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Plasmid Transfer between Strains of *Bacillus thuringiensis* Infecting *Galleria mellonella* and *Spodoptera littoralis*

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To determine the possibility of plasmid transfer occurring between strains of *Bacillus thuringiensis* in infected lepidopterous larvae, *Galleria mellonella* and *Spodoptera littoralis* were infected with two or more strains of *B. thuringiensis* and the resulting bacteria from the dead insects were examined for plasmid transfer. Transfer rates of plasmids coding for crystal production and tetracycline resistance were high, reaching levels similar to those obtained in laboratory broth cultures. Transfer was higher in *G. mellonella* than *S. littoralis*, probably due to the greater ability of *B. thuringiensis* to colonize the larvae. In broth cultures, *B. thuringiensis* was also able to transfer plasmids into sporeforming bacteria present in soil samples. The results suggest that plasmid transfer between strains of *B. thuringiensis* occurs in nature, resulting in the production of new combinations of delta-endotoxins within populations of the bacteria.

The entomopathogen *Bacillus thuringiensis* is of special interest owing to its ability to produce at sporulation a delta-endotoxin protein crystal toxic to lepidopteran, dipteran, or coleopteran larvae (6, 7, 18). Strains of *B. thuringiensis* form the basis for a number of commercial microbial insecticides used to control insect pests worldwide.

Numerous studies have shown that *B. thuringiensis* contains a complex array of plasmids (14, 20) and that large plasmids in many strains contain genes coding for toxin production (10, 17, 19). It has been shown in *B. thuringiensis* and the closely related species *Bacillus cereus* that, when grown in mixed culture, plasmids are transferred between strains at high frequencies by a conjugationlike process (8). Plasmids from *B. thuringiensis* strains have also been transferred into *Bacillus subtilis* (16) and *Bacillus anthracis* (2). *B. thuringiensis* is widely distributed in nature, often found infecting insect larvae and commonly found in soil (24, 26, 28). With the release of plasmid recombinant strains (A. Bartlett, P. Jarrett, and H. D. Burges, Abstr. 20th Annu. Meet. Soc. Invertebr. Pathol. 1987, p. 73) and the potential release of genetically engineered strains of *B. thuringiensis*, experiments were performed to determine whether plasmid transfer could occur under conditions encountered by the bacteria in nature. To simulate such conditions, we infected larvae with two or more strains of *B. thuringiensis*, and the resulting bacteria isolated from the dead larvae were examined for plasmid transfer. Results showed that *B. thuringiensis* transferred plasmids in infected larvae at frequencies similar to those obtained in laboratory broth cultures. Plasmids could also be transferred to soil bacteria.

MATERIALS AND METHODS

Bacterial strains, mutants, and plasmids. *B. thuringiensis* subsp. *kurstaki* HD1, *B. thuringiensis* subsp. *thuringiensis* HD2, *B. thuringiensis* subsp. *aizawai* HD137, and *B. thuringiensis* subsp. *galleriae* HD240 were kindly supplied by H. T. Dulmage (Cotton Insects Research Laboratory, U.S. Department of Agriculture, Brownsville, Tex.). An acrystalliferous derivative of HD1 was isolated as described previously (15) and induced to streptomycin resistance (Str^r) (200 μg/ml) by repeated growth on increasing concentrations of streptomycin. Asporogenous mutants of HD240 were produced after treatment of spores with ethyl methanesulfonate (13).

Plasmid isolation and transformation. The tetracycline resistance (Tet^r) plasmid pBC16 (4) was isolated by the method of Gryczan et al. (11) and further purified by cesium chloride gradient centrifugation (23).

pBC16 was transformed into HD137, HD240, and HD240 Spo^- by the method of Heierson et al. (12). Plasmid analysis of *B. thuringiensis* strains and recombinants was performed as described by Jarrett (15). DNA preparations were analyzed on vertical 0.6% agarose gels (14 by 14 by 0.3 cm). Sodium dodecyl sulfate was added to the electrophoresis buffer to a final concentration of 0.1% (wt/vol), as this was found to improve resolution of the DNA bands. Samples were electrophoresed for 1 h at 5 mA followed by 3.5 h at 25 mA. Plasmid size was determined by comparison with the previously characterized *B. thuringiensis* HD2 (8).

