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## Research Note

# Transcriptomic study of the rat pinworm *Syphacia muris*

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**Summary**

*Syphacia muris* is a ubiquitous nematode parasite and common contaminant of laboratory rats. Although *S. muris* infection is considered symptomless, it has some effects on the host's immunity and therefore can interfere with experimental settings and interrupt final results. However, the molecular mechanisms involved in the alteration within the host's immunity remain unclear because of the absence of information about mRNA expressed in this parasite. In this study we performed the transcriptome profiling of *S. muris* by next-generation sequencing. After *de novo* assembly and annotation, 14,821 contigs were found to have a sequence homology with any nematode sequence. Gene ontology analysis showed that the majority of the expressed genes are involved in cellular process, binding, and catalytic activity. Although the rate of expressed genes involved in the immune system was low, we found candidate genes that might be involved in the alteration within the host's immunity by regulating the host's innate immune response.
**Keywords:** *Syphacia muris;* transcriptome; Gene ontology analysis

## Introduction

*Syphacia muris* is a common pinworm of rats. The life cycle of this parasite is direct and adult worms inhabit the cecum and colon. Mature females deposit the eggs in the perianal region of the host, causing widespread environmental contamination and making the control of this parasites quite difficult (Stahl, 1961; Baker, 1998). Although pinworms are thought to be nonpathogenic and symptomless since rats naturally infected with these parasites appear normal and healthy, there is the potential that *S. muris* can interfere with the experimental settings and later interrupt final results. Several research groups reported that infection with pinworms can alter the host's immune response (Sato *et al.*, 1995; Agersborg *et al.*, 2001; Bugarski *et al.*, 2006; Michels *et al.*, 2006; Ilić *et al.*, 2010; Trelis M *et al.*, 2013), however, the molecular mechanisms involved in the alteration within host's immunity during infection remain unclear. Investigation of mRNA expressed in *S. muris* provides us with a better understanding of molecular processes involved in parasite-host interactions. In this study we characterized the first global transcriptome of *S. muris* using next generation sequencing technology. This study provides fundamental data that will serve for future genomics studies and understanding the parasite-host relationship in *S. muris*.

## Materials and Methods

### *Animals*

8-week-old Wistar rats were obtained from Japan SLC, Inc. (Shizuoka, Japan). All animals maintained under conventional conditions with a 12 h on/off light cycle, commercial diet, and water *ad libitum*. All animal procedures were performed under the experimental animal guidelines of Azabu University.

### *Parasites*

*Syphacia muris* were obtained from the ceca and colons of naturally infected rats. These organs of infected rats were opened in a Petri dish with 0.8 % NaCl, and worms were collected and washed with PBS.

*RNA isolation from S. muris*

Total RNA was purified from *S. muris* using RNeasy Fibrous Tissue Mini Kit and QIAshredder (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Briefly, worms were suspended in RLT buffer containing 2-mercaptoethanol, then transferred into a QIAshredder. Homogenized lysate was harvested by centrifuge and treated by Proteinase K. After applying lysate onto a RNA Mini Column followed by DNase I treatment to remove contaminated genome DNA, total RNA was eluted from the column in RNase-free-water. High quality RNA was confirmed by NanoDrop measurement (Thermo Fisher Scientific, Massachusetts, USA).

*Next-generation sequencing and analysis*

Gene expressions in *S. muris* were identified by direct high-throughput sequencing using the next-generation sequencer GS FLX Titanium (Roche, Basel, Switzerland) (Roche Diagnostics, 2009; Young *et al.*, 2010). At first, first-strand cDNA synthesis was performed using the total RNA primed with random hexamer oligonucleotide. Afterwards, 454 adapters A (5'-GACCTTGGCT-GTCACTCAGTT-3') and B (5'-TGGCCTGTCACTCACTGCGA-3') were ligated to 5' and 3' ends of the cDNA, cDNA was amplified with PCR (12 cycles). Normalization was carried out by one cycle of denaturation and reassociation of the cDNA. Reassociated double-stranded cDNA was separated from the remaining single-stranded cDNA (normalized ss-cDNA) by purification on a hydroxylapatite column. The normalized ss-cDNA was amplified with PCR (9 cycles) and size-selected (500–1200 bp). This size-selected cDNA was sequenced with a GS FLX Titanium using a standard protocol (Margulies *et al.*, 2005). The 454 Life Sciences software (Roche) was used for image and signal processing.

*Assembly of sequencing reads and sequence annotations*

After removal of the sequencing linkers, pooled sequencing reads were subjected to assembling by MIRA Assembler Version3.4 software to generate contigs (Chevreux *et al.*, 1999). Homologies of the contig sequences to known protein sequences were analyzed by BLASTx against protein database (Altschul *et al.*, 1997). Functional classification was performed using the Blast2GO software.

