TRANSDUCTION OF *BACILLUS LICHENIFORMIS* AND *BACILLUS SUBTILIS* BY EACH OF TWO PHAGES[1]

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Received for publication 10 April 1963

ABSTRACT

TAYLOR, MARTHA J. (U.S. Army Biological Laboratories, Fort Detrick, Frederick, Md.) AND CURTIS B. THORNE. Transduction of *Bacillus licheniformis* and *Bacillus subtilis* by each of two phages. J. Bacteriol. **86**:452–461. 1963.—A second transducing bacteriophage, designated SP-15, was isolated from the same soil-sample culture filtrate that supplied the *Bacillus subtilis* transducing phage, SP-10, reported earlier from this laboratory. SP-10 and SP-15 differ serologically and in several other respects, but share the ability to propagate on *B. subtilis* W-23-S^r (streptomycin-resistant) and *B. licheniformis* ATCC 9945a, and to mediate general transduction in either species when propagated homologously. Attempts to transduce between the species have failed. SP-10 forms plaques readily on both W-23-S^r and 9945a; SP-15 forms minute plaques on W-23-S^r and has shown no evidence of any lytic activity on 9945a. Maximal recoveries of prototrophic colonies from mixtures of SP-10 with auxotrophs of either W-23-S^r or 9945a were obtained only when excess phage was neutralized by post-transduction treatment with specific phage antiserum. Such treatment was not necessary for maximal recovery of transductants effected by SP-15. Unlike SP-10, SP-15 propagated on W-23-S^r did not transduce *B. subtilis* 168 (indole$^-$). SP-15 transduced *B. licheniformis* more efficiently than did SP-10. Neither phage was able to transduce *B. licheniformis* as efficiently as it transduced *B. subtilis*. The differing influences of multiplicity of infection were compared for the two phages in both species.

[1] In conducting the research reported herein, the investigators adhered to *Principles of Laboratory Animal Care* as established by the National Society for Medical Research.

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Thorne (1961, 1962) reported transduction from auxotrophy to prototrophy in *Bacillus subtilis* by a temperate bacteriophage, SP-10, isolated from soil. Phage SP-10 is also temperate for *B. licheniformis*, and we have now shown that it mediates general transduction in this species. Another phage, designated SP-15, has now been derived from the same soil sample. SP-15 differs from SP-10 by serological and certain other biological criteria, but, like SP-10, it mediates general transduction in both *B. subtilis* and *B. licheniformis*. Comparison of transduction frequencies with the two phages, the failure to transduce between the two species, and, with SP-10, recovery of transductants that are lost without post-transduction treatment with phage antiserum are the subjects of this report. Transduction in *B. subtilis* has also been reported by Takahashi (1961), Ivanovics and Csiszar (1962), and Takagi and Ikeda (1962), but transduction in *B. licheniformis* has not been reported previously.

MATERIALS AND METHODS

Organisms. The strains of *B. subtilis* were W-23-S^r (a streptomycin-resistant mutant of wild-type W-23), W-23-S^r-M4 (histidine$^-$), and 168 (indole$^-$). These are the same strains used previously in transduction studies (Thorne, 1962). The strain of *B. licheniformis* was ATCC 9945a, and the auxotrophic mutants derived from this strain (Thorne, 1962) were M1 (thiamine$^-$), M2 (lysine$^-$), M3 (lysine$^-$), M5 (uncharacterized), and M8 (methionine$^-$).

Phage SP-10 and *B. subtilis* W-23-S^r-L9, lysogenic for SP-10, were described previously (Thorne, 1962). Isolation and characteristics of the other phage, SP-15, are described under Results.

Media and cultural conditions. Minimal medium (Thorne, 1962) supplemented with 1 g of L-glutamic acid per liter was used for scoring transductants. NBY medium was composed, per liter, of

8 g of Difco nutrient broth and 3 g of Difco yeast extract. NBYG medium was NBY with 1 g of glucose per liter. When solid media were desired, 15 to 25 g of agar per liter were added. All media with agar were cooled to 46 C for pouring, and 25 ml was the volume per plate.

Cells for transduction were grown in 250-ml Erlenmeyer flasks containing 25 or 50 ml of medium. NBY broth was used for strain 168, and minimal broth plus yeast extract (3 g/liter) was used for W-23-S^r-M4. The auxotrophic mutants of *B. licheniformis* were grown on Difco nutrient broth (8 g/liter) supplemented with 5 g of glycerol per liter (autoclaved separately) and the salts of minimal medium. Flasks were inoculated with 2.5 or 5 ml of a 16-hr culture and incubated on a shaker for 5 to 7 hr at 37 C. Viable counts were made by plating on NBY agar. Difco 1% peptone (w/v) was the diluent for cells and phage.

