

Transduction in *Bacillus cereus* by Each of Two Bacteriophages

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The ability of phage CP-51 to mediate transduction both homologously and heterologously in some of its hosts was investigated. CP-51 was shown to transduce *Bacillus cereus* strains 6464, 9139, and T in addition to 569 which was reported earlier from this laboratory. Furthermore, CP-51 grown on *B. thuringiensis* was shown to transduce some mutants of *B. cereus*. During this investigation, a second transducing phage for *B. cereus* 569 was isolated from lysates of phage CP-51 grown on *B. cereus* 6464. This phage, designated CP-53, is carried by wild-type strain 6464 possibly as prophage. All auxotrophic mutants of *B. cereus* 569 tested, those requiring tryptophan, histidine, methionine, and leucine, were transduced to prototrophy by CP-53. Electron micrographs of the two phages revealed that CP-51 has a tail core surrounded by a contractile sheath and CP-53 has a long flexible tail without a contractile sheath. CP-53 is stable in the cold, whereas CP-51 is rapidly inactivated at 4 C.

The discovery of a transducing phage for *Bacillus cereus* NRRL 569 and *B. anthracis* was reported by Thorne (7, 8). The phage was isolated from soil and was designated CP-51. In studies reported here, the transducing properties of CP-51 were investigated in several other strains. Data are presented to show that CP-51 transduces *B. cereus* strains 6464, 9139, and T. Transductions of one strain with phage grown on another strain were unsuccessful for the majority of crosses tested. However, crosses between strains 569 and 6464 were positive for some markers, and phage grown on *B. thuringiensis* was effective in transducing certain markers in these two strains.

During these investigations, a second transducing phage for *B. cereus* 569 was found in lysates of CP-51 propagated on strain 6464. This phage, designated CP-53, is apparently the same phage that Altenbern and Stull (2) found to be carried by strain 6464. Results presented here demonstrate that CP-53 mediates generalized transduction in strain 569.

An initial comparison of phages CP-51 and CP-53 was made. Such properties as particle morphology, susceptibility to inactivation by ultraviolet light, antigenic relatedness, and stability at 4 and 15 C were investigated.

MATERIALS AND METHODS

Organisms. The organisms used in this investigation included *B. anthracis*, *B. thuringiensis*, and several

strains of *B. cereus*. The parent strains and mutants derived from them are listed in Table 1. The mutants were isolated by the procedure of Goldberg et al. (3) in which diethyl sulfate is the mutagen.

Media and cultural conditions. The media used have all been described in detail previously. NBY medium (7) contained nutrient broth (Difco) and Difco yeast extract (Difco); PA medium (7) contained nutrient broth (Difco) and salts at pH 6. The minimal media M10, M10C, and M1E, used for scoring transductants, contained a base of salts, glucose, and glutamic acid at pH 7. M10 (9) also contained alanine, serine, threonine, leucine, isoleucine, and valine. M10C was prepared by adding 5 g of vitamin-free acid-hydrolyzed casein (Nutritional Biochemicals Corp., Cleveland, Ohio) to the glucose-salts-glutamic acid base. M1E consisted of the glucose-salts-glutamic acid base and 1% (v/v) of NBY broth. Solid media were prepared by adding 15 g of agar per liter. Difco peptone (1%, w/v) was used as diluent for viable cell counts and phage assays.

Spores were prepared by inoculating a potato agar slant (6) and incubating for 5 days at 37 C. Growth from a single slant was collected in 5 ml of sterile distilled water and the suspension was heated at 65 C for 30 min. This procedure yielded about 10⁹ spores per ml.

