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Differential Gene Transcription in Honeybee (*Apis cerana*) Larvae Challenged by Chinese Sacbrood Virus (CSBV)

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Abstract

Honey bees are economically important social insects. They are suffering from all kinds of pathogens, especially the viruses. In response to pathogens, different immune pathways such as Toll, Imd, Jak-Stat and RNAi are involved. In the present study, the transcription analysis of 32 immune-related genes from *Apis cerana* challenged by Chinese sacbrood virus (CSBV), the most widely distributed virus in *A. cerana*, was carried out by qRT-PCR to provide cues for the antiviral mechanism and the effective control of bee viruses. The expression level of 22 genes were statistically changed, including 11 up-regulated genes (*cactus-2*, *lys-2*, *vir*, *s3a*, *mta1*, *faa*, *vhdl*, *co-1-iv*, *ago-1*, *ago-3*, *aub*) in which 3 (*ago-1*, *ago-3*, *aub*) were related to RNAi pathway, and 11 down-regulated genes (*kenny*, *pgrp-lc*, *pgrp-s2*, *abaecin*, *lys-1*, *lys-3*, *domeless*, *tepa*, *mlc*, *dscam*, *rpl8*) related to Toll, Imd, and Jak-Stat pathways. The results indicated that CSBV infection in *A. cerana* may activate the whole immunity systems, including the RNA-based antiviral immunity system. This work constituted the first report, under laboratory conditions, about induction of immune related genes in response to CSBV.

Introduction

Insects have diverse mechanisms to combat infection by pathogens (Evans et al., 2006), including cellular and humoral immune defenses. The innate immune response of insect actions include the secretion of antimicrobial peptides, phagocytosis, melanization and the enzymatic degradation of pathogens (Hoffmann, 2003; Hultmark, 2003), which result from triggering the four non-autonomous pathways, Toll (transmembrane signal transducing pathway serves in both immunity and development), Imd (immune deficiency), JAK/STAT (the Janus kinase/signal transducers and activators of transcription) and JNK (intracelular signalling pathways) (Boutros *et al.*, 2002). Viral double-stranded RNA is also recognized as a pathogen-associated molecular pattern and processed into small interfering RNAs (siRNAs) by the host ribonuclease Dicer. After amplification by host RNA-dependent RNA polymerases in some cases, these virus-derived siRNAs guide specific antiviral immunity through RNA interference and related RNA silencing effector mechanisms (Ding, 2010).

Apis mellifera and *Apis cerana* are the major honey bee species in the global beekeeping industry (Gallai et al., 2009; Garibaldi *et al.*, 2011). They are heavily infected by different vital viruses (Chen et al., 2004, 2006; Ai et al., 2012). The *A. mellifera* genome (Honeybee Genome Sequencing Consortium, 2006) revealed that honey bees possess homologues of members of the four pathways implicated in humoral immune responses (Evans et al., 2006).

Chinese sacbrood virus (CSBV) is the most stricken pathogen of *A. cerana*, which results in severe and deadly infections of the colony and eventually losses of the entire colony (Yan et al., 2008; Liu et al., 2010; Han et al., 2013). This virus was first observed in Guangdong Province, China in 1972 and spread to the whole China and the counties of Southeast Asia (Liu et al., 2010). Some efforts have been made to study this viruse, such as diagnostic methods (electron microscopy, enzyme-linked immunosorbent assay and reverse transcription-polymerase chain reaction (RT-PCR)) (Yan et al., 2009), and the control of this disease by RNA interference (Liu et al., 2010). It is interesting that *A. mellifera* is not sensitive to CSBV in general beekeeping practice

(Zhang et al., 2001). Furthermore, *A. mellifera* may develop cellular and humoral immune responses to various pathogens such as bacteria (Chan et al., 2009; Lourenço et al., 2013), viruses (Azzami et al., 2012), microsporidian (Antúnez et al., 2009; Chaimanee et al., 2012) and *Varroa* mites (Gregorc et al., 2012). However, no information on the expression of immune-related genes from *A. cerana* after infection of CSBV is reported.

