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Transduction in *Bacillus subtilis* by Bacteriophage SPP1

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Lysates of the virulent bacteriophage SPP1 were shown to be capable of mediating generalized transduction. Suppressible mutants of this bacteriophage (*sus*) were capable of transduction at a lower multiplicity of infection than virulent SPP1. Linkage analysis demonstrated that bacteriophage SPP1 transduced segments of the genome equal in size to that transferred by SP10. This bacteriophage should be useful in analyzing the regions of the genome where PBS1 appears to give anomalous results.

The ability of certain bacteriophages to act as vectors in the transfer of bacterial genes was observed initially by Zinder and Lederberg in 1952 (27). The division of transduction into two classes (generalized and specialized) is based on the number and location of the bacterial genes that can be mobilized (11). In specialized transduction, only a limited number of bacterial genes are transferred and these are generally very close to the prophage attachment site on the bacterial chromosome. In generalized transduction, however, all or at least a wide range of bacterial genes can be mobilized by the infecting bacteriophage (11). Although some generalized transducing particles contain bacteriophage DNA (11, 14), the majority carry only bacterial DNA (6, 11, 13, 22).

In *Bacillus subtilis*, generalized transduction has been performed primarily by bacteriophages SP10 and PBS1 (20, 21, 26; *In* R. C. King, ed., *Handbook of Genetics*, in press), whereas specialized trandsuction has only recently been reported for temperate bacteriophage ϕ105 (J. A. Shapiro, D. H. Dean, and H. O. Halvorson, Abstr. Annu. Meet. Amer. Soc. Microbiol. G225, p. 57, 1974). In our present study, we report the use of *B. subtilis* bacteriophage SPP1 as a generalized transducing particle.

MATERIALS AND METHODS

Strains and methods of propagation. The bacterial strains utilized are listed in Table 1. Lysates of bacteriophage PBS1 were prepared as previously described (9, 25, 26; King, ed., *Handbook of Genetics*, in press). To prepare lysates of bacteriophage SP10, RUB815 was grown at 37 C in M broth to an optical density of 50 Klett Units (Klett-Summerson colorimeter, filter 66) and this culture was infected at a multiplicity of infection (MOI) of 10. Incubation was continued at 37 C with aeration until lysis occurred. The lysates were centrifuged at 1,200 rpm in a Sorvall SP type centrifuge to remove cellular debris. The supernate was treated with DNase I (50 μg/ml, Worthington Biochemical Corp., Freehold, N.J.) and filtered through a type HA membrane filter (0.45 μm, Millipore Corp., Bedford, Mass.). Lysates of bacteriophage SPP1 were made either by the plate method (23) or by infecting a culture of *B. subtilis* in M broth (optical density of 50 Klett Units) with a MOI of 100 at 37 C. The lysate was processed as described for SP10. Bacteriophages SP10 and SPP1 were assayed on M agar in a 2-ml overlay of semisolid M agar with strains RUB815 and RUB818 as indicators, respectively. PBS1 was assayed as previously described (9).

Media. Penassay broth (PB, antibiotic medium no. 3, Difco) was used in growth experiments. M broth contains per liter of water: tryptone (Difco), 10 g; yeast extract (Difco), 5 g; and sodium chloride, 9.9 g. The M broth was supplemented with magnesium chloride (5 mM), calcium chloride (5 mM), and manganese chloride (0.5 mM). M semisolid agar was made by adding 7 g of agar to 1 liter of M broth. Minimal medium consisted of Spizizen minimal salts (19) supplemented with glucose (22 mM) and 1.4% agar. Bacterial cultures were maintained on tryptose blood agar base.