Spores infected with phage CP-53 were prepared by picking a turbid plaque into 50 ml of PA broth in a 250-ml flask and incubating at 37 C on a rotary shaker (250 rev/min) for 48 hr. The spores were pelleted by centrifugation, washed twice with sterile distilled water, and resuspended in 5 ml of sterile

TABLE 1. Characteristics of the organisms used^a

Designation	Tentative genotype	Origin or reference
<i>B. cereus</i> 569 569 UM5 569 UM7 569 UM10 569 UM23 569 UM26	Wild type, prototrophic Prototrophic, rough <i>his-1</i> <i>trp-1</i> <i>met-4</i> <i>met-4 leu-2</i>	NRRL 569 Spontaneous from 569 DES treatment of 569 UM5 DES treatment of 569 UM5 DES treatment of 569 UM5 DES treatment of 569 UM23
<i>B. cereus</i> 6464 6464 UM4 6464 UM17 6464 UM19 6464 UM22 6464 UM35 6464 UM39	Wild type, prototrophic Prototrophic, cured of CP-53 <i>met/cys-1</i> <i>leu-1</i> <i>ade-1</i> <i>ncn-5</i> <i>ncn-5 arg-1</i>	ATCC 6464 Altenbern and Stull (2) DES treatment of 6464 DES treatment of 6464 DES treatment of 6464 DES treatment of 6464 UM4 DES treatment of 6464 UM35
<i>B. cereus</i> 9139 9139 UM4 9139 UM9	Wild type, prototrophic <i>met-2</i> <i>lys-1</i>	ATCC 9139 DES treatment of 9139 DES treatment of 9139
<i>B. cereus</i> T T UM3 T UM4 T UM16 T UM24	Wild type, prototrophic <i>met/cys-1</i> <i>met-1</i> <i>met-1 his-2</i> <i>pab-2</i>	I. D. Goldberg DES treatment of T DES treatment of T NTG treatment of T UM4 DES treatment of T
<i>B. cereus</i> var. <i>alesti</i> <i>B. cereus</i> 7004 <i>B. cereus</i> 9592 <i>B. cereus</i> 11950 <i>B. thuringiensis</i> 1328 <i>B. anthracis</i> Sterne <i>B. anthracis</i> Sterne UM10	Wild type, prototrophic Wild type, prototrophic Wild type, prototrophic Wild type, prototrophic Wild type Wild type <i>trp-1</i>	Uffen and Canale-Parola (10) ATCC 7004 ATCC 9592 ATCC 11950 NRS 1328 Fort Detrick Fort Detrick

^a Abbreviations: *his*, histidine; *trp*, tryptophan; *met*, methionine; *leu*, leucine; *ncn*, nicotinamide; *arg*, arginine; *cys*, cysteine; *ade*, adenine; *pab*, para-aminobenzoic acid; DES, diethyl sulfate; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

distilled water. The spore suspension was then heated at 65 C for 30 min. About 5×10^8 spores per ml were obtained by this method.

Propagation and assay of phage. CP-51 was propagated and assayed as described earlier by Thorne (7). CP-53 was propagated by inoculating 5×10^7 infected spores of *B. cereus* 569 into 25 ml of NBY broth and incubating for 5 hr at 37 C on a rotary shaker. A 6-ml sample of the culture was then exposed to ultraviolet (UV) light for 60 sec. Five ml of the induced culture was added to 20 ml of fresh NBY broth and incubated on a shaker at 37 C for 90 min. The culture was filtered through a AA membrane (Millipore Corp., Bedford, Mass.). The filtrate containing the phage was assayed by the agar-overlay method (1), using a 2-ml overlay of NBY soft agar (0.5% agar) on a NBY agar base. *B. cereus* 6464 UM4 was used as indicator (2×10^7 spores/lawn) and the plates were incubated at 30 C for 18 hr. The filtrates routinely contained 2×10^8 to 5×10^9 plaque-forming units (PFU)/ml. Phage preparations were stored at 15 C. However, as will be shown, CP-53 was also stable at 4 C and probably could be stored at that temperature as well.

Transductions. Recipient cells for transductions were grown by inoculating 25 ml of NBY broth in a 250-ml flask with 5×10^7 spores and incubating for 4 to 5 hr at 37 C on a rotary shaker (250 rev/min). Viable cell counts were made by plating appropriate dilutions on NBY agar.

Transductions were performed by adding 0.1 ml of phage suspension and 0.9 ml of cell culture to 18-mm Pyrex tubes and incubating at 37 C for 30 min on a reciprocating shaker in a water bath. After incubation, 0.1-ml samples of the transduction mixture were plated on the appropriate minimal agar plates. When CP-51 was the phage being used, an equal volume of phage antiserum was plated with the transducing phage, antiserum was omitted. Duplicate samples were plated, and controls to test for spontaneous revertants were always included in each test. Plates were incubated at 37 C and transductants were scored after 18 hr when plated on M10C or after 40 hr when plated on M10 or M1E.