In the present study, the transcription analysis of the immune-related genes from *A. cerana* challenged by CSBV was conducted to provide cues for the antiviral mechanism of the honey bees and the effective control of the bee viruses.

Materials and Methods

Honey Bees

Second instar larvae of *A. cerana* were obtained from a single mated honeybee queen in a healthy apiary at Conghua, Guangdong Province (113°17' E, 23°8'' N), China. To obtain age controlled larvae, the queen was caged on a comb and left to lay eggs for six hours. Twenty hours after larval eclosion, or ninety-two hours after oviposition, the comb containing second instar larvae was retrieved from the colony, and placed in the laboratory for treatments at 32°C - 34°C. All the larvae used in this study were detected by RT-PCR method for the absence of the following viruses, black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), acute bee paralysis virus (ABPV), deformed wing virus (DWV), kashmir bee virus (KBV), CSBV and Israeli acute paralysis virus (IAPV), with the primers from Ai et al. (2012). RNA was extracted using Trizol Reagent (Invitrogen) and cDNA synthesis was performed using PrimeScript™ 1st Strand cDNA Synthesis Kit with DNA eraser (TaKaRa, Japan). Amplification profile of PCR consisted of an initial 2-min denaturation at 94 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C, 1 min at 72 °C and finally 7-min cycle at 72 °C. No signs of clinical American foulbrood or viral disease were observed in these larvae.

Virus

CSBV-infected larvae with typical symptom were collected from an apiary at Xinhua Village from Hunan Province, and kept at -80 °C for less than 10 days before use. The presence of CSBV virus was confirmed by observing the morphological symptom under electronic microscopy and RT-PCR according to Chen et al. (2004, 2006) and Liu et al. (2010). To obtain CSBV, the larvae (approximately 0.2 g) infected by CSBV were ground in 6 mL sterile phosphate-buffered solution (PBS) (1x: 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.4) with a sterile grinder. The resulting solution was centrifuged at 12,000 rpm at 4 °C for 10 min, and the supernatant was further passed through a 0.45 μm cell filter first, then through a 0.22 μm cell filter (Ghosh *et al.*, 1999). CSBV concentration was quantitated by absolute quantification assay (Liu *et al.*, 2010). CSBV polyprotein gene (*sbv1*) was amplified to detect copy number of CSBV according to Liu et al. (2010). PCR reactions were carried out in triplicate in an Mx3000PTM Real-Time PCR System (Stratagene, California, USA), using SYBR_Green (Brilliant II SYBR-Green QPCR Master Mix; Stratagene, California, USA). CSBV concentration for the experiments was 7.20×10^4 copies/mL.

Viral challenge and sample collection

Second instar larvae were collected from a comb with a special grafting tool and transferred to a 96-well tissue culture plate filled with 30 μL of basic diet prepared according to Patel *et al.* (2007). The larvae were maintained in an incubator (Sanyo, Tokyo, Japan) at 33°C - 34°C and 75% relative humidity. Twenty-four hours later, each larva was fed with 30 μL CSBV suspension or PBS for 24 h. Then, twenty larvae were picked from each replicates, sterilized with 75% ethanol (prepared with 0.2% Diethylpyrocarbonate (DEPC) -Water) for 5 min, washed with DEPC-Water for three times, immediately frozen in liquid nitrogen, and kept at -80°C until total RNA isolation (Liu et al., 2010; Zhang et al., 2010). Three replicates with 50 larvae in each were used for both treatments.

Gene selection and primers

A total of 32 genes from *A. cerana* were selected for quantitative analysis according to references on the innate immune pathways in insects (Toll, Imd, JAK/STAT and RNAi pathways) (Table 1) (Christophides et al., 2004; Clem et al., 2005; Dostert et al., 2005; Royet et al., 2005; Yang & Cox-Foster, 2005; Evans et al., 2006; Ding et al., 2010). Primers and Genebank accession numbers of the corresponding genes was summarized in Table 1.

