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RESEARCH ARTICLE

cDNA cloning and characterization of the carboxylesterase *pxCCE016b* from the diamondback moth, *Plutella xylostella* L.



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Abstract

Carboxylesterase is a multifunctional superfamily and can be found in almost all living organisms. As the metabolic enzymes, carboxylesterases are involved in insecticides resistance in insects for long time. In our previous studies, the enhanced carboxylesterase activities were found in the chlorantraniliprole resistance strain of diamondback moth (DBM). However, the related enzyme gene of chlorantraniliprole resistance has not been clear in this strain. Here, a full-length cDNA of carboxylesterase *pxCCE016b* was cloned and exogenously expressed in *Escherichia coli* at the first time, which contained a 1693 bp open reading frame (ORF) and encoded a protein of 542 amino acids. Sequence analysis showed that this cDNA has a predicted mass of 61.56 kDa and a theoretical isoelectric point value of 5.78. The sequence of deduced amino acid possessed the classical structural features: a type-B carboxylesterase signature 2 (EDCLYLVNYTK), a type-B carboxylesterase serine active site (FGDPENITIFGESAG) and the catalytic triad (Ser186, Glu316, and His444). The real-time quantitative PCR (qPCR) analysis showed that the expression level of the *pxCCE016b* was significantly higher in the chlorantraniliprole resistant strain than in the susceptible strain. Furthermore, *pxCCE016b* was highly expressed in the midgut and epidermis of the DBM larvae. When the 3rd-instar larvae of resistant DBM were exposed to abamectin, alpha-cypermethrin, chlorantraniliprole, spinosad, chlorfenapyr and indoxacarb insecticides, the up-regulated expression of *pxCCE016b* was observed only in the group treated by chlorantraniliprole. In addition, recombinant vector pET-*pxCCE016b* was constructed with the most coding region (1293 bp) and large number of soluble recombinant proteins (less than 48 kDa) were expressed successfully with prokaryotic cell. Western blot analysis showed that it was coded by *pxCCE016b*. All the above findings provide important information for further functional study, although we are uncertainty whether the *pxCCE016b* gene is actually involved in chlorantraniliprole resistance.

Keywords: *Plutella xylostella*, carboxylesterase, chlorantraniliprole, insecticide resistance, *pxCCE016b*

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1. Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.), is one of the most important cruciferous pests in China (Li *et al.* 2016) and around the world (Talekar and Shelton 1993). DBM cost the world economy about 4–5 billion USD per

year in losses (Furlong *et al.* 2013). It is well known that DBM has developed various levels of resistance to all major classes of insecticides used (Zhao *et al.* 2002; Heckel *et al.* 2004; Sayyed and Wright 2006; Whalon *et al.* 2008). As the first commercial insecticide of new anthranilic diamide class, the chlorantraniliprole was applied as an ideal reduced-risk rotation insecticide for insecticide resistance management (IRM) program (Hannig *et al.* 2009; Silva *et al.* 2012). In China, chlorantraniliprole was recommended as an effective rotation insecticide for the control of DBM since 2008. However, DBM developed highly resistance to this insecticide in the field populations in Guangdong Province because of the intensive usage during 3–5 years (Wang *et al.* 2010; Wang and Wu 2012). The similar situation has happened in Philippine Islands (Edralin *et al.* 2011).

