Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 6951–6955, November 1982
Genetics

Transfer of *Bacillus thuringiensis* plasmids coding for δ-endotoxin among strains of *B. thuringiensis* and *B. cereus*

(agarose gel electrophoresis/immunological analysis/intraspecific and interspecific hybrids/*Bacillus* taxonomy)

JOSÉ M. GONZÁLEZ, JR., BARBARA J. BROWN, AND BRUCE C. CARLTON

Department of Molecular and Population Genetics, University of Georgia, Athens, Georgia 30602

Communicated by Norman H. Giles, July 12, 1982

ABSTRACT **The recently discovered high-frequency transfer of plasmids between strains of *Bacillus thuringiensis* was used to study the genetic relationship between plasmids and production of the insecticidal δ-endotoxin crystal. Three strains of *B. thuringiensis* transmitted the Cry$^+$ (crystal-producing) phenotype to Cry$^-$ (acrystalliferous) *B. thuringiensis* recipients. Agarose gel electrophoresis showed that one specific plasmid from each donor strain was always present in Cry$^+$ "transcipients." The size of the transmissible crystal-coding plasmid varied with the donor strain, being 75 MDal (megadaltons) in size in HD-2, 50 MDal in HD-73, and 44 MDal in HD-263. Immunological analysis showed the Cry$^+$ transcipients to be hybrid strains, having flagella of the recipient serotype and crystals of the donor serotype. These results demonstrate that the structural genes for the δ-endotoxin are plasmid borne. Crystal-coding plasmids also transferred into two strains of the related species *Bacillus cereus* and yielded transcipients that produced crystals of the same antigenicity as the donor strain.**

The Gram-positive sporulating bacterium *Bacillus thuringiensis* is of special scientific and economic interest because it produces an insecticidal toxin (known as the δ-endotoxin) lethal to larvae of a wide range of lepidopterans as well as some dipterans (1). δ-Endotoxin appears during sporulation as a crystalline inclusion (the parasporal crystal) that is phase-refractile, proteinaceous, and bipyramidal in most strains (2). Natural isolates of *B. thuringiensis* have been classified into at least 19 varieties on the basis of their flagellar and crystal antigens and their spectra of insecticidal activity (1, 3, 4). The closely related species *B. cereus* is distinguished by inability to produce the δ-endotoxin (1).

Several inconclusive studies have attempted to determine whether the toxin genes are located on extrachromosomal plasmids, which are apparently ubiquitous in strains of *B. thuringiensis* (5, 6). We have recently implicated specific plasmids in the production of δ-endotoxin by several strains of *B. thuringiensis*, through analysis of mutants cured of individual plasmids (7). These curing studies could not show whether the plasmids carried the actual toxin gene(s) or, possibly, regulatory genes controlling the expression of chromosomal toxin genes. A means of genetic transfer was needed to show how a crystal-coding plasmid affected the phenotype of an acrystalliferous (Cry$^-$) strain.

We recently discovered that certain *B. thuringiensis* plasmids could be transmitted between two strains during growth in mixed culture, at frequencies of up to 75% of recipient colonies examined (8). Preliminary results showed that one plasmid previously implicated in toxin production could transfer into a Cry$^-$ strain of a different flagellar serotype, yielding crystalliferous (Cry$^+$) transcipients (by "transcipients" we mean recipient cells that have acquired one or more plasmids from a donor strain*). These transcipients, though Cry$^+$, still exhibited the basic plasmid pattern, colony morphology, and flagellar serotype of the original Cry$^-$ recipient (8).

In this study, we describe the electrophoretic and immunological analysis of Cry$^+$ transcipient strains derived by transfer of three different transmissible crystal-coding plasmids into Cry$^-$ strains of both *B. thuringiensis* and *B. cereus*. Our findings indicate that these transmissible plasmids indeed carry the structural genes for the δ-endotoxin crystal.

MATERIALS AND METHODS

Strains. The prototype strains used in this study are listed in Table 1, along with their serological characteristics and their plasmid complements. The plasmid patterns of these strains are shown in Fig. 1. The prototype *B. thuringiensis* strains HD-2, HD-73, and HD-263 were kindly provided by H. Dulmage. *B. cereus* NRRL 569 was obtained from K. Bernhard. *B. cereus* T was from our *Bacillus* stock collection. All cultures were grown at 30°C.