Spores of *B. licheniformis* 9945a were prepared as described by Thorne et al. (1954). Spores of the *B. subtilis* strains and auxotrophic mutants of *B. licheniformis* were grown in potato medium (Thorne, 1962). Spores lysogenic for SP-15 were produced in 250-ml Erlenmeyer flasks containing 20 ml of PA broth (medium as used by Thorne, 1962, for assaying plage but without agar) inoculated with 0.2 ml of macerated soft agar (PA for *B. subtilis* and Difco nutrient agar for *B. licheniformis*) in which SP-15 and wild-type spores had been mixed and then incubated at 37 C for 16 hr. After 72 hr of shaken culture at 37 C, the spores that had formed were concentrated by centrifugation, heated at 65 C for 60 min to inactivate free phage and vegetative cells, and stored in mother liquor at 4 C.

Propagation of phage. Phage was prepared by methods similar to those described previously (Thorne, 1962), except that the PA broth was used for preparation of phage from lysogenic strains, and nutrient broth (8 g/liter) plus agar (7 or 25 g/liter) was used when phage was propagated on wild-type bacteria by the agar layer method. All preparations were filtered through ultrafine fritted-glass filters to remove bacteria. Phage suspensions were preserved by adding glycerol (15%, v/v) and storing at −15 C. All preparations of phage were tested for bacterial contamination by adding about 0.5 ml to a NBY agar plate and incubating at 37 C for 24 hr.

Assay for phage. SP-10 was assayed by the agar layer technique as described previously (Thorne, 1962). For SP-15, the same medium (referred to here as PA medium) was used, but the plaques were so small that a modification of the procedure was necessary. Freshly poured plates of PA agar were layered immediately before use with 3 ml of very soft PA agar (5 g/liter) containing 2×10^8 spores of *B. subtilis* W-23-S^r. Lab-Tek plastic petri-plate bottoms (100 × 15 mm; Lab-Tek Plastics Co., Westmont, Ill.) covered with glass lids provided an optically uniform bottom through which the plaques could be counted by use of 1 × objective and 12 × oculars of a dissecting microscope. Appropriately diluted phage was applied to the agar surface in 4 or 5 drops (0.005 ml) from a 0.1-ml serological pipette, and the plates were then covered with a glass lid. After 15 min, the glass lids were replaced with unglazed porcelain covers. After most of the moisture had been absorbed (30 to 60 min) at room temperature, the glass lids were placed on top of the porcelain covers, and the plates were then transferred to an incubator at 37 C. This procedure allowed maximal formation of visible plaques by conserving an optimal amount of moisture and permitting, at the same time, sufficient air exchange. The plastic, glass, or unglazed porcelain lids alone did not control these factors adequately. Plaques were counted after 20 hr. Up to 100 SP-15 plaques per 0.005 ml could be counted accurately. This procedure was also satisfactory for assaying SP-10, if the number of infective particles did not exceed 20 per 0.005 ml. Phage titers are expressed as plaque-forming units (PFU)/ml.

Phage antiserum. Antiserum to SP-10 was prepared by subcutaneous injection of rabbits with a phage preparation (1.3×10^{10} PFU/ml) propagated on *B. licheniformis* 9945a by the agar overlay method. Phage was stored frozen with glycerol until injected. A total of six injections of 5 ml each was given at 3- to 4-day intervals, and the rabbits were bled from the heart 5 days after the last injection. Highly active antiserum resulted. For antiserum to SP-15, a similar series of six injections was given, both subcutaneously and intradermally, with a preparation containing 1.3×10^9 PFU/ml. However, the resulting sera were only weakly active. Booster injections of 10^{11} PFU each were given intradermally 3 and 4 weeks after the first series, and highly active serum was collected after another week. A supply of preimmune serum was taken from each rabbit before phage was injected. The sera were sterilized by filtration through fritted glass, heated at 56 C for

30 min to inactivate complement, and stored at −15 C.

Assay of antisera. To determine whether an antiserum was of sufficiently high titer to neutralize excess phage after transduction, we combined a sample of transduction mixture with an equal volume of undiluted antiserum and placed approximately 0.001 ml on a phage assay plate seeded with spores of *B. subtilis* W-23-S^r. A control sample prepared with preimmune serum was placed on the same plate. After 16 hr at 37 C, the plates were examined for the lysis of the lawn. For qualitative tests of antiserum specificity, we added 0.1 ml of each antiserum or of the corresponding preimmune serum to 0.9 ml of suspensions of SP-10 and SP-15 containing 10^7 PFU/ml. After 10 min at room temperature, three or four tenfold serial dilutions were made, and 0.005 ml of each was spotted on a phage assay plate prepared with spores of *B. subtilis* W-23-S^r. Plates were examined for plaques after 16 and 24 hr at 37 C.