Plasmid transfer between strains of *B. thuringiensis* and analysis of recombinants. The method of plasmid transfer in broth cultures was similar to that described by Gonzalez et al. (8). Crystal-producing donor strains HD137 Tet^r and HD240 Tet^r containing pBC16 and the acrystalliferous, streptomycin-resistant HD1 recipient, HD1 Str^r Cry^-, were grown separately at 25 and 30°C without shaking for 18 h in 5 ml of brain heart infusion broth (Oxoid, Ltd., London, England) in 50-ml flasks. Donor and recipient cultures (100 μl) were then added to 5 ml of fresh broth and incubated at 40 rpm on an orbital shaker at 25 and 30°C. Each of the strains was incubated separately as a control. After 24 h, cultures were diluted and spread onto nutrient agar plates (Oxoid) alone or containing streptomycin (50 μg/ml), tetracycline (25 μg/ml), or both. Plates were incubated for 48 h at 25°C before counting. Colonies were examined for crystal

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TABLE 1. Transfer of pBC16 and crystal-coding plasmids between strains of *B. thuringiensis* in broth cultures

Donor and recipient strains	Colony counts/ml					
	Total	No. Str^r	% Str^r producing crystals[a]	No. Tet^r	No. Tet^r + Str^r	% Str^r + Tet^r producing crystals[a]
25°C						
HD240 Tet^r	5×10^8	<2		3.6×10^8	<2	
HD240 Tet^r + HD1 Str^r Cry^-	6.5×10^8	3.9×10^8	59.4	ND[b]	1.3×10^5	90.8
HD137 Tet^r	4.2×10^8	<2		2.8×10^8	<2	
HD137 Tet^r + HD1 Str^r Cry^-	5.6×10^8	4.4×10^8	4.0	ND	4.1×10^5	45.0
HD1 Str^r Cry^-	1.2×10^9	1.4×10^9		<2	<2	
30°C						
HD240 Tet^r	ND	<2		ND	<2	
HD240 Tet^r + HD1 Str^r Cry^-	6.7×10^8	4.2×10^8	45.0	1.4×10^8	3.9×10^4	85.4
HD137 Tet^r	ND	<2		ND	<2	
HD137 Tet^r + HD1 Str^r Cry^-	1.1×10^9	6.7×10^8	6.25	2.3×10^8	3.3×10^6	38.0
HD1 Str^r Cry^-	ND	ND		<2	<2	

[a] A minimum of 50 colonies were examined per experiment.
[b] ND, Not determined.

production by phase-contrast microscopy on a minimum of 50 colonies per experiment.

Plasmid transfer from *B. thuringiensis* into sporeforming soil bacteria. Soil samples (0.5 g) were added to 4.5 ml of sterile preheated water, vortexed, and incubated at 65°C for 15 min to kill vegetative cells. A 100-μl sample of the heated soil suspension and a loopful of the HD240 Tet^r Spo^- asporogenous donor containing pBC16 were grown separately without shaking for 18 h in 5 ml of nutrient sporulation medium (21) at 25°C. A 100-μl sample of the donor and 100 μl of each soil culture were inoculated in 5 ml of fresh broth and incubated on an orbital shaker at 40 rpm for 24 h and then at 200 rpm for a further 48 h to allow sporulation. Donor and recipients were grown individually as controls. Cultures were heated at 65°C for 20 minutes to kill remaining vegetative cells and plated onto nutrient agar with or without tetracycline (25 μg/ml). Plates were incubated at 25°C for 48 h before counting. Colonies growing on tetracycline plates were examined microscopically for crystal production.

Infection of insects. Donors HD137 Tet^r and HD240 Tet^r were grown on nutrient agar plates containing 25 μg of tetracycline per ml for 48 h at 30°C. HD1 Str^r Cry^- was grown as above without tetracycline in the medium. Cultures were removed from the surface of the plates and washed twice in cold deionized water by centrifugation and stored in 15% glycerol at −70°C. Viable spore counts were made by pour plating on nutrient agar.