Isolation of Mutants. Procedures for isolating partially cured (including Cry$^-$) mutants of HD-2 and HD-73 have been described (7). All five strains listed in Table 1 are streptomycin-sensitive (Str^S). Streptomycin-resistant (Str^R) mutants were isolated on nutrient agar or Spizizen's minimal salts/Casamino acids/glucose (SCG) agar plates (7) containing streptomycin sulfate at 100 μg/ml, either spontaneously, after growth at 42°C, or after ethyl methanesulfonate mutagenesis [carried out as described by Carlton and Brown (12)].

Plasmid Transfer Conditions. A Cry$^+$ donor and a Cry$^-$, usually Str^R, recipient strain were grown separately in Difco nutrient broth in a shaker–incubator for 12–14 hr. To obtain actively growing vegetative cells, the cultures were diluted 1:100 into fresh nutrient broth (2 ml) and incubated with shaking for 6–8 hr. Ten microliters of each culture was mixed in 1 ml of nutrient broth in a culture tube and the mixture was incubated with gentle shaking for 18–96 hr. The mixed culture was streaked either on SCG agar (when the recipient was Str^S), and recipient colonies were detected by their distinctive morphology (8), or on nutrient agar containing streptomycin (when the recipient was Str^R). The proportion of Cry$^+$ transcipients was usually high enough (≈10–20%) that they could be detected by examining from 5 to 50 recipient colonies for crystal production (by phase-contrast microscopy).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: Cry$^+$, crystal-producing; Cry$^-$, acrystalliferous; MDal, megadaltons; Str^S, streptomycin-sensitive; Str^R, streptomycin-resistant.

* We have used "transcipient" and not a more explicit term (such as "transconjugant") because the actual transfer mechanism has not been fully characterized (8).

Table 1. Properties of wild-type strains of *B. thuringiensis* and *B. cereus*

Strain no. and variety name	Flagellar serotype*	Crystal serotype†	Plasmids, no.‡	Plasmid masses, megadaltons (MDal)‡	Ref.
HD2-1 = *B. thuringiensis* var. *thuringiensis*	1	*Thu*	10	5.2, 6.2, 7.2, 7.6, 32, 37, 54, 57, 75, ≈150	7
HD73-1 = *B. thuringiensis* var. *kurstaki*	3ab	K-73	6	4.9, 5.2, 5.4, 7.5, 50,§ 50	7
HD263-1 = *B. thuringiensis* var. *kurstaki*	3ab	K-1	11	≈1.4, 4.9, 5.0, 5.2, 5.4, 7.5, 43, 44, 60, ≈115, ≈130	This study
BC569-1 = *B. cereus* NRRL 569	—	Cry^-	2	8.0, ≈150	This study
BCT-1 = *B. cereus* T	—	Cry^-	1	≈120	This study

* According to deBarjac and Bonnefoi (3). The flagellar serotypes of the two *B. cereus* strains are unclassified. Cells of strains BC569 and BCT did not crossreact with antisera against *B. thuringiensis* flagella of serotype 1 or 3ab (data not shown).
† According to Krywienczyk *et al.* (9, 10).
‡ The number of plasmid size classes in strains HD263-1, BC569-1, and BCT-1, and their masses, were determined by agarose gel electrophoresis by using the electron microscopic sizes of plasmids of HD2-1 as molecular weight markers (7).
§ This plasmid is present in lower copy number than the crystal-coding 50-MDal plasmid and comigrates with it (7).

Analysis of Recipient Colonies on Agarose Gels. Plasmid patterns were generated by using a modified Eckhardt method (7, 11) with further alterations. A 20-slot Teflon comb was used to form smaller slots (4 mm wide, 3 mm deep, 2.2 mm apart). The concentrations of lysozyme and RNase A were 1 mg/ml and 50 μg/ml, respectively. The $NaDodSO_4$ solution was adjusted to pH 8.0 with HCl. The volumes of $NaDodSO_4$ solution and spheroplast suspension layered in each slot were scaled down to 12 μl and 6 μl, respectively. Gels were 0.5% agarose (low electroendosmosis, Sigma) in Tris borate buffer (5) and were run at constant current in three stages: 1 hr at 3.0 mA, then 30 min at 7.0 mA, and finally 3–4 hr at 28 mA (≈110 V).

Purification and Solubilization of Endotoxin Crystals. Confluent cultures were grown on Difco nutrient agar plates for 3–4 days. Spores and crystals were collected and washed three times with deionized water, sonicated briefly, and immediately fractionated on step gradients of Renografin-60 (Squibb). A typical gradient included 4 ml of 75%, 5 ml of 85%, and 1 ml of 100% Renografin-60 with a 2-ml sample layered on top. Centrifugation was at 13,000 rpm (Beckman SW 41 rotor) for 2 hr at 20°C. The resulting three bands were removed with a pasteur pipette, diluted with deionized water, and centrifuged. The middle band was usually >95% crystals. Phase-contrast microscopy was used to monitor crystal purification. If required, crystals were refractionated.

