A comprehensive transcriptome data of normal and Nosema ceranae-stressed midguts of Apis mellifera ligustica workers

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Abstract

Honeybees are pivotal pollinators of crops and wild flora, and of great importance in supporting critical ecosystem balance. Nosema ceranae, a unicellular fungal parasite that infects midgut epithelial cells of honeybees, can dramatically reduce honeybee population and productivity. Here, midguts of Apis mellifera ligustica workers at 7 d and 10 d post inoculation (dpi) with sucrose solution (Ac7CK and Ac10CK) and midguts at 7 dpi and 10 dpi with sucrose solution containing N. ceranae spores (Ac7T and Ac10T) were sequenced using strand-specific cDNA library construction and next-generation sequencing. A total of 1956129858 raw reads were gained in this article, and after quality control, 1946489304 high-quality clean reads with a mean Q30 of 93.82% were obtained. The rRNA-removed clean reads were then aligned to the reference genome of Apis mellifera with TopHat2. For more insight please see "Genome-wide identification of long non-coding RNAs and their regulatory networks involved in Apis mellifera ligustica response to Nosema ceranae infection" [1]. Raw data were deposited in NCBI Sequence Read Archive (SRA) database under the BioProject number PRJNA406998. These data can be used for comparative analysis to identify differentially expressed coding RNAs and non-coding RNAs involved in A. m. ligustica responses to N. ceranae

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stress, and for investigation of molecular mechanisms regulating host \textit{N. ceranae} -response. © 2019 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Data

\textit{N. ceranae} spores (Fig. 1A) \cite{1} were purified using Percoll discontinuous density gradient centrifugation. As Fig. 1B \cite{1} shown, \textit{A. m. ligustica} workers were starved for 2 h and then each artificially inoculated with 50\% sucrose solution containing \textit{N. ceranae} spores. The shared transcriptome data are from deep sequencing of untreated and \textit{N. ceranae}-stressed midguts of \textit{A. m. ligustica} workers. On average, more than 23.32 Gb raw reads were gained from each group (Table 1) \cite{1}. The quality control of transcriptome data shows clean reads in each group are over 99.42\% (Table 1) \cite{1}; in addition, Q20 (quality score is 20) and Q30 (quality score is 30) of clean reads in each group are above 96.98\% and 93.34\%, respectively (Table 1) \cite{1}. Moreover, Pearson correlation coefficients of different biological replicas in each group are more than 91.19\% (Fig. 2) \cite{1}. The raw data were deposited in the Sequence Read Archive (SRA) database (http://www.ncbi.nlm.nih.gov/sra/) and connected to BioProject PRJNA406998.

2. Experimental design, materials, and methods

2.1. \textit{N. ceranae} spore purification

Fresh spores were isolated from naturally-infected foragers from a colony located at Fuzhou city, Fujian province, China, following the method described by Cornman et al. \cite{2} with some minor
modifications [3]. Firstly, bees were kept in −20 °C for 5 min followed by separation of midguts with clean dissection tweezers, homogenization in distilled water, filtration in four layers of sterile gauze, and then three times of centrifugation at 8000 rpm for 5 min at −4 °C; secondly, the supernatant was discarded as the spores remained in the sediment, the re-suspended pellet was further purified on a discontinuous Percoll gradient (Solarbio, China) consisting of 5 mL each of 25%, 50%, 75% and 100% Percoll solution, the spore suspension was overlaid onto the gradient and centrifuged at 14000 rpm for 90 min at 4 °C; thirdly, the spore pellet was carefully extracted with a sterile syringe followed by centrifugation again on a discontinuous Percoll gradient to gain clean spores (Fig. 1A) [1], which were immediately frozen in liquid nitrogen and stored at −80 °C until deep sequencing; fourthly, a portion of spores were subjected to PCR identification and proved to be mono-specific using previously described primers [4]; finally, the spore concentration was determined by counting using a CL kurt counter (JIMBIO, China). The spore suspension was freshly prepared before use.

2.2. Experimental design and sample collection

Frames of sealed brood obtained from a healthy colony of A. m. ligustica located in the teaching apiary of College of Bee Science, Fujian Agriculture and Forestry University were kept in an incubator at 34 ± 0.5 °C, 50% RH to provide newly emerged Nosema-free honeybees. The emergent workers were carefully removed, confined to cages in groups of 20, and kept in the incubator at 32 ± 0.5 °C, 50% RH. The bees were fed ad libitum with a solution of sucrose (50% w/w in water). One day after eclosion, the honeybees were starved for 2 h and 20 workers per group were each immobilized and then fed with 5

