Intraspecific and Interspecific Plasmid Transfer in *Bacillus thuringiensis* subsp. *alesti* by the Mating System¹

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Bacillus thuringiensis has great economic importance and interest for microbial control due to the production of the delta-endotoxin (parasporal inclusion, crystal). The crystal produced during sporulation is insecticidal to a wide range of lepidopterans as well as some dipterans and coleopterans. It was revealed that the certain *B. thuringiensis* plasmids carrying the crystal protein genes were transferred by mixed culture not only into *B. thuringiensis* strains (GONZÁLEZ et al., 1982; GONZÁLEZ and CARLTON, 1984) but also into *Bacillus anthracis* and *Bacillus cereus* (BATTISTI et al., 1985; REDDY et al., 1987). The mating system is expected to expand the insect host range of *B. thuringiensis* although the mechanism of the plasmid transfer is not fully analyzed. In this paper, we describe the intraspecific and interspecific transfer of plasmid(s) of the type strain of *B. thuringiensis* subsp. *alesti*.

The mating system reported by BATTISTI et al. (1985) was used in this study. The crystalliferous type strain of subsp. *alesti* which is sensitive to streptomycin was used as a donor strain. In the intraspecific plasmid transfer, two isolates (JR-02S and JR-03S) of streptomycin resistant mutants obtained by N-methyl-N'-nitro-N-nitrosoguanidine (NTG) treatment of an acrySTALLIFEROUS strain derived from the type strain of subsp. *alesti* were used as recipient strains. The growth rate of the strain JR-02S was faster than that of the strain JR-03S (Fig. 1). A streptomycin resistant mutant obtained by NTG treatment of *B. subtilis* strain Y-12S was used as a recipient strain in the interspecific plasmid transfer. Plasmid transferents appeared on LB agar plate containing streptomycin (100 µg/ml) were examined by microscopic observa-

Fig. 1. Growth curve of the type strain of *B. thuringiensis* subsp. *alesti* (○), acrySTALLIFEROUS strains JR-02S (■) and JR-03S (▲).

tion and plasmid analysis. The extraction of plasmids was carried out by the method of BATTISTI et al. (1985) and plasmid profiles were analyzed by the 0.7% agarose gel electrophoresis.

GONZÁLEZ et al. (1981) reported that the large molecule of plasmid (105 Mdaltan in size) in *B. thuringiensis* strain HD-4 belonging to subsp. *alesti* was eliminated in its acrySTALLIFEROUS strains. In contrast, our experiment revealed that the largest plasmid molecule pQU1 (115 Mdaltan in size) in the type strain of subsp. *alesti* was eliminated, but the 105 Mdaltan plasmid was maintained in the acrySTALLIFEROUS strains JR-02S and JR-03S which showed the same plasmid profiles (Fig. 2).

In the intraspecific mating experiment, it was observed that acrySTALLIFEROUS strains were converted into the crystalliferous strain at the frequency of $9-32 \times 10^{-2}$ (Table 1), whereas the frequency of the spontaneous mutation of streptomycin resistance in the crystalliferous type strain was less than 1.0×10^{-8} . Furthermore, it was revealed that all crystalliferous recipient strains possessed pQU1 which was eliminated in acrySTALLIFEROUS recipient strains (Fig. 2). These results indicated that pQU1 was a transmissible plasmid and carried the crystal

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Fig. 2. Plasmid analysis of *B. thuringiensis* subsp. *alesti* transferents. (1) Crystalliferous type strain; (2) acrySTALLIFEROUS strain JR-03S; (3) and (4) crystalliferous transferents obtained by the mating system. Arrow shows pQU1 and white circle shows 105 Mdaltan plasmid.

Table 1. Frequency of crystal-forming transferents by the mating system

Recipient strains	Number of crystal-forming colonies/colonies of recipients examined
JR-02S	8/85
JR-03S	35/110

protein gene in the type strain of subsp. *alesti*.

Two acrySTALLIFEROUS mutants showing different growth curves were used as recipient strains in the mating experiment. It was demonstrated that the frequency of the crystal production in the strain JR-03S showing the slow growth rate was about three times higher than that of the strain JR-02S after mating (Table 1). The results suggested that the plasmid transfer correlated with the growth rate of recipient cells.

The interspecific plasmid transfer by mating was reported from the strain YAL UM1 belonging to subsp. *alesti* into *B. cereus* and *B. anthracis* without

Fig. 3. Plasmid patterns of the type strain of subsp. *alesti* (A) and the transferent of *B. subtilis* strain Y-12S (B).

any modification (REDDY et al., 1987). In the present study, the plasmid transfer of the type strain of *alesti* was also attempted by using a plasmid-free *B. subtilis* strain Y-12S derived from the strain Marburg 168 as the recipient strain.

No crystalliferous recipient was observed among 114 recipients examined after mating. Three demonstrating the same plasmid profile (Fig. 3) showed the same requirements of leucine and arginine as *B. subtilis* strain Y-12S and did not possess any flagellar antigen of *B. thuringiensis*. The newly acquired plasmids in the recipients were possibly transferred from the donor strain although plasmids in recipients did not correspond to any plasmid molecules in the donor strain. These plasmids were not stable and eliminated spontaneously by the subculturing of transferents.

The modified plasmid molecule in the recipient strain was reported in the intraspecific mating between *B. thuringiensis* subsp. *thuringiensis* and subsp. *kenyae* (FISCHER et al., 1984). In the present study, it was found that a plasmid in the type strain of subsp. *alesti* transferred without modification in the intraspecific mating, but the modification and/or the elimination of plasmids occurred when plasmid(s) transferred interspecifically into *B. subtilis*.

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