Crystal Solubilization and Antibody Preparation. Crystals were solubilized for antigen–antibody reactions by incubating for 2 hr at 37°C in 0.05 M $NaHCO_3$ buffer at pH 10.2 and stored at −20°C. Antigen preparations for rabbit inoculations were solubilized by titration to pH 12 with NaOH, activated at 28°C for 72 hr, and stored at −20°C. Young rabbits were immunized with solubilized crystals from strains HD-1, HD-73, and HD-2, which produce crystals of K-1, K-73, and *Thu* type, respectively (Table 1; refs. 9 and 10). The immune serum was stored in 1-ml aliquots at −20°C.

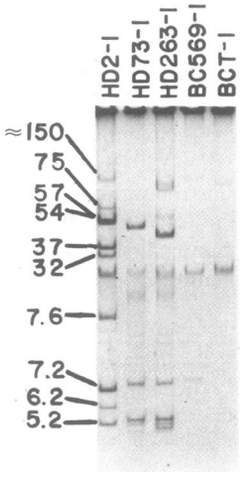

FIG. 1. Plasmid patterns of the wild-type strains listed in Table 1, by using a modified Eckhardt method of agarose gel electrophoresis (described in the text and in refs. 7 and 11). The molecular weights (in MDal) of the plasmids of HD2-1 are listed on the left margin as size references; the 7.6-MDal plasmid is present only as the open circular form (5, 7).

Flagellar Antigen Preparation. Highly flagellated *B. thuringiensis* cells were obtained by a slightly modified Craigie (13) procedure. After several passages on Craigie tubes containing nutrient broth with 0.2% Bacto-agar, cultures were inoculated into 100 ml of nutrient broth in a 500-ml flask and incubated on a rotary shaker for 5–8 hr. The cells were fixed with an equal volume of 0.6% formalized saline and refrigerated overnight. The cells were pelleted by centrifugation, washed, resuspended in 3–5 ml of 0.6% formalized saline, and stored at 4°C.

Immunological Assays. Crystal serology was done on Ouchterlony gels containing 1% Ionagar no. 2 (Oxoid, Basingstoke, England) dissolved in 0.01 M $NaPO_4$ buffer with 0.85% NaCl and formed on GelBond film (FMC, Rockland, ME). One to 5 μl of antigen and antiserum were pipetted into 2-mm diameter wells and allowed to diffuse at room temperature for approximately 24 hr. The gels were soaked in 0.3 M NaCl for 24 hr and 0.15 M NaCl for an additional 24 hr to help remove nonprecipitated proteins; they were then stained with 0.1% Brilliant Blue R (Sigma) in 10% acetic acid/30% methanol for 30 min at room temperature and then were destained in 7% acetic acid.

For flagellar serotyping, the formalin-treated cells were diluted in 0.6% formalized saline to 20–30 × 10^8 cells per ml. Then 0.1 ml of the appropriate antiserum dilution was mixed with 0.9 ml of the diluted cell suspension and incubated at 37°C for 2 hr. Agglutination was observed visually.

RESULTS

Isolation of Mutant Donor and Recipient Strains. The variants of *B. thuringiensis* and *B. cereus* used as donors and recipients in plasmid transfer experiments were derived as shown in Fig. 2. Some mutants were described elsewhere (7). In mutants HD73-4 and HD73-6, the 50-MDal lower copy-number plasmid, apparently decreased in size, no longer comigrates with the 50-MDal crystal-coding plasmid on agarose gels, so that it was possible to tell which of the two plasmids was present in a transcipient. The wild-type strain HD2-1 was a poor donor of crystal production; partially cured mutants such as HD2-5 and HD2-13 were better donors and were used instead. All of

the recipient strains used, except HD2-19 and HD2-41 (8), were Str^R mutants, so that recipient cells could usually be directly selected after mating with Str^S donors.

Transfer of the Crystal-Coding Plasmid of Strain HD-73. Fig. 3*A* shows the results of various matings by using HD-73 variants as donors. The donor HD73-4 transmitted the 50-MDal plasmid into HD2-19 to yield the Cry^+ transcipient HD2-19-1. The donor HD73-6 transferred the 50-MDal plasmid into HD2-41 to yield the Cry^+ transcipients HD2-41-1, HD2-41-2, and HD2-41-3. Fig. 3*A* shows that Cry^+Str^S HD73-6 can transfer crystal-producing ability into the Str^R, and naturally Cry^-, *B. cereus* recipient BC569-2 to yield Cry^+Str^R transcipients such as BC569-2-2, which has acquired the 50-MDal and four smaller plasmids. Mating of HD73-6 with BCT-4 yielded no Cry^+ transcipients, although the 43-, 6.7-, and 4.9-MDal plasmids transferred at high frequencies (≈50% of the recipient cells) as in transcipient BCT-4-6.