For *Galleria mellonella*, bacteria were added to an artificial diet that contained no antibiotics as described by Burges et al. (5) to a final concentration of 10^8 donor and recipient spores per g of diet. Fourteen-day-old larvae were added to the treated food and held singly in glass tubes at 30°C for 5 days. For infection of *Spodoptera littoralis*, larvae fed on cotton leaves for 8 days at 25°C were placed onto fresh cotton leaves treated with bacterial suspensions in 0.1% Triton X-100 in deionized water at the rate of 5×10^6 donor and recipient spores per cm^2. Larvae were kept in 5-cm-diameter petri dishes for 6 days on treated food at 25°C. To enumerate the *B. thuringiensis* spores in the dead insects from each of the treatments, larvae were suspended in 1 ml of sterile deionized water and homogenized in a glass tissue grinder and the suspensions were heated at 65°C for 15 min. Samples were appropriately diluted and plated onto nutrient agar alone or containing streptomycin (50 μg/ml), tetracycline (25 μg/ml), or both. Plates were incubated for 48 h at 25°C before counting and microscopic examination.

RESULTS

Transfer of plasmids in broth cultures. When donor HD137 Tet^r or HD240 Tet^r was grown with recipient HD1 Str^r Cry^-, the previously acrystalliferous mutant gained the ability to produce crystals at frequencies of between 4 and 60% (Table 1). Similar rates of plasmid transfer were observed when bacteria were grown at either 25 or 30°C. Plasmid analysis of the Cry^+ transcipients showed that acquisition of a 50-megadalton (MDa) plasmid from HD240 Tet^r and a 45-MDa plasmid from HD137 Tet^r resulted in crystal production (Fig. 1 and 2). In addition, many of the Cry^+ transcipients also contained a number of other smaller plasmids from the donors. Of 20 Cry^+ transcipient colonies from matings with HD240 Tet^r, all contained a plasmid of 5.4 MDa and 6 contained a linear 11-MDa element of DNA. Transfer from HD137 Tet^r of an 11.0-MDa linear element of DNA and plasmids of 4.0 and 8.0 MDa was associated with transfer of the Cry^+ plasmid, although in 5 of the 10 Cry^+ transcipient colonies examined, the 45-MDa plasmid was transferred alone.

All Tet^r transcipients were found to contain a plasmid of the same mobility as pBC16 (Fig. 1 and 2). Transfer frequency of Tet^r was lower than that for Cry^+ (Table 1). Transfer of pBC16 was always accompanied by transfer of other plasmids from the donor strains when plasmid profiles were analyzed—the 5.4-MDa plasmid from HD240 Tet^r and either the 4.0- or 45-MDa plasmid from HD137 Tet^r (Fig. 1 and 2). A total of 38 to 91% of the Tet^r transcipients also produced crystals and contained one of the large plasmids coding for Cry^+.

Transfer of streptomycin resistance from the recipient to the two donor strains was not observed. From 92 transcipient Str^r colonies that were Cry^+, Tet^r, or both, all had HD1 Str^r plasmid profiles plus a number of transmissible plasmids from the donor strains. These results, plus the high stability of streptomycin resistance, indicate that it is chromosomally located in HD1 Str^r Cry^- and would therefore be transmissible at lower levels if at all.

Transfer of plasmids in infected *G. mellonella* larvae. Microscopic examination of larvae killed by *B. thuringiensis*

