Cloning of cytochrome P450, *CYP6P5,* and *CYP6AA2* from *Anopheles minimus* resistant to deltamethrin

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ABSTRACT: Two new genes in the cytochrome P450 (*CYP6*) family 6 with complete coding sequences were cloned and sequenced from deltamethrin-resistant *Anopheles minimus*, a major malaria vector in Thailand. *CYP6P5* encodes a protein of 508 amino acids, while *CYP6AA2* contains 505 residues. Each encoded protein contains a hydrophobic N-terminal region and a highly conserved heme-binding region typical of P450s. Alignments of deduced amino acid sequences with other insect P450 genes indicate a high degree of identity to insect *CYP6* genes. Comparative mRNA expression studies using semi-quantitative RT-PCR analysis indicated that the relative amount of CYP6AA2 transcript was greater in the deltamethrin-resistant *An. minimus* compared to the susceptible strain. The expression of CYP6AA2 in deltamethrin-resistant mosquitoes is associated with development of deltamethrin resistance in *An. minimus* mosquito. The CYP6P5 transcript is equally expressed in both resistant and susceptible mosquitoes. ***Journal of Vector Ecology* 28 (2): 150-158. 2003.**

Keyword Index: Cytochrome P450 monooxygenases, cloning, insecticide resistance, deltamethrin, *Anopheles minimus.*

INTRODUCTION

The cytochrome P450 monooxygenases (P450s) or CYPs constitute a family of enzymes involved in the metabolism of a wide variety of endogenous and exogenous compounds such as steroids, fatty acids and xenobiotics (Feyereisen 1999). These divergent enzymes have multiple and overlapping substrate specificities. Examining P450 gene diversity in insects has revealed numerous P450 forms. For example, 17 genes of the *CYP4* family were identified from *Anopheles albimanus* mosquitoes (Scott et al. 1994), 14 P450 genes were identified from the Mediterreanean fruit fly *Ceratitis capitata* (Danielson et al. 1999), and 8 *CYP4* genes from *Helicoverpa armigera* (Pittendrigh et al. 1997). Recently, sequencing of *Drosophila melanogaster* genome has uncovered total P450 diversity in this species, with 90 P450 genes found (Adams et al. 2000).

Insect P450s have been implicated in insect growth, development, reproduction, insecticide resistance and tolerance to plant toxins (Feyereisen 1999, Hodgson and Kulkarni 1983, Scott et al. 1998). The cytochrome P450 enzymes confer insecticide resistance in populations of insects via an increased level of P450 activities resulting from elevated expression of P450 genes. An example is provided by CYP6D1, a P450 isolated from the house fly, *Musca domestica* (Tomita and Scott 1995). CYP6D1 was previously shown to metabolize pyrethroids at a higher level in a Learned Pyrethroid Resistant strain (Wheelock and Scott 1992), leading to increased pyrethoid detoxification and resistance. Consequently, the levels of CYP6D1 transcript and protein are elevated in the pyrethroid resistant flies compared to a susceptible strain (Kasai and Scott 2000, Tomita et al. 1995). In the multi-resistant Rutgers strain of house fly, the CYP6A1 mRNA level was higher than in the susceptible strain (Carino et al. 1994, Feyereisen et al. 1995). Other instances have shown that resistance is associated with increased expression of certain P450 mRNAs. This includes the over-expression of *Cyp6a2* in the RDDT^R insecticide-resistant strain of *Drosophila melanogaster* compared to the Canton^S sensitive strain (Brun et al. 1996), *CYP6B7* in a pyrethroid-resistant strain of *Helicoverpa armigera* (Ranasinghe and Hobbs 1998), *CYP6F1* in pyrethroid resistant *Culex quinquefasciatus* Say (Kasai et al. 2000), *CYP9A1* in a thiodicarb-selected resistant population of *Heliothis virenscens* (Rose et al. 1997), and *CYP4G8* in a pyrethroid-resistant strain of