The 50-MDal toxin plasmid also transferred between strains of HD-73. Mating of the Cry^+Str^S HD73-6 with the Cry^-Str^R HD73-21 yielded Cry^+Str^R transcipients carrying the 50-MDal plasmid (not shown).

Transfer of the Crystal-Coding Plasmid of Strain HD-263. We had no mutational evidence indicating which plasmid might code for crystal production in HD-263. However, when HD-263 was grown with Cry^- strains of HD-2 or with BC-569, the Cry^+ phenotype was transmitted at high frequency (≈10–20% of recipient cells). Fig. 3*B* shows transcipients generated with HD263-1 as plasmid donor. Mating Cry^+Str^S HD263-1 with Cry^-Str^R HD2-62 generated the Cry^+Str^R transcipients HD2-62-3, HD2-62-4, and HD2-62-5. All have a new 44-MDal plasmid corresponding to the mobility of a plasmid in HD263-1, suggesting that this plasmid codes for crystal synthesis. Some small plasmids also transferred into HD2-62-4 and HD2-62-5. Mating HD263-1 with Cry^-Str^R BC569-2 yielded Cry^+Str^R transcipients like BC569-2-1, which now carries the 44-MDal plasmid and three smaller plasmids from HD263-1. Like HD-73, HD-263 did not transmit crystal production to BCT-4; only the three small plasmids were transferred, as in transcipient BCT-4-10. Mating HD263-1 with Cry^-Str^R HD73-21 yielded Cry^+Str^R transcipients like HD73-21-1, which acquired the 44-, 7.5-, 5.4-, and 5.0-MDal plasmids of HD-263 (Fig. 3*C*).

Transfer of the Crystal-Coding Plasmid of Strain HD-2. We previously reported that 19 of 20 Cry^- mutants of HD-2 lost the 75-MDal plasmid, implicating this plasmid in crystal production (7). The Cry^- mutant HD2-19 was the only exception; it *retained* the 75-MDal plasmid but instead had lost the 54-MDal plasmid. Its loss of crystal production could be due to (*i*) mutation of the 75-MDal plasmid, (*ii*) loss of the 54-MDal plasmid, or (*iii*) mutations of chromosomal genes required for crystal synthesis. These alternatives were tested by replacing the 75-MDal plasmid of HD2-19 with a "normal" 75-MDal plasmid from a Cry^+ variant of HD-2. As shown in Fig. 3*D*, HD2-19 was first cured of its 75-MDal plasmid to give the Cry^- strain HD2-41, which was then mutagenized with ethyl methanesulfonate to give the Cry^-Str^R recipient HD2-61. Mating of Cry^+Str^S HD2-13 with Cry^-Str^R HD2-61 yielded Cry^+Str^R transcipients such as HD2-61-1 at frequencies of 20–40% of the final recipient population. Transcipient HD2-61-1 has acquired the 75-MDal plasmid from HD2-13 and is Cry^+, although it lacks the 54-MDal plasmid. These results indicate that HD2-19 is Cry^- because of mutation of the 75-MDal plasmid and not because of chromosomal mutation or loss of the 54-MDal plasmid.

Mating of HD-2 strains with *B. cereus* strain T resulted in low-frequency transfer of the 75-MDal plasmid (<0.1% of recipient cells). Mating Cry^+Str^S HD2-5 with plasmidless, Cry^-Str^R BCT-4 yielded the Cry^+Str^R transcipient BCT-4-12, containing only the 75-MDal plasmid (Fig. 3*E*). Cry^+Str^S HD-2 strains also transferred some plasmids into Cry^-Str^R recipi-

