JOURNAL OF BACTERIOLOGY, June 1985, p. 1238–1243
0021-9193/85/061238-06$02.00/0

Vol. 162, No. 3

Plasmid Transduction by *Bacillus subtilis* Bacteriophage SPP1: Effects of DNA Homology Between Plasmid and Bacteriophage

INGEBORG DEICHELBOHRER, JUAN C. ALONSO, GERHILD LÜDER, AND THOMAS A. TRAUTNER*

Max-Planck-Institut für molekulare Genetik, D-1000 Berlin 33, Federal Republic of Germany

Received 19 December 1984/Accepted 4 March 1985

Any SPP1 DNA restriction fragment cloned into *Bacillus subtilis* plasmid pC194 or pUB110 increased the transduction frequency of the plasmid by SPP1 100- to 1,000-fold over the transduction level of the plasmid alone. This increment was observed irrespective of whether a fragment contained the SPP1 packaging origin (*pac*). Furthermore, an SPP1 derivative into whose genome pC194 DNA had been integrated transduced pC194 DNA with a greatly enhanced frequency. Transduction enhancement mediated by DNA-DNA homology between plasmid and SPP1 was independent of the extent of homology (size range analyzed, 0.5 to 3.9 kilobases) and the recombination proficiency of donor or recipient.

SPP1 is a virulent *Bacillus subtilis* phage. The phage DNA with an M_r or 28.6×10^6 is partially circularly permuted and terminally redundant (19, 20, 22). To account for these properties of the mature phage DNA, we have assumed that a packaging mechanism similar to the one first described by Tye et al. (31) for P22 is also operative for SPP1: SPP1 DNA is packaged sequentially from concatemeric molecules. Maturation originates preferably within a region of 5 base pairs (bp) (6) termed *pac*, in line with the terminology used by Jackson et al. (10). The nucleotide sequence of about 1,000 bp surrounding the SPP1 *pac* region has been determined (6). Not only phage DNA but also *B. subtilis* chromosomal and plasmid DNAs can be packaged into SPP1 particles, although with very low efficiency. Therefore, the virulent SPP1 is also a transducing phage (4, 7, 33). The molecular weight of transducing DNA contained in SPP1 particles is identical to that of infective phage DNA. Such DNA is organized as a linear, concatemeric array of unit-length plasmid molecules (4).

In this communication we first report an analysis to determine the significance of the *pac* region. To establish whether *pac* represents a signal for effective DNA packaging, we have cloned SPP1 DNA restriction fragments containing the *pac* sequence into plasmids. We subsequently determined the effect of the presence of *pac* on the efficiency of plasmid transduction. These studies led to experiments in which the effects of DNA-DNA homology between plasmid and transducing SPP1 particles were investigated. The results presented suggest that it is the spurious interaction between plasmid and infecting phage which leads to the generation of concatemeric, i.e., packable plasmid DNA and that the frequency of this interaction can be greatly enhanced by providing DNA-DNA homology.

MATERIALS AND METHODS

Media. The media used were as described previously (4).

Bacterial strains, plasmids, and phages. The bacterial strains used are listed in Table 1. The plasmids and phages used are listed in Table 2 and Fig. 1.

Preparation of plasmid-transducing SPP1 lysates. Transducing phage stocks were obtained either from plate lysates as described previously (4) or from one-cycle lysates in liquid TY medium containing 5 μg of antibiotic per ml. The stocks obtained by either procedure had titers of about 10^{10} PFU.

SPP1 transduction. SPP1 transduction was performed as described previously (4). If not mentioned otherwise, the recipient strain was *B. subtilis* 222 (*rec*$^+$).

Preparation of competent cells and transformations. Competent cells were prepared and transformations were performed as previously described (24).

Isolation of plasmid and phage DNA. Preparative amounts of plasmid DNA were isolated by the CsCl-ethidium bromide centrifugation method described by Canosi et al. (5). Analytical amounts of plasmid DNA for restriction mapping were prepared by the alkali method (2). Plasmid DNA was digested with restriction endonucleases purchased from either Boehringer GmbH, Mannheim, Federal Republic of Germany, or Bethesda Research Laboratories, Gaithersburg, Md., and used as directed by the manufacturers. Digests were subjected to either analytical or preparative gel electrophoresis (5). Endonuclease-generated fragments were purified from gels by electroelution (32). Ligation of fragments with T4 DNA ligase (Boehringer) to construct recombinant plasmid molecules was done by the procedure of Maniatis et al. (12). Phage DNA was prepared as previously described (18).