Transduction. The recipient cells were grown in PB at 37 C with vigorous aeration (250 rpm) to an optical density of approximately 130 Klett Units. The MOI was usually 1 to 5 for PBS1, 5 for SP10, and 30 for SPP1. Infected bacteria were incubated for 10 min at 37 C, centrifuged in a Sorvall SP type centrifuge at 1,200 rpm, resuspended in minimal salts (twice the original volume), centrifuged, and resuspended in minimal salts (half the original volume). The bacteria were plated on minimal agar supplemented with amino acids (16 μg/ml) and incubated at 37 C for 36 to 48 h. In addition to the supplements of amino acids, it was found that 0.1 ml of a 0.5% solution of casein hydrolysate (Nutritional Biochemical Co., Cleveland) resulted in an increased number of transductants. This amount of casein hydrolysate stimulated the growth of transductants but cannot support the growth of auxotrophs. The transductants were picked, placed on selective media, and again incubated at

TABLE 1. *Strains of Bacillus subtilis*

Strains	Genotype[a]	Remarks
W23	Wild type	Obtained from K. Bott, sensitive to SP10
RUB815	*Ery*[b]	Isolated from W23[c]
BR85	*trpC2, argC4*	Obtained from B. Reilly
BR151	*lys-3, trpC2, metB10*	Standard competent strain used in our laboratory
TV173	*hisB, metB10, su-3*	Obtained from M. J. Tevethia
RUB805	*hisB, metB10, su-3, Ery*	Isolated from TV173
RUB806	*lys-3, trpC2, metB10, su-3, Ery*	Transformation of BR151 with DNA from RUB805 using congression (10)
RUB818	Wild type	Three separate transformations of BR151 with DNA from a wild-type 168
RUB826	*Ery, su-3*	Transformation of RUB818 with DNA from RUB806
RUB834	*aroB, trpC2, hisB, tyrA, metB10*	Transformation of BR151 with DNA from SB202 obtained from A. T. Ganesan
RUB836	*trpC2, metB10, hisA1*	Transformation of BR151 with DNA from RUB1402
RUB843	*trpC2, hisA1, argC4*	Transformation of RUB836 with DNA from BR85
RUB1402	*dal, hisA1, metB10, leu-8*	Obtained from M. Dul

[a] Symbols as previously described (26).
[b] Erythromycin resistant.
[c] Spontaneously arose on a tryptose blood agar base plate (Difco) supplemented with 80 μg of erythromycin.

37 C for 24 h before determining the extent of linkage between selected and nonselected markers.

Mutagenesis. Suppressible mutants of bacteriophage SPP1 were obtained essentially by the method described by Spatz and Trautner (18). The bacteriophage were incubated at 37 C for 30 h in 0.1 M sodium phosphate buffer (pH 6.0) containing 1 mM EDTA (Sigma Chemical Co., St. Louis) and 0.4 M hydroxylamine (hydroxylamine hydrochloride, Mallinckrodt Chemical Works, St. Louis). The bacteriophage were appropriately diluted and plated on indicator strain RUB806. The top soft agar from the dilution that yielded almost confluent lysis (more than 2,000 plaque forming units) was suspended in PB, collected, centrifuged, treated with DNase, and filtered as described in the section on strains and methods of propagation. Approximately 3% of the bacteriophage could lyse the permissive strain RUB806, but not the nonpermissive strain BR151.

RESULTS

Transduction with SPP1. Lysates of virulent bacteriophage SPP1 and suppressible mutants of this bacteriophage were utilized for transduction at various MOI. Optimal transduction was accomplished with a MOI of approximately 30 and 9 for SPP1 and SPP1*sus6*, respectively (Fig. 1). The efficiency of transduction for the *trpC2* marker was 10^{-8} for bacteriophage SPP1 and 10^{-7} for the suppressible mutant SPP1*sus6*. The ability of the transductants to survive in the presence of excess virulent bacteriophage (MOI of greater than 100) is an interesting observation that will be discussed later.

Studies with bacteriophages SP10 and PBS1 have shown that these two bacteriophages are