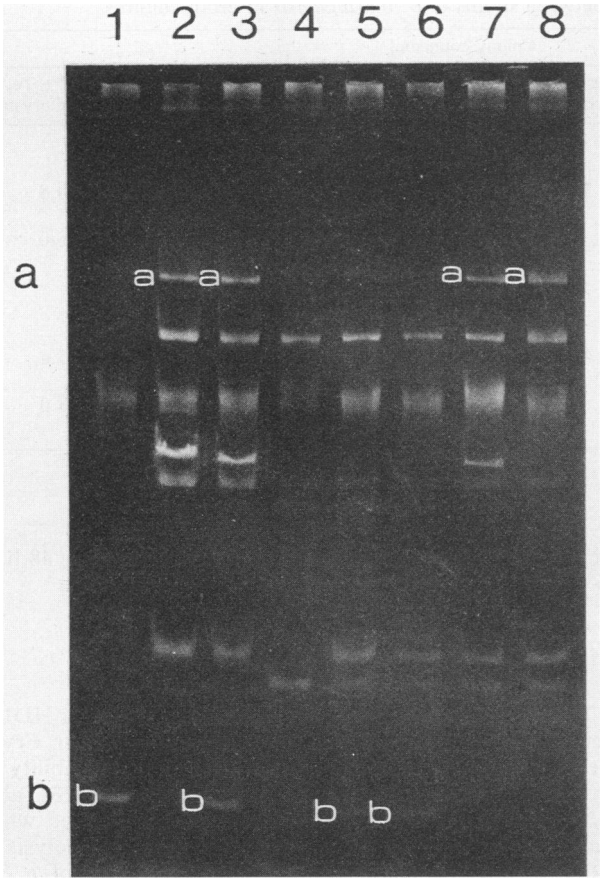

FIG. 1. Plasmid profiles of donor, recipient, and transcipient strains in experiments with donor HD240 Tet^r. Plasmid bands: a, crystal-coding plasmid from donor; b, pBC16. Lanes: 1, pBC16; 2, HD240; 3, HD240 Tet^r donor; 4, HD1 Str^r Cry$^-$ recipient; 5, HD1 Str^r Cry$^-$ Tet^r transcipient; 6, HD1 Str^r Cry$^-$ Tet^r transcipient; 7, HD1 Str^r Cry$^+$ transcipient; 8, HD1 Str^r Cry$^+$ transcipient.

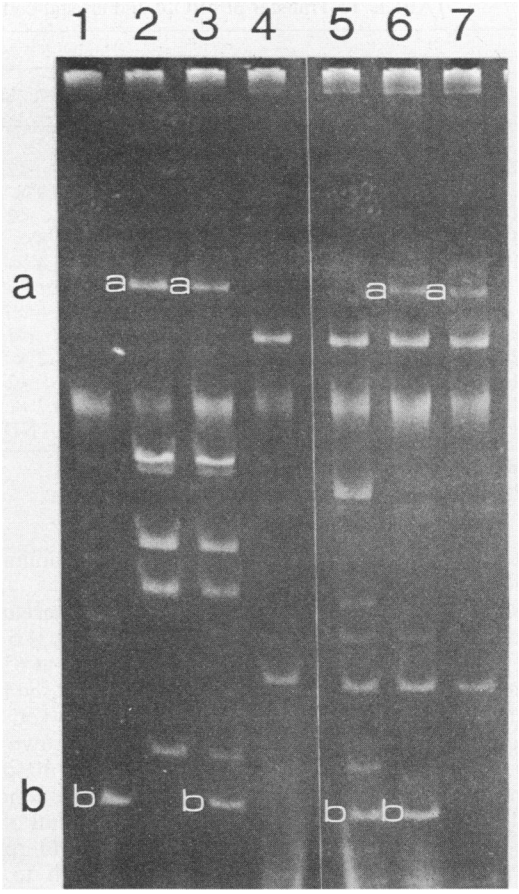

FIG. 2. Plasmid profiles of donor, recipient, and transcipient strains in experiments with donor HD137 Tet^r. Plasmid bands: a, crystal-coding plasmid from HD137 Tet^r donor; b, pBC16. Lanes: 1, pBC16; 2, HD137; 3, HD137 Tet^r donor; 4, HD1 Str^r Cry$^-$ recipient; 5, HD1 Str^r Cry$^-$ Tet^r transcipient; 6, HD1 Str^r Cry$^+$ Tet^r transcipient; 7, HD1 Str^r Cry$^+$ transcipient.

showed that the bacteria were able to grow and sporulate in insect cadavers. The nonpathogenic recipient HD1 Str^r Cry$^-$ alone was unable to multiply in larvae, although when fed in combination with a lethal dose of a pathogenic strain it could multiply, sporulate, and often outcompete the pathogenic strain (Table 2).

A high level of plasmid transfer occurred between donor and recipient strains after larvae were fed an equal dose of HD240 Tet^r and HD1 Str^r Cry$^-$ (Table 2). A total of 30 to 42.4% of the Str^r colonies had gained the ability to produce crystals, and all 20 Str^r Cry$^+$ isolates examined contained a 50-MDa plasmid. Transfer of Tet^r occurred at frequencies of 6.5×10^{-6} to 1.1×10^{-4}, with greater than 58 to 61% of these colonies producing crystals as a result of cotransfer of the crystal-coding plasmid. When larvae were fed 100-fold fewer HD1 Str^r Cry$^-$ spores compared with HD240 Tet^r, transfer of crystal production was reduced to less than 1.2%, although, surprisingly, transfer of Tet^r increased by approximately 1,000-fold.

