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Silencing of cytochrome P450 *CYP6B6* gene of cotton bollworm (*Helicoverpa armigera*) by RNAi

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Abstract

RNA interference (RNAi) induced through double-stranded RNA (dsRNA) has been used widely to study gene function in insects. In this paper we demonstrate the efficacy of RNAi in the cotton bollworm, *Helicoverpa armigera*. Using *CYP6B6* as the target gene, which is expressed in the fat baby and midgut of the lepidopteran pest *H. armigera*, we constructed the vector which expressed dsRNA of CYP6B6. Northern blot analysis showed that dsRNA expressed in the *Escherichia coli* (HT115) was target gene. The results also showed that the gene expression level and protein expression level of *H. armigera* larvae fed with dsRNA expressed by *E. coli* were significantly lower than those of all controls, but the gene expression level was more obvious than that at the protein level; significant lethality differences were also found between HT115 bacteria containing L4440-dsC1 treatment and HT115 bacteria containing L4440 vector or CK (ddH_2O) in instar larvae on 4 day when continuous feeding, 32.45% mortality was recorded in the group of feeding HT115 bacteria containing L4440-dsC1 on 10 day. Our results suggest that the RNAi pathway can be exploited to control insect pests.

Keywords: RNA interference, *Helicoverpa armigera*, *CYP6B6*, double-stranded RNA

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Introduction

Helicoverpa armigera (Lepidoptera: Noctuidae) is a major insect pest in a wide range of agricultural and commercial crops in many parts of the world including China. An increase in insecticide detoxification by P450 and other enzymes is a common mechanism of insecticide resistance (Hemingway & Karunaratne, 1998; Scott, 1999; Enayati *et al*., 2005). The cytochrome P450-dependent monooxygenases are components of an extremely important metabolic system because of their involvement in regulating the titers of endogenous compounds such as hormones, fatty acids and steroids, and in the catabolism and anabolism of xenobiotics such as drugs, pesticides and plant toxins (Scott, 1999). Monooxygenases are found in virtually all aerobic organisms, including organisms as diverse as insects, plants, mammals, birds and bacteria (Stegeman & Livingstone, 1998). Insect monooxygenases can be detected in a wide range of tissues. Higher monooxygenase activities are usually associated with the midgut, fat bodies and Malpighian tubules (Brun *et al*., 1996). Inducibility is a general characteristic of cytochrome P450 (Harrison *et al*., 2001). Induction of cytochrome P450 genes by chemicals such as allelochemicals and insecticides, has been well documented (Feyereisen, 2005). The link between exogenous compounds acting as induction agents and the induced enzymes that metabolize them has been established in both mammalian and insect detoxification systems (Willoughby *et al*., 2006). Xenobiotic inducible genes may be more likely to be involved in resistance (Le Goff *et al*., 2006). Therefore, reducing the expression of detoxifying enzymes is a key in pest control.