RESULTS

Transducibility of pC194/SPP1 hybrid plasmids by SPP1 in the presence and absence of the *pac* region. To determine whether the presence of the *pac* region of SPP1 affected the transducibility of pC194 DNA, the *pac*-containing *Hin*dIII DNA fragment 1 of SPP1delX was ligated into the unique *Hin*dIII site of pC194 (Fig. 2). *B. subtilis* 222 cells were transformed with the ligation mixture. Plasmids were isolated from chloramphenicol-resistant (Cm^r) transformants. All plasmids obtained possessed deletions within cloned DNA, of which some eliminated the *pac* site. Transducing SPP1 phage stocks were grown on such cells. The transduction frequencies for the Cm^r marker of pC194 from such phage stocks are shown in Fig. 2. It is evident that stocks grown on cells with hybrid plasmids show a 100- to 1,000-fold increment of transducing activity over the level observed with stocks grown on cells with pC194 alone. This dramatic increment in transduction frequency was observed irrespective of whether the SPP1 *pac* site had been deleted. *pac*-containing plasmids, however, were consistently trans-

* Corresponding author.

TABLE 1. List of strains

Strain	Genotype[a]	Source
222	*trpC2 arg*	This laboratory
BD170	*trpC2 thr-5*	D. Dubnau
BD194 (1A43)	*trpC2 recA1*	B.G.S.C[b]
BD191	*trpC2 thr-5 recB2*	D. Dubnau
BD193 (1A45)	*trpC2 thr-5 recB3*	B.G.S.C.
BD1641	*trpC2 tyrA1 hisB2 aroB2 recD41*	G. Mazza
BD224	*trpC2 thr-5 recE4*	D. Dubnau
BD1633	*trpC2 tyrA1 hisB2 aroB2 recF33*	G. Mazza
PB1639	*trpC2 tyrA1 hisB2 aroB2 recG39*	G. Mazza
BD241	*trpC2 thr-5 recL16*	B.G.S.C.
GSY2252	*metA2 add-5*[c]	C. Anagnostopoulos

[a] Names of all *rec* strains (except *recE4*) are given in accordance with the classification previously (17).
[b] B.G.S.C., Bacillus Genetic Stock Center, Columbus, Ohio.
[c] ATP-dependent DNAse deficient mutant described as *recE5* (17).

duced with a higher frequency than that observed with SPP1 containing plasmids without the *pac* site.

Transduction enhancement is a consequence of homology between phage and plasmid DNAs and is replicon independent. Other restriction fragments of SPP1 and of other phages were also cloned into either the *Hin*dIII site of pC194 or the *Eco*RI site of plasmid pC1943 or pUB110, and then the transducibility of these plasmids was measured. The data shown in Table 3 indicate that every SPP1 DNA fragment tested, but not fragments of other DNA cloned into pC194, greatly enhances the transducibility of such hybrid plasmids. In contrast to observations with P22 (27), no correlation was found between the size of inserted SPP1 DNA and transduction enhancement. Similar effects to those described with pC194/SPP1 hybrids were observed with pUB110/SPP1 hybrids (Table 3), although the absolute transduction values of pUB110 and its derivatives were significantly reduced. Transduction enhancement therefore appears to be predominantly a consequence of DNA-DNA homology between phage and plasmid. It is independent both of the presence of the *pac* region in cloned SPP1 DNA and of the transduced plasmid.

Reciprocity in transduction enhancement. Provided DNA-DNA homology was sufficient to cause transduction enhancement, one would also anticipate enhanced transduction of plasmid DNA alone if SPP1 contained at least part of such plasmid DNA. To analyze this situation we cloned pC194 DNA, linearized by a partial *Mbo*I digest, into the unique *Bam*HI site of phage SPP1v. After transfection of *B. subtilis* 222 cells with the ligated DNA, a hybrid pC194/SPP1v phage which contained the complete pC194 plasmid (SPP1v32) was recovered. Stocks of this hybrid phage grown on cells with pC194 transduced *B. subtilis* cells to Cm^r with a 100-fold higher efficiency than that of pC194-free SPP1 phage (Table 4). An even higher transduction frequency with this phage was observed with stocks grown on cells with plasmid pBD302, which also had homology to SPP1 (Table 4). Furthermore, similar transduction enhancement was observed irrespective of whether transducing phages had been produced on rec^+ or *recE4* cells. These results indicate that plasmid-phage DNA homology alone is indeed sufficient to enhance the frequency of plasmid transduction.