Phage antiserum. Antisera to phages CP-51 and CP-53 were prepared in rabbits by intravenous injection.

tions of phage suspensions having 10^{10} or more PFU/ml (5).

Ultraviolet irradiation. UV light exposure was carried out with two General Electric germicidal lamps (15 w). A petri dish with 6 ml of the phage suspension was placed 40 cm below the source on a rotating platform (200 rev/min).

RESULTS

Host range of CP-51. The host range of this phage was investigated by spotting various dilutions of phage on soft NBY agar overlays containing spores of the prospective host and looking for zones of lysis after incubation for 18 hr at 37 C. By this method, several strains of *B. cereus* as well as *B. anthracis* and *B. thuringiensis* proved to be susceptible to the phage. *B. subtilis*, *B. licheniformis*, *B. brevis*, and *B. megaterium* were not susceptible. Attempts were made to propagate CP-51 on strains of *B. cereus* which gave no lysis by the spot test method and on those which did. About 10^6 PFU was added to soft NBY agar overlays containing spores, and the plates were incubated at 37 C for 18 hr. The overlays were macerated and suspended in NBY broth, and the debris was removed by centrifugation. The supernatant fluid was filtered to remove cells and then assayed for PFU. The results of this experiment are found in Table 2. *B. cereus* strains 7004 and 9592, did not serve as hosts. In general, high yields of phage were produced on all susceptible hosts. Similar results were found in other experiments in which the phage was propagated at 30 C.

Transduction by CP-51. Previously, Thorne presented evidence for transduction by CP-51

using mutants of *B. cereus* 569 and the Sterne strain of *B. anthracis* (7, 8). Table 3 gives the results of transduction experiments involving *B. cereus* strains 6464, 9139, and T. Pretreatment of the phage preparations with heat (65 C for 30 min) or antiserum to CP-51 (37 C for 15 min) resulted in no transductants, whereas treatment with deoxyribonuclease (100 μ g/ml for 30 min at 37 C) had no effect on the number of transductants recovered. These data demonstrate that CP-51 mediated genetic exchange in these strains. The frequency of transduction was about 5×10^{-7} for the mutants of T, 1.5×10^{-6} for mutants of 9139, and 5×10^{-7} for mutants of 6464 except for 6464 UM22 which transduced at a frequency of 2×10^{-6} .

To determine how closely the various hosts of

TABLE 2. Ability of CP-51 to grow on various organisms

Host strain	Phage yield (PFU/ml) ^a
<i>Bacillus cereus</i> 569	3×10^{11}
<i>B. cereus</i> T	3×10^{11}
<i>B. cereus</i> 6464	4×10^{11}
<i>B. cereus</i> 9139	4×10^{11}
<i>B. cereus</i> var. <i>alesti</i>	3×10^{11}
<i>B. cereus</i> 7004	No growth
<i>B. cereus</i> 9592	No growth
<i>B. anthracis</i> Sterne	3×10^{10}
<i>B. thuringiensis</i> 1328	4×10^{10}

^a Plaque-forming units were assayed against *B. cereus* 569.

TABLE 3. Transduction of *B. cereus* strains 6464, 9139, and T by CP-51

Recipient	Colonies/ml		
	Without phage	With phage grown on ^a	
		Prototroph	Mutant
6464 UM39 (Ncn ⁻ Arg ⁻)	20 (Ncn ⁺)	140 (Ncn ⁺)	0 (Ncn ⁺)
6464 UM17 (Met/Cys ⁻)	0	190	185
6464 UM19 (Leu ⁻)	15	145	NT ^b
6464 UM22 (Ade ⁻)	10	435	NT
9139 UM4 (Met ⁻)	5	350	0
9139 UM9 (Lys ⁻)	0	275	260
T UM3 (Met/Cys ⁻)	25	150	NT
T UM16 (His ⁻ Met ⁻)	0 (His ⁺)	145 (His ⁺)	0 (His ⁺)
T UM24 (Pab ⁻)	5	155	150

^a Phage was grown on either the wild-type or a mutant of the homologous strain. The mutants on which phage was grown are 6464 UM39, 9139 UM4, and T UM16. Transduction mixtures contained 0.9 ml of cells (about 3×10^8 colony-forming units) and 0.1 ml of phage (about 2.5×10^8 PFU). Transductants were scored on M1E. When recipients were double mutants, transductants were scored for only one marker; the unselected marker was added to the M1E plate at a final concentration of 20 μ g/ml.

