

论著

淡色库蚊细胞色素P450抗性相关基因克隆与初步鉴定

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摘要

[目的]探讨淡色库蚊细胞色素P450与溴氰菊酯抗药性的关系。[方法]采用一对昆虫细胞色素P450简并引物,以反转录聚合酶链反应从淡色库蚊扩增特异片段,T/A直接克隆法筛选阳性克隆,对新序列进行cDNA芯片和逆Northern分析。[结果]从淡色库蚊对溴氰菊酯敏感品系和抗性品系获得112个阳性克隆,其中24个阳性克隆测序后显示为细胞色素P450新序列,由GenBank登录上网;经国际细胞色素P450命名委员会鉴定分别属CYP4家族CYP4C、CYP4D、CYP4H和CYP4J等4个亚家族;24个CYP4cDNA片段中,来自敏感品系的2个片段(NYDS3和NYDS5)和来自抗性品系的4个片段(NYDR6、NYDR9、NYDR15和NYDR17)在两品系间存在差别,cDNA芯片信号亮度值均是抗性品系大于敏感品系,倍数在3.1~9.7范围内;NYDR17仅与抗性探针杂交;逆Northern再鉴定,获得了与cDNA芯片一致的结果。[结论]淡色库蚊CYP4与溴氰菊酯抗性相关;在淡色库蚊溴氰菊酯抗性机理中,可能存在P450基因点突变而导致的特异表达。

关键词 [淡色库蚊](#) [杀虫剂抗性](#) [细胞色素P450](#) [基因克隆](#) [cDNA芯片](#) [逆Northern](#)

分类号

CLONING AND IDENTIFICATION OF CYTOCHROME P450 RESISTANCE RELATED GENES IN THE MOSQUITO, CULEX PIPIENS PALLENS

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Abstract

[Objective] To inquire into the relationship between cytochrome P450 and deltamethrin resistance. [Methods] 24 new cDNA sequences encoding cytochrome P450 were amplified respectively from deltamethrin susceptible and resistant strains of Culex pipiens pallens with a pair of degenerate primers according to the conservative amino acid sequences of CYP4 in insects by RT PCR and the Direct Cloning Method, and then were identified by cDNA chip and reverse Northern. [Results] 112 positive clones were obtained, of which 24 were shown to be new sequences encoded for cytochrome P450. They have been lodged in GenBank and were appraised by the Nomenclature Committee of Cytochrome P450, belonging to the subfamily CYP4C,CYP4D,CYP4H and CYP4J in CYP4 family. The hybrid signal values of 6 P450 sequences (NYDS3, NYDS5, NYDR6, NYDR9, NYDR15 and NYDR17) were 3.1~9.7 times higher in the resistance probe than in the susceptible probe, and NYDR17 only reacted with the resistance probe. The result of reverse Northern in NYDR15 was similar to that of cDNA chip. [Conclusion] CYP4 is related to deltamethrin resistance and the specific expression caused by point mutation of cytochrome P450 gene may exist in deltamethrin resistant Cx.pipiens pallens .

Key words [Cx.pipiens pallens](#) [insecticide resistance](#) [cytochrome P450](#) [gene cloning](#) [cDNA chip](#) [reverse Northern](#)

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