For determining *K* values of antisera, we modified the procedures of Adams (1959) and Duff and Wyss (1961). The dilution of antiserum that would inactivate 90 to 99% of the phage particles in 5, 7, or 10 min in a suspension containing a suitable number of PFU/ml was determined by adding 0.1 ml of appropriate dilutions of antiserum or preimmune serum to 0.9 ml of phage. At the end of 5, 7, or 10 min, 0.1 ml of each mixture was added to 9.9 ml of ice-cold peptone diluent, and these suspensions were assayed for active phage. Replicate experiments revealed that the degree of variation among *K* value determinations by this method was of the order of ± 10%. Phages to be compared for serological relatedness were reacted against an appropriate dilution of antiserum for a convenient time.

Transduction. Equal amounts of phage suspension and 5- to 7-hr broth cultures of recipient cells, usually in a final volume of 1 ml, were incubated in 30-ml cotton-plugged vaccine vials on a shaker for 45 min at 37 C. Peptone diluent with 15% glycerol (v/v) replaced phage in control vials and was used when diluted phage was required. When serum or deoxyribonuclease and $MgSO_4$ were included in transduction mixtures, equal amounts of peptone diluent or of distilled water, respectively, were included in control mixtures with and without phage. After incubation, undiluted or appropriately diluted samples (usually 0.1 ml) were spread on minimal agar plates that had been freshly poured and dried with unglazed porcelain covers at 46 C for 4 hr. When experiments included post-transduction treatment with antiserum, the standard procedure was to mix equal parts of serum or control reagent and the sample when spreading on the plate. Plates were incubated at 37 C and scored daily until no more colonies appeared. The low numbers of spontaneously reverted mutants to prototrophy (0 to 40/ml on control plates) were subtracted from transductant scores.

Demonstration of role of phage in mediating transduction. To eliminate transformation as a contributory mechanism toward prototroph production, either deoxyribonuclease was included in the transduction mixture according to Thorne (1962) or the phage preparations were reacted for 1 hr at 37 C with deoxyribonuclease (100 μg/ml in 0.005 M $MgSO_4$). This was usually done before the addition of glycerol for frozen storage. To ascertain that prototrophs did not appear in the absence of active phage, each host-phage system was set up at least once with phage that had been neutralized with specific antiserum and was controlled with phage in preimmune serum from the same rabbit, according to the procedure of Thorne (1962).

RESULTS

Plaque morphology. Plaques were classified as follows, according to size and appearance on W-23-S^r lawns. Type A: turbid, 0.5 to 1 mm in diameter, with dense growth in the center of a less dense ring, produced by temperate SP-10; type B: clear, of any size up to 1.3 mm in diameter, produced by virulent mutants of SP-10; type C: turbid, 0.05 to 0.1 mm in diameter, produced by SP-15.

A, B, and C are sharply different types. Usually, SP-10 assay plates contained some smaller turbid plaques (0.25 to 0.5 mm in diameter). These were small, probably because they were late in developing, since propagation of phage from single plaques of A or from the smaller plaques produced the same population pattern: predominately A, along with some of the smaller plaques and a few of type B. Propagation from single plaques of B resulted in phage that produced only plaque type B. Single plaques of C propagated poorly or not at all but, when pure populations were derived by another method, C bred true.

Isolation of SP-15. SP-10 was originally isolated

(Thorne, 1962) from a filtrate prepared after incubation of broth inoculated with soil and cells of *B. subtilis* W-23-S^r. The filtrate was tested for phage by the usual assay procedure. Three plaques of type A were selected, and phage was propagated from each of them. These phage preparations will be referred to here as I, II, and III. From preparation I, SP-10 was purified by propagating a single turbid plaque on W-23-S^r and then preparing spore stocks from lysogenic colonies isolated from the propagation broth. One of these spore stocks was called *B. subtilis* strain W-23-S^r-L9. Upon culture, this strain released only SP-10 (type A plaques with a small proportion of virulent mutant plaque type B). Further purification by serial isolation and propagation from single type A plaques altered no characteristics of the phage from W-23-S^r-L9. Several months later, phage that produced plaques of type A was purified by serial isolation and propagation from single plaques from preparation III, and was shown to be indistinguishable from SP-10 by several criteria including serological activity, transducing ability, storage stability, and characteristics of propagation.

When phage preparations I, II, and III, or unpurified progeny derived from them, were re-examined, plaques of type C, as well as type A, were found. For example, progeny from preparation III propagated on *B. licheniformis* 9945a gave initial PFU titers on W-23-S^r of 3×10^{10} type A and 5×10^7 type B plaques/ml. After storage for 1 year at 4 C, the titers were 3×10^2 type A, 0 type B, and 1.6×10^7 type C. Some of the lysed lawn showing no plaques of type A was used as seed for propagation of type C. Routine culture and harvest yielded a suspension containing 10^9 PFU of type C per ml and no type A or B. This phage, designated SP-15, readily propagated on wild-type *B. subtilis* and *B. licheniformis* to yield suspensions containing 10^9 to 10^{10} PFU/ml. Spores of the two organisms lysogenic for SP-15 were also obtained, and were convenient sources of the phage. Although SP-15 propagated on both organisms, neither visible plaques nor any evidence of lytic activity was produced on lawns of *B. licheniformis*. Therefore, *B. subtilis* was always used as the indicator.

