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Molecular cloning and nucleotide sequence of CYP6BF1 from the diamondback moth, *Plutella xylostella*

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Abstract

A novel cDNA clong encoding a cytochrome P450 was screened from the insecticide-susceptible strain of *Plutella xylostella* (L.) (Lepidoptera:Yponomeutidae). The nucleotide sequence of the clone, designated CYP6BF1, was determined. This is the first full-length sequence of the CYP6 family from *Plutella xylostella* (L.). The cDNA is 1661bp in length and contains an open reading frame from base pairs 26 to 1570, encoding a protein of 514 amino acid residues. It is similar to the other insect P450s in gene family 6, including CYP6AE1 from *Depressaria pastinacella*, (46%). The GenBank accession number is AY971374.

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Introduction
Cytochrome P450 genes form a superfamily and nucleotide sequences of more than 1958 these genes have been registered in the DNA database. The P450 genes are classified into thirty-six gene families based on the comparison of deduced amino acid sequences (Nelson, 2005; Zhou and Huang, 2002). Cytochrome P450s play an important role in metabolism of host plant chemicals, and in degradation of various insecticides such as pyrethroids, organophosphorus compounds, carbamates etc. (Tsukamoto, 1983; Oppenoth, 1985; Scott and Wen, 2001; Andersen et al., 1994).

The effects of cytochrome P450 monoxygenase inhibitors on the toxicity of permethrin in the permethrin-resistant strain of *Culex quinquefasciatus* have been studied, as well as the quantities of the enzymes and the degradation of permethrin by the enzymes in permethrin-susceptible and resistant strains (Kasai et al., 1998; Zhou et al., 2001; Qiu and Leng, 1999). Although there are numerous reports on the occurrence of cytochrome P450s in houseflies (Nelson et al., 1993), only one nucleotide sequence of a cytochrome P450 has been reported from the Diamondback Moth, *Plutella xylostella* (L) (Lepidoptera : Yponomeutidae) (Shen et al., 2003). In order to elucidate the mechanisms of insecticide susceptibility in *P. xylostella*, we cloned and sequenced cytochrome P450 cDNAs. Here we report the nucleotide sequence encoding a cytochrome P450 and its deduced primary structure. This cytochrome P450 belongs to the CYP6 family (Nelson et al., 1993).

Materials and Methods

Biological materials
The insecticide susceptible strain of *P. xylostella* was collected from the Wuhai Vegetable Academy of the P.R. of China in 2004 and cultured without exposure to insecticides. They were reared at 20 ± 10 C, and a photoperiod of 16:8 (L:D).

Preparation of the specific primers
Five whole bodies of the third-instar larvae of the *P. xylostella* were disrupted in TRIzol reagent (Invitrogen, www.invitrogen.com). The total RNA obtained was used for RT-PCR, and construction of cDNA fragments. The first strand cDNA was synthesized with Oligo(dT)18 at 70° C for 5 min in water and for 10 min on ice. It was then mixed with dNTP, Rnase- M-MLV and ddH2O at 42° C for 60 min, 95° C for 5 min. The reproducing system contained the cDNA template obtained above, dNTP, MgCl2, Taq DNAse and the pair of primers. The system was kept 94° C for 1 min, then 30 cycles of RT-PCR (94° C for 30 sec, 45° C for 30 sec, 72° C for 1 min), and was finally kept at 72° C for 5 min.

The nucleotide sequences of synthetic primers were the following:

for the forward primer and

for the reverse primer. The primers were designed as described by Kasai et.al (1998), Danielson and Fogleman (1997), and Liu and Zhang (2002). The resultant DNA fragment of about 250 base pairs (bp) was cloned into pGEM-T Easy Vector (Promega, www.promega.com) and positive clones were sequenced.

The amino acid sequence deduced from the nucleotide sequence showed that it is related to the CYP6 family. The PCR fragment was therefore used as a probe to screen the full-size CYP6 gene.

Full-length amplification of the gene
Using the fragment described above, pairs of the specific primers were designed as follows: 5'-GAGAGATTACAAAGACTACACGTCC-3' for the forward primer and 5'-CCGCCCCCAAGGCCAAATGTAGGTAT-3' for the reverse primer. Using the BD SMART RACE c DNA amplification kit (Clontech, www.clontech.com), 5' and 3'-cloned fragments were obtained. The RT-PCR products were purified directly from bands excised from agarose gels and cloned into pGEM-T Easy Vector (Promega). Positive clones were sequenced.

Gene analysis
Software including megas2, bioedit, and gene-explorer were used to analyze the gene sequences.
Results

The isolation and the characterization of the CYP6BF1

Using the specific primers, four positive clones were obtained, two of which were 3′-clones. By overlaying the cloned sequences a full sequence of the P450 CYP6BF1 gene was obtained. The cDNA is 1661 bp in length, including 25 nucleotides of 5′-untranslated region upstream of the ATG (Fig.1).

This open reading frame codes for a predicted translation product that is 514 amino acids in length. The predicted molecular mass was 59 kDa. The stop codon was found at nucleotide 1570, followed by 91 nucleotides of 3′-untranslated sequence, which includes the 26bp poly(A) tail. A poly(A) addition signal, AAATAAA, was present in a short untranslated region at the 3′ end. This gene

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was named CYP6BF1 (GenBank accession number:AY971374).

**Multiple alignment of members of the insect CYP6 family**

A BLAST search analysis indicated that CYP6BF1 exhibits similarity with other members of the CYP6 family, with amino acid identities of about 46-35% (Table 1). For example, it showed 46% identity to the CYP6AE1 from *Depressaria pastinacella* and 34% identity to the CYP6B8 from *Helicoverpa zea*.

| Genes         | Score | Expected     | Amino acid identity |
|---------------|-------|--------------|---------------------|
| CYP6AE1 D. pastinacella | 481   | 1.00E-134    | 46                  |
| CYP6B7 H. zea | 290   | 9.00E-77     | 33                  |
| CYP6A1 N. lugens | 288   | 3.00E-76     | 32                  |
| CYP6B2 H. zea | 286   | 8.00E-76     | 32                  |
| CYP6B6 H. armigera | 280   | 7.00E-74     | 32                  |
| CYP6A3 P. xylostella | 280   | 7.00E-74     | 32                  |
| CYP6B1 P. glaucus | 277   | 6.00E-73     | 32                  |
| CYP6B2 P. glaucus | 276   | 1.00E-72     | 33                  |
| CYP6A4 P. canadensis | 271   | 3.00E-71     | 31                  |

**Analysis of the dendrogram of cytochrome P450s from the insect CYP6 family**

From the dendrogram, the phylogenetic relationship of the CYP6BF1 to the other members of the insect CYP6 family is clear (Fig.2). CYP6BF1 is related to CYP6B subfamily, and is more distantly related to the CYP9G2, using *Drosophila melanogaster* and *Blattella germanica* as outgroups.

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