MOLECULAR CLONING IN E.COLI OF THE DNA FRAGMENTS SPECIFIC FOR ANOPHELES DIRUS C

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ABSTRACT

Molecular cloning in E.coli of An.dirus C DNA was based on a technique called "Deletion Enrichment". An.dirus C DNA was cut with EcoRI* and the DNA fragments of An.dirus C that shared homology to those of A and B species were removed by absorbing with the randomly sheared A and B DNA. The remaining DNA in double-stranded DNA fraction eluted from hydroxyapatite column was cloned into pUN121 at EcoRI site in the CI gene. About two thousands and five hundred recombinants were obtained by DMSO method. Nineteen clones were found to hybridized with An.dirus C DNA by using colony hybridization technique and six clones were used as probes to differentiate An.dirus C from others by Southern blot and dot blot hybridization. Four clones, namely pMUC 10, 11,19 and 20 could differentiate An.dirus C DNA from A,B and D species by Southern blot hybridization. Dot blot hybridization was not suitable because there were cross hybridization to An.dirus A when using pMUC 10, 11 and 20 as probes. There were cross hybridization at different level between An.dirus A,B,C and D when using pMUC 19 probe. The cross hybridization between An.dirus C and A when used pMUC 11 as probe came from the same sequence in the insert but the cross hybridization between An.dirus C and A when used pMUC 20 as probe came from different sequence in the insert. Like pMUC 20, the cross hybridization of An.dirus A,B,C and D when using pMUC 19 probe came from different sequence in the insert that could hybridize with each species. The probe, pMUC 11, was tested

on a limited number of F-1 derived from wild-caught females and it hybridized to the An.dirus C and A materials.