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RNA interference (RNAi), which is initially discovered in nematode *Caenorhabditis elegans*, is widely accepted as a powerful tool for gene function study (Fire *et al*., 1998). Most insect RNAi studies have used specific double-stranded RNAs (dsRNAs) as effectors to induce RNAi activity in recipient insects (Huvenne & Smagghe, 2010). The dsRNA effectors have been delivered either via intrathoracic injection or oral feeding. Injection of dsRNAs is widely used and proven to be effective in many insect species, such as the pea aphid (*Acyrthosiphon pisum*) (Amdam *et al*., 2003; Jaubert-Possamai *et al*., 2007), honeybee (*Apis mellifera*) (Amdam *et al*., 2003), flour beetle (*Triboliumc astaneum*) (Bucher *et al*., 2002) and grasshopper (*Schistocerca gregaria*) (Dong & Friedrich, 2005). Successful RNA knock-down effects via artificial feeding of dsRNAs have also been reported in insects including the Triatomine Bug (*Rhodnius prolixus*) (Araujo *et al*., 2006), light brown apple moth (*Epiphyas postvittana*) (Turner *et al*., 2006). The dsRNAs can be synthesized using an RNAi kit and produced in bacteria. Due to a high cost and short duration of synthetic siRNA *in vivo*, feeding of dsRNA is an even more attractive approach than hemocoel injection because it is non-invasive and will aid our design of new methods in pest control. In the case of feeding, the gut obviously is the primary target organ, representing environmental RNAi. Using genetically engineered *Escherichia coli* strain HT115 to express dsRNA is an economical way to produce large quantities of dsRNA. Besides, bacterial feeding is a non-disrupting technique preserving the integrity of the treated animals. *E. coli*-mediated delivery of dsRNA has been reported in *C. elegans* (Timmons & Fire, 2001), planarians (*C. elegans*) (Newmark *et al*., 2003), *Entamoeba histolytica* (Solis *et al*., 2009) and *Spodoptera exigua* (Tian *et al*., 2009). Successful RNAi experiments have been carried out in a number of lepidopteran species. One reported the knockdown of a pigment gene following dsRNA injection into *Bombyx mori* embryos (Quan *et al*., 2002), and another targeted a putative *Bacillus thuringiensis* toxin receptor in *Spodoptera litura* larvae (Rajagopal *et al*., 2002), and a third gene encode immune response protein in the tobacco hornworm *Manduca sexta* (Eleftherianos *et al*., 2006). In insects, midgut is generally thought to be the primary detoxification organ (Hodgson, 1985). As an important detoxification enzyme of P450 in the midgut of the cotton bollworm, it plays an important role in the degradation of toxic substances (Hlavica, 2011). *CYP6B6* transcription is highly inducible by deltamethrin at a low dose in both midgut and fat body. This observation suggests that *CYP6B6* may contribute to deltamethrin metabolism in this pest (Grubor & Heckel, 2007). At the same time, the *p*-nitroanisole *O*-demethylase activity of P450s and the expression of *CYP6B6* mRNA induced by two plant secondary substances, 2-tridecanone and quercetin, have been investigated in the cotton bollworm. The *O*-demethylase activity is higher in the fat body than that in the midgut of 6th instar larvae fed on artificial diets mixed with these allelochemicals. Similar results were obtained when induced by a combination of 2-tridecanone and quercetin at different concentrations (Liu *et al*., 2006). Therefore, silencing the *CYP6B6* expression can lead to reduced resistance to pyrethroids of cotton bollworm, and even to other toxic substances. Within the last decade, targeted down regulation of gene expression mediated by dsRNA, collectively known as RNAi, has proved to be invaluable for understanding gene functions and crop improvement.

Here we explored the feasibility of feeding dsRNA to larvae of *H. armigera* to achieve RNAi knockdown. The results of quantitative real-time polymerase chain reaction indicated that feeding dsRNA expressed by HT115 (DE3) bacteria containing L4440-dsC1 could induce RNAi in *H. armigera*. Similar results were also obtained by immunohistochemistry when detected the cytochrome P450 CYP6B6 gene expression in midgut. Current results suggest that the target gene can be silenced by feeding engineered bacteria with the expression of dsRNA. This study lays a useful foundation for management of the cotton bollworm.

Material and methods

Insect culture

The laboratory strain of *H. armigera* came from China Agricultural University and was reared on an artificial diet in the conditioned room and maintained at 26 ± 1°C, 70–80% relative humidity, with a photoperiod of 16:8 (L:D). Artificial composition composes as follows: corn flour 300 g, soybeans powder 100 g, yeast extract powder 100 g, citric acid 2.5 g, vitamin C 10 g, sorbic acid 1.5 g, vitamin B 1.5 g, erythromycin 0.05 g, propionic acid 5 ml, vitamin E and water 2.5 L. Adults were held under the same condition and supplied with a 10% sugar solution.

Bacterial strains, plasmids and reagents

The L4440 plasmid and HT115 (DE3) strain were obtained from Bo Xinwen of XinJiang Academy of Agricultural and Reclamation Science. rTag DNA polymerase, T4 DNA ligase, AMV Reverse Transcriptase XL, DL2000 DNA Marker, pMD18-simple-T vector D, *Bgl*II and *Pst*I from Takara (China, Dalian), and TRIzol reagent from Invitrogen (Invitrogen, USA), anti-CYP6B6 (prepared by our research team), secondary antibody (goat anti-rabbit), diaminobenzidine (DAB) chromogenic kit were purchased from Zhangshan Goldbridge, China and DIG-High Prime DNA Labeling and Detection Starter Kit II (Roche).

Design and synthesis of primers

Based on the cytochrome P450 *CYP6B6* sequence from the GenBank (accession no. AY950636) and predicted possible interference site through the online prediction software (http://zh.invitrogen.com/site/cn/zh/home/brands/ambion.html), we designed the primers by DNAMAN6.0 software, L4440-dsC1 sequence primers (5′–3′) F: GAAGATCTTCGTATTTTCCGGCAGTGA; R: AACTGCAGTTCTCAAAGCTCTCCACG the site of *Bgl*II and *Pst*I was underlined. The primers were synthesized by Shanghai Sangon Company.