Evidence that SP-10 and SP-15 are different phages. When phage populations derived from spores of *B. subtilis* W-23-S^r-L9 (lysogenic for SP-10) or from SP-10 purified by single-plaque passage were treated with SP-10 antiserum, no PFU remained. When mixed populations containing both SP-10 and SP-15 were treated with SP-10 antiserum, only SP-15 remained active.

Table 1 gives the results of an experiment designed to demonstrate the specificity of SP-10 and SP-15 antisera and to test whether SP-15 could be derived from SP-10 upon storage. SP-10 and progeny of phage preparation III (containing both SP-10 and SP-15) were propagated on *B. subtilis*. Assays before and after treatment with SP-10 antiserum revealed about equal numbers of PFU of types A + B (SP-10) and type C (SP-15) in the progeny from preparation III, and only types A and B in the progeny from SP-10. Samples of each suspension were stored at −15 C after addition of glycerol and at 4 C without glycerol. After 5 weeks, each of the four samples was assayed before and after treatment with SP-10 and SP-15 antisera. The results indicate that type C PFU were not present in SP-10 before storage, and they did not develop in this preparation as a result of storage. Type C was present in the other preparation before storage, and the titer of this type remained almost the same under both conditions of storage. Types A and B were present in both preparations, and their titers dropped considerably upon storage at 4 C. Antiserum to SP-10 neutralized all types A and B PFU and no type C. Antiserum to SP-15 neutralized all of type C and insignificant amounts of types A and B.

In another experiment, when SP-10 and SP-15 were assayed separately and in mixture, the expected proportions of each were detected in the mixture on the basis of differential counts of plaque types. Our conclusion is that SP-15 was present in the original filtrate derived from the soil sample but went unobserved at that time because plating conditions were inadequate for formation of type C plaques. The three plaques of type A that were isolated occurred in a lawn probably containing numerous PFU of SP-15, and some of these were inevitably contaminating the phage prepared from the type A plaques.

Results of quantitative serological tests, using phages SP-10 and SP-15 and their antisera, are given in Table 2. The data provide further evidence that the two phages are serologically distinct.

SP-10 and SP-15 responded differently to storage at 4 C. Titers of SP-10 preparations dropped rapidly when samples were stored at 4 C, but

TABLE 1. *Specificity of SP-10 and SP-15 antisera**

Phage	Temp for phage storage	Serum	PFU/ml of phage and serum mixtures		
			Plaque type A	Plaque type B	Plaque type C
	C				
Progeny of preparation III	−15	Preimmune	6.6×10^8	6.4×10^7	2.6×10^8
	4		4.4×10^6	4.5×10^5	1.8×10^8
	−15	SP-10 antiserum	0	0	2.9×10^8
	4		0	0	1.6×10^8
	−15	SP-15 antiserum	7.2×10^8	6.7×10^7	0
	4		5.8×10^6	3.8×10^5	0
SP-10	−15	Preimmune	5.9×10^8	7.1×10^6	0
	4		4.8×10^6	2.6×10^4	0
	−15	SP-10 antiserum	0	0	0
	4		0	0	0
	−15	SP-15 antiserum	4.3×10^8	2.2×10^6	0
	4		5.2×10^6	2.1×10^4	0

* Phage was stored for 5 weeks in peptone diluent at 4 C and in peptone diluent with 15% glycerol (v/v) at −15 C. A mixture of 0.9 ml of undiluted phage and 0.1 ml of serum was held for 10 min at room temperature before it was diluted and plated in 0.005-ml amounts on lawns of *B. subtilis* W-23-S^r. The titers of the frozen samples were adjusted for the dilution factor introduced by addition of glycerol.

TABLE 2. *Cross-neutralization tests between phages SP-10 and SP-15 and their antisera*

Phage	Dilution of serum	Reaction		Phage titer (PFU/ml)			Neutralization	
		Time	Temp	Control in preimmune serum	In antiserum to		Per cent	K*
					SP-10	SP-15		
		min	C					
SP-15	10	10	37	3.9×10^6	1.6×10^5		96	3
SP-15	100	10	37	5.0×10^6	4.0×10^6		20	+†
SP-15	500	5	37	8.6×10^6	8.5×10^6		0	+
SP-10	2000	10	37	6.7×10^6	1.8×10^5		97	719
SP-10	1000	10	25	1.1×10^7	1.0×10^5		99	460
SP-15	1000	10	25	5.0×10^6	2.6×10^6		48	+
SP-15	4000	7	25	1.2×10^9		5.3×10^7	96	1781
SP-10	4000	7	25	3.0×10^6		3.2×10^6	0	+
SP-10	40	7	25	3.0×10^3		1.4×10^3	53	+

* See text for method of determining K, the velocity constant of phage neutralization with antiserum.

