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论著

苏云金杆菌以色列亚种晶体蛋白CryIVD基因的克隆及表达产物的效果测定

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摘要

目的 克隆、表达苏云金杆菌以色列亚种晶体蛋白CryIVD基因并测定其杀蚊毒效。 方法 采用PCR技术 ,扩增得到CryIVD基因片段 ;通过双酶切及连接反应 ,将该基因克隆入大肠杆菌质粒 pUC18构建重组克隆及表达载体 ;转化E .coliDH5α,提取重组质粒进行酶切鉴定及DNA序列测定 ;以IPTG诱导表达CryIVD蛋白 ,SDS PAGE分析表达产物并进行杀蚊毒效测定。 结果 CryIVD基因成功克隆并在宿主菌中正确表达 ,表达产物对蚊幼的杀灭毒效测定显示其对淡色库蚊Ⅱ～Ⅲ龄健康幼虫的LC50 为 2 .3 8× 10 6 cells ml,对白纹伊蚊健康幼虫的LC50 为 1.6× 10 7cells ml。 结论 CryIVD基因被成功克隆和表达 ,且其表达产物有明显杀蚊幼活性。

关键词 苏云金杆菌以色列亚种 CryIVD基因 PCR 基因表达 蚊虫

分类号

Cloning and Expression of the CryIVD Gene of Bacillus thuringiensis subsp. israelensis and its Mosquito Larvicidal Activity

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Abstract

Objective To clone and express Bacillus thuringiensis subsp. isrealensis(B.t.i.) crystal protein CryIVD gene and determine its mosquito larvicidal activity. Methods The gene encoding CryIVD (2.0 kb or so) was amplified by PCR, the amplified fragment was inserted into E.coli plasmid pUC18 to construct the recombinant cloning and expression vector pUC18 CryIVD, which was named pUC18 1. The ligation was transformed into competent E.coli DH 5α and the recombinant vector pUC18 1 was confirmed by restriction enzyme digestion and DNA sequencing. After being inducted by IPTG, the expression of CryIVD gene in positive clone was detected by SDS PAGE and the mosquito larvicidal activity of CryIVD was also determined by standard bioassay. Results The results showed that the CryIVD gene was successfully cloned and expressed in E.coli DH 5α . Mosquito larvicidal activity of engineered E.coli (LC 50) to Cx.pipiens pallens and Ae.albopictus Ⅱ-Ⅲ instar larvae was 2.38×10 6 cells/ml and 1.6×10 7cells/ml respectively. Conclusion The CryIVD gene was successfully cloned and expressed, and a high mosquito larvicidal activity was observed.

Key words Bacillus thuringiensis subsp. israelensis CryIVD gene PCR gene expression mosquito

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