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Full-length transcriptome construction and systematic characterization of virulence factor-associated isoforms in Nosema ceranae

Mengyi Wang[1], Xiaoxue Fan[1], He Zang[1], Dafu Chen[2], Rui Guo[3]
[1]College of Bee Science and Biomedicine, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[2]National & Local United Engineering Laboratory of Natural Biotoxin, Fuzhou 350002, China
[3]Apitherapy Research Institute of Fujian Agriculture and Forestry University, Fuzhou 350002, China

Honeybees are an indispensable pollinator in nature with pivotal ecological, economic, and scientific value. However, a full-length transcriptome for Apis mellifera, assembled with the advanced third-generation Nanopore sequencing technology, has yet to be reported. Here, Nanopore sequencing of the midgut tissues of un-inoculated and Nosema ceranae-inoculated A. mellifera workers was conducted, and the full-length transcriptome was then constructed and annotated based on high quality long reads, followed by the improvement of sequences and annotations of the current reference genome of A. mellifera. In total, 5 942 745 and 6 664 923 raw reads were produced from midguts of workers at 7 days post-inoculation (dpi) with N. ceranae and 10 dpi, while 7,100,161 and 6,506,665 raw reads were generated from the midguts of corresponding uninoculated workers, respectively. After strict quality control, 6 928 170, 6 353 066, 5 745 048, and 6 416 987 clean reads were obtained, with a length distribution ranging from 1 kb to 10 kb. Additionally, 16 824, 17 708, 15 744, and 18 246 full-length transcripts were respectively detected, including 28 019 nonredundant ones. Among these, 43 666, 30 945, 41 771, 26 442, and 24 532 full-length transcripts could be annotated to the Nr, KOG, eggNOG, GO, and KEGG databases, respectively. For the first time, 501 novel genes (20,326 novel transcripts) were identified, among which 401 (20 255), 193 (13 365), 414 (19 186), 228 (12 093), and 202 (11 703) were respectively annotated to each of the aforementioned five databases. The expression and sequences of three randomly selected novel transcripts were confirmed by RT-PCR and Sanger sequencing. The 5′ UTR of 2082 genes, the 3′ UTR of 2029 genes, and both the 5′ and 3′ UTRs of 730 genes were extended. Moreover, 17 345 SSRs, 14 789 complete ORFs, 1 224 long non-coding RNAs (lncRNAs), and 650 transcription factors (TFs) from 37 families were detected. Findings from this work not only refine the annotation of the A. mellifera reference genome, but also provide a valuable resource and basis for relevant molecular and omics studies.