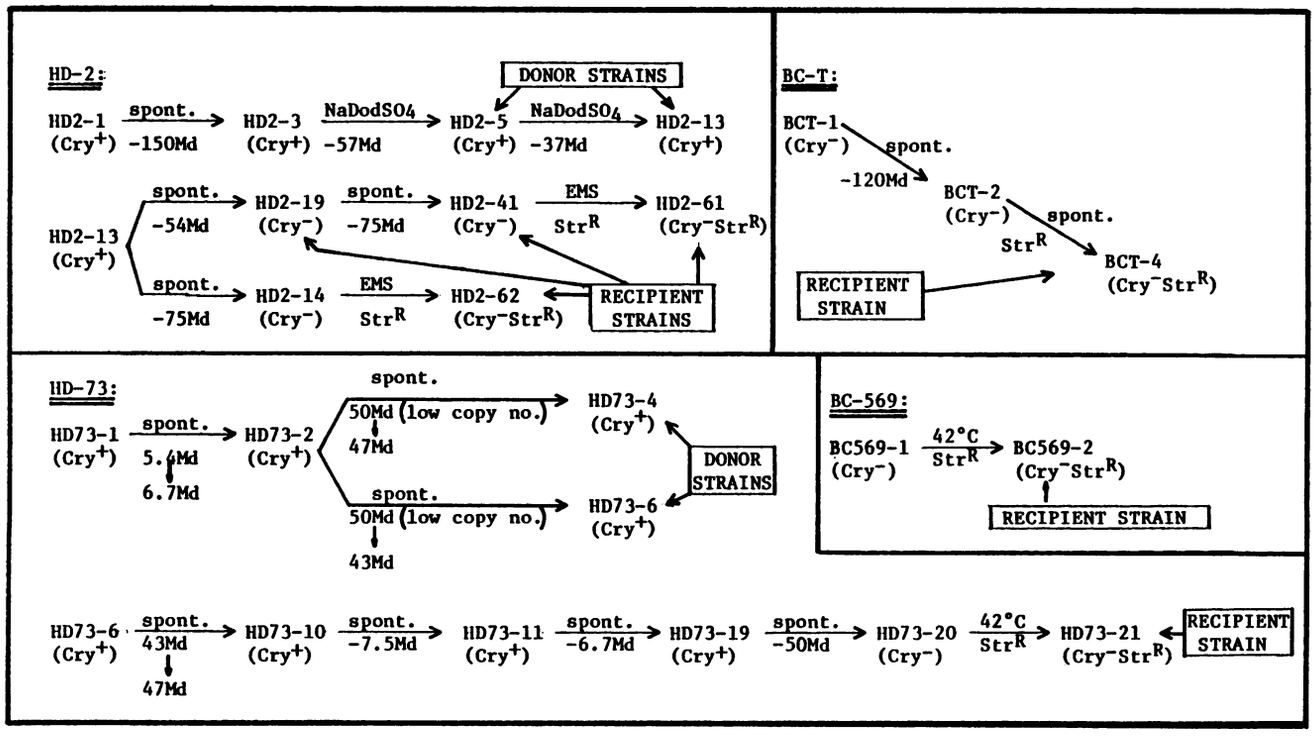

FIG. 2. Derivations of mutant donor and recipient strains of *B. thuringiensis* and *B. cereus*. The method of derivation is given above the arrow preceding each mutant: spont., the mutant arose spontaneously; $NaDodSO_4$, the mutant arose after growth in the presence of $NaDodSO_4$; 42°C, the mutant arose after growth at 42°C; EMS, ethyl methanesulfonate used as mutagen. The change in the plasmid array of each mutant is listed under each arrow as the mass (Md, MDal) of the plasmid that has been lost or altered in size.