† Expressed as per cent inactivation outside the range (90 to 99) for which equation for calculation of K is valid.

titers were maintained when samples were stored frozen with glycerol. On the other hand, SP-15 was quite stable under both of these storage conditions.

Inhibition of growth of transductants. During early experiments that established the ability of SP-10 to carry out general transduction from auxotrophy to prototrophy in *B. licheniformis*, several observations suggested that the presence of excess phage inhibited the appearance of trans-

ductant colonies on the minimal agar plates. Appropriate experiments revealed that most prototrophic cells did not survive, or at least did not produce colonies, when plated in mixture with phage and auxotrophs. One preliminary experiment with appropriate mixtures of *B. licheniformis* 9945a, 9945a M2 (lysine$^-$), phage SP-10, preimmune serum, and antiserum to SP-10 provided these facts. (i) SP-10, incubated for 45 min at 37 C with a mixture of 10^9 auxotrophs and 10^3 prototrophs, greatly reduced or entirely suppressed the number of prototrophs able to form colonies on minimal agar. (ii) Antiserum added to phage before the cells were introduced allowed the prototrophs to form colonies, and, as was to be expected, eliminated transductants. Table 3 shows the results of another experiment, which demonstrated the following. (i) Large numbers of auxotrophic cells in the absence of phage did not interfere with the ability of prototrophs to produce colonies promptly. These colonies were the number expected, and formed the mounds of glutamyl polypeptide characteristic of strain 9945a on this medium. (ii) The addition of SP-10 delayed the appearance of prototrophs and reduced their number. (iii) Post-transduction incubation with 0.5 ml of SP-10 antiserum permitted all the expected prototrophs and some transductants to prototrophy to appear. Similar treatment with preimmune serum did not have this effect. Another experiment demonstrated that, when 10^9 PFU of SP-10 were mixed with 5.6×10^2 prototrophic cells of *B. licheniformis* 9945a (auxotrophic cells were omitted) and the mixture was shaken at 37 C for 45 min, the expected number of colonies appeared promptly on minimal agar. However, most of the colonies failed to produce glutamyl polypeptide and were of the phenotype associated with colonies of 9945a lysogenic for SP-10. It was obvious that transduction frequencies under varying experimental conditions could not be evaluated without knowledge of influence of excess phage on recovery of transductants.

Effect of post-transduction treatment with antiserum. With *B. licheniformis* 9945a M2, we performed eight experiments in which mixtures of SP-10 and cells were treated after transduction with SP-10 antiserum or with peptone broth. (Preimmune serum was not used as the control reagent because its presence enhanced the inhibition phenomenon.) For the 48-hr scoring of plates, antiserum permitted 4- to 28-fold more transductant colonies to develop than did peptone; at the end of the experiments (usually 150 hr), the difference was 3- to 18-fold. In 27 experiments with *B. subtilis* W-23-S^r-M4 and SP-10, the difference with antiserum was 2- to 12-fold at 48 hr and at the end of the experiments. Data from typical experiments with the two species are shown in Fig. 1 and 2. In three experiments with *B. subtilis* 168 and SP-10, no effect of post-transduction treatment with antiserum was observed: no new colonies arose after 24 hr, and the total number of transductants was the same with peptone as with antiserum. As will be documented later, this inhibition did not occur with either species when transductions were mediated by SP-15.

TABLE 3. *Inhibition of growth of Bacillus licheniformis prototrophs in the presence of auxotrophs and SP-10 phage*

Additions to cell mixture*		Prototrophs/ml scored at		
SP-10†	Serum‡	24 hr	48 hr	144 hr
−	Peptone	640	640	650
+	Peptone	0	70	250
+	Preimmune	0	90	120
+	SP-10 antiserum	730	760	760

* Cells (1.5×10^9) of auxotroph 9945a M2 (lysine$^-$) and 5.2×10^2 strain 9945a prototrophic cells were shaken with or without phage at 37 C for 45 min in a total volume of 1 ml.

† Where indicated, 8.1×10^8 PFU of phage were added.

‡ Either serum or peptone diluent was added (0.5 ml) at the end of the shaking period, and, after 15 min, 0.15-ml samples were plated on minimal agar.