^b Not tested.

CP-51 were related, attempts were made to transduce each strain with phage grown on the other strains. As can be seen from the data in Table 4, the strains used rarely crossed with one another. *B. cereus* strains 6464 and 569 and *B. thuringiensis* did cross reasonably well for some markers, however. Possibly, failures of strains to cross-transduce were due to deoxyribonucleic acid (DNA) in homologies among the respective strains. Further investigation of this possibility is now underway.

Phage CP-53. A second transducing phage for *B. cereus* has been isolated from lysates of CP-51 grown on *B. cereus* 6464. This phage, which has a different plaque morphology from that of CP-51, has been designated CP-53. Presumably, CP-53 is the same phage reported by Altenbern and Stull (2) to be carried by strain 6464. It gave very turbid plaques on both *B. cereus* 569 and 6464 UM4, although occasionally spontaneous, clear plaque mutants were found. The plaques were rather indistinct, but the phage could be titrated reliably. By the spot-test method, only strain 569 and the cured strain 6464 UM4 were shown to be susceptible to CP-53. The other strains tested may not be hosts for the phage or they may be immune. Unsuccessful attempts were made to propagate CP-53 by picking a turbid plaque and using it as

inoculum in a sensitive lawn. Altenbern and Stull (2) showed that the phage could be induced in strain 6464 by UV irradiation. Hence, the method used to propagate the phage was to induce appropriate lysogens as described in Materials and Methods.

Evidence for transduction by CP-53. After isolation and purification of CP-53 by several single-plaque passages in *B. cereus* 569, infected spores were prepared as described above. Phage was then propagated on 569 by UV induction of infected cultures and used in transduction experiments. Table 5 shows the results of an experiment in which a tryptophan-requiring mutant of *B. cereus* 569 was transduced to prototrophy by phage grown on wild-type 569. As these data show, the phage mediated genetic exchange in this strain. Treatment which inactivated the phage prevented transduction. Further evidence of the transducing ability of CP-53 is found in Table 6, which shows that phage grown on a tryptophan auxotroph did not transduce that mutant but did transduce other mutants to prototrophy. In the same experiment, each of the recipients was transduced to prototrophy by phage grown on wild-type 569. Transducing frequencies ranging from 10^{-6} to 5×10^{-6} were obtained.

Effects of UV irradiation of CP-53 and plating

TABLE 4. Tests for cross-transduction among some hosts of CP-51^a

Recipient	Colonies/ml						
	Without phage	With phage propagated on prototrophic					
		569	6464 UM4	9139	T	Sterne	<i>thuringiensis</i>
569 UM10 (Trp ⁻)	5	720	0	0	0	0	0
569 UM7 (His ⁻)	10	850	180	0	0	0	160
569 UM26 (Met ⁻ Leu ⁻)	0 Met ⁺ , 10 Leu ⁺	460, 385	40, 0	0, 0	0, 0	NT ^b	180, 120
6464 UM39 (Ncn ⁻ Arg ⁻)	25	0	165	0	0	0	0
6464 UM17 (Met/Cys ⁻)	0	100	140	0	0	0	0
6464 UM19 (Leu ⁻)	15	0	145	0	0	NT	0
6464 UM22 (Ade ⁻)	10	450	435	0	0	NT	190
9139 UM4 (Met ⁻)	5	0	0	350	0	0	0
9139 UM9 (Lys ⁻)	0	0	0	275	0	NT	0
T UM3 (Met/Cys ⁻)	25	0	0	0	150	NT	0
T UM16 (His ⁻ Met ⁻)	25	0	0	0	180	0	0
T UM24 (Pab ⁻)	5	0	0	0	160	0	0
Sterne UM10 (Trp ⁻)	25	0 ^c	0	0	0	200	0

^a Transduction mixtures contained 0.1 ml of phage (2×10^8 PFU) and 0.9 ml of cells (about 3×10^8 Colony-forming units). Transductants were scored on M1E. If a double mutant was used as the recipient, the second requirement was added to the M1E plate at a final concentration of 20 μ g/ml. In the case of T UM16, only the *his* marker was scored, and only the *ncn* marker was scored in 6464 UM39.