Quantitative Real-time PCR (qRT-PCR)

Quantitative Real-Time PCR was carried out according to our previously described method with modifications (Zhang et al., 2010). Briefly, total RNA and cDNA were prepared as described above**.** Expression of *actin* gene (GI: 406122) was used as an internal control. The efficiency of each primer set was first validated by constructing a standard curve through five serial dilutions. PCR reactions were carried out in triplicate in an Mx3000PTM Real-Time PCR System (Stratagene, USA), using SYBRGreen (SYBR® Premix Ex Taq™ II, TAKARA, Japan). A control without template was included in all batches. The PCR program began with a single cycle at 95 °C for 10 min, 40 cycles at 95 °C for 15 s, 55 °C for 30 s and 72 °C for 45 s. Afterwards, the PCR products were heated to 95 °C for 30 s, cooled to 55 °C for 30 s and heated to 95 °C for 30 s, in order to measure the dissociation curves

and to determine a unique PCR product for each gene. mRNA levels were calculated relative to *actin* expression using the Mx3000PTM Software (version 4.1) (Agilent, USA). The fold change was calculated using the 2−ΔΔCt method. Each sample was analyzed independently and processed in triplicate. PCR products were diagnosed by 1% agarose electrophoresis. Positive samples were cloned to pMD-19T (TAKARA, Japan), then sent to Invitrogen Company China for sequencing with corresponding specific primers.

Data Analysis

Data were analyzed using SPSS statistical software (version 16.0) and the significance between treatments in each experiment was evaluated by One-Way ANOVA test followed by Tukey's test for pair comparisons at $p < 0.05$. The values are expressed as means ± S.D. ($p < 0.05$) was defined as statistically significant.

Results

Bee colonies did not present any visible clinical symptoms of any disease (i.e., American Foulbrood or Chalkbrood). *Nosema* spp. spores were not detected from the bee samples. No CSBV, BQCV, CBPV, DWV, KBV, SBV, or IAPV viruses were detected in all the larvae used in this study. CSBV was only present in the larvae fed with CSBV.

RNA expression levels of 32 genes were determined using qRT-PCR 24 h after CSBV challenge. A set of 32 genes showed differential expression in the larvae challenged by CSBV. Gene-specific amplification was confirmed for 32 primer pairs by sequencing, by a single peak in melt-curve analysis and a single band with the expected size in agarose gel electrophoresis. No primer-dimer formation was detected and the standard curves derived from five-fold serial dilutions of pooled cDNA from the larvae (CSBV-challenged/control) gave correlation coefficients greater than 0.995 and efficiencies between 80% and 103%. Among 32 differential expression genes, 11 genes (*cactus-2*,$P = 0.0269$; *lys-2*, $P = 0.0173$; *vir*, $P = 0.0451$; *s3a*, $P = 0.0399$; *mta1*, $P = 0.0219$; *faa*, $P = 0.0233$;*vhdl*, $P = 0.0400$;*co-1-iv*, $P = 0.0141$;*ago-1*, $P = 0.0018$; *ago-3*, $P = 0.0186$; *aub*, $P = 0.0142$) were up-regulated, 11 genes (*Kenny*, $P = 0.0057$; *pgrp-lc*, $P = 0.0418$; *pgrp*-s2, $P = 0.0352$; *abaecin*, $P = 0.0247$;*lys-1*, $P = 0.0048$;*lys-3*, $P = 0.0276$; *domeless*, $P = 0.0434$; *tepa*, $P = 0.0336$; *mlc*, $P = 0.0389$; *dscam*, $P = 0.0012$;*rpl8*, $P = 0.012$;) down-regulated, and 10 gene (*galectin*, $P = 0.2860$; *cactus-1*, $P = 0.1460$; *imd*, $P = 0.0991$; *basket*, $P = 0.1010$; *def2*, $P = 0.1070$; *tpi*, $P = 0.1200$; *ago-2*, $P = 0.0765$; *dicer-1*, $P = 0.0629$; *dicer*, $P = 0.2570$; *piwi*, $P = 1.9400$;) showed no significant difference in expression (ANOVA, Tukey's test , $p < 0.05$) (Fig 1).