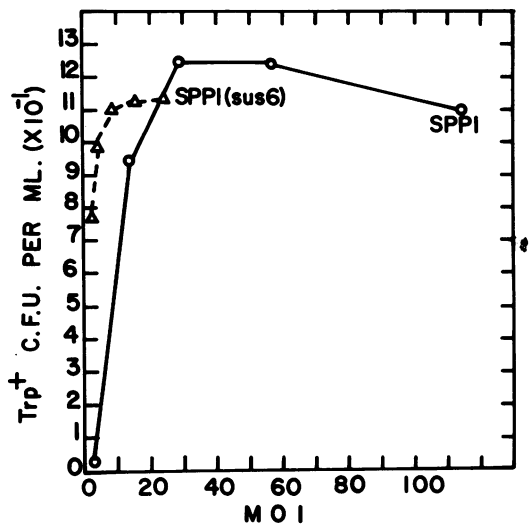

FIG. 1. *The number of Trp⁺ transductants obtained after incubation of strain BR151 with lysates of SPP1 (○) and SPP1sus6 (△) at different MOI as described.*

capable of establishing a "pseudolysogenic" or carrier state in *B. subtilis* (1, 3, 7). The bacteria in the carrier state continue to release bacteriophage, although they are resistant to further infection from the same bacteriophage. Eventually, the population becomes sensitive to infection and lysis (1, 3, 7). The carrier state, therefore, differs from lysogeny in the permanence of the relationship and in the lack of recombination between the bacterial chromosome and the viral genome that occurs in most

lysogenic systems (2). To investigate whether a carrier state exists after infection with bacteriophage SPP1, Trp^+ transductants from the experiment in Fig. 1 were grown in minimal medium supplemented with 1% casein hydrolysate (tryptophan free) until they ceased exponential growth. These bacteria were then tested for sensitivity to bacteriophage SPP1. All 10 of the transduced clones tested were as sensitive to the bacteriophage as the parental strain BR151.

Growth of B. subtilis after infection with bacteriophage SPP1. Cultures of *B. subtilis* growing in M broth at 37 C were infected with bacteriophage SPP1 at a MOI of 100 and 200. Growth was followed turbidimetrically with a Klett-Summerson colorimeter. The results from this type of infection experiment reveal that the infected cultures begin to lyse approximately 30 min after infection; however, the infected cultures increase in optical density after an additional 60 min (Fig. 2). The growth rate in the infected culture subsequently approximates the growth rate of the uninfected population. Bacteria isolated from the culture infected at a MOI of 200 were tested for their resistance to SPP1. Nine of the ten clones isolated were sensitive to SPP1. The one resistant clone failed to adsorb the bacteriophage.

Adsorption of bacteriophage SPP1 to B. subtilis. Strain BR151 was grown in PB, using the same procedures described for transduction in Materials and Methods. Bacteria were infected at a MOI of 4×10^{-6} with bacteriophage SPP1, incubated for 15 min at 37 C, centrifuged at 8,000 $\times$ *g* for 1 min, and the residual bacteriophage in the supernate was assayed as described. At this low MOI, 67% of bacteriophage SPP1 adsorb to strain BR151 (Table 2).

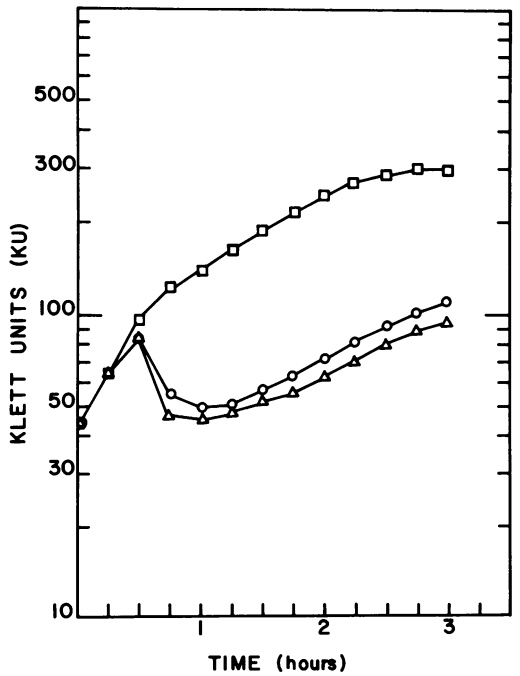

FIG. 2. *Growth of strain BR151 in PB after the addition of broth (□) or bacteriophage SPP1 at MOI of 100 (○) and 200 (△).*