^b Not tested.

TABLE 5. Evidence for transduction by phage CP-53^a

Treatment of phage	Colonies/ml
No phage.....	5
No treatment.....	180
65 C, 30 min.....	0
Preimmune serum 15 min at 37 C.....	170
Immune serum, 15 min at 37 C.....	10
Deoxyribonuclease (100 µg/ml), 30 min at 37 C.....	190

^a Transduction mixtures contained 0.9 ml of 569 UM10 cells (7.5×10^8 colony-forming units) and 0.1 ml of phage (4.5×10^8 PFU) propagated on wild-type 569. In those tests with serum and deoxyribonuclease, 0.1 ml of phage was incubated with an equal volume of additive before being added to the recipient cells. Transductants were scored on M10C.

TABLE 6. Specificity of transduction by phage CP-53 propagated on an auxotrophic mutant^a

Recipient	Colonies/ml		
	Without phage	With phage grown on	
		Prototroph	569 UM10 (Trp ⁻)
569 UM10 (Trp ⁻)	0	500	0
569 UM7 (His ⁻)	10	280	280
569 UM26 (Met ⁻ Leu ⁻)	20 Met ⁺ 4 Leu ⁺	880 Met ⁺ 260 Leu ⁺	800 Met ⁺ 250 Leu ⁺

^a Each phage preparation had 3×10^8 PFU/ml before UV treatment that inactivated 97% of the PFU. Transduction mixtures consisted of 0.9 ml of cells (about 2×10^8 colony-forming units) and 0.1 ml of phage. Transductants were scored on M1E. When Met⁺ transductants were scored, leucine was added to the plates, and when Leu⁺ transductants were scored, methionine was added to the plates. Final concentration of the amino acid was 20 µg/ml.

with phage antiserum on the recovery of transductants. Suspensions of phage CP-53 were exposed to UV irradiation and then used to transduce an auxotrophic mutant. Table 7 shows that inactivation of plaque-forming ability by UV treatment increased the recovery of transductants. Exposure of the phage suspension to UV for 150 sec gave the greatest recovery of transductants for the times tested; such treatment inactivated about 97% of the PFU.

When transduction mixtures were plated in the presence of antiserum to CP-53, no enhancement of the recovery of transductants was observed. In most cases, plating with antiserum resulted in fewer transductants. This may be due to continued adsorption of phage to cells after plating when antiserum was omitted; the presence of antiserum

would inactivate the remaining phage and prevent further adsorption.

Comparison of CP-51 and CP-53. A comparison of some characteristics of phages CP-51 and CP-53 was made. Electron micrographs of the two phages are shown in Fig. 1. These micrographs show that CP-51 has a tail core surrounded by a contractile sheath, and a distinct base plate. CP-53, on the other hand, has a long flexible tail with no contractile sheath and what appears to be a knob at the base of the tail.

The UV inactivation curves of the two phages are shown in Fig. 2. The figure shows that CP-51 was inactivated at a greater rate than CP-53. This result is consistent with the hypothesis that CP-51 contains more nucleic acid than CP-53, thus giving CP-51 a larger target size and hence greater sensitivity to UV light.

Antiserum prepared against each phage was checked for its ability to inactivate the PFU in preparations of each phage. The results of such an experiment, shown in Table 8, show that each antiserum was specific for the respective phage. Although *K* values for the antisera were not determined, it is obvious that the two phages are different antigenically.

Thorne reported that CP-51 was unstable in the cold but relatively stable at 15 C (7). Hence, the

TABLE 7. Effect of UV irradiation of phage CP-53 on the recovery of transductants^a

UV exposure (sec)	PFU inactivated (%)	Transductants per ml
0	0	380
30	50	450
60	63	470
90	85	560
120	91	635
150	97	655

^a Transduction mixtures contained 0.9 ml of cells (3.6×10^8 colony-forming units) and 0.1 ml of phage (5×10^8 PFU before UV) grown on wild-type 569. Recipient was 569 UM10 and transductants were scored on M10C.