Of the up-regulated expression genes, *cactus-2*, *lys-2* were genes related to Toll pathway; *ago-1*, *ago-3*, and *aub* were members of RNAi pathways; *Vhdl* and *co*-1-*iv* were related to cellular component and development; *mta1*, *faa*, and *s3a* were involved in cellular metabolic and regulated progress. Among the down-regulated genes, *kenny*, *pgrp-lc* were the important members in Imd pathways; Imd is specific for antimicrobial defense and normal development; *pgrp-s*2 coding peptidoglycan recognition protein critically was involved in sensing bacterial infection and activation of the Toll signaling pathway (Evans et al., 2006); *Abaecin, lys*-1, *lys*-2 and *lys*-3 were antimicrobial effectors of Toll pathway; *domeless*, *vir* and *tepa* were the members of Jak pathway; *mlc* was related to cellular component and development; *Dscam* was related to humoral immunity (Evans et al., 2006); *rpl*8 was involved in cellular metabolism and regulation.

No selected genes specific to RNAi pathway displayed down-regulated expression, although *ago-2, dicer-1*, *dicer*, and *piwi* showed no significant difference in expression.

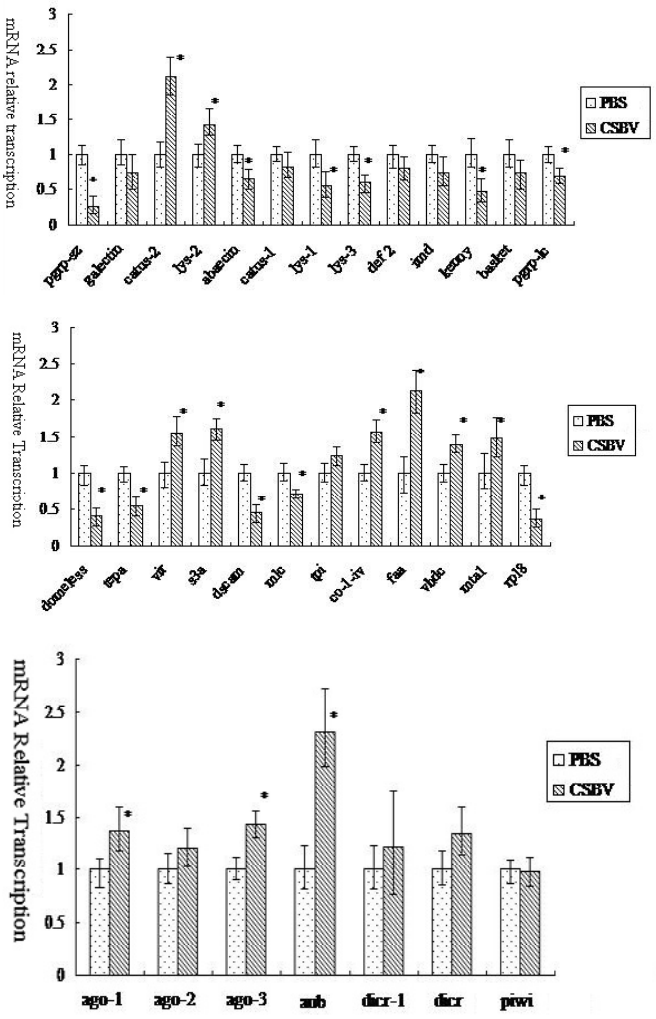

Fig 1 Relative transcription analysis of selected genes in CSBV infected *Apis cerana* larvae by qRT-PCR. CSBV - The larvae fed with CSBV for 24 h. PBS - The larvae fed with phosphate-buffered solution as control. Results are reported as an average of the triplicates plus the standard deviation. Bars with different letters indicated significant differences by Tukey's test at $P < 0.05$(*).

