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Authors: Li, Hongshan, Dai, Huaguo, and Wei, Hui

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

Hongshan Li[2], Huaguo Dai[1]and Hui Wei[1]

[1]College of Plant protection, Nanjing Agricultural University, Nanjing, 210095, China
[2]Yancheng Academy of Agricultural Sciences, Yancheng, 224002, China

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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Correspondence: lhs158@sohu.com
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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; Oppenooth, 1985; Scott and Wen, 2001; Andersen et al., 1994).

The effects of cytochrome P450 monooxygenase 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 susceptiblity 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 ddH_2O at 42° C for 60 min, 95° C for 5 min. The reproducing system contained the cDNA template obtained above, dNTP, $MgCl_2$, 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:

5'-CGGA(A/G)AC(A/G/C/T)(A/C/T)(C/T)(A/G/C/T)(A/C)G(A/G/C/T)AA(A/G)TA(T/C)CC- 3'

for the forward primer and

5'-CGGG(A/G/C/T)CC(A/G/C/T)(G/T)C(A/G/C/T)CC(A/G)AA(A/G/C/T)GG- 3'

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'-GAGAGATTTACAAAGACTACACGCTCC-3' for the forward primer and 5'-CCGTCCCCAAAGGGCAAGTAGGTAT-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 mega2, bioedit, and gene-explorer were used to analyze the gene sequences.

Fig 1. Nucleotide sequence and deduced amino acid sequence of CYP6 Cdna clone.