TABLE 8. Specificity of CP-51 and CP-53 antisera

Phage	Plaque-forming units/ml after treatment with ^a		
	Preimmune serum	CP-51 antiserum	CP-53 antiserum
CP-51	1.4×10^{11}	0	1.5×10^{11}
CP-53	3.6×10^{10}	3.5×10^{10}	7×10^7

^a Phage was incubated with an equal volume of undiluted serum for 15 min at 37 C before titrating against 569.

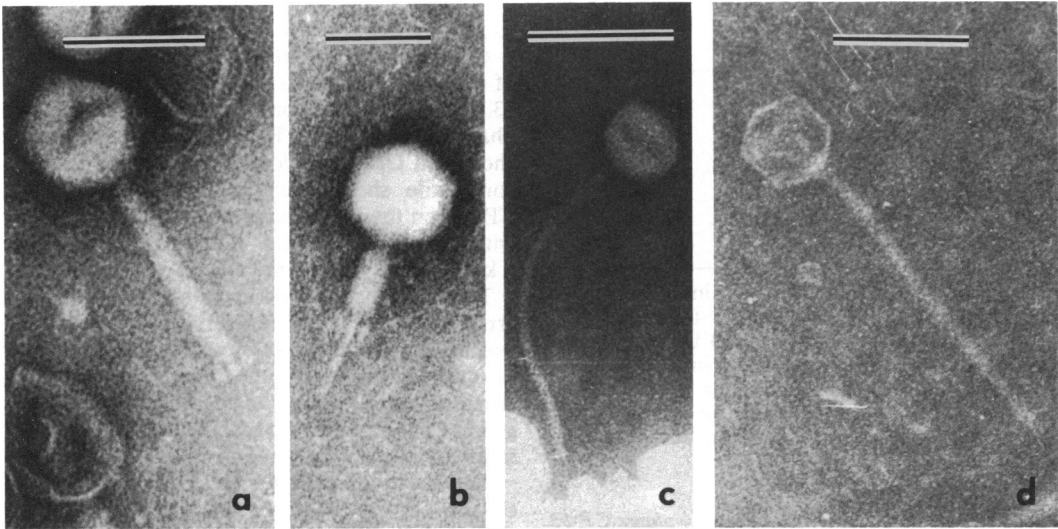


FIG. 1. (a) Phage CP-51, $\times 190,400$; (b) phage CP-51 after chilling at 4 C, $\times 141,000$; (c) phage CP-53, $\times 200,000$; (d) phage CP-53, $\times 190,400$. Phages were stained with phosphotungstic acid. Markers represent 100 nm. Micrographs were supplied by Stanley C. Holt.

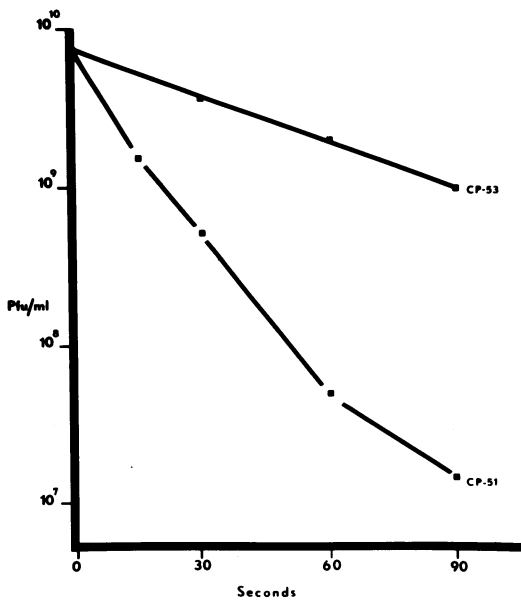


FIG. 2. UV inactivation of phages CP-51 and CP-53. A petri dish with 6 ml of phage suspension was placed on a rotary shaker 40 cm from the source and exposed to UV light. The samples were then assayed for PFU against 569.

