REFERENCES

1 Lehmann RG, Miller JR, Fontaine DD, Laskowski DA, Hunter JH and Cordes RC, Degradation of a sulfonamide herbicide as a function of soil sorption. *Weed Res* **32**:197– 205 (1992).

2 Malkos EV, Bioremediation of naphthalene in soils of varying textures, nutrient concentrations, and hydrocarbon contents *MS Thesis*, University of Illinois (1996).

3 Mervosh TL, Sims GK, Stoller EW and Ellsworth TR, Clomazone sorption in soil: incubation time, temperature and soil moisture effects. *J Agric Food Chem* **43**:2295– 2300 (1995).

4 Mervosh TL, Sims GK and Stoller EW, Clomazone fate in soil as affected by microbial activity, temperature, and soil moisture. *J Agric Food Chem* **43**:537– 543 (1995).

5 Wolt JD, Smith JK, Sims GK and Duebelbeis DO, Products and kinetics of cloransulam-methyl aerobic soil metabolism. *J Agric Food Chem* **44**:324– 332 (1996).

6 Bichat F, Sims GK and Mulvaney RL, Microbial utilization of heterocyclic nitrogen from atrazine. *Soil Sci Soc Am J* (1998) (in press).

7 Sims GK, Wolt JD and Lehmann RG, Bioavailability of sorbed pesticides and other xenobiotic molecules. *Proc Int Symp on Environ. Aspects of Microbiol*, Uppsala, Swedish Univ-Agric Sci. pp. 159–164 (1992).

Molecular cloning and gene expression of two cytochrome P450s from permethrin-resistant *Culex quinquefasciatus* larvae

Shinji Kasai,[1]† Indira S Weerashinghe,[2] Minoru Yamakawa[3] and Toshio Shono[1]*

[1]*Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan*

[2]*Department of Entomology, Medical Research Institute, Colombo, Sri Lanka*

[3]*Laboratory of Biological Defense, National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305-0851, Japan*

Abstract: As part of a continuing study of factors influencing the development of pesticide resistance in insects, two new cytochrome P450s of the CYP6 family have been identified.

Keywords: mosquito; *Culex quinquefasciatus*; resistance; cytochrome P450 monooxygenase; pyrethroid; permethrin; metabolism; synergist; PBO; CYP6E1

Culex quinquefasciatus Say is important as a vector of filariasis in many tropical countries. The prolonged use of organophosphates and carbamates for the control of these mosquito larvae has resulted in the development of resistance to these classes of pesticides. More recently, the extensive use of photostable pyrethroid insecticides to control agricultural pests and disease vectors has also brought about the development of resistance to this class in mosquitoes as well as many other insects of agricultural and medical importance.

* Correspondence to: Toshio Shono, Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

† Present address: Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY 14853, USA

Contract/grant sponsor: Science and Technology Agency, Japan

Contract/grant sponsor: Ministry of Agriculture, Foresty and Fisheries, Japan; contract/grant number: BMP97-V-1-5-1

(*Received 3 July 1998; accepted 5 January 1999*)

C quinquefasciatus larvae collected from Saudi Arabia (JPal-per) showed a high level of resistance (2 500-fold) to permethrin. In our previous study, a major contribution of P450 monooxygenases in the degradation of pyrethroids in the JPal-per strain was indicated by large differences in the synergistic effects of oxidase inhibitors such as PBO (piperonyl butoxide) and PTPE (2-propynyl 2,3,6-trichlorophenyl ether) on permethrin toxicity between the JPal-per and the susceptible (S) strain.[1] P450 monooxygenases in the microsomes of both larval guts and the remaining body parts metabolized permethrin to 4′-hydroxypermethrin. Furthermore, microsomes from the JPal-per strain were found to have a much greater ability to metabolize permethrin than the S strain, and this activity was inhibited by PBO and PTPE.[1] In order to elucidate the mechanisms of permethrin resistance in *C quinquefasciatus*, we attempted to clone and determine the nucleotide sequences of cytochrome P450 cDNAs.