The frequency of plasmid transfer when HD137 Tet^r was the donor strain was different from that with HD240 Tet^r. Colonies isolated from dead insects previously fed an equal number of HD1 Str^r Cry$^-$ and HD137 Tet^r spores showed that most of the bacteria isolated were Str^r (Table 2). These results indicated that HD1 Str^r Cry$^-$ was able to outgrow the donor even though, alone, HD137 Tet^r was able to grow and sporulate in infected larvae to a comparable level. The uneven growth of the two bacteria resulted in a low level of transfer of crystal-coding ability, with less than 1% of the transcipient colonies producing crystals. By comparison, larvae fed 100-fold fewer HD1 Str^r Cry$^-$ spores than HD137 Tet^r spores resulted in 4% of the HD1 Str^r Cry$^-$ spores acquiring the 45-MDa plasmid coding for crystal production. This was most probably due to the fact that, under these conditions, HD1 Str^r Cry$^-$ was unable to outgrow HD137 Tet^r, and therefore, a suitable ratio of donor to recipient was present in the insect to allow increased plasmid transfer. The transfer rate of Tet^r from HD137 Tet^r was not markedly affected by the differing ratio of spores fed to the larvae, and this implies that conditions required for optimal transfer of the crystal-coding plasmid and pBC16 are different.

When all three strains, HD137 Tet^r, HD240 Tet^r, and HD1 Str^r Cry$^-$, were fed to insects together in equal ratios, 26.3 and 31.8% (larvae 1 and 2, respectively) of the resulting HD1 Str^r Cry$^-$ colonies from the dead insects produced crystals (Table 2). Plasmid analysis of the crystalliferous transcipients showed that 9 of 16 colonies examined contained both

TABLE 2. Transfer of pBC16 and crystal-coding ability between strains of *B. thuringiensis* in infected *G. mellonella* larvae

Donor, recipient, and ratio of spores fed to larvae	Larva	No. of colonies/larva					
		Total	No. Str^r	% Str^r	No. Tet^r producing crystals[a]	No. Tet^r + Str^r	% Str^r + Tet^r producing crystals[a]
HD240 Tet^r	1	4.7×10^7	<10		ND[b]	ND	
	2	9.7×10^7	<10		ND	ND	
	3	1.6×10^8	<10		ND	ND	
	4	3.4×10^8	<10		1.5×10^8	<10	
HD240 Tet^r + HD1 Str^r Cry$^-$ (1:1)	1	4.8×10^7	3.7×10^7	30	ND	5.2×10^3	58.1
	2	9.8×10^7	6.2×10^7	42.4	ND	6.4×10^2	58.0
	3	8.4×10^7	4.5×10^7	40.8	2.0×10^7	4.4×10^3	60.6
HD240 Tet^r + HD1 Str^r Cry$^-$ (100:1)	1	1.5×10^8	2.6×10^7	<1.0	ND	4.5×10^6	ND
	2	7.3×10^7	1.6×10^7	1.2	5.0×10^7	3.3×10^6	2.0
HD137 Tet^r	1	4.6×10^7	<10		ND	<10	
	2	5.1×10^7	<10		ND	<10	
	3	5.2×10^7	<10		5.1×10^7	<10	
HD137 Tet^r + HD1 Str^r Cry$^-$ (1:1)	1	1.7×10^8	1.6×10^8	<1.0	ND	8.0×10^4	ND
	2	5.6×10^7	5.0×10^7	ND	ND	4.4×10^5	ND
	3	2.6×10^7	2.4×10^7	<1.0	ND	7.1×10^4	<1
	4	2.7×10^7	2.3×10^7	ND	1.2×10^6	5.0×10^5	ND
HD137 Tet^r + HD1 Str^r Cry$^-$ (100:1)	1	1.6×10^8	6.2×10^7	ND	ND	5.0×10^5	ND
	2	5.7×10^7	1.1×10^7	4.0	3.8×10^7	1.0×10^5	48.0
HD137 Tet^r + HD240 Tet^r + HD1 Str^r Cry$^-$ (1:1:1)	1	4.0×10^8	6.2×10^7	26.3	ND	4.1×10^5	70.0
	2	9.4×10^7	6.6×10^6	31.8	7.4×10^7	6.0×10^4	44.4
HD1 Str^r Cry$^-$	1	4.7×10^3	4.5×10^3		<10		
	2	6.4×10^3	6.1×10^3		<10		

[a] A minimum of 50 colonies were examined per experiment.
[b] ND, Not determined.

crystal-coding plasmids, the 45-MDa plasmid from HD137 and the 50-MDa plasmid from HD240, along with smaller plasmids from both donors (Fig. 3). The other seven contained only one of the plasmids.