## Results and Discussion

We sequenced normalized ss-DNA synthesized from *S. muris* RNA using the next-generation sequencer GS FLX Titanium. We gained a total of 630,068 sequence reads with an average length of 495 bp (Table 1). These reads were assembled by MIRA Assembler software after removal of adapters. This process resulted in 37,789 contigs of 41 to 16,567 bp with an average length of 828 bp. In these contigs, there are 6,569 "large contigs" which are defined as longer than 1 kb and consisting of more than 5 reads. The total contig length was 31,295,281 bp (Table 1). The size distribution of contigs is shown in Table 2. 16,425 contigs (43.5 %) were in the range of 500 to 799 bp, and 8,342 contigs (22.1 %) were longer than 1000 bp.

Table 1. Summary of *Syphacia muris de novo* sequence assembly

| | |
|---|---|
| Number of reads | 630,068 |
| Total read length (nucleotides) | 312,338,714 |
| Average read length (nucleotides) | 495 |
| Number of contigs | 37,789 |
| Total contig length (nucleotides) | 31,295,281 |
| Average contig length (nucleotides) | 828 |

Table 2. Size distribution of *Syphacia muris* contigs

| Size range (bp) | Number | Proportion (%) |
|---|---|---|
| <200 | 1,628 | 4.31 |
| 200 – 299 | 904 | 2.39 |
| 300 – 399 | 1,590 | 4.21 |
| 400 – 499 | 3,560 | 9.42 |
| 500 – 599 | 4,205 | 11.13 |
| 600 – 699 | 5,114 | 13.53 |
| 700 – 799 | 7,106 | 18.80 |
| 800 – 899 | 3,119 | 8.25 |
| 900 – 999 | 2,221 | 5.88 |
| 1000 – 1499 | 5,358 | 14.18 |
| 1500 – 1999 | 1,662 | 4.40 |
| 2000 – 2999 | 973 | 2.57 |
| 3000 – 3999 | 238 | 0.63 |
| 4000 – 4999 | 70 | 0.19 |
| 5000< | 41 | 0.11 |

We analyzed homologies of contigs to known protein using BLASTx. After cutting-off at 1 x $10^{-5}$ of E-value and removing overlapped results, 14,821 contigs from *S. muris* were found to have a sequence homology with nematode sequence. Significant homologies were detected with 58 genera of nematodes (Table 3). *Ascaris* (*A. lumbricoides*, *A. suum*), *Loa* (*Loa loa*), *Caenorhabditis* (*C. angaria*, *C. brenneri*, *C. briggsae*, *C. elegans*, *C. remanei*), and *Brugia* (*B. malayi*, *B. pahangi*) species were found to have high similarity with *S. muri*s. As shown in table3, *Ascaris* species are most similar to *S. muris* (31.89 %). The percentage level of similarity for *Caenorhabditis* and *Brugia* species to *S. muris* are 15.51 % and 14.91 %, respectively. Among these nematodes, *C. brenneri*, *C. remanei*, *B. malayi*, and *A. suum* were also shown to have an RNA sequence highly similar to that of *Angiostrogylus cantonesis*, the transcriptome analysis of which has recently been reported (Wang *et al.*, 2013). On the other hand, *Loa* species (*Loa loa*) have high similarity with *S. muri*s transcriptome (25.09 %, Table 3), but not with *A. cantonesis* (Wang *et al.*, 2013). *Loa loa*, the African eyeworm, is a filarial parasite of humans and its draft genome sequence and nematode phylogenomics were recently reported (Desjardins *et al.*, 2013). This study showed that many *L. loa* genes were systematically related to other filarial parasites,

Table 3. Fifty nine Nematode genera which have sequence homologies with transcriptome of *Syphacia muris*