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1 GCAGTTGGATATTTCCACCGGTGCC ATG CCG TAC CTC GAT GTC GCT GTA GCT TTA CTA GCT GCC TTC
                           M   P   Y   L   D   V   A   V   A   L   L   A   A   F
68   ATC GCG TTC ACC TTG TGG ACC AAC CGA AGA TGG AAC TAC TGG AAG AAA CAG AAC GTC AAG
      I   A   F   T   L   W   T   N   R   R   W   N   Y   W   K   K   Q   N   V   K
128  TAC TTG ACG CCG ATC CCT TTC CTG GGG AAC GTG GCT GAT GTG ATC TTC CAG AGG GAC ACC
      Y   L   T   P   I   P   F   L   G   N   V   A   D   V   I   F   Q   R   D   T
188  TTC GGA GCC GTG ACG CAA CGG ATC TGC CAG CAG TTC CCC GAT GAA GCT GTG GTC GGC ATG
      F   G   A   V   T   Q   R   I   C   Q   Q   F   P   D   E   A   V   V   G   M
248  TTC TAC TGC AGC AAC CCT GCA GCC CTC GTA CAG TGC CCT GAC ATG CTC AAG ACA GTC ATG
      F   Y   C   S   N   P   A   A   L   V   Q   C   P   D   M   L   K   T   V   M
308  GTC AAG GAC TAC GCC TAC  TGC TCC AGT AAG GAG GTC TCC GTC CAC AGC CAC AAG GAA CCC
      V   K   D   Y   A   Y   C   S   S   K   E   V   S   V   H   S   H   K   E   P
368  ATG ACC AAG AAC ATG TTC TTC ACC TTC GGA GAC AAG TGG AAG CTC ATC CGG CAG AAC CTC
      M   T   K   N   M   F   F   T   F   G   D   K   W   K   L   I   R   Q   N   L
428  ACG CCG GTC TAC ACG TCC GCC AAA ATG AAG AAC ATG TTT CCA CTG GTA CAG GAT TGC TGC
      T   P   V   Y   T   S   A   K   M   K   N   M   F   P   L   V   Q   D   C   C
488  AGA ATA TTC CAG AAG GTT CTC GAT GAT GAG ATA GGA AAG GGC CGG GTG GTG GAA GTG AAG
      R   I   F   Q   K   V   L   D   D   E   I   G   K   G   R   V   V   E   V   K
548  TCT TTG ATA GCT CGG TAT ACT ATG GAC TGT ATA ACT TCG TGT GCA TTC GGC GTC GAC TCT
      S   L   I   A   R   Y   T   M   D   C   I   T   S   C   A   F   G   V   D   S
608  GGC ACG ATG TCG AAG GGC GAG GAA GGG AAC CCT TTC ACA GAA ACA GGT CAC CTT TTA TTT
      G   T   M   S   K   G   E   E   G   N   P   F   T   E   T   G   H   L   L   F
668  GAT GAA AGA CCA ATT GCA GGC GTG AAG AAT GTC CTC AGA TAC GGC TAC CCT TCC TTC TTC
      D   E   R   P   I   A   G   V   K   N   V   L   R   Y   G   Y   P   S   F   F
728  TAC AGC GTG GGA TTG GAG CTC TAT TCC AGC AAA ATT TAC CGT TTC TTC CGA TCT GTT ATA
      Y   S   V   G   L   E   Y   S   S   K   I   Y   R   F   F   R   S   V   I   L
788  CTT GAC GTT ATA AAC AGT CGT AAC GGC GCC AAA TCT TCG AGG AAT GAC ATG GTG GAT CTT
      L   D   V   I   N   S    R   N   G   A   K   S   S   R   N   D   M   V   D   L
848  ATT TCC GAT TGG AAG AAG AAC AAA TAC ATA ACG GGA GAC AGT ATT GAT AAT GGC ATA GAC
      I   S   D   W   K   K   N   K   Y   I   T   G   D   S   I   D   N   G   I   D
908  GGT GGA AAC AAG AAG GTG CGT ATC GAA GTC GAC GAC GAA CTT TTG GTG AGC CAA TGT GTG
      G   G   N   K   K   V   R   I   E   V   D   D   E   L   L   V   S   Q   C   V
968  CTG TTC TTC CAA GCT GGC TTC CAG CCA AGT GCG CTG ACA TCG GCG TAC CTG TTG TAC GAG
      L   F   F   Q   A   G   F   Q   P   S   A   L   T   S   A   Y   L   L   Y   E
1028 TTG GCA AAG AAC CAG GAC ATC CAA GAG AGG GTG TTG GCT GAA GTG GAC GAG TAC TGG AGC
      L   A   K   N   Q   D   I   Q   E   R   V   L   A   E   V   D   E   Y   W   S
1088 ACT CGG GAC GAG GTG CAG ACC GAC TGC GTG ACC GCC CTG CCT TTC CTC GCC CAG TGC ATG
      T   R   D   E   V   Q   T   D   C   V   T   A   L   P   F   L   A   Q   C   M
1148 GAG GAA TCC CTC CGC ATG TAT CCT CCA GTC TCG GTG CTC ATG AGA GAG ATT TAC AAA GAC
      E   E   S   L   R   M   Y   P   P   V   S   V   L   M   R   E   I   Y   K   D
1208 TAC ACG CTA CCG AAT GGT GTG CAT CTA AAG AAG GGG ATG ATG ATA CAT ATT CCT GTT TAT
      Y   T   L   P   N   G   V   H   L   K   K   G   M   M   I   H   I   P   V   Y
1268 CAT TTG CAT CAC AAT CCG AAG TAT TTC CCG GAG CCC GAG GTG TTT CGT CCG GAG CGG TTT
      H   L   H   H   N   P   K   Y   F   P   E   P   E   V   F   R   P   E   R   F
1328 TCT GAA GAA GGA CGG AAA AGT ATT GTC CCG TAT ACC TAC TTG CCC TTT GGG GAC GGG CCG
      S   E   E   G   R   K   S   I   V   P   Y   T   Y   L   P   F   G   D   G   P
1388 AGG ATG TGT ATA GGC TAC CGT TTC GCA AGG CTA GAG ATC TTC TCC AGC CTA GCA GTT CTG
      R   M   C   I   G   Y   R   F   A   R   L   E   I   F   S   S   L   A   V   L
1448 TTG AAG AAA TAC CGA GTG GAG CTG GCC CCC CAC ATG CCG AGG AAG CTG CAG TTC TTG ACC
      L   K   K   Y   R   V   E   L   A   P   H   M   P   R   K   L   Q   F   L   T
1508 ACG TCC CGG GTG CTC ACC AGC ATC CAC GGC ATA CAC CTG CGG CTG GTG GAC AGA GTC AAC
      T   S   R   V   L   T   S   I   H   G   I   H   L   R   L   V   D   R   V   N
1568 TAG AAATATAAATTGCGGATTCAGTAAAATAAAATTAAAAGTAATGCTGAATAAATTGTATAGTCTCTAAAAAAAA
1644 AAAAAAAAAAAAAAAAA
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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, AATAAA, was present in a short untranslated region at the 3' end. This gene

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*.

Table 1. Blast analysis of the insect Cytochrome P450 CYP6 family.

