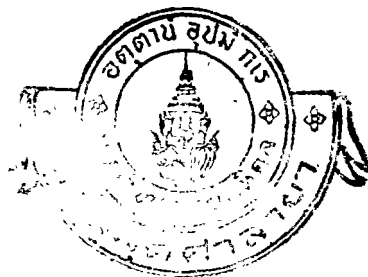


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CLONING OF PLASMIDS

FROM BACILLUS THURINGIENSIS VAR. ISRAELENSIS

IN ESCHERICHIA COLI

BY

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ABSTRACT

The recent emerging larvicidal agent Bacillus thuringiensis var. israelensis (B.t.i.) has been extensively studied throughout, due to its, potent larvicidal activity against the mosquito larvae, There are at least four different-size plasmids in the B.t.i. The two smaller plasmids which were 5.4 and 6.6 kb in size were individually cloned into pBR 322. The recombinant plasmids were named pBR 322 :: pBti 4, and pBR 322 :: pBti 3, respectively. The 5.4 Kb plasmid was found to contain single sites for Bam HI and Kpn I ; more than one sites of the EcoRI, Msp I, and Pvu II ; and lacked the following restriction enzyme sites, Pst I, Sac II, Sma I, and Xba I. The 6.6 Kb plasmid had the single sites of the Bam HI, FnuD II, Pst I, Pvu II, Sau 961, and Sma I ; many sites for Acc I, Hind III, Hpa II, Rsa I, and Msp II ; but no site for Ava II, EcoRV, Sall, and XhoI.

The preliminary study showed that the 6.6 plasmid was related to the toxin production, However, the evidence obtained by insect toxicity assays and typical double immunodiffusion experiment revealed that the extract of E. coli harbouring the recombinant plasmid, pBR 322 :: pBti3 did not contain the biological active toxic protein at a detectable level.