Linkage studies. Representative bacterial strains were used to determine the relative linkage obtained by transduction with SPP1, SPP1*sus6*, SP10, and PBS1. The linkage between *lys-3* and *trpC2* obtained with SP10 transduction of strain BR151 was slightly greater than that obtained with SPP1 (Table 3). No linkage was obtained between *trpC2* and *metB10* with SPP1, whereas these two markers demonstrated a cotransfer frequency of 3.8% in SP10-mediated transduction. Further linkage studies, using other recipient strains and additional genetic markers (Table 4), indicate that bacteriophages SP10, SPP1, and SPP1*sus6* are capable of transducing approximately the same size of bacterial chromosome, whereas bacteriophage PBS1 transduces a significantly larger piece of DNA. We were unable to detect linkage of markers *hisA1* and *argC4* with PBS1 transduction, even though these two markers were linked when bacteriophage SPP1 was used as the transducing agent.

DISCUSSION

Generalized transduction in B. subtilis. Bacteriophages SP10 and PBS1 have been most widely used in transduction studies in *B. subtilis*. However, these two bacteriophages

TABLE 2. *Adsorption of bacteriophage SPP1 to BR151*

Expt no.	PFU[a]/ml		Adsorption (%)
	Control[b]	Experimental[c]	
1	192	65	
	231	72	67.6
2	185	66	
	180	57	66.3

[a] Plaque-forming units.

[b] PB (1 ml) was added to approximately 2,000 PFU/ml. The mixture was incubated for 15 min at 37 C and the number of PFU was determined.

[c] Between 4×10^8 and 5×10^8 bacteria (BR151 grown in PB) were added to PB containing approximately 2,000 PFU/ml. The mixture was incubated at 37 C for 15 min and the number of PFU was determined.

TABLE 3. *Transduction of B. subtilis strain BR151*

Transducing bacteriophage	Strain	Selected marker	Unselected marker	Cotransfer[a] (%)
SPP1	RUB818	*trpC2*	*metB10*	0/260 (0)
SPP1	RUB818	*trpC2*	*lys-3*	2/260 (0.8)
SPP1*sus 6*	RUB826	*trpC2*	*metB10*	0/104 (0)
SPP1*sus 6*	RUB826	*trpC2*	*lys-3*	2/104 (1.9)
SP10	RUB815	*trpC2*	*metB10*	4/104 (3.8)
SP10	RUB815	*trpC2*	*lys-3*	3/104 (2.9)
PBS1	RUB818	*trpC2*	*metB10*	145/260 (55.8)
PBS1	RUB818	*trpC2*	*lys-3*	171/260 (65.8)

[a] The number of the transductants carrying the unselected marker, numerator, the total number of transductants, denominator, and percentage of cotransfer, (parentheses) are provided for each cross.

TABLE 4. *Transduction with bacteriophages SPP1, SPP1sus-6, PBS1, and SP10*

Transducing bacteriophage	Strain	Recipient strain	Selected marker	Unselected marker	Cotransfer[a] (%)
SPP1	RUB818	RUB843	*argC4*	*hisA1*	11/260 (4.2)
PBS1	RUB818	RUB843	*argC4*	*hisA1*	0/260 (0)
SPP1	RUB818	RUB834	*tyrA*	*trpC2*	298/312 (95.5)
PBS1	RUB818	RUB834	*tyrA*	*trpC2*	311/312 (99.6)
SPP1	RUB818	RUB834	*tyrA*	*metB10*	0/312 (0)
PBS1	RUB818	RUB834	*tyrA*	*metB10*	187/312 (59.9)
SPP1	RUB818	RUB834	*trpC2*	*hisB*	151/156 (96.8)
SPP1*sus 6*	RUB826	RUB834	*trpC2*	*hisB*	98/104 (94.2)
SP10	RUB815	RUB834	*trpC2*	*hisB*	94/104 (90.4)
PBS1	RUB818	RUB834	*trpC2*	*hisB*	156/156 (100)

[a] The number of the transductants carrying the unselected marker, numerator, the total number of transductants, denominator, and percentage of cotransfer, (parentheses) are provided for each cross.