Plasmid transfer in *S. littoralis*. The number of *B. thuringiensis* spores isolated from dead *S. littoralis* larvae ranged from 5.0×10^5 to 9.2×10^7 per larva (Table 3).Microscopic examination showed that *B. thuringiensis* was able to grow and sporulate in infected larvae, even though larvae also contained a large number of nonsporeforming bacteria, presumably from the normal gut flora of the larvae, which resulted in a lower number of *B. thuringiensis* spores isolated per insect than in *G. mellonella*.

The number of transcipient colonies producing crystals isolated from larvae fed an equal dose of HD1 Str^r Cry$^-$ and HD240 Tet^r ranged from <1.0 to 26.0%. Plasmid profiles of crystal-producing transcipients showed that all those examined acquired a 50-MDa plasmid along with a number of other smaller plasmids. In larvae in which transfer of Tet^r was demonstrated, the frequency of transfer ranged between 2.3×10^{-5} and 3.8×10^{-5}. Transfer frequencies varied between individual larvae. The two larvae (numbers 1 and 3) containing the greatest numbers of spores showed the highest rate of genetic exchange, whereas no transfer was demonstrated in the two larvae (numbers 2 and 4) containing the lowest number of spores, indicating that a critical level of *B. thuringiensis* growth is required in an insect to permit high-level plasmid transfer.

When larvae were fed a lethal dose of HD137 Tet^r and HD1 Str^r Cry$^-$, no transfer of crystal-coding ability or tetracycline resistance was detected from donor to recipient. The absence of genetic exchange was thought to be due to the poor ability of HD137 Tet^r to colonize the infected insect, as the majority of spores isolated from insects were Str^r. Numbers of HD137 Tet^r spores in infected larvae reached high levels only with the addition of tetracycline to the insect food, presumably reducing the growth of the natural larval bacteria and permitting HD137 Tet^r to grow and sporulate under less competitive conditions (Table 3).

Plasmid transfer from *B. thuringiensis* into soil bacteria. An asporogenous mutant (Spo$^-$) of HD240 Tet^r was selected which contained the full parental complement of plasmids and the same level of plasmid-transferring ability as the parent (Table 4). When HD240 Tet^r Spo$^-$ was grown in broth cultures together with populations of sporeforming soil bacteria, both crystal-coding ability and tetracycline resistance were transferred (Table 4). Plasmid profiles of Tet^r colonies contained a plasmid of the same mobility as pBC16, and those producing crystals contained a 50-MDa plasmid (Fig. 4). No colonies from soil 1 were found to produce crystals before growth with HD240 Tet^r Spo$^-$ or after growth with an acrystalliferous strain of HD1 containing pBC16, indicating that transfer of the 50-MDa plasmid from HD240 Tet^r Spo$^-$ was responsible for crystal synthesis. Of a total of 1,200 tetracycline transcipients, all had a typical *B. cereus* morphology, suggesting that the transfer of *B. thuringiensis* plasmids into less closely related *Bacillus* species than *B. cereus* occurs at a lower level or does not occur.

