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Mini-Tn10 transposon mutagenesis to explore bacteriocin antibiotic coding genes and corresponding conjugative plasmid in *Bacillus thuringiensis*

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Bacillus thuringiensis strain BUPM4 is known for its ability to produce a bacteriocin, called Bacthuricin F4 (BF4), which inhibits the growth of several Gram-positive bacteria and particularly Bacillaceae. This study aimed to use the insertional transposon mutagenesis approach for disrupting and thus identifying genes associated with BF4 synthesis. Here, the mini-Tn10 transposon was used to generate a library of B. *thuringiensis mutants*. Twenty thousand clones were screened for the search of mutants with affected bacteriocin synthesis. By molecular hybridization, it was demonstrated that the mini-Tn10 transposition occurred in different sites. Clone MB1, containing a mini-Tn10 single-copy insertion, lost the BF4 synthesis, but maintained its immunity to BF4. The flanking sequences surrounding the mini-Tn10 insertion were cloned and sequenced. The bacteriocinogenic plasmid pIBF4 from Bacillus thuringiensis responsible of Bacthuricin F4 synthesis was isolated and characterized. It has a molecular weight of 19.1kb. Ninety-five percent of cells retained the pIBF4 plasmid after 200 generations, demonstrating its high stability. pIBF4 was successfully transferred to Bacillus thuringiensis HD1CryB strain with a transfer frequency of 10-8 transconjugants per donor cell. The study of the recipient host range revealed that pIBF4 is specifically transferable to Bacillus thuringiensis strains with variable transfer frequencies depending on the recipient host strain.

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