RNA isolation and cDNA synthesis

Total RNA was extracted using TRIzol according to the manufacturer's instructions. The quality and concentration of RNA samples were examined by agarose gel electrophoresis and spectrophotometer analysis. RNA was digested by DNase I in order to eliminate the genomic DNA contamination. cDNA was synthesized by reverse transcription in 20 µl of reactions containing 1 µg of total RNA, 2 µl of oligo (dT)18 primer (50 mM), added the RNase free water to 6 µl at 72°C for 10 min, and then inserted ice for 2 min, added 0.5 µl of RTase

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M-MLV (RNase H-) and 4 µl of 5 × M-MLV buffer, 0.5 µl of 20 U RNase inhibitor, 4 µl of dNTP mixture (2.5 mM each), 5 µl of DNase and RNase free water and at 42°C for 1 h, and then at 72°C for 15 min, then inserted ice for 5 min. Three independent RNA preparations representing three biological replicates were used for cDNA synthesis.

Vector construction and dsRNA preparation

In order to construct a plasmid that expresses dsRNA corresponding to *CYP6B6* from the midgut in the cotton bollworm, 377 bp (L4440-dsC1), was amplified by PCR using cDNA as a template. The primers were shown in 2.1.3. Amplification reactions comprised 35 cycles of 94°C for 40 s, 58°C for 40 s and 72°C for 45 s, with a final extension step of 72°C for 10 min. PCR products were confirmed by separation on 1% agarose gels and purified by DNA gel extraction kit (Sangon, Shanghai). The PCR product was then cloned into the pMD18-simple-T vector with the *Bgl*II and *Pst*I sites, and transformed into $DH_{5\alpha}$ competent strains, positive cloning vector was sent for sequencing (Sangon, Shanghai). After sequencing the positive clones to ensure insertion, the L4440-dsC1 was digested from pMD18-simple-T, and cloned to the L4440 plasmid, and then the recombinant vector L4440-dsC1 was transformed into competent HT115 (DE3) cells for the respective dsRNA expression. To produce dsRNA, single colonies of HT115 (DE3) bacteria containing L4440-dsC1 or L4440 vector was grown for 14 h with shaking in LB with 50 mg ml^{-1} ampicillin plus 12.5 mg ml^{-1} tetracycline at 37°C. The culture was diluted 100-fold in 5 ml of 2 × YT medium and allowed to grow to $OD_{595} \approx 0.4$. Synthesis of T7 polymerase was induced by addition of isopropyl β-D-thiogalactopyranoside (IPTG) to 0.5 mM and the bacteria were incubated with shaking for an additional 4 h at 37°C. The expressed dsRNA was extracted as described previously. The length of the dsRNA was confirmed by electrophoresis on 1% agarose gel, and then the dsRNA expression was further verified by the Northern bolt analysis. To obtain the respective dsRNA expressed in the HT115, bacterial cells were collected from 10 ml IPTG-induced culture by centrifugation at 4000 rpm for 5 min, and then used for *H. armigera* feeding bioassays. The HT115 (DE3) was treated in the same way.

An unrelated gene of the *D. melanogaster, white gene* (*DmWhite*) (GenBank accession no. X51749) was selected as a control dsRNA. The recombinant plasmid for *DmWhite* dsRNA expression protocol was the same as that for L4440-dsC1. The PCR primers used to amplify the fragment (419 bp) of *DmWhite* were as follows: forward primer *DmWhite* F: (CAGATCTGTCATCTTTTTGGGCCAA, under-lined letters indicate a *Bgl* site) and reverse primer *DmWhite* R: (ACTGCAGTGAGCAGGAATGGTATGATAA, spanning nucleotides 1347–1768; under-lined letters indicate a *Pst*I site).

Analysis of bacteria accumulation in the larval gut

To confirm that bacterially expressed dsRNA was introduced into the larval gut through ingestion, the bacteria, colored by FD&C Blue, were coated onto the surface of the insect artificial diet. Third instar day two larvae were allowed to feed on the colored diet. After feeding on the diet containing FD&C Blue for 6 h, 12 h, and 24 h, the larvae were dissected and their gut was examined with Nikon SMZ800 to determine whether the colored bacteria accumulated in the gut. All samples were photographed using a Nikon SMZ800.