possess certain limitations. Bacteriophage PBS1 is flagella specific and does not lyse strains of *B. subtilis* exceptionally well (9, 20, 26; King, ed., *Handbook of Genetics*, in press). Therefore the use of bacteriophage PBS1 is limited to highly motile recipient cells and by the difficulties involved in obtaining high titer lysates. In addition, PBS1 is not satisfactory for fine structure analysis. Bacteriophage SP10, on the other hand, is smaller and would lend itself readily to fine structure analysis. However, bacteriophage SP10 cannot normally replicate in *B. subtilis* 168 and must be propagated on *B. subtilis* W23 (21). Therefore, all SP10-mediated transduction of *B. subtilis* 168 (the most competent strain of *B. subtilis*) must involve heterologous DNA (26; King, ed., *Handbook of Genetics*, in press). Bacteriophage SPP1 can be utilized as a generalized transducing particle, and it can be grown readily on *B. subtilis* 168 and its derivatives (12). A distinct further advantage is that this bacteriophage can successfully infect bacteria lacking glucosylated teichoic acid (R. E. Yasbin, V. C. Maino, and F. E. Young, manuscript in preparation). Therefore, bacteriophage SPP1 can be used in the fine structure mapping in the region of the chromosome where the genes that determine glucosylation of the teichoic acid appear to cluster (25, 26). Previously, consistent mapping by transduction in this region has not been possible due to the inability of bacteriophage SP10 to attach to these bacterial mutants and to certain inconsistencies in PBS1-mediated transduction (5, 24–26; King, ed., *Handbook of Genetics*, in press).

From the linkage studies in Tables 3 and 4 and from previously published reports (26; King, ed., *Handbook of Genetics*, in press), it has been shown that bacteriophage PBS1 is capable of transducing between 5 to 8% of the bacterial chromosome, whereas bacteriophages SP10 and SPP1 transduce approximately 1% of the same chromosome. However, as mentioned earlier, we have demonstrated linkage of *hisA1* and *argC4* (4.2%) with SPP1-mediated transduction, whereas no linkage was observed in PBS1-mediated transduction. In earlier studies with bacteriophage PBS1, it was noted that linkage between *hisA1* and *argC4* could only occur in some of the constructed strains (25). Replication analysis indicated that the order of replication of markers was *thr*, *cysB*, *hisA1*, and *argC4* (5). On the other hand, some recent

studies by Lepesant-Kejzlarová et al. (8) suggested that *hisA1* is linked to *narA1*. These results require a major translocation of some markers on the map of *B. subtilis* such that *hisA1* replicates before *cysB* and *thr*. Linkage studies utilizing the SPP1 transduction system may be helpful in resolving these discrepancies.

This additional transducing system enlarges the scope of genetic manipulations with *B. subtilis*. Additionally, the discovery that transduction can be achieved with bacteriophage SPP1 and coliphage T1 (4) raises the possibility that many more virulent bacteriophages may be capable of transduction. Drexler found that he could achieve transduction with bacteriophage T1 by using suppressible mutants (4). Similarly, we have noticed that the efficiency of transduction with bacteriophage SPP1 can be increased by the use of suppressible mutants (Fig. 1). We have examined the transduction efficiency of three separate mutants of bacteriophage SPP1 that have been shown to be different by complementation (A. T. Ganesan, personal communication). The results presented with the mutant SPP1*sus6* are representative.

Surprisingly, we have also been able to demonstrate transduction with wild-type bacteriophage SPP1. These results indicate that the infected bacteria must be able to survive even in the presence of excess bacteriophage. The reason for this survival is presently not understood, but may be attributable to a failure of the majority of the bacteriophage to attach successfully to the bacteria. Even at low multiplicities of infection, bacteriophage SPP1 does not completely attach to the bacteria under the conditions used for transduction (Table 2). In any case, the development of a transducing system from a virulent bacteriophage has some important implications. Schmieger (15–17) has obtained mutants of bacteriophage P22 altered in their ability to act as transducing vectors. In these mutants, the ratio of transducing particles to bacteriophages increases or decreases depending on the nature of the mutant (16, 17). Our results, together with these other studies, suggest that most bacteriophages can be developed into efficient transducing systems by the isolation of appropriate mutations.

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