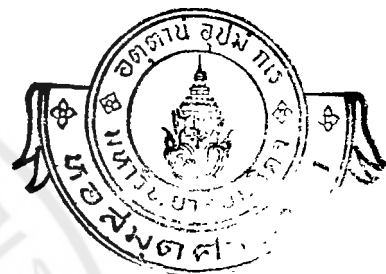


TRANSFER OF PLASMIDS IN BACILLUS THURINGIENSIS
SUBSP. ISRAELENSIS

BY

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SUMMARY

The two selective markers plasmid was constructed, designated pCT 20, by ligation of plasmid pC 194 and plasmid pTp 5. The hybrid plasmid was transformed into B. subtilis where it stably maintained and expressed both chloramphenicol resistance and tetracycline resistance phenotype simultaneously. This plasmid might be very useful in future experiment on genetic manipulation in B.ti.

The transformation of B. thuringiensis subsp. israelensis (B.ti.) with various plasmid vectors including pCT 20 was carried out by using either protoplast or competent cell transformation but successful result could not be obtained. A lysozyme sensitive mutant i.e. B. thuringiensis O 016, was isolated and could be shown to be effectively transformed with plasmid pC 194 and pHV 33 using protoplast transformation technique.

The plasmid pC 194 from B. thuringiensis strain O 016-194 could be transferred to B.ti. by the new method of "conjugation-like" process. The plasmid pBC 16 from B. cereus could also be transferred to B.ti. with high frequency by using conjugation like process. Additionally, both plasmids, pC 194 and pBC 16, could be transferred between strains of B.ti. which resulted in B.ti. transipient strain that harbored and expressed both plasmids.

The kinetics of plasmid transfer between B. cereus and B.ti. showed that the maximal number of transipient could be obtained after 18 h of mating. Results obtained in this study on the development of appropriate plasmid vector together with the conjugation-like process of gene transfer in B.ti. could lead to future development of effective gene transfer system in B. thuringiensis subsp. israelensis.