Nature of the transducing DNA. Canosi et al. previously showed (4) through DNA-DNA hybridization that pC194-transducing DNA had the M_r of mature SPP1 DNA and consisted exclusively of a concatemer of unit-length plasmid molecules.

Analogous results were obtained with transducing DNAs derived from the SPP1 stocks described here, which gave highly increased transduction frequencies. In Fig. 3 *Sma*I-*Kpn*I DNA digests of SPP1 WT (nontransducing) and a pBD302-transducing lysate (lanes D and E) as well as *Eco*RI digests of DNAs of SPP1 and the pC194-transducing phage SPP1v32 (lanes F and G) are compared. These restriction enzymes were chosen because they digest phage DNA but not transducible plasmid DNA. In both cases the restriction patterns of the two DNAs differ in that the transducing lysates contain only one additional band at the position of unit-genome-length SPP1 DNA. That only this band contained plasmid DNA was verified by DNA-DNA hybridization experiments (data not shown).

The amounts of transducing DNA in the various lysates correspond roughly to the transducing capacity. In the case of pBD302, transducing DNA represents approximately 10%

TABLE 2. List of plasmids and phages

Plasmid or phage	Description	Reference or source
Plasmid		
pC194	Naturally occurring plasmid conferring Cm^r.	9
pUB110	Naturally occurring plasmid conferring neomycin resistance.	11
pC1943	As pC194, with the *Hae*III site substituted by an *Eco*RI site	U. Günthert, unpublished data
pBD302	Representative of pC194 linearized with *Hin*dIII and joined with *Hin*dIII fragment 1 of SPP1delX. Other derivatives obtained include plasmids pBD306, pBD307, pBD305, pBD310, pBD311, pBD318, and pBD331 (Fig. 2).	This work
pBG55	pUB110 linearized with *Eco*RI joined with *Eco*RI fragment 5 of SPP1. Analogous constructs were plasmids pBG59, pBG60, pBG61, and pBG66 (Fig. 1).	This work
pBG3	pC1943 linearized with *Eco*RI joined with *Eco*RI fragment 3 of SPP1 (Fig. 1). Analogous constructs were pBG5, pBG6, pBG7, pBG8, pBG10, pBG11, pBG12, pBG13, and pBG14.	This work
pBC58	pC194 linearized with *Hin*dIII joined with *Hin*dIII fragment 2 of ϕ105 phage.	1
pBC14	pC194 linearized with *Hin*dIII joined with a *Hin*dIII fragment of 6.5 kilobase pairs (kbp) of SPβ phage.	U. Canosi, unpublished data
pBC1	pC194 linearized with *Hin*dIII joined with a 2.6-kbp *Hin*dIII fragment of *B. subtilis* chromosomal DNA.	3
pBC9	As pBC1, but the chromosomal fragment cloned into pC194 has a size of 470 bp.	3
Phage		
SPP1	Wild type	23
SPP1v	SPP1delX with a unique *Bam*HI restriction site.	8
SPP1v32	SPP1v digested with *Bam*HI and joined with a partial *Mbo*I digest of pC194.	This work