Determination of CYP6B6 *silencing by qRT-PCR*

To monitor transcriptional levels of *CYP6B6* in larvae after feeding the genetically engineered bacteria, the third instar larva were fed on different treatment artificial food after starvation 2 h. RNA in the midgut was extracted as described previously. Three independent RNA preparations representing three biological replicates were used for cDNA synthesis. The transcription levels of *H. armigera CYP6B6* after feeding the genetically engineered bacteria were quantified by real-time PCR (RT-PCR) using a 7500 Real Time PCR System (Applied Biosystems, USA) and Real Master Mix SYBR Green PCR kit (Invitrogen, China). The elongation β-actin was used as a reference gene to normalize the target gene expression levels among samples. RT-PCR of each cDNA sample and template-free was performed in triplicate. All the primers sets used in this study were listed as follows. Specificity of the PCR amplification was checked by a melt curve analysis and by sequencing the PCR products. RT-PCR was run in a 25 µl of reaction system containing 12.5 µl of Real Master Mix/SYBR solution, 0.5 µl of each of forward and reverse primer (10 µM), 1 µl of cDNA template, 10.5 µl of ddH_2O and used the following cycling parameters: 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 62°C for 30 s and 72°C for 30 s. The relative expression levels of target genes were calculated by the comparative C_T method. Primers of *CYP6B6* and β-actin (5′–3′): *CYP6B6*: F: TTCAAACTTATACCATGTCCACAATT, R: CCAATTGACGGAGCTCTAGAATCA; *DmWhite*: F: CCTCATCTTTTTGGGCCAACAA; R: TCGCCGTGAAGACCAGTGG; β-actin: F: ATCATCGACGCTCCCGGACA, R: TAGCTGCTTGACTCCGAGGGTG.

Analysis of CYP6B6 *transcription in the larval midgut at the protein level*

To monitor translational levels of CYP6B6 transcription in larval gut after feeding the genetically engineered bacteria, the third instar larvae were fed on artificial food treated with genetically engineered bacteria. The level of CYP6B6 protein was determined in transverse sections of the body treated at different time, after fixation, dehydration, embedding, sectioning, dewaxing, incubating with antibody and staining with DAB.

The effect of dsRNA expressed on the larval development

In order to elucidate the effect of the dsRNA containing in the diet on the larval growth, the larva were fed on the artificial food that coated water, HT115 (DE3) bacteria containing L4440 vector and the recombination bacteria which express the dsRNA, respectively, the larva were feeding continuously, and the artificial food, HT115 (DE3) bacteria containing L4440 vector and the recombination bacteria were replaced every 24 h, at the same time we recorded larva mortality and calculated mortality rate daily.

Results

Construction of plasmid expressing dsRNA

A plasmid that expresses dsRNA corresponding to cotton bollworm *CYP6B6*, L4440-dsC1 (377 bp) fragment, and the control L4440-ds*w* (419 bp) were amplified by PCR using cDNA as a template and cloned into the pMD18-simple-T

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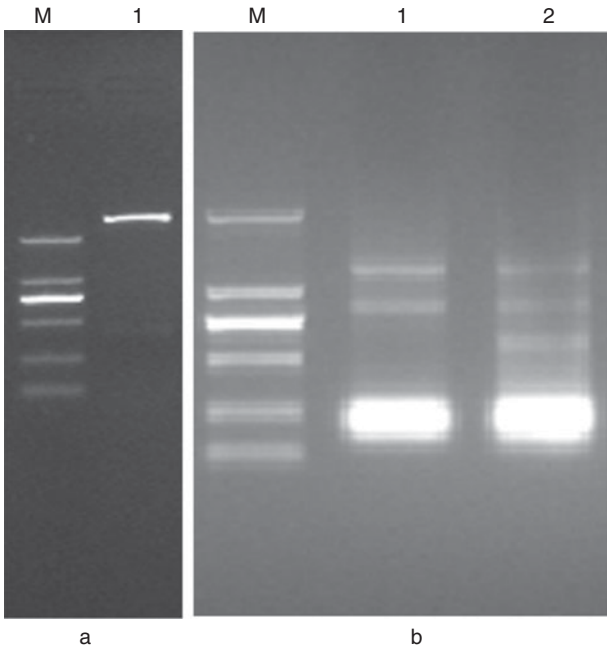

Fig. 1. Identification of recombinant plasmid of L4440-dsC1 (a) and agarose gel electrophoresis of dsRNA produced by induced bacteria strain *E. coil* HT115(DE3) (b). (a) M: DNA marker DL2000 1: L4440-dsC1. (b) M: DNA marker DL2000 1: L4440 vector 2: L4440-dsC1.