Genes	Score	Expected	Amino acid identity
CYP6AE1 *D. pastinacella*	481	1.00E-134	46
CYP6B8 *H. zea*	298	3.00E-79	34
CYP6B7 *H. armigera*	290	9.00E-77	33
CYP6AY1 *N. lugens*	288	3.00E-76	32
CYP6B27 *H. zea*	286	8.00E-76	32
CYP6B6 *H. armigera*	280	7.00E-74	32
CYP6N3V3 *A. albopictus*	280	7.00E-74	32
CYP6B16 *P.glaucus*	277	6.00E-73	32
CYP6B21 *P.glaucus*	276	1.00E-72	33
CYP6B14 *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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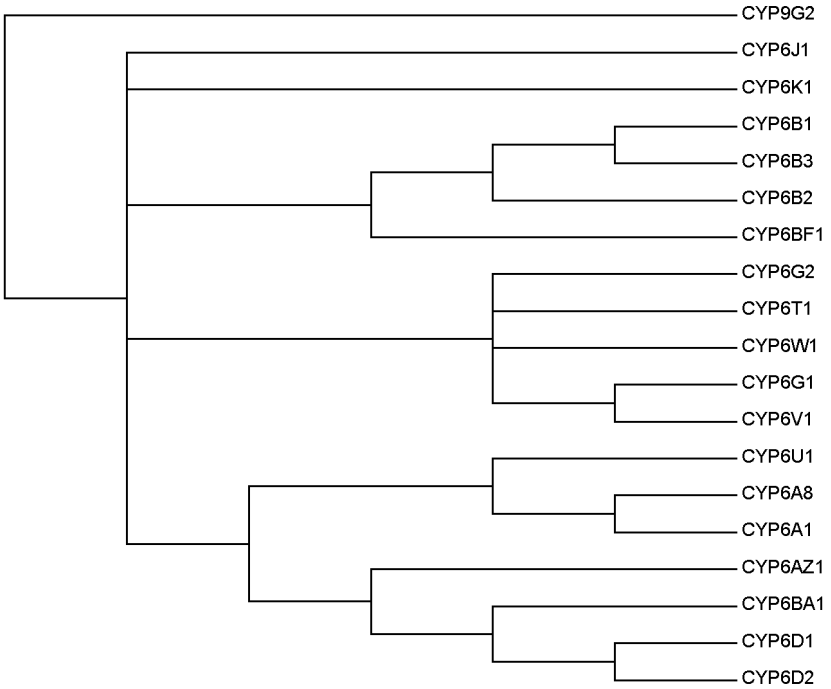
References

Andersen JF, Utermohlen JG, Feyereisen R. 1994. Expression of house fly CYP6A1 and NADPH-cytochrome P450 reductase in *Escherichia coli* and reconstitution of an insecticide metabolizing P450 system. *Biochemistry* 33:2171-2177.

Fig 2. Dendrogram of cytochrome P450s from insect CYP6 family *Plutella xylostella*: CYP9G2, CYP6Pz. *Drosophila melanogaster*: CYP6A8, CYP6D1, CYP6D2, CYP6G2, CYP6G1, CYP6U1, CYP6V1, CYP6W1, CYP6T1. *Blattella germanica*: CYP6J1, CYP6K1. *Papilio glaucus*: CYP6B3. *Helicoverpa armigera* : CYP6B2. *Papilio polyxenes* : CYP6B1. *Musca domestica*: CYP6A1. *Mayetiola destructor*: CYP6AZ1, CYP6BA1.



Danielson PB, Fogleman JC. 1997. Isolation and sequence analysis of cytochrome P450 12B1: the first mitochondrial insect P450 with homology to 1 alpha, 25 dihydroxy-d324-hydroxylase. *Insect Biochemistry and Molecular Biology* 27:595-604.

Kasai S, Shono T, Yamakawa M. 1998. Molecular cloning and nucleotide sequence of a cytochrome P450 Cdna from a pyrethroid-resistant mosquito, *Culex quinquefasciatus Say*. *Insect Moleucular Biology* 7:185-190.

Liu NN, Zhang L. 2002. Identification of two new cytochrome P450 genes and their 5'-flanking regions from the housefly, *Musca domestica*. *Insect Biochemistry and Moleucular Biology* 32:755-764.

Nelson DR. 2005. P450 families and subfamilies http://drnelson.utmem.edu/P450.stats.all.2005.htm

Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsalus IC, Gotoh O, Okuda K, Nebert DW. 1993. The p450 superfamily - update on new sequences, gene-mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA and Cell Biology* 12: 1-51

Oppenooth, F.J., 1985. Biochemistry and genetics of insecticide resistance. In: *Comprehensive insect Physiology*, *Biochemistry and Pharmacology* (Kerkut,G.A. and Gilbert, L.I., eds), Vol. 12, pp.731-774. Pergamon Press, Oxford.

Qiu XH, Leng XF. 1999. Expression regulation of cytochrome P450 genes and the molecular basis of P450 monooxygenase-mdiated insecticide resistance in insect. *Chinese Journal of Pesticide Science*, 1(1):7-14.

Scott JG, Wen ZM. 2001. Cytochrome P450 of insect: the tips of the iceberg. *Pest Management Science* 57:958-967.

Shen BC, Jin ZP, Zhao, DX. 2003.Construction of a full-length cDNA library of the diamondback moth, *Plutella xylostella*. *Zoological Research* 24:215-219.

Tsukamoto, M. 1983. Methods of genetic analysis of insecticide resistance. In: *Pest Resistance to Pesticides* (Georghiou, G.P. and Saito,T.,eds), pp.71-98. Plenum Press, New York.

Xu HX, Li PJ. 2002. Research process of cytochrome P450 in organisms. *Agro-environmental Protection*, 21(2):189-191.

Zhou GL, Huang JL. 2002. Diversity and evolution of CYP6 family in insects. *Entomological Knowledge*, 39(4): 246-251.

Zhou GL, Huang JL, Wu Y. 2001.Molecular cloning and sequence analysis of three new full lengths cDNAs of cyochrome P450 from *Aedes albopictus*. *Entomologia Sinica*?8:141-154.