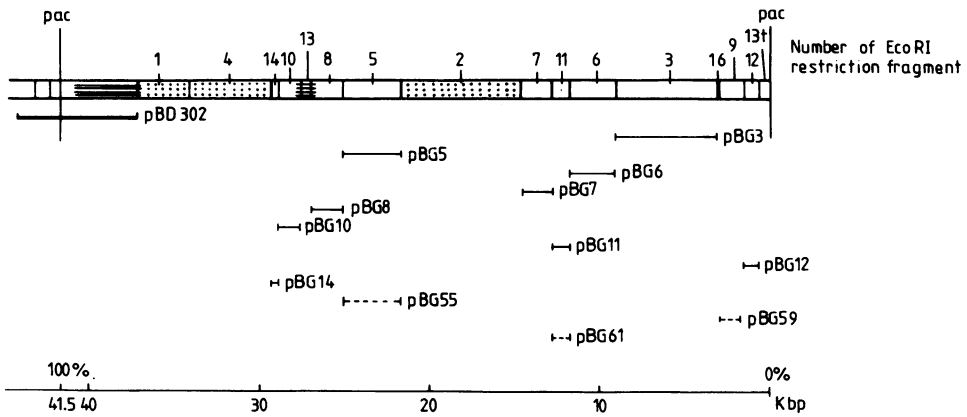

FIG. 1. *Eco*RI restriction map of SPP1 with indications of the fragments cloned into the *Hin*dIII site of pC194 (heavy line) or the *Eco*RI site of pC1943 (thin line) and pUB110 (dotted line). Regions not analyzed in transduction enhancement are shaded. Cross-lined regions in the map describe dispensable regions of the SPP1 genome. *pac* describes the location of the origin of SPP1 DNA packaging.

of the total DNA, which is significantly more than the amount of plasmid-transducing DNA observed with SPP1v32 and still more than the amount of pC194-transducing lysates. Therefore the enhancement of the transduction of plasmid carrying SPP1 DNA is due to the larger amount of transducing particles in such lysates.

Independence of homology-mediated transduction enhancement from known Rec functions. It was conceivable that genetic recombination within the region of plasmid-phage DNA homology was involved in the generation of transducing DNA. To analyze this possibility, we determined the transduction frequency of SPP1 lysates grown on various recombination-deficient cells containing plasmid pBD302 (Tables 4 and 5). Obviously the generation of highly transducing particles is not dependent on the recombination proficiency of the donor cell. Also, in the recipient, establishment of the plasmid is *rec* independent (Table 5) as well as the conversion of the multimeric to the monomeric plasmid form (data not shown). Analogous results were obtained with plasmids not containing the *pac* region (data not shown). In this respect, plasmid transduction, even with plasmids containing DNA homologous to phage DNA, is distinct from the transduction of bacterial markers (4, 7).

DISCUSSION

Facilitation of plasmid transduction or an increment in the frequency of plasmid transduction as described here in SPP1 has been observed in several instances, in which homologous DNA was shared between a transducing phage and plasmid DNA (14, 21, 25–27). Here we have shown that this effect of plasmid-phage DNA homology can also be generated by the use of chimeric phages which contain plasmid DNA.

In phages in which DNA molecules are terminated by unique sequences and maturation involves nucleolytic cleavages at such sequences, the choice of phage DNA fragment inserted into a plasmid in the facilitation of transduction plays a decisive role. Although any restriction fragment of SP02 DNA cloned into pUB110 or pC194 will facilitate the transducibility of such plasmids, the highest transduction frequency is observed with the fragment which carries the DNA sequence of the cohesive ends of phage SP02 (13, 14). The termini of such transducing multimeric plasmid DNA molecules have the cohesive ends of phage SP02, indicating that packaging of multimeric plasmid DNA originated in these plasmids with the same sequences as in vegetative phage maturation. The same situation pertains to the transduction by phage lambda of ColE1/lambda chimeric plasmids with a *cos* sequence (28). In P22 transduction, any phage DNA fragment contained in pBR322 will render the plasmids transducible. However, in the absence of functioning *rec* systems of the host (*recA*$^-$) and phage (*erf*$^-$), only *pac*-carrying plasmids are transducible (25). A similar pronounced effect due to the presence of the *pac* region could not be observed in experiments we performed with SPP1. In this case, the presence of the *pac* site within a transducible

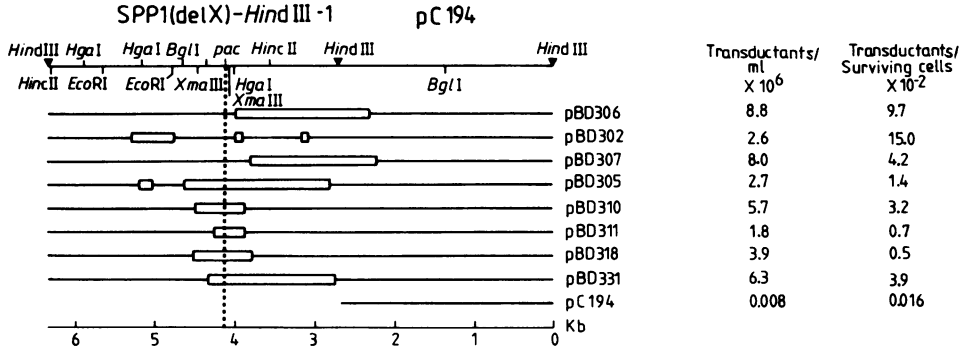

FIG. 2. Restriction map, extent of deletions, and transduction frequency of various pC194/SPP1*delX* *Hin*dIII fragment 1 hybrids. Deletions are indicated by bars. Characteristics of the deletions were derived from an estimate of the M_r of the cloned *Hin*dIII fragment and the deletion of mapped restriction sites.

