SPECIES SPECIFIC DNA PROBE FOR IDENTIFICATION OF SPECIES D OF THE ANOPHELES DIRUS COMPLEX

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ABSTRACT

Failure to correctly and quickly differentiate four sibling species of Anopheles dirus, the major malaria vector in Thailand and Southeast Asia, has hindered our understanding of their vectorial capacity. This study developed DNA probe for identification of Anopheles dirus species D among the four cryptic species. Genomic DNA library of Anopheles dirus species D was screened for species specific DNA fragment by the negative hybridization to the DNA of species A,B and C in colony hybridization, Southern blot hybridization and dot blot hybridization techniques. From 7,000 recombinant library, 9 specific clones were obtained. Three of them, namely pMU-D9, pMU-D10 and pMU-D76, contained small specific DNA pieces of 115bp, 289 bp and 124 bp with high repeat of about 1.8×10^4, 3.8×10^3 and 1.8×10^4 times in the mosquito's genome, respectively. These specific sequences should sit very closely in the genome of mosquito.

One clone, pMU-D76, could correctly identify the 43 Anopheles dirus D among the 184 samples of F-1 derived from wild-caught female of Anopheles dirus mosquito by direct and indirect (sandwich) hybridization processes. This probe was available to detect one in a hundred portions of a single male and female mosquito. It was used for identification of Anopheles dirus D at larval stage and in pinned-samples. This DNA probe also offers an alternative means of using non-radioisotope detection system in identification of Anopheles dirus sibling species D.