Cytochrome P450 microsomal monooxygenase-mediated detoxification is a major mechanism by which insects develop resistance to insecticides. Cytochrome P450 genes form a superfamily, and the nucleotide sequences of more than 220 genes have been registered in the DNA data base.[2] These P450 genes are classified into 36 gene families based on the comparison of deduced amino acid sequences.[2] Most of the cytochrome P450s contain a conserved amino acid sequence in the vicinity of the C-terminus, which is involved with a heme-binding region. We designed oligonucleotide primers from this conserved region using sequence data previously reported for CYP6 (cytochrome P450 family 6) isoforms obtained from insecticide-resistant insects including house fly (*Musca domestica* L),[3] cotton bollworm (*Helicoverpa armigera* Hübn),[4] and fruit fly (*Drosophila melanogaster* Meig).[5] A polymerase chain reaction (PCR) was performed using degenerate primers and larval gut cDNA as a template. A cDNA encoding a cytochrome P450 was cloned and found to belong to CYP6 family. We screened a cDNA library constructed from JPal-per larvae using this partial sequence as a probe and determined the complete sequence. This novel P450, designated CYP6E1 was the first reported full-length sequence of a mosquito P450 cDNA.[6]

The deduced amino acid sequence of CYP6E1 was compared to those of cytochrome P450s from other insects (Table 1). CYP6D1 is known to be involved in metabolism of pyrethroid compounds[7] and CYP6A1,[8] CYP6A2[5] and CYP6B2[4] are also cloned from insecticide-resistant insects. The overall homology in amino acid sequence of CYP6E1 to those of CYP6A1, CYP6A2, CYP6B2 and CYP6D1 was 38.9, 35.7, 28.7 and 31.3%, respectively. Percentage divergence showed that CYP6E1 is related to CYP6A and CYP6C subfamilies (Table 1). However, since expression of CYP6E1 was very low and was not found to be very different between permethrin-susceptible and -resistant JPal-per strains, we re-screened the

Table 1. Percentage similarity and divergence of eight cytochrome P450s from various insects[a]

	CYP6A1[b]	*CYP6A2*[b]	*CYP6B1*[b]	*CYP6B2*[b]	*CYP6C1*[b]	*CYP6C2*[b]	*CYP6D1*[b]	*CYP6E1*[b]
CYP6A1	—	46.7	28.1	28.8	33.3	38.9	25.4	38.9
CYP6A2	78.8	—	31.9	31.7	34.5	38.5	27.4	35.7
CYP6B1	136.4	129.0	—	53.0	27.6	27.7	27.7	29.5
CYP6B2	136.8	126.3	68.3	—	27.4	27.4	25.4	28.7
CYP6C1	120.9	119.9	152.4	149.1	—	65.7	27.6	34.1
CYP6C2	104.6	107.7	145.3	146.1	44.2	—	27.7	38.3
CYP6D1	151.3	147.7	147.5	154.5	144.9	135.2	—	31.3
CYP6E1	100.1	111.5	140.5	143.8	116.7	100.7	128.7	—

[a] Figures in upper right and lower left portions of the table indicate percentage similarity and percentage divergence of amino acid sequences, respectively which were calculated using DNA star software (Madison, WI) via Clustal method of alignment.
[b] CYP6A1,[8] CYP6C,[9] CYP6C2[9] and CYP6D1[3] are derived from *Musca domestica*, CYP6A2[5] from *Drosophila melanogaster*, CYP6B1[10] is from *Papilio polyxes* and CYP6B2[4] from *Helicoverpa armigera*.

library and identified another cytochrome P450 cDNA from *C quinquefasciatus* larvae.

The PCR products of *c*250 bp were amplified, and 35 and 29 clones from permethrin-suseceptible and -resistant JPal-per strains, respectively, were sequenced. Alignments of the deduced amino acid sequences from these clones revealed that there were seven distinct sequences. One of these clones, which accounted for more than 57% of the total clones, was used as a probe to re-screen the cDNA library. Another full-length cDNA was cloned and designated CYP6F1. Northern blot analysis revealed that the *CYP6F1* gene in JPal-per strain appeared to be expressed more strongly than that in the S strain suggesting a possible involvement of this gene in resistance to pyrethroids. This result supports our previous finding that the content of cytochrome P450 was about 2.7 times higher in JPal-per strain than in the S strain.[1] Results of Southern blotting indicated that *CYP6F1* genes from susceptible and JPal-per strains contain different *Eco* RI and *Eco* RV digestion sites, suggesting that these two strains have different genome structures.