TABLE 3. Effect of the presence of SPP1 DNA in plasmids on transduction

Plasmid carried by donor strain 222	Size of SPP1 insert (kbp)	No. of transductants/ml	No. of transductants/ surviving cell
pC1943		2.0×10^4	8.0×10^{-5}
pBG3	5.9	1.2×10^6	2.4×10^{-2}
pBG5	3.4	3.1×10^6	7.0×10^{-2}
pBG6	2.7	6.7×10^6	2.7×10^{-2}
pBG7	1.9	2.5×10^6	6.6×10^{-2}
pBG8	1.8	2.3×10^6	5.5×10^{-2}
pBG10	1.3	8.1×10^6	5.4×10^{-2}
pBG11	1.1	7.4×10^6	1.3×10^{-1}
pBG12	0.9	5.5×10^6	1.4×10^{-1}
pBG13	0.7	1.8×10^6	3.4×10^{-2}
pBG14	0.5	8.5×10^6	2.4×10^{-2}
pUB110		3.7×10^2	3.7×10^{-6}
pBG55	3.4	9.4×10^4	6.4×10^{-3}
pBG59	1.5	2.5×10^4	4.7×10^{-4}
pBG61	1.1	6.3×10^3	1.0×10^{-4}
pBG66	1.5 + 1.1	3.8×10^5	2.2×10^{-2}
pBC1		3.2×10^3	2.9×10^{-4}
pBC9		3.4×10^3	3.7×10^{-4}
pBC58		2.3×10^4	7.2×10^{-4}
pBC14		8.0×10^3	8.3×10^{-4}
pC194		4.8×10^3	2.6×10^{-4}

plasmid produced only a marginal enhancement of transduction frequency over the highly elevated background mediated by any cloned SPP1 DNA fragment. Here also, none of the *rec* functions of the host analyzed selectively affected the transducibility of *pac*-containing plasmids, contrary to the situation in P22 (25). These results indicate that in SPP1 the *pac* sequence itself is neither an exclusive nor a highly preferred packaging signal. We must assume that any terminus of a DNA molecule sufficiently large to be encapsulated into an SPP1 head represents a potential packaging origin. The *pac* region might then represent a preferred end of SPP1 DNA, presumably generated during SPP1 DNA replication.

In SPP1 and many other transducing phages, plasmid DNA is organized as headful-sized concatemeric DNA (SPP1 [4, this communication]; T4 [29]; P22 [25]; Mu [30]). Here we have shown that the proportion of defective phages containing such DNA could be greatly enhanced when DNA-DNA homology is provided between phage and plasmid genomes. We suggest that such homology could facilitate efficient synapsis between phage and plasmid. Within the joining region between the two replicons, the phage replication

TABLE 4. Effects of the presence of pC194 DNA in SPP1 on transduction

Donor strain	Relevant genotype	Bacteriophage used	No. of transductants/ml	No. of transductants/surviving cell
BD170	*rec*$^+$	SPP1v32	<10	$<10^{-8}$
BD170(pC194)	*rec*$^+$	SPP1	1.4×10^4	3.5×10^{-5}
BD170(pC194)	*rec*$^+$	SPP1v32	1.8×10^6	4.0×10^{-3}
BD170(pBD302)	*rec*$^+$	SPP1	8.6×10^6	1.7×10^{-2}
BD170(pBD302)	*rec*$^+$	SPP1v32	2.1×10^7	4.0×10^{-2}
BD224	*recE4*	SPP1v32	<10	$<10^{-8}$
BD224(pC194)	*recE4*	SPP1	4.5×10^4	1.0×10^{-4}
BD224(pC194)	*recE4*	SPP1v32	2.2×10^6	5.5×10^{-3}
BD224(pBD302)	*recE4*	SPP1	6.2×10^6	1.3×10^{-2}
BD224(pBD302)	*recE4*	SPP1v32	1.1×10^7	1.4×10^{-2}

