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Transductional Selection of Cloned Bacteriophage φ105 and SP02 Deoxyribonucleic Acids in *Bacillus subtilis*

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The *Bacillus subtilis* temperate bacteriophages φ105 and SP02 are incapable of transduction of the small, multicopy drug resistance plasmids pUB110 and pCM194. Cloning endonuclease-generated fragments of φ105 or SP02 DNA into each of the plasmids renders the chimeric derivatives susceptible to transduction specifically by the phage whose deoxyribonucleic acid is present in the chimera. The majority of phage deoxyribonucleic acid fragments identified that render plasmids transducible by φ105 or SP02 appear to be internal fragments, not fragments containing the cohesive ends. However, the highest overall transduction frequency was observed in SP02-mediated transduction of a derivative of pUB110 containing a 1.6-megadalton *Eco*RI fragment that likely contains the SP02 cohesive ends (plasmid pPL1010). The transducing activity present in a φ105 transducing lysate had a buoyant density slightly greater than infectious particles, whereas the majority of transducing particles in an SP02(pPL1010) transducing lysate had a buoyant density slightly less than infectious particles. Although no detectable change in plasmid structure resulted from transduction by φ105 or SP02, deoxyribonucleic acid isolated from a purified SP02(pPL1010) transducing lysate contained no detectable monomeric pPL1010, but did contain a form of pPL1010 of higher molecular weight than the monomer.

φ105 and SP02 are temperate bacteriophages infectious for *Bacillus subtilis* 168 (12). The two phages are heteroimmune, and both show only weak neutralization by antiserum prepared against the heterologous phage (2). SP02 had been reported to not complement several mutations in φ105 (21), and the DNA from the two phages shows less than 15% homology in hybridization analysis (6). Thus, the two phages seem very distantly related or unrelated. Because these phages are reasonably well characterized, we chose to use them in examining a phenomenon termed "selective plasmid transduction" previously demonstrated with the less well-studied *B. pumilus* temperate phage φ75 (4).

φ75 mediates transduction of the 4.4-megadalton (Md) plasmid pPL10 which specifies the production of, and immunity to, a bacteriocin-like activity in *B. pumilus* (5, 15). In contrast, φ75 does not transduce any of several other plasmids tested, such as pUB110 or pCM194 (Lovett, unpublished data). Homology between pPL10 and φ75 DNA was detected by hybridizing nick-translated (17) φ75 *Hpa*II-generated DNA fragments to pPL10 (R. Taylor and P. S. Lovett, unpublished data). No homology was detected between φ75 DNA and the plasmids not susceptible to transduction. The transduction specificity of φ75 thus appeared related to homology with a specific plasmid. In the present study we demonstrate that cloning fragments of phage DNA into each of several plasmids renders the constructed plasmids susceptible to transduction specifically by the phage whose DNA is present in the construct.

MATERIALS AND METHODS

Bacteriophage and host cells. φ105 was obtained from B. Reilly. ρ14 and ρ10, which are related to φ105 (19) and SP02, were obtained from D. Dean. φ105 mutants *EN9*(Ts), *D13*(Sus), and *C19*(Sus) and a suppressor-containing strain of *B. subtilis*, strain GB43(Su-3), were provided by A. Garro (22). *B. subtilis* strains 1A1000 (cured of SPβ) and 1A304 (cured of SPβ and noninducible for PBSX) were obtained from the *Bacillus* Genetic Stock Center at Ohio State University. φ105 and SP02 plaque-forming units were assayed on *B. subtilis* strain BR151 (*trpC2 metB10 lys-3*). All incubations were at 37°C. The media used for φ105 assays were as described for φ75 (4). SP02 was assayed for plaque formation on M medium: 1% tryptone (Difco), 0.5% yeast extract, 0.9% sodium chloride, 5 mM $MgCl_2$, 5 mM $CaCl_2$, and 5 mM $MnCl_2$ (20). Noble agar (Difco) was added to 1.8 and 0.6% for bottom and top agar, respectively. Propagation and purification of phages through CsCl gradient centrifugation were as previously described for phage φ75 (4).

Plasmids and enzymes. Plasmids pUB110 (3 Md) and pCM194 (2 Md) specify resistance to neomycin (Neo^r) and chloramphenicol (Chr^r), respectively (11).