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**Table 1**

| Sample       | Raw reads     | Clean reads (%) | Q20 (%)     | Q30 (%)     |
|--------------|---------------|-----------------|-------------|-------------|
| Am7CK1       | 160844082     | 160049106 (99.51%) | 23340144349 (97.41%) | 22521956996 (94.00%) |
| Am7CK2       | 129878194     | 129283918 (99.54%) | 18891245674 (97.56%) | 18239412915 (94.19%) |
| Am7CK3       | 113683898     | 113165446 (99.54%) | 16535666991 (97.52%) | 15943589999 (94.03%) |
| Am7T1        | 152323278     | 151668484 (99.57%) | 22161043664 (97.55%) | 21387125499 (94.15%) |
| Am7T2        | 200417896     | 199313090 (99.45%) | 28948504448 (97.11%) | 27829913730 (93.35%) |
| Am7T3        | 126667596     | 126053962 (99.52%) | 18386919122 (97.38%) | 17719616862 (93.85%) |
| Am10CK1      | 160537248     | 159765346 (99.52%) | 23262715888 (97.27%) | 22443038732 (93.84%) |
| Am10CK2      | 149230808     | 148494716 (99.51%) | 21633348548 (97.28%) | 20852891752 (93.77%) |
| Am10CK3      | 131386354     | 130619802 (99.42%) | 18959297638 (96.98%) | 18248516385 (93.34%) |
| Am10T1       | 249473666     | 248333982 (99.54%) | 36162922479 (97.32%) | 34857597435 (93.81%) |
| Am10T2       | 208589832     | 207574770 (99.51%) | 30251988213 (97.34%) | 29139831253 (93.77%) |
| Am10T3       | 173097006     | 172166682 (99.46%) | 25113348781 (97.38%) | 24175449594 (93.74%) |

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Fig. 1. Artificial inoculation of A. m. ligustica worker with purified N. ceranae spores. A: Microscopic observation of purified N. ceranae spores (400 times magnification). B: Artificial inoculation of A. m. ligustica worker.
μL of 50% sucrose solution containing $1 \times 10^6$ spores of *N. ceranae*, as shown in Fig. 1B [1]. Those individuals that did not consume the total amount of solution were discarded from the assay. After feeding, bees were isolated for 30 min in individual vials in the growth chamber to ensure that the sugar solution was not transferred among honeybees and the entire dosage was ingested. Control bees were inoculated in an identical manner using a 50% sucrose solution (w/w in water) without *N. ceranae* spores. Three replicate cages of 20 honeybees each were used in *N. ceranae*-treated and control groups. Each cage was checked every 24 h and any dead bees removed. *N. ceranae*-treated and control workers’ midguts were respectively harvested at 7 d or 10 d post inoculation (dpi), immediately frozen in liquid nitrogen and kept at $-80^\circ{\text{C}}$ until deep sequencing. *N. ceranae*-treated groups at 7 dpi and 10 dpi with sucrose solution containing *N. ceranae* spores were termed as Am7T (Am7T-1, Am7T-2, Am7T-3) and Am10T (Am10T-1, Am10T-2, Am10T-3); control groups at 7 dpi and 10 dpi with sucrose solution without *N. ceranae* spores were termed as Am7CK (Am7CK-1, Am7CK-2, Am7CK-3) and Am10CK (Am10CK-1, Am10CK-2, Am10CK-3).

Fig. 2. Pearson correlation coefficients between different replicates within each *N. ceranae*-treated and control group.
2.3. RNA extraction, strand-specific cDNA library construction and deep sequencing

Total RNA of the six midgut samples from *N. ceranae*-treated groups and six midgut samples from control groups were respectively extracted using Trizol (Life Technologies) according to the manufacturer's instructions, and examined via 1% agarose gel electrophoresis. Next, rRNAs were removed to retain mRNAs and ncRNAs, which were fragmented into short fragments by fragmentation buffer (Illumina) and reverse transcription into cDNA with random primers. Second-strand cDNA were synthesized by DNA polymerase I, RNase H, dNTP (dUTP instead of dTTP), and buffer. The cDNA fragments were purified using QiaQuick PCR extraction kit (QIAGEN, Germany), end repaired, poly(A) added, and ligated to Illumina sequencing adapters. UNG (Uracil-N-Glycosylase) (Illumina, USA) was then used to digest the second-strand cDNA. Ultimately, the digested products were size selected by agarose gel electrophoresis, PCR amplified, and sequenced on Illumina HiSeq™ 4000 platform (Illumina, USA) by Gene Denovo Biotechnology Co. (Guangzhou, China). All RNA sequencing data produced in our study are available in NCBI SRA database and connected to BioProject PRJNA406998.

2.4. Quality control and mapping of reads

Since reads produced from the sequencing machines included raw reads containing adapters or low quality bases which would affect the following assembly and analysis, raw reads were further filtered by removing reads containing adapters, more than 10% of unknown nucleotides (N), and more than 50% of low quality (Q-value ≤ 20) bases to obtain high quality clean reads. Quality control of transcriptome data is presented in Table 1 [1].

Short reads alignment tool Bowtie2 [5] was used for mapping reads to ribosome RNA (rRNA) database. The mapped reads were removed and the remaining reads were used in assembly and further analysis. The rRNA removed reads of each sample were then mapped to reference genome of *Apis mellifera* (assembly Amel_4.5) using TopHat2 (version 2.0.3.12) [6] following alignment parameters: (1) maximum read mismatch is two; (2) the distance between mate-pair reads is 50 bp; (3) the error of distance between mate-pair reads is ±80 bp.

2.5. Transcripts assembly

Transcripts were assembled using software Cufflinks [7], which together with TopHat2, allow researchers to identify novel genes and novel splice variants of known ones. The program reference annotation based transcripts (RABT) was preferred. Cufflinks constructed faux reads according to reference to make up for the influence of low coverage sequencing. During the last step of assembly, all of the reassembles fragments were aligned with reference genes and then similar fragments were removed. Cuffmerge was used to merge transcripts from different replicas of a group into a comprehensive set of transcripts, and the transcripts from multiple groups were then merged into a finally set of transcripts. Pearson correlations between every biological replicas in each sample group were calculated and shown in Fig. 2 [1].

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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