McManus 2007). The lipid content of the present species (Fig. 1) confirms earlier reports. The parenchyma of the cestodes is the most important tissue for the storage of lipids (Frayha and Smyth 1983). Lipids have also been reported from the calcareous corpuscles and the organs of the reproductive system as well as the excretory canals (Chowdhury and De Rycke 1976; Parshad and Guraya 1977). Lipids are the main components of the cestode eggs and thereby the gravid proglottids housing enormous number of eggs (Chowdhury and De Rycke 1976) account for substantial amount of the lipid content of the parasite. Earlier reports on rRNA of cestodes show that it has a G + C content of around 50 % (Smyth and McManus 2007). In the present investigation the A + T and G + C contents of 28S rRNA gene sequence of *C. cuneata* (HQ610177) were determined and recorded as 49.82 and 50.18 % respectively. The G + C content does not seem to vary much from that of other cestodes. The phylogenetic tree showed that *C. cuneata* branched with its closest cluster comprising of *Raillietina tunetensis* (EU665459), *Raillietina australis* (AF286914) *Fuhrmannetta malakartis* (EU665457) and *Raillietina sonini* (EU665462) with 99 % bootstrap support (Fig. 3).

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