Plasmid pPLFHL is a 3.6-Md derivative of pUB110. It was constructed by inserting a 0.9-Md *Eco*RI fragment specifying Chr^r into the *Eco*RI site in pUB110. The plasmid was then digested with *Hae*II to remove a 0.25-Md fragment that spans the *Bgl*II site on pUB110. The removal of the 0.25-Md *Hae*II fragment renders pPLFHL neomycin sensitive. The origin of the Chr^r gene on the 0.9-Md *Eco*RI fragment of pPLFHL was pCM194 (11). pCM194 and pSL103 (14) were joined in vitro at the respective *Hin*dIII sites. When this composite plasmid was introduced into strain BR151 by transformation, a rearrangement occurred resulting in a plasmid that was neomycin and chloramphenicol resistant, but lacked the *trp* complementing activity characteristic of pSL103 (14). The 0.9-Md *Eco*RI fragment in this rearranged composite plasmid was homologous with pCM194 (by hybridization), and specified chloramphenicol transacetylase. The 0.9-Md fragment appears to be incapable of autonomous replication in BR151.

T4-induced DNA ligase and restriction enzymes were purchased from Bethesda Research Laboratories or Biolabs. Each was used according to specifications provided with the enzymes.

Single-colony lysis gel electrophoresis. Individual colonies of plasmid-containing BR151 were transferred with toothpicks to 25 μl of lysozyme buffer (16) containing 200 μg of lysozyme per ml and incubated for 15 min at 37°C. A 100-μl amount of sodium dodecyl sulfate buffer (16) was added, and incubation was continued for 15 min. A 40-μl amount of 5 M NaCl was added, and each tube was vigorously agitated, held at 2°C for 30 min, and centrifuged for 15 min in an Eppendorf centrifuge. A 50-μl quantity of each supernatant fraction was placed in the wells of a 1% horizontal agarose gel and subjected to electrophoresis for 20 h at room temperature and stained with ethidium bromide.

Transductional cloning. φ105 DNA (20 μg) was suspended in 100 μl of *Eco*RI buffer. Twice the volume of *Eco*RI necessary for complete digestion was added (the amount of enzyme was empirically determined). Digestion was at 37°C for 30 min. Five micrograms of a limit *Eco*RI digest of pUB110 was added to the digested phage DNA, and the mixture was held at 65°C for 15 min. T4-induced DNA ligase (0.01 U), 10 mM dithiothreitol, and 1 mM ATP were added. The mixture (120 μl) was incubated for 18 to 20 h at 13°C. The DNA was dialyzed against TES buffer (0.02 M Tris, 0.1 M NaCl, 5 mM EDTA, pH 7.5), and the entire mixture (ca. 0.1 ml) was shaken for 1 h at 37°C with 1 ml of a competent culture (3) of strain BR151 (φ105). After transformation, the cells were added to 10 ml of Penassay broth (Difco) containing 10 μg of neomycin sulfate per ml. After overnight growth at 37°C, a portion of the culture was diluted into fresh Penassay broth grown to early exponential phase, and induced with mitomycin C (4). At 3 h after induction, the culture had cleared. The lysate was incubated for 10 min at 37°C with 20 μg of pancreatic DNase per ml and centrifuged at low speed. The resulting supernatant fraction was sterilized by filtration. The titer of the induced lysates was generally 10^8 to 2×10^9 plaque-forming units per ml. A 0.5-ml amount of the lysate was shaken at 37°C with 5×10^8 recipient cells [e.g., BR151 or BR151(φ105)] for 35 min, and 0.3-ml portions were spread onto tryptose blood agar base containing neomycin sulfate (10 μg/ml). After 24 h of incubation, about 10 to 20 transductants appeared on each plate. All such transductants that we have examined by the single-colony lysis technique (about 300) contained plasmids of higher molecular weight than the vector (see Fig. 1). Variations in this standard procedure were as follows. When SP02 DNA was used instead of φ105 DNA, the transformation recipient was lysogenic for SP02. SP02 lysogens were induced in liquid M medium. The use of restriction enzymes other than *Eco*RI required different buffers (11) and different vector plasmids. *Hin*dIII inserts were made in pCM194. *Hae*II inserts were made in pPLFHL.