Table 1 - Locus names, gene annotation, primers and genebank accession numbers for the selected genes

Gene Name	Gene ID	Gene annotation	Primers	Regulation[1]	References[2]			
					References	host	pathogens	Regulation
galectin	GB10026	cellular regulation	F: ACCACCTTCAGGTCCTGGTT R: CCCAGAGAGCCAAGGTTTCC	D	-			
co-1-iv	GB14564	collagen	F: GGTTACGTTCGTTCCCGGTT R: TACCTTGCTCGCCCCTGTAA	U	-			
tpi	gi|148224276	Glycolytic enzyme	F: CATGGGTAATTCTTGGTC R: CATGGGTAATTCTTGGTC	E	-			
faa	GB13982	hydrolase	F: AGGGATATTTGGTTTGATTGG R: GTGGAGAAGATGCTCTGGGAT	U	Zhang *et al.*, 2010	*A.cerana*	*Varroa destruvtor*	D
pgrp-lc	GB17188	Imd pathway	F: TCCGTCAGCCGTAGTTTTTC R: CGTTTGTGCAAATCGAACAT	D	George *et al.*, 2002	*Anopheles*	*E. coli*	U
kenny	GB17106	Imd pathway	F: GCTGAACCAGAAAGCCACTT R: TGCAAGTGATGATTGTTGGA	D	Evans *et al.et al.*, 2006	*A. mellifera*	*E. coli* *P. larvae*	D
imd	GB18606	Imd pathway	F: TGTTAACGACCGATGCAAAA R: CATCGCTCTTTTCGGATGTT	E	Evans *et al.et al.*, 2006	*A. mellifera*	*E. coli* *P. larvae*	D
basket	GB16401	Imd pathway	F: AGGAGAACGTGGACATTTGG R: AATCCGATGGAAACAGAACG	E	Evans *et al.et al.*, 2006	*A. mellifera*	*E. coli* *P. larvae*	D
dscam	GB11871	Immunoglobulin	F: TTCAGTTCACAGCCGAGATG R: ATCAGTGTCCCGCTAACCTG	D	Evans *et al.et al.*, 2006	*A. mellifera*	*E. coli* *P. larvae*	D
tepa	GB18789	Jak pathway	F: CAAGAAGAAACGTGCGTGAA R: ATCGGGCAGTAAGGACATTG	D	Evans *et al.*, 2006	*A. mellifera*	*E. coli* *P. larvae*	D
domeless	GB16422	Jak pathway	F: TTGTGCTCCTGAAAATGCTG R: AACCTCCAAATCGCTCTGTG	D	Xi *et al.*, 2008	*Aedes aegypti*	(dengue virus serotype 2) DENV-2	U
vir	100872984	Jak pathway	F: GGGTAGTGTAGGCAGAGGGG R: CTTGTGTCCAAGGGCGACTT	U	Dostert *et al.*, 2005	*Drosophila*	Drosophila C Virus (DCV)	U
vhdl	GB726182	Lipoprotein	F: GCATCACCTTCTGACCAACC R: ACCTCGTCCAACATCCTTCT	U	Zhang *et al.*, 2010	*A. cerana*	*Varroa*	U
mlc	GB409881	Myosin regulatory	F: AATCTCTTCGCATCTCGC R: CGCATCGTTGACTTCCTT	D	Scharlaken *et al.*, 2008	*A. mellifera*	*E.coli*	D

1 Regulation in this study; 2 Regulation, challenged hosts and pathogens from the references; U: up-regulated genes; D: down-regulated genes; E: no differential expression genes.

(Continuation)

