

Global discovery, expression pattern, and regulatory role of miRNA-like RNAs in *Ascosphaera apis* infecting the Asian honeybee larvae

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Ascosphaera apis, a specialized fungal pathogen, causes lethal infection in honeybee larvae. miRNA-like small RNAs (milRNAs) are fungal small RNAs similar to miRNAs, which have been shown to regulate fungal hyphal growth, spore formation, and pathogenesis. Based on our previously obtained transcriptome data, differentially expressed miRNA-like RNAs (DEmilRNAs) in *A. apis* infecting the *Apis cerana cerana* worker 4-, 5-, and 6-day-old larvae (Aa-4, Aa-5, and Aa-6) were screened and subjected to trend analysis, followed by target prediction and annotation as well as investigation of regulatory networks, with a focus on sub-networks relative to MAPK signaling pathway, glycerolipid metabolism, superoxide dismutase, and chitinase. A total of 606 milRNAs, with a length distribution ranging from 18 nt to 25 nt, were identified. The first nucleotide of these milRNAs showed a bias towards U, and the bias pattern across bases was similar across the three groups. There were 253 68 up- and 54 down-regulated milRNAs shared by the above-mentioned three groups. Additionally, the expression and sequences of three milRNAs were validated by stem-loop RT-PCR and Sanger sequencing. Trend analysis showed that 79 DEmilRNAs were classified into three significant profiles (Profiles 4, Profiles 6, and Profiles 7). Target mRNAs of DEmilRNAs in these three significant profiles were engaged in 42 GO terms such as localization, antioxidant activity, and nucleoid. These targets were also involved in 120 KEGG pathways including lysine biosynthesis, pyruvate metabolism, and biosynthesis of antibiotics. Further investigation suggested that DEmilRNAs-targeted mRNAs were associated with MAPK signaling pathway, glycerolipid metabolism, superoxide dismutase, and chitinase. Moreover, the binding relationships between aap-milR10516-x and ChsD as well as between aap-milR-2478-y and mkh1 were confirmed utilizing a combination of dual-luciferase reporter gene assay and RT-qPCR. Our data provide new insights into the *A. apis* proliferation and invasion, lay a basis for illustrating the DEmilRNA-modulated mechanisms underlying the *A. apis* infection, and offer candidates for functional dissection.