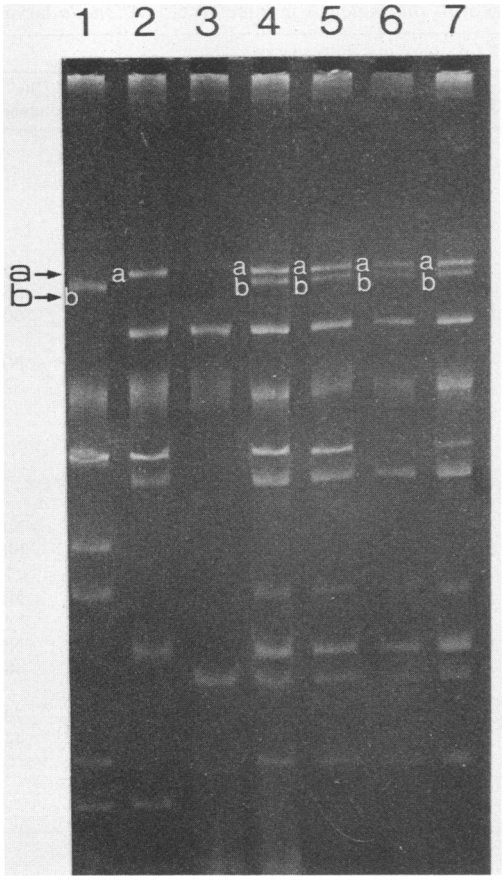

FIG. 3. Plasmid profiles of donor, recipient, and transcipient strains. Plasmid bands: a, crystal-coding plasmid from donor HD240 Tet^r; b, crystal-coding plasmid from donor HD137 Tet^r. Lanes: 1, HD137 Tet^r donor; 2,HD240 Tet^r donor; 3, HD1 Str^r Cry$^-$ recipient; 4 to 7, HD1 Str^r Cry$^+$ transcipients isolated from *G. mellonella* larvae.

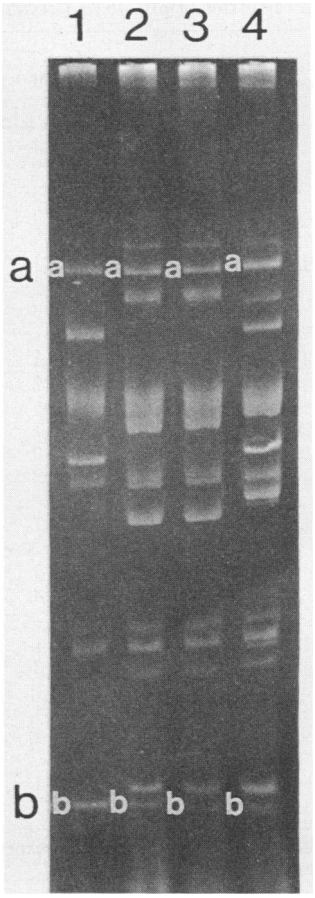

FIG. 4. Plasmid profiles of donor and transcipient strains. Plasmid bands: a, crystal-coding plasmid from HD240 Tet^r Spo$^-$ donor; b, pBC16. Lanes: 1, HD240 Tet^r Spo$^-$ donor; 2 to 4, Cry$^+$ Tet^r transcipients from soil.

DISCUSSION

When grown in broth cultures, *B. thuringiensis* has the ability to transfer plasmids at high frequencies by a conjugationlike plasmid transfer process first described by Gonzalez et al. (8). Results presented here showed for the first time that plasmid transfer can also occur under conditions similar to those which may be encountered by the bacteria in the environment. When larvae were infected with two or more strains of *B. thuringiensis*, the resulting bacteria isolated from dead insects showed that plasmid transfer had occurred at levels similar, in some cases, to those obtained in broth cultures in these experiments and for different strains by other workers (1, 8, 10). The donor strains HD137 Tet^r and HD240 Tet^r were both able to transfer Cry$^+$ and Tet^r plasmids to HD1 Str^r Cry$^-$. Transfer of the 45-MDa plasmid from HD137 Tet^r resulted in crystal formation at 25°C but not at 30°C when expressed in HD1 Str^r Cry$^-$. A similar phenomenon has been recorded with other toxin-coding plasmids in *B. thuringiensis* (25). In contrast, transfer of the 50-MDa plasmid from HD240 Tet^r resulted in crystal formation at both 25 and 30°C. Both donors also have a plasmid of approximately 120 MDa which contains genes coding for delta-endotoxins, although transfer of these plasmids was not observed in the matings performed.

Levels of plasmid transfer varied in infected larvae and were shown to depend on insect species, ability to colonize the larvae, bacterial strain, and dosage rate fed to the larvae. Rates of transfer were higher in *G. mellonella* than in *S. littoralis*. This was thought to be due to the greater ability of the bacteria to grow in *G. mellonella*, resulting in an increased chance of cell-to-cell contact required for plasmid transfer (9). The reason for the increased colonization by *B. thuringiensis* in *G. mellonella* is thought to be due to the lower numbers of gut bacteria found in untreated larvae compared with *S. littoralis* (P. Jarrett, unpublished data), resulting in a less competitive environment for growth and sporulation. Growth of *B. thuringiensis* also plays an essential role in the pathology of the bacteria in *G. mellonella* (22), unlike *S. littoralis* (27).