When excess SP-10 phage was neutralized after transduction so that transductant colonies began to appear promptly on minimal agar plates, new transductant colonies continued to appear for as long as 150 hr after plating (Fig. 1 and 2). This is in contrast to the results obtained with phage SP-15. When transduction was mediated by this phage, colonies developed promptly (85 to 100% in 24 hr), and the results were not changed by plating with antiserum. This delayed appearance of transductants when phage SP-10 was used was not caused by transduction on the plate because it was most pronounced when the ratio of antiserum to phage was highest. It could not be attributed to "feeder" colonies because late transductants appeared at random on sparsely populated plates, and because they grew promptly and vig-

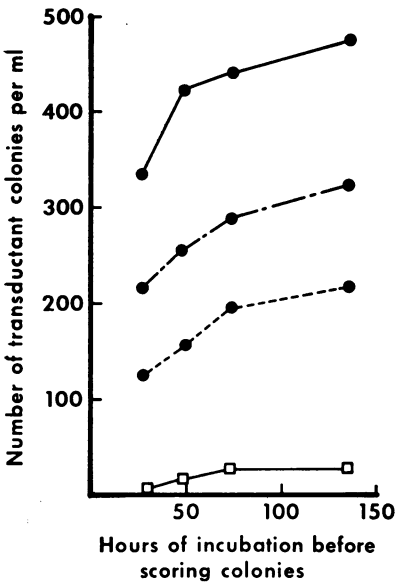

FIG. 1. *Effect of post-transduction treatment with phage antiserum on yield of Bacillus licheniformis transductants. Phage SP-10 was mixed with 1.5 × 10^9 cells of 9945a M2 (lysine⁻) in a final volume of 1 ml, and the mixture was shaken for 45 min at 37 C. Before plating, each mixture was combined 1:1 with undiluted antiserum or with peptone diluent and held 15 min at 37 C. Then 0.2 ml was spread on minimal agar plates in triplicate. Symbols:* ● = *plated with antiserum;* □ = *plated with peptone diluent. Three multiplicities of infection were used:* —— = *1.4;* — — — = *0.7;* - - - - = *0.4.*

orously when subcultured onto minimal agar. We believe that we ruled out transformation on the plate by transducing with phage that had been treated previously with deoxyribonuclease, as well as by adding deoxyribonuclease to the antiserum for post-transduction treatment. Moreover, recent work of Takahashi (1962) demonstrated that when auxotrophs of *B. subtilis* became competent for transformation on agar plates the competence was transient and at a maximum 150 min after the cells were spread.

Comparison of transduction of B. subtilis with phages SP-10 and SP-15. Table 4 gives a compilation of results from several experiments comparing frequencies of transduction of *B. subtilis* mediated by SP-10 and SP-15. As was shown previously (Thorne, 1962), SP-10 transduced strain 168 (indole⁻) as well as auxotrophs of strain W-23-S^r. SP-15, however, transduced the auxotroph of W-23-S^r but did not transduce strain 168. The highest frequencies for transduction of W-23-S^r-M4 were 6.8 × 10^{-6} per PFU for SP-10 and 2.7 × 10^{-6} per PFU for SP-15. The multiplicity of infection (ratio of PFU to cells placed in transduction flasks) was more critical for SP-15 than for SP-10. With SP-10, frequencies of transduction were comparable when multip icities of infection of 0.9, 1.2, and 6.5 were used. With SP-15, the frequency was greatly reduced when the multiplicity of infection was high. Experiments involving SP-15 obtained from lysogenic spore cultures did not show as efficient transduction as those in

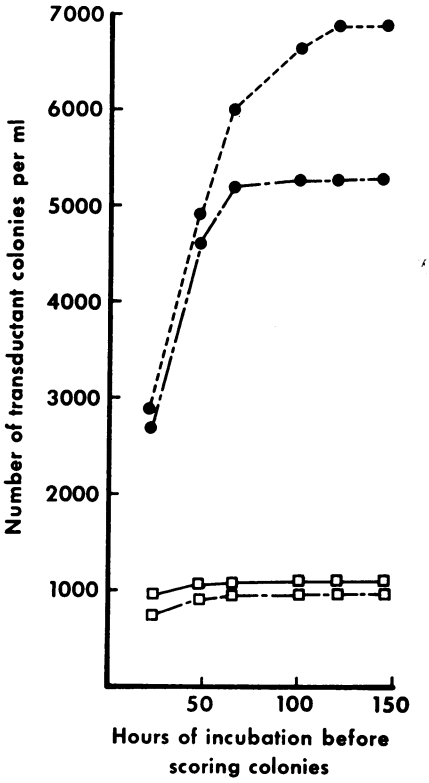

FIG. 2. *Effect of post-transduction treatment with phage antiserum on yield of Bacillus subtilis transductants. Phage SP-10 (10^9 PFU) and 1.6 × 10^8 cells of W-23-S^r M4 (histidine⁻) were mixed in a volume of 1 ml and shaken for 45 min at 37 C. Samples (0.1 ml) were plated undiluted or at dilutions of 1:2 or 1:10 on minimal agar. Either 0.1 ml of SP-10 antiserum or peptone diluent was spread on the plate with the sample. Symbols:* ● = *plated with antiserum;* □ = *plated with peptone diluent;* —— = *plated undiluted;* — — — = *diluted 1:2;* - - - - = *diluted 1:10. Transductants/ml were adjusted for the volume and dilution plated.*