Plasmid isolation and characterization. Methods for isolation of plasmid DNA by dye-buoyant gradient centrifugation and electrophoresis of plasmids and DNA fragments through horizontal agarose gels have been described in detail (16).

Hybridization to DNA molecules resolved by electrophoresis. Intact or digested DNA was applied to a 1% agarose gel (30 by 15 by 0.75 cm) and electrophoresed at 36 V for 18 h at room temperature (16). Bands were visualized by staining the gel with ethidium bromide (~1 μg/ml in water) and photographed under a UV light source (Ultra-Violet Products, Inc.). The portion of the gel to be blotted to nitrocellulose was cut from the large slab and pressed onto a nitrocellulose strip for 36 to 48 h at room temperature (23). The strip was dried at 37°C for 30 min, placed at 80°C for 2 h in a vacuum oven, and then incubated in Denhardt buffer (9) for 6 h at 65°C. Plasmid pUB110 (5 μg) was linearized with *Eco*RI and nick translated with a commercially available kit (Bethesda Research Laboratories). The ^{32}P-labeled pUB110 (about ~10^6 cpm) was placed in boiling water for 5 min and quickly chilled. DNA was incubated with the nitrocellulose strip at 65°C for about 48 h in the following buffer: 4× SSCP (480 mM NaCl, 60 mM sodium citrate, 52 mM KH_2PO_4, 4 mM EDTA, pH 7.2), 0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.2% sodium dodecyl sulfate. The strip was then washed with 4× SSC (1× SSC, 0.15 M NaCl–0.015 M sodium citrate) and 2× SSC buffer (9), dried at 37°C, and exposed to X-ray film with an intensifier screen for up to 2 days at −80°C.

RESULTS

Transductional cloning of plasmids containing φ105 or SP02 fragments. In the initial experiments *Eco*RI-generated fragments of φ105 DNA were joined in vitro to *Eco*RI-digested plasmid pUB110, and the mixture was used to transform strain BR151. Several of the resulting Neo^r transformants contained plasmids that complemented the mutation in three replication-deficient mutants of φ105. By this approach we cloned φ105 *Eco*RI fragments G [complements mutations *EN9*(Ts)] and E [complements mutations *D13*(Sus) and *C19*(Sus)] (10). pUB110 containing either the G or E fragment was transduced by φ105 at frequencies on the order of

10^{-5} to 10^{-7} transductants per plaque-forming unit, whereas ϕ105 did not detectably transduce pUB110 or pUB110 containing segments of the tryptophan gene cluster cloned from *B. pumilus*, *B. subtilis*, or *B. licheniformis* (13). The apparent specificity of ϕ105 transduction indicated that the phage could identify plasmid derivatives containing at least certain insertions of ϕ105 DNA. Accordingly, we developed the transductional cloning procedure to identify ϕ105 DNA fragments (and SP02 DNA fragments) that rendered the constructed plasmids sensitive to transduction by the homologous phage (see Materials and Methods).

Table 1 shows several of the *Eco*RI-, *Hin*dIII-, and *Hae*II-generated fragments of ϕ105 and SP02 cloned into three vector plasmids and identified by transductional cloning. Each of the constructed plasmids contained a phage DNA fragment which corresponded to a fragment of either SP02 or ϕ105 DNA as determined by digesting both the plasmids and phage DNA with the endonuclease used in the plasmid construction and electrophoresis of both digests through a 1% agarose gel (Fig. 1; see also 22, 25). The smallest segments cloned which conferred transducibility were 0.25 and 0.29 Md (Table 1). The largest phage DNA insert cloned by this method consisted of three *Hin*dIII fragments (possibly resulting from partial digestion of the phage DNA) having a combined molecular mass of about 5.84 Md (Table 1). In these cloning experiments, certain phage DNA fragments were detected much more frequently than others. For example, the *Eco*RI-G (0.7 Md) and -E (3.48 Md) fragments of ϕ105 (22) accounted for 52 of 65 transductants in one experiment. This is unexplained since the efficiency of transduction by ϕ105 of pUB110 containing either the E or G fragments was not significantly greater than the transduction frequency of pUB110 containing the *Eco*RI fragment I (0.25 Md) (Table 1, reference 22). Moreover, the growth rate of BR151 cells harboring the pUB110 derivatives