| Nematodae genus | Number | Proportion (%) | Nematodae genus | Number | Proportion (%) |
|---|---|---|---|---|---|
| *Ascaris* | 4,727 | 31.89 | *Setaria* | 4 | 0.03 |
| *Loa* | 3,718 | 25.09 | *Strongyloides* | 4 | 0.03 |
| *Caenorhabditis* | 2,299 | 15.51 | *Syphacia* | 4 | 0.03 |
| *Brugia* | 2,210 | 14.91 | *Globodera* | 3 | 0.02 |
| *Wuchereria* | 1,559 | 10.52 | *Heterodera* | 3 | 0.02 |
| *Trichinella* | 50 | 0.34 | *Heterorhabditis* | 3 | 0.02 |
| *Haemonchus* | 21 | 0.14 | *Litomosoides* | 3 | 0.02 |
| *Angiostrongylus* | 20 | 0.13 | *Aelurostrongylus* | 2 | 0.01 |
| *Onchocerca* | 18 | 0.12 | *Chabertia* | 2 | 0.01 |
| *Enterobius* | 15 | 0.1 | *Cooperia* | 2 | 0.01 |
| *Steinernema* | 12 | 0.08 | *Dracunculus* | 2 | 0.01 |
| *Dirofilaria* | 11 | 0.07 | *Heliconema* | 2 | 0.01 |
| *Toxocara* | 10 | 0.07 | *Labiostomum* | 2 | 0.01 |
| *Wellcomia* | 9 | 0.06 | *Oesophagostomum* | 2 | 0.01 |
| *Ancylostoma* | 8 | 0.05 | *Punctodera* | 2 | 0.01 |
| *Bursaphelenchus* | 8 | 0.05 | *Spauligodon* | 2 | 0.01 |
| *Teladorsagia* | 8 | 0.05 | *Teratocephalus* | 2 | 0.01 |
| *Baylisascaris* | 7 | 0.05 | *Ascaridia* | 1 | 0.01 |
| *Contracaecum* | 7 | 0.05 | *Diplogasteroides* | 1 | 0.01 |
| *Pristionchus* | 7 | 0.05 | *Heligmosomoides* | 1 | 0.01 |
| *Anisakis* | 6 | 0.04 | *Mecistocirrus* | 1 | 0.01 |
| *Meloidogyne* | 6 | 0.04 | *Parascaris* | 1 | 0.01 |
| *Bunostomum* | 5 | 0.03 | *Parastrongyloides* | 1 | 0.01 |
| *Dictyocaulus* | 4 | 0.03 | *Plectus* | 1 | 0.01 |
| *Ditylenchus* | 4 | 0.03 | *Syngamus* | 1 | 0.01 |
| *Koerneria* | 4 | 0.03 | *Thaumamermis* | 1 | 0.01 |
| *Metastrongylus* | 4 | 0.03 | *Trichostrongylus* | 1 | 0.01 |
| *Necator* | 4 | 0.03 | *Tylopharynx* | 1 | 0.01 |
| *Ostertagia* | 4 | 0.03 | *Uncinaria* | 1 | 0.01 |

*B. malay* and *Wuchereria bancrofti*, but not to *C. elegans*. Phylogenetic profiling also revealed that *L. loa* has low communality with nonparasitic nematodes (Desjardins *et al*., 2013). This report and our results suggest that *S. muris* is genetically relatively closely-related to filariae, although further genomics study is required.

Gene Ontology (GO) annotation was further carried out for the annotated nematode genes in terms of biological process, cellular component and molecular functions The sequences were categorized with 16 biological processes, 9 cellular components and 11 molecular function categories in GO level2 (Fig. 1). In the biological process, the most highly represented GO terms were cellular process (27.5 %), including genes involved in such signal transduction; and the metabolic process (26.1 %), including genes involved in such translation. In cellular component, the majority of GO terms were associated with the cell (37.8 %), including proteins associated with cytoplasm; and organelle (25.2 %), including mitochondrion. Regarding molecular function, major GO terms were binding (43.5 %), including genes involved in such purine ribonucleoside triphosphate binding; and catalytic activity (39.7 %), including genes involved in such ATPase activity, GTPase activity and helicase activity. Our results that genes associated with metabolic process are abundantly expressed in *S. muris* is consistent with the report of proteomic analysis of *S. muris* (Sotillo *et al*., 2012). Likewise in that study, we also found metabolic enzymes such as enolase (concerned with glycolysis), phosphoenolpyruvate carboxykinase (concerned with gluconeogenesis) in the metabolic process category.

We previously reported that *S. muris* infection delayed the onset of type 2 diabetes mellitus (T2DM) in WBN/K-*Lepr*$^{a}$ (*fa/fa*) rats, a new model rat of the obese T2DM with pancreatitis (Akimoto *et*