which the phage was propagated on wild-type W-23-S^r. The nature of this difference has not been studied. Table 4 also shows the effect of post-transduction treatment with antiserum on the number of transductant colonies: with SP-10, the number of colonies was increased by the use of antiserum, but with SP-15 such use of antiserum did not affect the yield. Treatment of phage with antiserum before mixing with cells prevented transduction. Treatment of phage with deoxyribonuclease or addition of deoxyribonuclease to transducing mixtures did not affect the number of transductants obtained with either of the two phages.

Transduction of B. licheniformis by phages SP-10 and SP-15. Transduction by SP-10 is shown in Table 5. All five of the auxotrophic mutants were transduced to prototrophy at comparable frequencies. Experiments with M2 (lysine$^-$) revealed that, although lower multiplicities of infection tended to give higher frequencies of transduction per PFU, the frequencies did not vary more than about twofold over a range of multiplicities of infection varying from 0.7 to 8. The highest frequency obtained (1.6×10^{-7} per PFU) was lower by a factor of about 40 than the highest frequency obtained in similar experiments with SP-10 and *B. subtilis* (Table 4). Table 5 also demonstrates the effect of post-transduction treatment with SP-10 antiserum on the number of transductant colonies. The addition of antiserum to phage before mixing with cells prevented transduction.

Transduction of *B. licheniformis* 9945a M2 by phage SP-15 is shown in Table 6. In other experi-

TABLE 4. *Comparison of transduction of Bacillus subtilis by phages SP-10 and SP-15*

Recipient cells		Phage	Ratio of PFU to cells	Transductants	
Strain	No. ($\times 10^8$)/ml			Per ml	Per PFU
W-23-S^r M4	7.0	SP-10	0.9	1760	2.7×10^{-6}
	6.2	SP-10	1.2	2180	2.9×10^{-6}
	6.2	SP-10	1.2	5100*	6.8×10^{-6}*
	1.6	SP-10	6.5	610	6.1×10^{-7}
	1.5	SP-10	6.5	6270*	6.3×10^{-6}*
168	6.5	SP-10	1.0	690	1.1×10^{-6}
	5.5	SP-10	1.4	1285	1.7×10^{-6}
	5.5	SP-10	1.4	1195*	1.6×10^{-6}*
W-23-S^r M4	7.0	SP-15†	0.7	1330	2.7×10^{-6}
	7.0	SP-15†	1.0	1090	1.6×10^{-6}
	6.7	SP-15	0.9	207	3.5×10^{-7}
	6.7	SP-15	1.8	0‡	0‡
	6.7	SP-15	1.8	143	1.2×10^{-7}
	6.7	SP-15	1.8	93*	7.8×10^{-8}*
	6.7	SP-15	5.4	77	2.1×10^{-8}
	6.7	SP-15	9.0	93	1.6×10^{-8}
168	14.0	SP-15†	0.4	0	0
	7.0	SP-15†	1.2	0	0

* From the same experiment as shown on line above, but samples were plated with an equal volume of antiserum specific for the phage.

† Indicates that the phage was propagated on wild-type strain W-23-S^r. All other phage preparations were filtrates of broth-cultured spores of W-23-S^r lysogenic for SP-10 or SP-15.

‡ Phage was neutralized with antiserum before mixing with cells.

TABLE 5. *Transduction of Bacillus licheniformis by phage SP-10**

Recipient cells		Ratio of PFU to cells	Transductants	
Strain	No. ($\times 10^8$)/ml		Per ml	Per PFU
9945a M1	8.8	8.0	480	7×10^{-8}
9945a M2	8.8	8.0	503	7×10^{-8}
9945a M3	12.6	5.4	483	7×10^{-8}
9945a M5	8.4	8.3	440	6×10^{-8}
9945a M8	10.0	7.0	370	5.3×10^{-8}
9945a M2	11.0	2.7	320	1.1×10^{-7}
	11.0	1.4	165	1.1×10^{-7}
	11.0	0.7	134	1.6×10^{-7}
	5.5	5.4	195	6.5×10^{-8}
	5.5	2.8	125	8.3×10^{-8}
	5.5	1.4	120	1.6×10^{-7}
9945a M2	15.0	4.0	26	4.3×10^{-9}†
	15.0	4.0	404	6.7×10^{-8}
	15.0	2.0	286	9.5×10^{-8}
	15.0	1.0	220	1.5×10^{-7}

* Phage was propagated on wild-type 9945a by agar layer method. Titrations for PFU were with *B. subtilis* W-23-S^r. With the exception of the one example shown, excess phage was neutralized after transduction by mixing with an equal volume of phage antiserum.