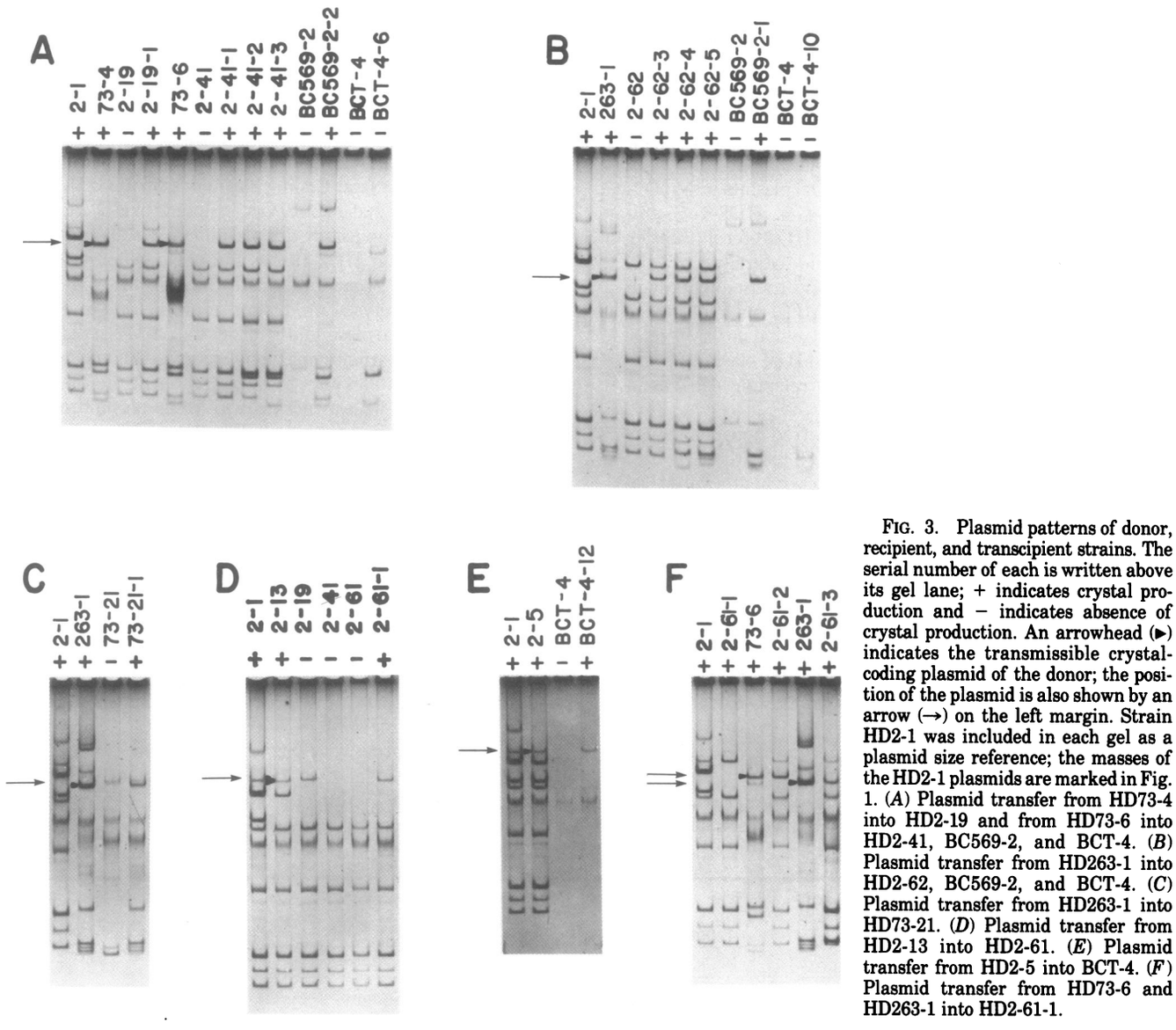

FIG. 3. Plasmid patterns of donor, recipient, and transcipient strains. The serial number of each is written above its gel lane; + indicates crystal production and − indicates absence of crystal production. An arrowhead (▸) indicates the transmissible crystal-coding plasmid of the donor; the position of the plasmid is also shown by an arrow (→) on the left margin. Strain HD2-1 was included in each gel as a plasmid size reference; the masses of the HD2-1 plasmids are marked in Fig. 1. (*A*) Plasmid transfer from HD73-4 into HD2-19 and from HD73-6 into HD2-41, BC569-2, and BCT-4. (*B*) Plasmid transfer from HD263-1 into HD2-62, BC569-2, and BCT-4. (*C*) Plasmid transfer from HD263-1 into HD73-21. (*D*) Plasmid transfer from HD2-13 into HD2-61. (*E*) Plasmid transfer from HD2-5 into BCT-4. (*F*) Plasmid transfer from HD73-6 and HD263-1 into HD2-61-1.

ents HD73-21 and BC569-2 at high frequency (not shown) but no Cry^+Str^R transcipients were detected.

Production of Strains Carrying Two Distinct Crystal-Coding Plasmids. The Cry^+ transcipient HD2-61-1, carrying the 75-MDal plasmid of HD-2, was used as recipient. Fig. 3*F* shows the results of mating HD73-6 and HD263-1 with HD2-61-1. The transcipient HD2-61-2 acquired the 50-MDal crystal-coding plasmid from HD73-6, and the transcipient HD2-61-3 acquired the 44- and 5.4-MDal plasmids from HD263-1. The 50- and 44-MDal plasmids both appear to be compatible with the 75-MDal plasmid, because these strains can be subcultured without loss of the relevant plasmids.