mta1	gi|110756803	NURD	F: CATCTCTGTGCTTCTCCTC R: ACTCGATCTGGTTGTTTTC	U				
s3*a*	gi|66547340	Ribosomal protein	F: GTGCGGGTAGTAAGAGCGAAGAAG R: GTGCGGGTAGTAAGAGCGAAGAAG	U	-			
***rpl*8**	GB17629	Ribosomal protein	F: TGGATGTTCAACAGGGTTCATA R: CTGGTGGTGGACGTATTGATAA	D	-			
***ago*-1**	GB122654	RNAi pathway	F: TGGCCCAGATCAAGTAGAGC R: AATTTGATAGCGTTTGTGGTGAT	U	Ding, 2010			
***ago*-2**	GB15464	RNAi pathway	F: GAGCTATTGCGCGCTTAGAGA R: TATCAACAATGGTGCCCGCC	E	Ding, 2010	*Drosophila*	Flock house virus (FHX)	mutant
***ago*-3**	GB19389	RNAi pathway	F: CCCAATGCCCGATGTGATGT R: TCCATTCTGCAGGCTTTGCC	U	Ding, 2010; Keene *et al*., 2004	*Anopheles gambiae*	O'nyong-nyong virus	mutant
aub	GB10293	RNAi pathway	F: TGGTTGGGATGTTTTGCCACA R: TCGCATAGCACCTCTTCCCA	U	Ding, 2010			
dicer	GB15170	RNAi pathway	F: AGCAGTAGCTGATTGTGTGGA R: TTCAGAAGCGCAAGGCATGT	U	Ding, 2010			
***dicer*-1**	GB11966	RNAi pathway	F: AAGAGCTCCAGATGCCCTGT R: TGCATCACCTCCATCAAGTGG	E	Ding, 2010			
piwi	412427	RNAi pathway	F: TGCAAAAGAAACAGTGCTGGA R: TCGCATAGCACCTCTTCCCA	E	Ding, 2010			
abaecin	GB18323	Toll pathway	F: CAGCATTCGCATACGTACCA R: GACCAGGAAACGTTGGAAAC	D	Evans *et al*., 2006	*A. mellifera*	*E. coli* *P. larvae*	U
cactus-1	GB10655	Toll pathway	F: CACAAGATCTGGAGCAACGA R: GCATTCTTGAAGGAGGAACG	E	Evans *et al*., 2006	*A. mellifera*	*E. coli* *P. larvae*	U
***cactus*-2**	GB13520	Toll pathway	F: TTAGCAGGACAAACGGCTCT R: CAGAAAGTGGTTCCGGTGTT	U	Xi *et al*., 2008	Aedes aegypti	(dengue virus serotype 2) DENV-2	D
***def*2**	GB10036	Toll pathway	F: GCAACTACCGCCTTTACGTC R: GGGTAACGTGCGACGTTTTA	E	Evans *et al*., 2006;	*A. mellifera*	*E. coli;* *P. larvae*	U
***lys*-1**	GB10231	Toll pathway	F: GAACACACGGTTGGTCACTG R: ATTTCCAACCATCGTTTTCG	D	Evans *et al*., 2006	*A. mellifera*	*E. coli* *P. larvae*	U
***lys*-2**	GB15106	Toll pathway	F: CCAAATTAACAGCGCCAAGT R: GCAATTCTTCACCCAACCAT	U	Evans *et al*., 2006	*A. mellifera*	*E. coli* *P. larvae*	D
***lys*-3**	GB19988	Toll pathway	F: TCGATGAATGCGAAGAAAATC R: TCGATGAATGCGAAGAAAATC	D	Evans *et al*., 2006	*A. mellifera*	*E. coli* *P. larvae*	U
***pgrp*-s2**	GB19301	Toll pathway	F: TAATTCATCATTCGGCGACA R: TGTTTGTCCCATCCTCTTCC	D	Evans *et al*., 2006	*A. mellifera*	*E. coli* *P. larvae*	U
actin	GB406122	Reference gene	F: ATGCCAACACTGTCCTTTCTGG R: GACCCACCAATCCATACGGA	-				

Discussion

As social animals, honey bees are at considerable risk from parasites and pathogens (Gregory et al., 2005; Yang & Cox-Foster, 2005; Evans, 2006; Azzami et al., 2012). Specifically, increased genetic relatedness and the high population densities of honey bee societies can strongly favour pathogen spread and epizootic outbreak. However, compared to the sequenced *Drosophila* and *Anopheles* genomes, honey bees are relatively immunologically deficient (i.e., express fewer immune response proteins) (Evans et al., 2006). Usually, in response to bacterial pathogens, fungal pathogens, parasite or acaricides, the expression level of antimicrobial peptides were up-regulated in the infected bees (Gregory et al., 2005; Evans, 2006; Scharlaken et al, 2008; Aronstein et al., 2010; Dussaubat et al, 2012; Gregorc et al, 2012; Hamiduzzaman et al, 2012; Garrido et al., 2013). The up-regulated genes were detected from *A. mellifera* after response to bacteria (*ERp60*, *obp17*, *proPO*, *HSP*s; *lys*, *hymenoptaecin, transferrin-1abaecin*, *defensin*, *hymenoptaecin, pgrp-s3, B-glc-2, cactus-2, dorsal-1B, relish*) (Scharlaken et al., 2008; Chan et al., 2009; Lourenço et al., 2013), microsporidian *(abaecin*, *defensin*, *hymenoptaecin*) (Antúnez et al., 2009; Chaimanee et al., 2012) and *Varroa* mites (*pgrp-sc, abaecin*, *defensin1*, *hymenoptaecin*) (Gregorc et al., 2012). However, infection of *A. mellifera* with ABPV produced neither elevated levels of specific antimicrobial peptides (AMPs), such as hymenoptaecin and defensin, nor any general antimicrobial activity, such as nodulation (Azzami et al., 2012). These data suggest that bees use a distinct mechanism to counter different viral infections. The present results showed that *A. cerana* produced expression changes of immune-related genes when responding to CSBV infection. RNAi based antiviral pathway might be very important for *A. cerana*.