ACKNOWLEDGEMENTS

This work was supported by Enhancement of Center of Excellence, Special Coordination Funds for Promoting Science and Technology, Science and Technology Agency, Japan, and in part by Grants-in-Aid (Bio Media Program, BMP 97-V-1-5-1) of the Ministry of Agriculture, Forestry and Fisheries, Japan.

REFERENCES

1 Kasai S, Weerashinghe IS and Shono T, monooxygenases are an important mechanism of permethrin resistance in *Culex quinquefasciatus* Say larvae. *Arch Insect Biochem Physiol* **37**:47–56 (1998).
2 Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez PJ, Coon MJ, Gunsalus IC, Gotoh O, Okuda K and Nebert DW, The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. *DNA Cell Biol* **12**:1–51 (1993).
3 Tomita T and Scott JG, cDNA and deduced protein sequence of CYP6D1: the putative gene for a cytochrome P450 responsible for pyrethroid resistance in house fly. *Insect Biochem Molec Biol* **25**:275–283 (1995).
4 Wang X-P and Hobbs AA, Isolation and sequence analysis of a cDNA clone for a pyrethroid-inducible cytochrome P450 from *Helicoverpa armigera*. *Insect Biochem Molec Biol* **25**:1001–1009 (1995).
5 Waters LC, Zelhof AC, Shaw BJ and Ch'ang LY, Possible involvement of the long terminal repeat of transposable element 17.6 in regulating expression of an insecticide resistance-associated P450 gene in *Drosophila*. *Proc Natl Acad Sci USA* **89**:4855–4859 (1992).
6 Kasai S, Shono T and Yamakawa M, Molecular cloning and nucleotide sequence of a cytochrome P450 cDNA from a pyrethroid-resistant mosquito, *Culex quinquefasicatus* Say. *Insect Mol Biol* **7**:185–190 (1998).
7 Wheelock GD and Scott JG, The role of cytochrome $P450_{lpr}$ in deltamethrin metabolism by pyrethroid-resistant and susceptible strains of house flies. *Pestic Biochem Physiol.* **43**:67–77 (1992).
8 Feyereisen R, Koener JF, Farnsworth DE and Nebert DW, Isolation and sequence of cDNA encoding a cytochrome P450 from an insecticide-resistant strain of the house fly, *Musca domestica*. *Proc Natl Acad Sci USA* **86**:1465–1469 (1989).
9 Cohen MB and Feyereisen R, A cluster of cytochrome P450 genes of the CYP6 family in the house fly. *DNA Cell Biol* **14**:73–82 (1995).
10 Cohen MB, Schuler MA and Berenbaum MR, A host-inducible cytochrome P450 from a host-specific caterpillar: molecular cloning and evolution. *Proc Natl Acad Sci USA* **89**:10920–10924 (1992).

Control of clubroot of crucifers by *Phoma glomerata* and its product epoxydon

Tsutomu Arie,*[1] Yumiko Kobayashi,[1] Yoshiki Kono,[2] Okada Gen[1] and Isamu Yamaguchi[1]
[1]*The Institute of Physical and Chemical Research (RIKEN), Hirosawa, Wako, Saitama 351-0198, Japan*
[2]*Ibaraki University, School of Agriculture, Ami, Ibaraki 300-0332, Japan*

Abstract: *Phoma glomerata* strain JCM9972 con-

* Correspondence to: Tsutomu Arie, Institute of Physical and Chemical Research (RIKEN), Hirosawa, Wako, Saitama 351-0198, Japan
Contract/grant sponsor: Science and Technology Agency, Japan
(*Received 3 July 1998; accepted 5 January 1999*)