stability of CP-53 was compared with that of CP-51 during storage at both 15 and 4 C (Table 9). As the data indicate, CP-53 was stable at both temperatures, whereas CP-51 rapidly lost titer at the lower temperature. Chilling of the two phages in an ice bath for 45 min gave dramatic evidence

of this difference in stability (Table 10). Although the titer of CP-53 remained constant, the titer of CP-51 declined by 65%. The instability of CP-51 in the cold seems to be due to contraction of its

TABLE 9. Effect of temperature on the stability of phages CP-51 and CP-53^a

Days	Per cent of original PFU remaining			
	CP-51		CP-53	
	4 C	15 C	4 C	15 C
0	100	100	100	100
1	13.3	100	100	100
3	1.3	100	100	100
7	0.7	100	100	100
28	0.03	50	100	100

^a Phages were grown on strain 569 and stored in NBY broth. The original lysates were diluted 1:10 before storage.

TABLE 10. Stability of phages CP-51 and CP-53 to cold shock^a

Phage	Plaque-forming units/ml	
	Before cold shock	After cold shock
CP-51	1.2×10^9	4.2×10^8
CP-53	4.3×10^9	4.3×10^9

^a Phage suspensions in NBY broth were titrated, placed in an ice bath for 45 min, and titrated again. *B. cereus* 569 was the indicator.

tail sheath, resulting in the loss of DNA from the phage (C. B. Thorne, *unpublished data*).

DISCUSSION

Phage CP-51 has been shown to transduce *B. cereus* strains 6464, 9139, and T. The fact that it transduced *B. cereus* T should prove useful as it might facilitate genetic analysis of the extensively studied sporulation processes in this strain. Although no mutants of *B. thuringiensis* were available to use as recipients, the data indicate that CP-51 grown on this strain contains transducing particles since it did transduce mutants of *B. cereus* 569 and 6464. Thus, it seems probable that this species should also be transducible by the phage.

Phage CP-51 was able to transduce each host that was tested. Since nontransducing phages are known for *B. cereus*, the results described above support the hypothesis that it is a property of the bacteriophage and not the host which determines whether transducing particles are formed after infection of a bacterium by a phage. If the bacteriophage possesses a property which enables it to act as a transducing phage, then it should act as a transducing phage for all of its hosts. One could envision that this property is a reflection of the mechanism for packaging DNA into a protein coat (4). If the mechanism is specific (i.e., if it recognizes only phage DNA), then that phage could not form transducing particles. However, if the mechanism is not specific (i.e., if it packages DNA regardless of source), then that phage would form transducing particles. According to such a model, a phage with a nonspecific packaging mechanism would be able to form transducing particles in any bacterial strain that served as host.

While performing these experiments, we found that the frequencies of transduction were often quite variable. Closer investigation showed that the age of the recipient culture was critical; old cultures yielded few transductants. The loss of transductants with increasing age of the recipient culture may be due to the onset of sporulation. Two plausible explanations for this loss of transductants came to mind. The sporulating cell may become compartmentalized early in the sporulation process. If the transducing particle injected its nucleic acid into a compartment not containing the genome of the cell, no transduction would occur. Alternatively, it is known that DNA replication stops in *B. cereus* during the early stages of sporulation (11). If, in *B. cereus*, re-

combination requires replication of the genome, then a cell undergoing sporulation could not be transduced because the transducing fragment would not be integrated into the bacterial chromosome. Investigations of the optimal age of the culture for recovery of transductants indicated that, when starting with 5×10^7 spores in 25 ml of broth, 4- to 5-hr cultures gave the greatest number of transductants. The exact time varied slightly from mutant to mutant, but all gave the best results at mid-log phase of growth.

The results presented here demonstrate that phage CP-53 mediates transduction in *B. cereus* 569. Mutants representing four different auxotrophic markers have been tested and each was transduced to prototrophy by the phage. Other markers have not yet been tested. Investigations are currently underway to determine whether CP-53 will transduce mutants of 6464.

The data presented here indicate that CP-51 might contain more DNA than CP-53 (greater sensitivity to UV). This prediction is being investigated by examining the ability of the two phages to cotransduce linked markers and by physically determining the molecular weights of their genomes.

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