In the present study, after challenged by CSBV, *A. cerana* larvae produced 3 down-regulated (*pgrp*-s2, *abaecin*, *lys*-1) and 2 up-regulated (*cactus*-2, *lys*-2) genes for Toll pathway. However, in response to *Paenibacillus larvae*, the genes (*pgrp*-s2, *abaecin*, *lys*-1 and *lys*-3) in *A. mellifera* were strongly up-regulated, while *cactus*-2 and *lys*-2 were down-regulated (Evans et al., 2006). The differences in gene expression patterns of Toll pathway may be related with the bee species and responding pathogens. Moreover, from the gene regulation pattern, to a certain extent, it seemed that the Toll pathway was suppressed by CSBV infection.

Of 4 genes (*imd*, *basket*, *kenny*, *pgrp-lc*) related to Imd pathway, 2 genes (*kenny* and *pgrp-lc*) were down-regulated and others (*Imd* and *basket*) showed no significant difference in expression. PGRP-LC is the activator of the Imd signaling process, which elicited by peptidoglycan, fungi and *Varroa* mites (Werner et al., 2003; Stenbak et al., 2004; Gregorc et al., 2012). It was apparent that CSBV infection suppressed the expression of *pgrp-lc*.

Among 3 genes related to Jak/STAT pathway, 2 genes (*domeless*, *tepa*) were up-regulated, but *vir* (virus-induced RNA) was down-regulated. *Vir* was not induced by pathogenic bacteria or fungi in *Drosophila* (Dostert et al., 2005). But Jak kinase Hopscotch was involved in the control of the viral load in infected flies (Dostert et al., 2005).

Previous studies also indicated that RNA interference as an intrinsic defense against viral infection play a major role in antiviral immunity in insects, e.g. *D. melanogaster*, *Anopheles gambiae*, *Bombyx mori*, *Caenorhabditis elegans* (Keene et al., 2004; Galiana-Arnoux et al., 2006, 2007; van RiJ. et al., 2006; Kemp & Imler, 2009; Azzami et al., 2012; Xu et al., 2012). Central to the RNAi mechanism are the slicing enzymes of the Argonaute (AGO) family (five members in *Drosophila*), which mediate highly specific cleavage of target RNA molecules. The specificity of AGO enzymes is achieved by their association with small RNAs, which guide them to complementary sequences (Ding, 2010). Three RNAi pathways, involving different members of the AGO family (AGO-1, AGO-2, AGO-3), have been defined in *Drosophila* (Kemp & Imler, 2009) In RNA silencing, AGO proteins are the effector molecules of specific gene silencing, the specificity of which is determined by the AGO-bound siRNA (Kemp & Imler, 2009; Ding, 2010). In our results, *ago*-1, *aub*, and *ago*-3 in *A. cerana* larvae were significantly up-regulated after CSBV infection, although *ago*-2, *dicer*-1, *dicer* and *piwi* showed no significant expression difference, indicating that in honey bees there may be a RNA-based antiviral immunity system, though it can active the whole immunity systems. Future studies are of importance to reveal the specific role of RNAi in the antiviral response of honey bees.

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