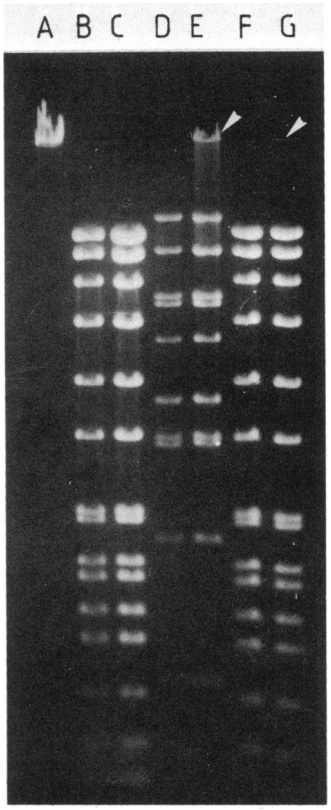

FIG. 3. Electropherograms of restriction enzyme digests of various SPP1 DNAs. Lanes: (A) WT DNA, undegraded; (B) WT DNA, *Eco*RI; (C) WT DNA, pC194 transducing, *Eco*RI; (D) WT DNA, *Kpn*I and *Sma*I; (E) WT DNA, pBD302 transducing, *Kpn*I and *Sma*I; (F) v32 DNA, *Eco*RI; (G) v32 DNA, pC194 transducing, *Eco*RI. Positions of nondegraded transducing DNA are indicated by arrows. Phage SPP1v32 is derived from SPP1delX, which contains a deletion of 3.2 kilobases within its *Eco*RI fragment 1. This deletion is compensated by the insert of pC194 (2.9 kilobases). Therefore, the *Eco*RI restriction patterns of SPP1 WT and SPP1v32 appear to be identical. Phage stocks were prepared on strain BD224 or its plasmid-containing derivatives.

apparatus might switch templates and thus induce phage-type (i.e., concatemeric) replication of plasmid DNA, which could then be encapsulated into phage heads. To account for the presence of concatemeric DNA in plasmid-transducing phages generated without providing homology, it is suggested that such synapsis would also occur, albeit at a much lower frequency, as a consequence of small regions of natural homology between plasmid and phage. One would visualize such a mechanism as dependent on *rec* functions controlling the alignment of homologous DNA. Indeed, generations of plasmid-transducing phages has been found to be *recA* dependent in T4- and P22-mediated plasmid transduction (25, 29). Furthermore, the observation in the case of P22 that transduction efficiency and extent of shared DNA-DNA homology are correlated would be compatible with this suggestion. The *rec* independence of the generation of transducing concatemers in SPP1 (Table 5), which was also observed with a *recE4 xin-1* SPβ^- strain (data not shown), might indicate either that none of the identified *rec* functions control this step or that an SPP1 specified recombination system is operating, or both. It was also surprising to find transduction enhancement to be independent of the extent of DNA-DNA homology. In this case it is assumed that the efficiency of recombination in *B. subtilis* is so high that even

TABLE 5. Dependence of transduction on recombination proficiency

Donor strains containing pBD302	Relevant genotype of donor	Recipient strain	No. of transductants/ml	No. of transductants/surviving cell
BD170[a]	*rec*$^+$	222	3.0×10^7	1.0×10^{-1}
BD194	*recA1*	222	3.1×10^7	1.2×10^{-1}
BD191	*recB2*	222	2.8×10^7	8.9×10^{-2}
BD193	*recB3*	222	1.7×10^7	5.5×10^{-2}
PB1641	*recD41*	222	3.2×10^7	1.0×10^{-1}
PB1633	*recF33*	222	2.8×10^7	1.5×10^{-1}
BD1639	*recG39*	222	2.7×10^7	1.0×10^{-1}
BD241	*recL16*	222	1.5×10^7	7.8×10^{-2}
BD224	*recE4*	222	1.0×10^7	5.2×10^{-2}
GSY2252	*add-5*	222	3.6×10^7	1.0×10^{-1}
222	*rec*$^+$	222	1.5×10^7	1.0×10^{-1}
222	*rec*$^+$	BD194	3.4×10^7	1.0×10^{-1}
222	*rec*$^+$	BD191	1.1×10^7	1.0×10^{-1}
222	*rec*$^+$	BD193	6.2×10^6	5.6×10^{-2}
222	*rec*$^+$	PB1641	1.3×10^7	3.6×10^{-2}
222	*rec*$^+$	PB1633	7.0×10^6	7.0×10^{-2}
222	*rec*$^+$	PB1639	2.4×10^6	3.0×10^{-1}
222	*rec*$^+$	BD241	1.6×10^7	5.0×10^{-2}
222	*rec*$^+$	BD224	5.3×10^6	3.1×10^{-2}
222	*rec*$^+$	GSY2252	7.7×10^6	1.7×10^{-1}