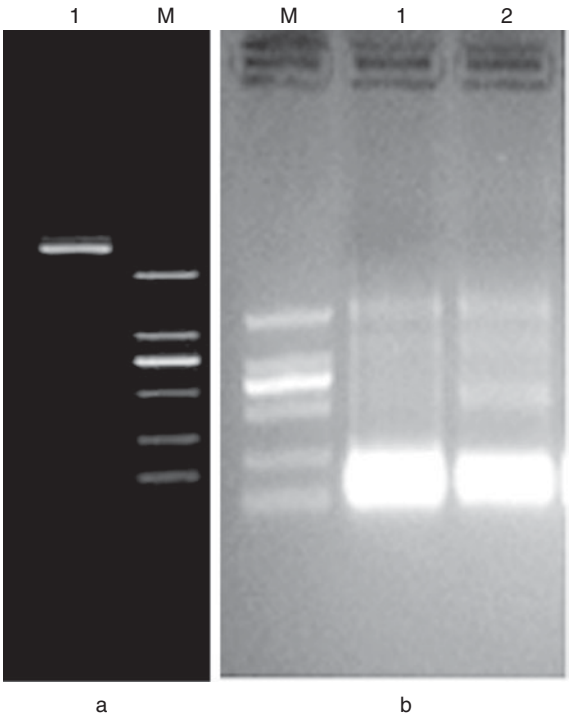

Fig. 2. Identification of recombinant plasmid of L4440-ds*w* and agarose gel electrophoresis of dsRNA produced by induced bacteria strain *E. coil* HT115(DE3). (a) M: DNA marker DL2000 1: L4440-ds*w*: (b) M: DNA marker DL2000 1: L4440 vector 2: L4440-ds*w*.

vector, and confirmed by digestion with *Pst*I and *Bgl*II (figs 1a and 2a). Positive clones were sent for sequencing (Sangon, Shanghai). The L4440-dsC1 and ds*w* digested from pMD18-simple-T were sub-cloned to the L4440 plasmid. The dsRNA were produced by bacteria strain HT115 containing recombinant plasmid L4440-dsC1 and L4440-ds*w* (figs 1b and 2b). Northern-blot analysis showed that the genetically engineered bacteria were able to express corresponding dsRNA, but the bacteria (HT115 and the bacteria containing L4440) were not (fig. 3).

Ingestion and accumulation of bacteria in the gut of H. armigera *larvae*

To confirm that bacterially expressed dsRNA was able to be introduced into the larval gut through ingestion, an experiment was performed with larvae feeding artificial diet coated with bacteria that were colored with FD&C Blue. The colored bacteria were easily detected inside the larvae (fig. 4). After 6 h of feeding on the diet containing colored bacteria, the blue diet was observed in the gut of larvae (fig. 4a); blue color accumulated in the gut 24 h later (fig. 4c). These results demonstrated that bacteria were ingested by larvae and accumulated in the gut.

Ingestion of bacterially expressed dsRNA reduces CYP6B6 *mRNA level*

The expression level of *CYP6B6* was detected by qRT-PCR using the bacterially expressed ds*w* as a negative control. The qRT-PCR results showed that *CYP6B6* mRNA was substantially decreased at 36 h in the larvae that ingested L4440-dsC1,

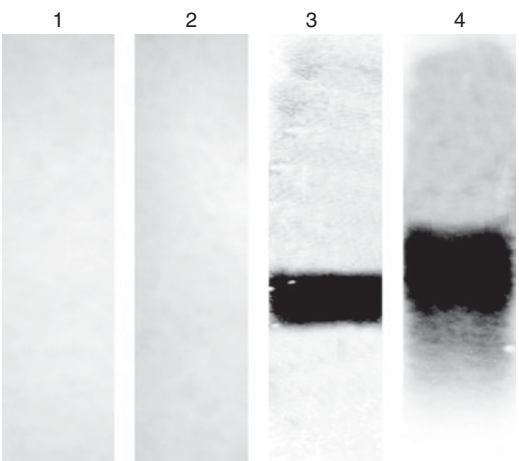

Fig. 3. Northern-blot analysis of dsRNA expression by different recombinant plasmids. Total RNAs (20 µg/sample) from HT115 (DE3) (lane 1), HT115 (DE3) bacteria containing L4440 vector (lane 2), L4440-dsC1 (lane 3) and L4440-ds*w* (lane 4) were electrophoresed on 12% polyacrylamide gel under denaturing conditions, blotted and hybridized with digoxin dsC1 (lane 1, lane 2, and lane 3) and ds*w* (lane 4) DNA oligonucleotide probes at 37°C.

compared with the negative controls (HT115 strain, HT115 bacteria containing L4440 and L4440-ds*w*), the expression level reduced to 32.04% on 36 h, 13.8% on 48 h and 11.8% on