Immunology of Transcipient Strains. Ouchterlony double-diffusion assays of δ-endotoxin crystals isolated from various transcipients are shown in Fig. 4. Control experiments showed that solubilized δ-endotoxin crystals from strain HD-2 reacted strongly with homologous anti-HD-2 crystal antiserum and weakly with antisera against crystals from strains HD-1 and HD-73. In contrast, neither K-1 nor K-73 crystals showed significant crossreactivity with antiserum against HD-2 crystals. As shown in Fig. 4, the solubilized crystals isolated from transcipients obtained by using the *kurstaki* strains HD-73 or HD-263 as donors and HD-2 or BC-569 strains as recipient crossreacted with antisera against *kurstaki*-type crystals but not with antiserum against HD-2 recipient-type crystals (BC-569 has never been known to produce crystals). The transcipient BCT-4-12, carrying the 75-MDal toxin plasmid from strain HD-2, makes crystals that crossreacted with anti-HD-2 crystal antiserum. The transcipient HD2-61-2 (Fig. 3*F*), which contains two *different* toxin plasmids, produces crystals having the antigenicity of *both* donor and recipient-type crystals (Fig. 4); HD2-61-3 (Fig. 3*F*) gave identical results (not shown).

In all cases in which donor and recipient were of different flagellar serotypes, antiserum against recipient-type flagella agglutinated the transcipient cells, which did not react with antiserum against donor-type flagella. BCT-4 transcipients were poorly motile and were not tested for flagellar antigens.

DISCUSSION

We had previously demonstrated that strains of *B. thuringiensis* could transfer plasmids by an efficient, conjugation-like process and that HD-73 could donate a 50-MDal plasmid coding for crystal synthesis (8). We had also reported the loss of δ-endotoxin production in mutants of HD-2 and HD-73 that had been cured of their 75- and 50-MDal plasmids, respectively (7). We

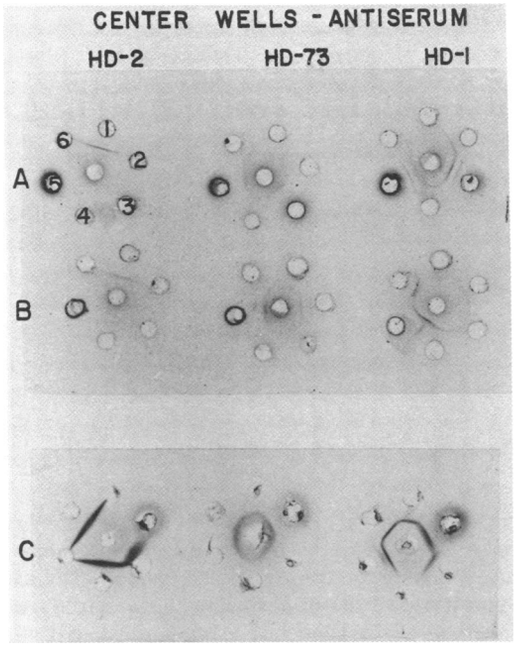

FIG. 4. Double-diffusion serological analysis of crystal proteins. The antisera were in the center wells. Those on the left-hand, middle, and right-hand sections of each panel contained antiserum against HD-2, HD-73, and HD-1 crystals, respectively. The outside wells contain crystals solubilized in $NaHCO_3$ buffer. The samples are: (*A*) 1, HD2-1; 2, HD73-6; 3, HD2-19-1; 4, HD2-41-1; 5, HD2-41-2; and 6, HD2-41-3; (*B*) 1, HD2-1; 2, HD73-6; 3, BC569-2-2; 4, HD263-1; 5, HD2-62-3; and 6, BC569-2-1; (*C*) 1, HD263-1; 2, HD73-21-1; 3, HD2-1; 4, BCT-4-12; 5, HD73-6; and 6, HD2-61-2.

have now eliminated the possibility that this association might be fortuitous by demonstrating that transfer of either of these two plasmids (or of the 44-MDal plasmid of HD-263) into Cry^- *B. thuringiensis* or *B. cereus* recipients invariably yields Cry^+ transcipients. Other plasmids of *B. thuringiensis* transferred between strains at detectable frequencies but only these three plasmids converted the Cry^- recipients into crystal producers.

Furthermore, the immunological properties of the crystals produced by transcipients match those of the plasmid donor strain and not those of the recipient strain. When HD-73 and HD-263 were mated with Cry^- HD-2 mutants, the crystals produced by the Cry^+ transcipients were of the same serotype as of the donor strains (Fig. 3 *A* and *B*; Fig. 4). Because the antigenic specificity (and therefore, the structural proteins) of transcipient crystals is independent of the strain used as a recipient and is determined instead by the plasmid acquired from the donor strain, we conclude that the structural gene(s) coding for the protein(s) of δ-endotoxin crystals in HD-73 and HD-263 reside on the implicated transmissible plasmids.