[a] Identical transduction values were observed with strain SB202 (*rec*$^+$), from which *rec*$^-$ strains of the PB series were derived.

the smallest homology generated (500 base pairs in the transduction of pBG14) is sufficient to guarantee maximal plasmid-phage interaction. A prediction of the model is that transducing plasmid DNA would be synthesized exclusively after phage infection. Evidence for postinfective synthesis of pBR322 DNA transduced by T4 has been obtained by Mattson et al. (15). Preliminary results from our own laboratory indicate that this is also true for plasmid DNA transduced by SPP1 (J. C. Alonso, G. Lüder, and T. A. Trautner, manuscript in preparation).

Other types of plasmid-transducing phages have functional phage genomes into which plasmids can be integrated. Such cointegrates involving plasmid pBR322 have been discovered for P22 (21) and T4 (16). No particles of this type were discovered for SPP1 in previous studies (4) or in the studies reported here. However, SPP1/pC194 cointegrates have been constructed in vitro. Phage SPP1v32, which gave enhancement of pC194 transduction, was described in this paper. Among the constructs which led to the isolation of SPP1v32, we discovered another SPP1v derivative containing pC194. When this phage is grown in plasmid-free *B. subtilis* cells, the hybrid genome is resolved, resulting in a pC194-like replicon. This indicates that in SPP1 also, plasmid-phage cointegrates could represent potentially transducing genomes. The details of plasmid resolution will be described in another communication.