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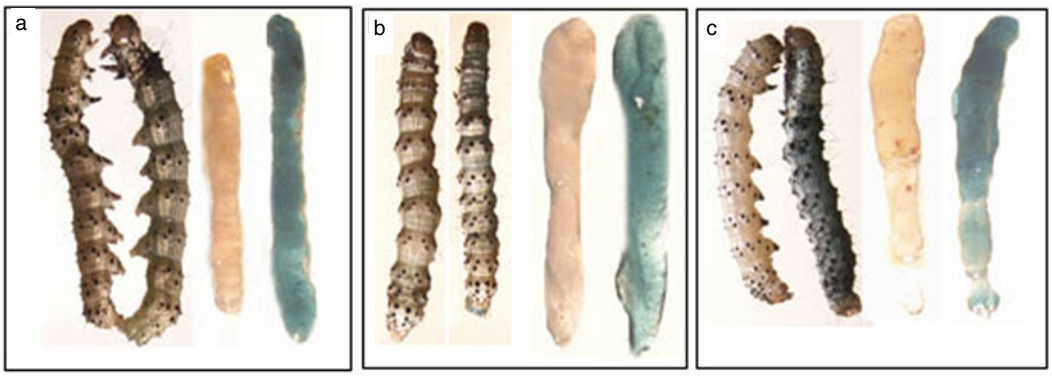

Fig. 4. Analysis of bacteria accumulation in the larvae gut of *H. armigera*. The gut from 3rd instar larvae fed on the diet containing FD&C Blue colored bacteria normal artificial diet at different time. (a) 6 h, (b) 12 h and (c) 24 h.

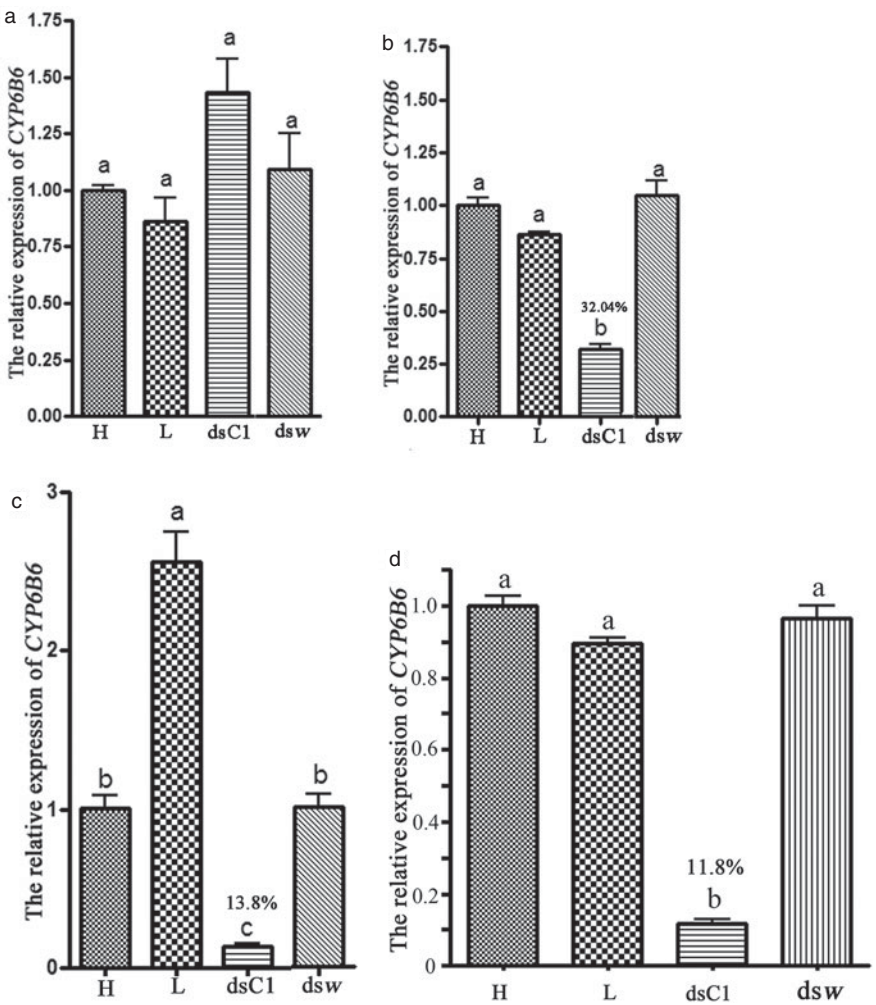

Fig. 5. Effects of ingesting bacterially expressed dsRNA on transcription of *CYP6B6*. The relative expression of CYP6B6 after 3rd instar larvae feeding different bacteria strain at (a) 12 h, (b) 36 h, (c) 48 and (d) 72 h. H indicates HT115 (DE3) strain and L indicates HT115 (DE3) bacteria containing L4440 vector; dsC1 indicates HT115 (DE3) bacteria containing L4440-dsC1; ds*w* indicates HT115 (DE3) bacteria containing L4440-ds*w*. Bars sharing the same letter for each group are not significantly different at $P > 0.05$.