Two strains of *B. cereus* were converted into crystal producers by means of plasmid transfer (Fig. 3 *A*, *B*, and *E*). The *B. cereus* transcipients produced crystals of the same serotype as the donor's crystal. As far as we know, *B. cereus* has never produced a parasporal crystal and it is unlikely that these strains could be carrying "silent" δ-endotoxin genes. Their conversion to donor-type crystal production by acquiring a toxin plasmid from HD-2, HD-73, or HD-263 is further evidence that these three transmissible plasmids each carry structural genes necessary for crystal synthesis. The ease of plasmid transfer between *B. thuringiensis* and *B. cereus* supports existing taxonomical data that suggest that these two species are very closely related (1, 14). Because production of a parasporal crystal is the principal difference between *B. thuringiensis* and *B. cereus*, the generation of Cry^+ *B. cereus* transcipients such as BC569-2-1, BC569-2-2, and BCT-4-12 further blurs this distinction. These transcipients might be classified as novel isolates of *B. thuringiensis* if their origins were not known.

Generally, each flagellar serotype of *B. thuringiensis* produces a characteristic δ-endotoxin crystal of unique antigenicity (1, 9). However, Krywienczyk *et al.* (10) recently reported the existence of serotype-1 strains (var. *thuringiensis*) that produce crystals either of K-1 or of mixed K-1/*Thu* antigenicity. Our results may explain this, because several of our transcipients have flagella of serotype-1 but make crystals of K-1 or mixed K-1/*Thu* antigenicity. Therefore, the apparent hybrids reported by Krywienczyk *et al.* (10) may have arisen through the natural transfer of toxin plasmids between varieties having different flagellar serotypes.

Although the actual transfer mechanism of *B. thuringiensis* plasmids—suggested in a preliminary study to be conjugation-like (8)—needs to be clarified, we were able to use this phenomenon to analyze the role of plasmids in δ-endotoxin production. We believe that this transfer system may play an important role in expanding our knowledge of *Bacillus* genetics.

We thank Dr. H. T. Dulmage and his associates for *B. thuringiensis* strains and for some of the flagellar antisera used in these experiments. Michael A. Von Tersch provided valuable assistance in preparing and isolating antisera against the δ-endotoxins. Terry Davis and Colleen McElfresh aided us with numerous retypings of the manuscript. This work was supported by research grants from Abbott Laboratories, North Chicago, IL, and Shell Development Company, Modesto, CA.

1. Dulmage, H. T. (1979) in *Genetics in Relation to Insect Management*, eds. Hoy, M. A. & McKelvey, J. J., Jr. (Rockefeller Foundation, New York), pp. 116–127.
2. Bulla, L. A., Jr., Kramer, K. J. & Davidson, L. I. (1977) *J. Bacteriol.* **130**, 375–383.
3. deBarjac, H. & Bonnefoi, A. (1973) *Entomophaga* **18**, 5–17.
4. Ohba, M., Ono, K., Aizawa, K. & Iwanami, S. (1981) *J. Invertebr. Pathol.* **38**, 184–190.
5. González, J. M., Jr., & Carlton, B. C. (1980) *Plasmid* **3**, 92–98.
6. Clark, B. D., Perlak, F. J., Chu, C.-Y. & Dean, D. H. (1982) in *Comparative Pathobiology*, eds. Bulla, L. A., Jr., & Cheng, T. C. (Plenum, New York), Vol. 5, in press.
7. González, J. M., Jr., Dulmage, H. T. & Carlton, B. C. (1981) *Plasmid* **5**, 351–365.
8. González, J. M., Jr., & Carlton, B. C. (1982) in *Genetic Exchange: A Celebration and a New Generation*, eds. Streips, U. N., Goodgal, S., Guild, W. R. & Wilson, G. (Dekker, New York), pp. 85–95.
9. Krywienczyk, J., Dulmage, H. T. & Fast, P. G. (1978) *J. Invertebr. Pathol.* **31**, 372–375.
10. Krywienczyk, J., Dulmage, H. T., Hall, I. M., Beegle, C. C., Arakawa, K. Y. & Fast, P. G. (1981) *J. Invertebr. Pathol.* **37**, 62–65.
11. Eckhardt, T. (1978) *Plasmid* **1**, 584–588.
12. Carlton, B. C. & Brown, B. J. (1981) in *Manual of Methods for General Bacteriology*, ed. Gerhardt, P. (Am. Soc. Microbiol., Washington, DC), pp. 222–242.
13. Craigie, J. (1931) *J. Immunol.* **21**, 417–511.
14. Gordon, R. E., Haynes, W. C. & Pang, C. H.-N. (1973) *The Genus Bacillus* (U.S. Dept. of Agriculture, Washington, DC), Agriculture Handbook No. 427.