TABLE 1. *Partial list of endonuclease fragments of ϕ105 and SP02 isolated by the transductional cloning method*[a]

Plasmid designation	Phage DNA/vector plasmid	Insert fragment size (Md)[b]	Transduction frequency by homologous phage (10^{-7})[c]
pPL1000	ϕ105/pUB110	0.25	3
pPL1001	ϕ105/pUB110	0.70	20
pPL1002	ϕ105/pUB110	3.48	100
pPL1003	ϕ105/pCM194	1.22	100
pPL1004[d]	ϕ105/pCM194	1.62	1,000
pPL1005	ϕ105/pCM194	0.72	700
pPL1006	ϕ105/pCM194	3.44, 1.91	2,000
pPL1007	ϕ105/pCM194	2.71	6,000
pPL1008	ϕ105/pCM194	2.71, 1.91, 1.22	30
pPL1009	ϕ105/pCM194	0.98	800
pPL1010	SP02/pUB110	1.67	100,000
pPL1011	SP02/pPLFHL	0.29	100
pPL1012	SP02/pPLFHL	1.57	300
pPL1013	SP02/pPLFHL	1.06, 0.98, 0.33	200
pPL1014	SP02/pPLFHL	0.60	10

[a] The transductional cloning procedure is described in the text. *Eco*RI inserts were made in pUB110, *Hin*dIII inserts were made in pCM194, and *Hae*II inserts were made in pPLFHL.

[b] The size of phage DNA insert in vector plasmid was estimated by agarose gel electrophoresis of each constructed plasmid after digestion with the endonuclease used in the construction. The standards were fragments of lambda DNA resulting from complete *Hin*dIII digestion.

[c] Transductants per plaque-forming unit. Recipients were lysogenic for homologous phage. Transduction frequency by heterologous phage was below 10^{-9}.

[d] The ϕ105 DNA insert in pPL1004 confers immunity to ϕ105.

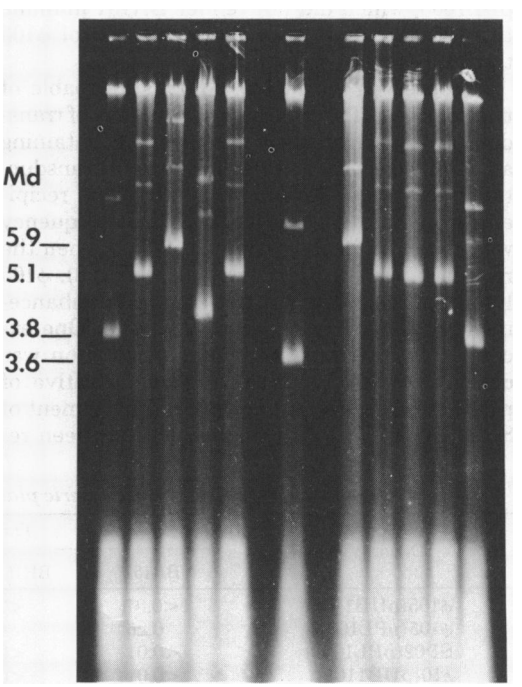

FIG. 1. *Single-colony lysis gel of chloramphenicol-resistant BR151 transductants resulting from transductional cloning of HaeII-generated SP02 DNA fragments in pPLFHL. The center well contained a lysate prepared from a colony carrying the vector plasmid pPLFHL. All other wells contained lysates prepared from SP02-generated transductants.*

containing *Eco*RI fragments of φ105 DNA did not differ.