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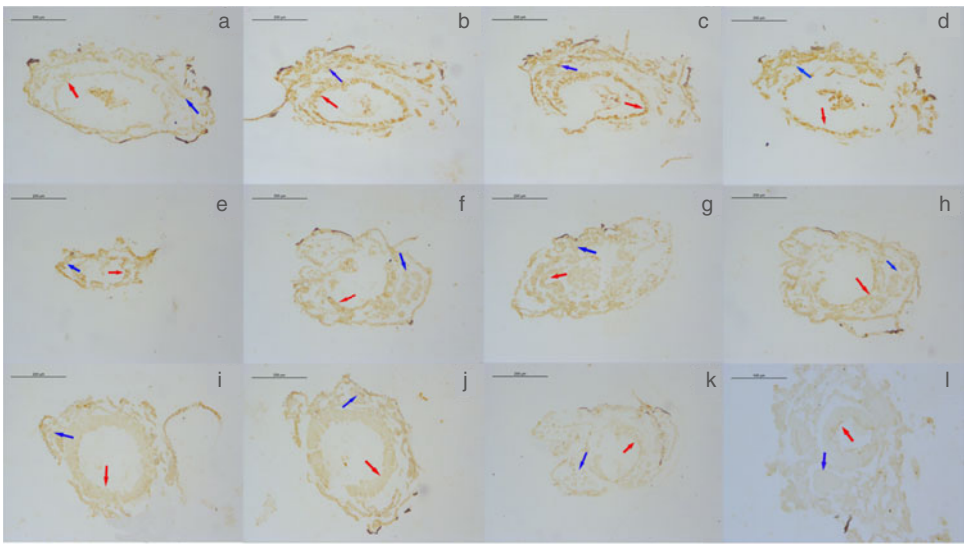

Fig. 6. Effects of ingesting bacterially expressed dsRNA on transcription of CYP6B6 by immunohistochemistry. Larvae feeding HT115 (DE3) strain (a, b, c and d), bacteria containing blank plasmid L4440 (e, f, g and h), and bacteria containing L4440dsC1 (i, g, h and l) at 12 h, 36 h and 48 h and 72 h. (The bar is 200 μm.)

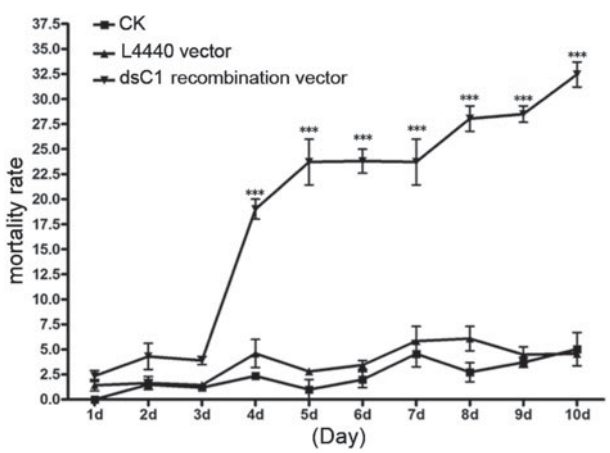

Fig. 7. The mortality rate of the larvae by fed on HT115 (DE3) bacteria containing L4440-dsC1 ($P < 0.0001$).

72 h compared with the negative control of HT115 bacteria strain, and the transcription of *CYP6B6* to minimum after feeding for 72 h, but the transcription level was not significantly affected after feeding for 48 h. These results suggest that the RNAi response induced by ingestion of dsRNA requires an accumulation of dsRNA in the larvae and the gene expression can be suppressed by continuously feeding.

Ingestion of bacterially expressed dsRNA reduces CYP6B6 *protein level*

The expression level of CYP6B6 protein was determined after ingestion of bacterially expressed dsRNA at 12 h, 36 h and 48 h and 72 h, by fixed, dehydrated, embedded, sliced, dewaxed, antibody incubated and colored DAB (fig. 6). The expression of the protein CYP6B6 of midgut (red arrow) and fat body (blue arrow) were obviously reduced after feeding of the dsRNA expressed by L4440-dsC1 strain for 48 h (fig. 6l). But negative groups treated with the bacteria strain HT115 (fig. 6a–d) and HT115 bacteria containing L4440 vector (fig. 6e–h) had not changed.