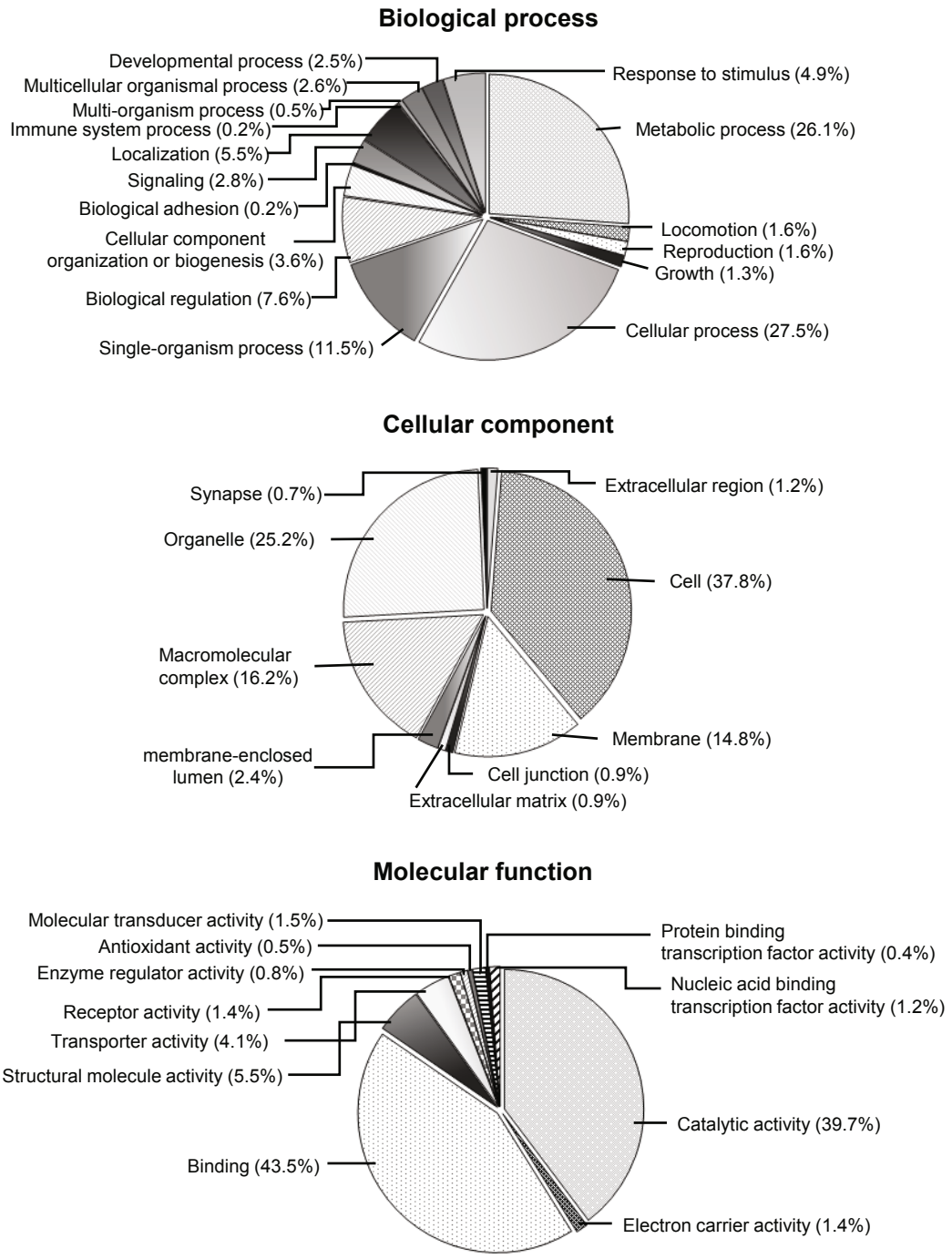


Fig. 1. Gene ontology (level2) for *Syphacia muris* transcriptome under biological process (a), cellular component (b), and molecular function (c)

*al.*, 2012, Taira *et al.*, 2015). This delay was considered to be due to *S. muris* infection having some pancreatitis-suppressing effect on *fa/fa* rats. Inflammatory cytokine production in *fa/fa* rats appear to be associated with developing pancreatitis, and we detected more macrophage infiltration into cecum of f*a/fa* rats than normal rats (data not shown). *S. muris* infection may inhibit inflammatory cytokine production from host immune cells such as macrophages, but the mechanism of immunosuppressing function by *S. muris* remains unclear. In the present study the percentage of gene annotated immune system process under biological process was only 0.2 % (Fig. 1), including not only identified proteins but also unnamed protein products. GO annotation showed that these unnamed protein products might be involved in an innate immune response or Fc receptor signaling pathway (data not shown). Several Fc receptors are expressed on the cell surface of macrophages, and more recently, Ng *et al.* (2015) reported that FcγRIIb signaling was involved in the development of atherosclerosis with inflammatory cytokine response by macrophages. This report and our results raise the possibility that inflammatory cytokine production from macrophages via Fc receptor signaling may cause pancreati-

tis, and yet-unnamed protein expressed in *S. muris* may attenuate this signaling pathway.

This study provides the first comprehensive transcriptomic analysis of *S. muris* and suggests the existence of proteins which have immune-regulating effects on host immune cells. Further studies will be needed to characterize these candidate proteins and to more fully understand the molecular mechanisms by which *S. muris* modulates the host's immune responses.

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