In order to delay resistance evolution in the field, it is important to study the relative resistance mechanisms of DBM. The major types of mechanisms, by which DBM become resistant to insecticides, are frequently associated with increased activities of detoxifying enzymes, reduced target site or nerve sensitivity, and decreased cuticular penetration (Eziah *et al.* 2009). The target site mutation resistance has been shown to be involved in resistance to chlorantraniliprole (Trocza *et al.* 2012). In addition, through synergism of enzyme inhibitors experiment, enzyme activities analysis and biochemical response test to insecticide-induced stress, detoxifying enzymes dependent resistance is observed in resistant strains of DBM from China (Wang and Wu *et al.* 2012; Hu *et al.* 2014a). Our previous studies on the transcriptome analysis in DBM also suggested that detoxifying enzymes were the major metabolic factors responsible for chlorantraniliprole resistance (Lin *et al.* 2013). Based on transcriptome analysis, we identified a novel P450 gene: *CYP321E1* (GenBank accession no. KC 626090) from DBM, RNA interference (RNAi) indicated that this gene was related to chlorantraniliprole resistance (Hu *et al.* 2014b). Meanwhile, an up-regulation carboxylesterase gene cDNA fragment: Unigene35058_yong_A, was also obtained from DBM EST database. The tag-based digital gene expression (DGE) analysis gave us an opinion that this gene may also play a role in chlorantraniliprole resistance of DBM (Lin *et al.* 2013).

Esterase is one of the major detoxifying enzymes in insects, insecticide resistance mediated by which has been found in many different insects (Zhang *et al.* 2010). As reported, the mainly molecular basis of this kind of resistance mechanism to some normal used insecticides is gene amplification, up-regulation or main coding sequence mutation (Li *et al.* 2007). Among them, up-regulation of esterase genes has been found in *Myzus persicae*, *Nilaparvata lugens* and *Aphis gossypii* (Small and Hemingway 2000; Bizzaro *et al.* 2005; Cao *et al.* 2008). In DBM, no relative

resistant gene has been cloned and characterized although studies suggested that carboxylesterases may be involved in chlorantraniliprole resistance.

In this paper, a full-length cDNA of *pxCCE016b* from DBM was identified and analyzed. Expression pattern of *pxCCE016b* and exposure experiments to several normal used insecticides were investigated. In addition, recombinant protein of *pxCCE016b* cDNA was expressed in *Escherichia coli* and then Western blot analysis was conducted. Our findings here provide important information for further functional study, although we are uncertainty whether the *pxCCE016b* is actually involved in chlorantraniliprole.

2. Results

2.1. cDNA cloning of *pxCCE016b*

Using the specific primers, the core sequence of *pxCCE016b* about 600 bp was obtained firstly. Based on it, 5'/3' rapid-amplification of cDNA ends (RACE) PCR reactions were performed respectively, and then the full-length cDNA sequence of *pxCCE016b* gene was obtained by overlapping three fragments. This cDNA is 1773 bp long (GenBank accession no. KM008609) with an open reading frame (ORF) of 1693 bp (Fig. 1), encoding 542 amino acids. Analysis of deduced amino acid sequence showed that this cDNA has a molecular weight of 61.56 kDa and a theoretical isoelectric point value of 5.78. But no signal sequence was observed.

2.2. Sequence analysis

Sequence analysis indicated that the translated amino acid of *pxCCE016b* had high homology to type-B carboxylesterases from other lepidopteran insects in the GenBank database (mostly >50%). Among them, *pxCCE016b* shared 53% identity in its deduced amino acid with that of *CCE016b* from *Helicoverpa armigera* (GenBank accession no. ADF43479), but whose function is still unrevealed (Teese *et al.* 2010).

Multi-sequence alignment analysis also showed that the amino acid sequence encoded by the *pxCCE016b* contains characteristic conservative regions of carboxylesterases: a type-B carboxylesterase signature 2 motif (EDCLYLNVTYK), a type-B carboxylesterase serine motif (FGGDPENITIFGE-SAG) and the catalytic triad (Ser186, Glu316, and His444). Furthermore, the common sequence GX SXG at the active site of carboxylesterase serine motif was also observed (Fig. 2).

Here, the phylogenetic tree, generated by 10 amino acid sequences of different insect carboxylesterases, showed that the current gene was grouped with *CCE016* (Fig. 3). Although its overall identity to *CCE016b* is only 53%, they are still considered to the same family for its conserved sequence in salient characteristics region.

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