The effect of bacterially expressed dsRNA on the larval development

The larvae were fed on artificial food that coated water, HT115 bacteria containing L4440 vector and the recombination bacteria which expressed the dsRNA every day, the result showed that with the continuous feeding for 4 days, cotton bollworm larval mortality rate increased significantly, compared with the control group (fig. 7). In the HT115 bacteria containing L4440-dsC1 treatment, average 32.45% mortality was recorded in the group of feeding HT115 bacteria containing L4440-dsC1, which was significantly higher compared with all other treatments (average 5.03% of CK mortality and average 4.59% of HT115 bacteria containing L4440-vector mortality) ($P < 0.0001$).

Discussion

RNAi occurs widely in eukaryotic organisms and previous studies have demonstrated that feeding-based RNAi can specifically induce an RNAi response in several insect species, such as *S. exigua* (Chen *et al.*, 2008), *H. armigera* (Mao *et al.*, 2007) and *Diabrotica virgifera virgifera* (Alves *et al.*, 2010). RNAi using bacteria to deliver dsRNA is an efficient method. This method makes use of bacteria that are deficient in RNaseIII, an enzyme that normally degrades most dsRNAs in the bacterial cell. Bacteria deficient of RNaseIII were engineered to produce high quantities of specific dsRNA segments (Timmons & Fire, 1998). Successful RNA knock-down effects via artificial feeding of dsRNAs have been reported in insects including the Triatomine Bug (*Rhodnius Prolixus*) (Araujo *et al.*, 2006), light brown apple moth (*E. postvittana*) (Turner *et al.*, 2006), tsetse fly (*Glossina morsitans morsitans*) (Walshe *et al.*, 2009).

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Regarding Lepidoptera, successful RNA knock-down effects via artificial feeding of dsRNAs have also been reported, such as diamondback moth (*Plutella xylostella*) (Bautista *et al.*, 2009), beet armyworm (*S. exigua*) (Tian *et al.*, 2009). RNAi will continue to be a useful tool to ascribe functions to the many newly identified genes.

Here, we have demonstrated the feasibility of RNAi via feeding and shown that it can specifically inhibit the transcription level of a target gene in *H. armigera*. Knockdown of the target gene *CYP6B6* could suppress its transcription and enzyme content. Compared with the control the expression of target gene was obviously suppressed after feeding for 36 h, but the CYP6B6 protein expression was obviously decreased at 48 h by immunohistochemistry (fig. 7j–m). CYP6B6 transcript levels dropped gradually and not instantaneously post feeding. This may be due to the lag time required for the dsRNA to enter midgut cells and the dsRNA processing to mediate the full-stoppage after the RNAi process. This may be because the lepidopteran gut is a hostile environment for nucleic acids as it has an alkaline pH (Terra *et al.*, 1996) and presumably contains numerous RNases. Different insect midgut environments may require different concentrations of dsRNA to trigger gene silencing. Initiation of RNAi by dsRNA feeding may therefore require some level of optimization with various concentrations of dsRNA tested. Variation in the extent of silencing over different concentrations of dsRNA has also been observed when dsRNA is injected (Tomoyasu & Denell, 2004). Interestingly, such conditions do not seem to prevent the ability of ingested dsRNA to initiate RNAi. In this instance, the ingested dsRNA must remain sufficiently intact in the insect midgut lumen to enter the midgut cells and initiate silencing of *CYP6B6*. The cytochrome P450-dependent monooxygenase is an important metabolic system because of its involvement in regulating the titers of endogenous compounds such as hormones, fatty acids and steroids. Therefore, if the P450 gene expression of cotton bollworm is suppressed, its growth and development will be effected. Our results also proved that when continuously feeding dsRNA, both the target gene and target protein were inhabited, and the continuous feeding for four days, the cotton bollworm larval mortality rate increased significantly, compared with the control group.

In summary, our results demonstrate that RNAi by feeding dsRNA is possible in the cotton bollworm. It will be interesting to see whether oral delivery of dsRNA is generally applicable across other lepidopteran species. Oral delivery of dsRNA offers the advantage of being a less invasive method of inducing RNAi compared with injection. Such a technique is amenable to high throughput initiation of RNAi, which may enable the analysis of phenotypic changes associated with the silencing of a wide variety of insect genes.

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