In broth cultures, *B. thuringiensis* was able to transfer Cry$^+$ and Tet^r to sporeforming bacteria from soil samples. All resulting transcipients were found to have a typical *B. cereus* morphology. As *B. thuringiensis* is often found infecting insect larvae and *B. cereus* and *B. thuringiensis* are common in soil (24, 26, 28), it is likely that plasmids are transferred between strains of *B. thuringiensis* and between *B. thuringiensis* and *B. cereus* in infected larvae in nature. Whether the potential for plasmid transfer in nature is restricted to just between the two closely related species (3)

TABLE 3. Transfer of pBC16 and crystal-coding ability between strains of *B. thuringiensis* in infected *S. littoralis* larvae

Donor, recipient, and ratio of spores fed to larvae	Larva	No. of colonies/larva					
		Total	No. Str^r	% Str^r producing crystals[a]	No. Tet^r	No. Tet^r + Str^r	% Str^r + Tet^r producing crystals[a]
HD240 Tet^r	1	8.6×10^7	<10		ND[b]	<10	
	2	6.7×10^6	<10		ND	<10	
	3	2.6×10^7	<10		2.0×10^7	<10	
HD240 Tet^r + HD1 Str^r Cry$^-$ (1:1)	1	2.2×10^7	8.6×10^6	2.0	ND	2.0×10^2	ND
	2	1.0×10^7	4.4×10^6	<1.0	ND	<10	ND
	3	4.4×10^7	1.8×10^7	26.0	ND	6.8×10^2	95.0
	4	8.2×10^6	5.0×10^7	<1.0	2.6×10^7	<10	ND
HD137 Tet^r	1	2.2×10^6	<10		ND	<10	
	2	1.1×10^6	<10		ND	<10	
	3	5.0×10^5	<10		3.7×10^5	<10	
HD137 Tet^r + HD1 Str^r Cry$^-$ (1:1)	1	9.2×10^7	9.0×10^7	ND	ND	<10	
	2	2.0×10^7	1.7×10^7	ND	ND	<10	
	3	1.5×10^7	1.0×10^7	<1.0	4.0×10^5	<10	
HD137 Tet^r	1	4.9×10^7	<10		3.8×10^7		
HD1 Str^r Cry$^-$	1	ND	3.0×10^4		<10		
	2	5.2×10^4	5.0×10^4		<10		

[a] A minimum of 50 colonies were examined per experiment.
[b] ND, Not determined.

is not known, although in broth cultures transfer of plasmids at lower levels than reported here has been shown to occur from *B. thuringiensis* into *B. subtilis* (16), *B. anthracis* (1), *Bacillus coagulans*, and *Bacillus megaterium* (P. Jarrett, unpublished data).

Plasmid profiles of Cry$^+$ recipients from *G. mellonella* infected with two donor strains showed that many contained the Cry$^+$ plasmids from both of the donors. These results show that the potential exists for new combinations of crystal toxins to be produced in nature. Many strains of *B. thuringiensis* contain a complex array of plasmids (14, 20) which includes many coding for delta-endotoxin synthesis. One of the major reasons for the complex activity spectrum of *B. thuringiensis* (6) may be growth of more than one strain of *B. thuringiensis* in susceptible larvae or in other suitable environments permitting plasmid transfer, creating new combinations of delta-endotoxins.

TABLE 4. Transfer of pBC16 and crystal-coding ability from the *B. thuringiensis* asporogenous mutant HD240 Tet^r Spo$^-$ to soil bacteria

Donor and recipient	Colony counts/ml		
	Total[a]	No. Tet^r	% Tet^r producing crystals[b]
Soil 1	1.24×10^8	<5	
Soil 1 + HD240 Tet^r Spo$^-$	8.65×10^7	6.7×10^3	3
Soil 2	9.4×10^7	<5	
Soil 2 + HD240 Tet^r Spo$^-$	1.78×10^7	1.1×10^2	<1
HD240 Tet^r Spo$^-$	<1	<1	
HD240 Tet^r Spo$^-$ + HD1 Str^r Cry$^-$	1.1×10^8	5.5×10^5	92

[a] Counts after samples were heated at 65°C for 10 min.
[b] A minimum of 50 colonies were examined per experiment.

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