LITERATURE CITED

1. **Bensi, G., A. Iglesias, U. Canosi, and T. A. Trautner.** 1981. Plasmid transformation in *Bacillus subtilis*: the significance of partial homology between plasmid and recipient cell DNAs. Mol. Gen. Genet. **184**:400–404.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Canosi, U., A. Iglesias, and T. A. Trautner.** 1981. Plasmid transformation in *Bacillus subtilis*: effects of insertion of *Bacillus subtilis* DNA into plasmid pC194. Mol. Gen. Genet. **181**:434–440.
4. **Canosi, U., G. Lüder, and T. A. Trautner.** 1982. SPP1-mediated plasmid transduction. J. Virol. **44**:431–436.
5. **Canosi, U., G. Morelli, and T. A. Trautner.** 1978. The relationship between molecular structure and transformation efficiency of some *S. aureus* plasmids isolated from *B. subtilis*. Mol. Gen. Genet. **166**:259–267.
6. **Deichelbohrer, I., W. Messer, and T. A. Trautner.** 1982. Genome of *Bacillus subtilis* bacteriophage SPP1: structure and nucleotide sequence of *pac*, the origin of DNA packaging. J. Virol. **42**:83–90.
7. **Ferrari, E., U. Canosi, A. Galizzi, and G. Mazza.** 1978. Studies on transduction process by SPP1 phage. J. Gen. Virol. **41**:563–572.
8. **Heilmann, H., and J. N. Reeve.** 1982. Construction and use of SPP1v, a viral cloning vector for *Bacillus subtilis*. Gene **17**:91–100.
9. **Iordănescu, A.** 1975. Recombinant plasmid obtained from two different compatible staphylococcal plasmids. J. Bacteriol. **124**:597–601.
10. **Jackson, E. N., H. J. Miller, and J. L. Adams.** 1978. *Eco*RI restriction endonuclease cleavage site map of bacteriophage P22 DNA. J. Mol. Biol. **118**:347–363.
11. **Lacey, R. W., and I. Chopra.** 1974. Genetic studies of a multiresistant strain of *Staphylococcus aureus*. J. Med. Microbiol. **124**:597–601.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Marrero, R., F. A. Chafari, and P. S. Lovett.** 1981. SP02 particles mediating transduction of a plasmid containing SP02 cohesive ends. J. Bacteriol. **147**:1–8.
14. **Marrero, R., and P. S. Lovett.** 1980. Transductional section of cloned bacteriophage ϕ105 and SP02 deoxyribonucleic acid in *Bacillus subtilis*. J. Bacteriol. **143**:879–886.
15. **Mattson, L. T., G. Van Houwe, A. Bolle, and R. Epstein.** 1983. Fate of cloned bacteriophage T4 DNA after phage T4 infection of clone-bearing cells. J. Mol. Biol. **170**:343–355.
16. **Mattson, T., G. Van Houwe, and R. Epstein.** 1983. Recombination between bacteriophage T4 and plasmid pBR322 molecules cloned T4 DNA. J. Mol. Biol. **170**:357–379.
17. **Mazza, G., and A. Galizzi.** 1978. The genetics of DNA replication, repair and recombination in *Bacillus subtilis*. Microbiologia **1**:111–135.
18. **Montenegro, M. A., H. Esche, and T. A. Trautner.** 1976. Induction of mutations in *B. subtilis* phage SPP1 by growth on host cells carrying a mutator DNA polymerase III. Mol. Gen. Genet. **149**:131–134.
19. **Morelli, G., C. Fisseau, B. Behrens, T. A. Trautner, J. Luh, S. W. Ratcliff, D. P. Allison, and A. T. Ganesan.** 1979. The genome of *B. subtilis* phage SPP1. The topology of DNA molecules. Mol. Gen. Genet. **168**:153–164.
20. **Morelli, G., M. A. Montenegro, G. Hillenbrand, E. Scherzinger, and T. A. Trautner.** 1978. The genome of *B. subtilis* phage SPP1: assignment of 5′-3′-polarity to the complementary strands of SPP1 DNA. Mol. Gen. Genet. **164**:93–97.
21. **Orbach, M. J., and E. N. Jackson.** 1982. Transfer of chimeric plasmids among *Salmonella typhimurium* strains by P22 transduction. J. Bacteriol. **149**:985–994.
22. **Ratcliff, S. W., J. Luh, A. T. Ganesan, B. Behrens, R. Thompson, M. A. Montenegro, G. Morelli, and T. A. Trautner.** 1979. The genome of *Bacillus subtilis* phage SPP1: the arrangement of restriction endonuclease generated fragments. Mol. Gen. Genet. **168**:165–172.
23. **Riva, S. C.** 1969. Asymmetric transcription of *B. subtilis* phage SPP1 DNA *in vitro*. Biochem. Biophys. Res. Commun. **34**:824–830.
24. **Rottländer, E., and T. A. Trautner.** 1970. Genetic and transfection studies with *B. subtilis* phage SP50. I. Phage mutants with restricted growth on *B. subtilis* strain 168. Mol. Gen. Genet. **108**:47–60.
25. **Schmidt, C., and H. Schmieger.** 1984. Selective transduction of

recombinant plasmids with cloned *pac* sites by Salmonella phage P22. Mol. Gen. Genet. **196**:123–128.
26. **Schmieger, H.** 1982. Packaging signals for phage P22 on the chromosome of *Salmonella typhimurium*. Mol. Gen. Genet. **187**:516–518.
27. **Schmieger, H.** 1984. pac sites are indispensible for *in vivo* packaging of DNA by P22. Mol. Gen. Genet. **195**:252–255.
28. **Shimada, K., K. Omene, T. Nakamura, and Y. Takagi.** 1978. Recombination in hybrid ColE1 DNAs as analyzed by λ-mediated transduction. Cold Spring Harbor Symp. Quant. Biol. **43**:991–998.
29. **Takahashi, H., and H. Saito.** 1982. Mechanism of pBR322 transduction mediated by cytosine-substituting T4 bacteriophage. Mol. Gen. Genet. **186**:497–500.
30. **Teifel-Greding, J.** 1984. Transduction of multi-copy plasmid pBR322 by bacteriophage Mu. Mol. Gen. Genet. **197**:169–174.
31. **Tye, B. K., J. A. Huberman, and D. Botstein.** 1974. Nonrandom circular permutation of phage P22 DNA. J. Mol. Biol. **85**:501–532.
32. **Yang, R., C.-A. Lis, J. Lis, and R. Wu.** 1979. Elution of DNA from agarose gels after electrophoresis. Methods Enzymol. **68**:176–182.
33. **Yasbin, R. E., and F. E. Young.** 1974. Transduction in *Bacillus subtilis* by bacteriophage SPP1. J. Gen. Virol. **14**:1343–1348.