† Plated from same vial as transductants in the line below, but plating was with peptone diluent instead of antiserum.

ments, the four other auxotrophic mutants of *B. licheniformis* (M1, M3, M5, and M8) were transduced at the same frequency as M2. The frequencies of transduction obtained with SP-15 were higher than those obtained with SP-10. However, *B. licheniformis* was transduced at a lower frequency by each of the phages than was *B. subtilis* W-23-S^r. With SP-15, post-transduction treatment with antiserum was not necessary for the formation of maximal number of transductant colonies. Treatment of phage with deoxyribonuclease or addition of deoxyribonuclease to transduction mixtures did not affect the number of *B. licheniformis* transductants obtained with either phage. Control experiments showed that treatment of SP-15 with antiserum rendered it ineffective in transduction.

TABLE 6. *Transduction of Bacillus licheniformis by phage SP-15**

Recipient cells		Ratio of PFU to cells	Transductants	
Strain	No. ($\times 10^8$)/ml		Per ml	Per PFU
9945a M2	11.0	1.10	840	7.3×10^{-7}
	11.0	0.54	287	5.0×10^{-7}
	11.0	0.27	217	7.5×10^{-7}
	11.0	0.14	80	5.6×10^{-7}
9945a M2	18.0	1.6	1090	3.8×10^{-7}
	18.0	1.6	1190	4.1×10^{-7}†
	18.0	1.6	0‡	0‡

* Phage was propagated on wild-type 9945a by agar layer method. Titrations for PFU were with *B. subtilis* W-23-S^r. The transduction mixtures were plated without antiserum, with the exception of the one example shown.

† Plated from same vial as transductants in line above, but with an equal volume of SP-15 antiserum.

‡ Phage was neutralized with antiserum before mixing with cells.

Table 7 summarizes the evidence that phages SP-10 and SP-15 mediated general transduction from auxotrophy to prototrophy in *B. licheniformis*, and that either phage propagated on an auxotroph transduced another auxotroph but not the one on which it was propagated.

Failure of cross transduction. Under optimal conditions of phage and cell concentration and recovery of transductants, we attempted without success to transduce between the two species. In each experiment, cells were successfully transduced by phage propagated on the homologous prototroph, but no transductants were obtained when either phage propagated on one of the species was reacted with auxotrophic cells of the other species. Several different auxotrophs of each species were tested.

DISCUSSION

The two phages, SP-10 and SP-15, share the ability to mediate general transduction from auxotrophy to prototrophy in mutants of *B. subtilis* W-23-S^r and *B. licheniformis* 9945a. SP-10, but not SP-15, also transduced strain 168 of *B. subtilis*. This, in itself, is evidence that the two phages are different from each other. Other evidence includes differences in plaque morphology, serological activity, storage stability, and the manner in which excess phage (SP-10 but not SP-15) influenced recovery of transductants.

Two examples given here show that a test for plaque formation is not a suitable screening method for transducing systems. SP-10 neither

TABLE 7. *General transduction in Bacillus licheniformis with SP-10 and SP-15*

Strain for phage propagation	Recipient strain (auxotrophs)	Phage	Transduction*
9945a	9945a M1, 2, 3, 5, 8	SP-10	+
9945a	9945a M1, 2, 3, 5, 8	SP-15	+
9945a M8 (met$^+$)†	9945a M8 (met$^-$)	SP-15	+
9945a M2 (lysine$^+$)†	9945a M2 (lysine$^-$)	SP-10	+
9945a M2 (lysine$^-$)	9945a M2 (lysine$^-$)	SP-10	−
	9945a M8 (met$^-$)	SP-10	+
	9945a M8 (met$^-$)	SP-15	+
	9945a M5 (?$^-$)	SP-15	+
	9945a M2 (lysine$^-$)	SP-15	−

* Plus indicates that transduction occurred, minus that transduction did not occur.

† Rendered prototrophic by transduction.

propagated nor produced plaques on *B. subtilis* 168, yet it transduced this strain when propagated on strain W-23-S^r. On the other hand, SP-15 propagated well on *B. licheniformis* and transduced auxotrophic mutants, but it did not produce visible plaques at high dilutions, nor show lytic activity at low dilutions, on lawns of this species under several conditions tested.

The failure to achieve cross transduction between *B. subtilis* and *B. licheniformis* is perhaps not surprising, since the two species differ with respect to the base composition of their deoxyribonucleic acid (Marmur and Doty, 1962).

ACKNOWLEDGMENTS

The graciousness of R. A. Altenbern in giving of his time and advice during discussions concerning this work is deeply appreciated.

The assistance of Samuel C. Hamilton is gratefully acknowledged.

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