Specificity and enhancement of φ105 and SP02 plasmid transduction. φ105 and the closely related homoimmune phages ρ10 and ρ14 (19) transduced derivatives of pCM194 and pUB110 containing φ105 DNA inserts at a frequency of about 10^{-6} to 10^{-8} transductants per plaque-forming unit when the recipient BR151 was not lysogenic for the phages. The transduction frequency generally increased 10- to 20-fold when the recipient was lysogenic for φ105, ρ10, or ρ14, although occasionally higher increases were observed (Table 2). This enhancement was not observed when the recipient was lysogenic for SP02 (Table 2). It seems possible that the enhancement of transduction observed with lysogenic recipients is due, at least in part, to protection of the plasmid transductants to the killing activity of infectious phage in the donor lysate. This interpretation is supported by the fact that BR151 carrying pPL1004, a derivative of PCM194 which renders the cells immune to φ105 but not SP02, caused an enhancement in the number of φ105-mediated transductants (Table 3). By contrast, the pCM194 derivative pPL1003, which did not render BR151 immune to φ105, did not enhance the number of φ105 transductants (Table 3).

As observed with φ105, SP02 is incapable of transduction of pUB110. The frequency of transduction of derivatives of this plasmid containing an SP02 insert ranged from 10^{-2} to $^{-7}$ transductants per plaque-forming unit when the recipients were not lysogenic for SP02. This frequency was generally increased 10- to 20-fold when the recipient was an SP02 lysogen (Table 3). φ105 lysogenic recipients did not show this enhancement of SP02 transduction (Table 3). One exception to the enhancement phenomenon was observed. Plasmid pPL1010 is a derivative of pUB110 containing a 1.6-Md *Eco*RI fragment of SP02 DNA. This *Eco*RI fragment has been reported to span the SP02 cohesive ends (25). SP02 transduction of pPL1010 was approximately the same regardless of whether the recipient was an SP02 lysogen or a nonlysogen.

Preincubation of φ105 or SP02 transducing lysates with φ105 or SP02 antiserum sufficient to neutralize greater than 99% of the plaque-forming units eliminated the transducing activity of the lysates. Thus, the transducing activity appears associated with the corresponding phage. However, *B. subtilis* 168 and most mutant derivatives are known to be lysogenic for two other phages, the inducible defective phage PBSX and the temperate phage SPβ (10, 24). Therefore, we introduced a derivative of pCM194 carrying a φ105 and DNA insert and a derivative of pPLFHL containing an SP02 insert (pPL1005 and pPL1011) into BR151 by transformation. Both of the resulting transformants

TABLE 3. *Effect on recovery of φ105 and SP02 transductants with lysogenic recipients and a recipient carrying a cloned φ105 immunity fragment*

Plasmid in donor cell[a]	Recipient	Transductants/plaque-forming unit (10^{-7})[b]	
		φ105	SP02
pPL1001	BR151	0.25	<0.01
pPL1001	BR151(φ105)	3.3	<0.01
pPL1001	BR151(pCM194)	0.20	<0.01
pPL1001	BR151(pPL1004)	33	<0.01
pPL1001	BR151(pPL1003)	0.20	<0.01
pPL1012	BR151	<0.01	0.20
pPL1012	BR151(SP02)	<0.01	3.0
pPL1012	BR151(φ105)	<0.01	0.31

[a] Donor cells (strain BR151) were lysogenized with SP02 or φ105 and induced with mitomycin C. SP02 and φ105 lysates had infectious titers of 2×10^9 plaque-forming units per ml.

[b] Transductions were as described in Table 2. pPL1012 transductants were selected on tryptose blood agar base containing 10 μg of chloramphenicol per ml.

TABLE 2. *Transduction of chimeric plasmid pPL1001 by phages φ105, ρ10, and ρ14*[a]

Donor lysate	Transductants/plaque-forming unit (10^{-7}) for:				
	BR151	BR151(φ105)	BR151(ρ10)	BR151(ρ14)	BR151(SP02)
φ105(pUB110)	<0.01	<0.01	<0.01	<0.01	<0.01
φ105(pPL1001)	0.26	4	12	12	0.52
SP02(pPL1001)	<0.01	<0.01	<0.01	<0.01	<0.01
ρ10(pUB110)	<0.01	<0.01	<0.01	<0.01	<0.01
ρ10(pPL1001)	11	60	28	13	5
ρ14(pUB110)	<0.01	<0.01	<0.01	<0.01	<0.01
ρ14(pPL1001)	10	11	30	35	16

[a] A 0.5-ml amount of phage lysate was shaken at 37°C with approximately 3×10^8 recipient cells in a total volume of 1 ml for 35 min. Aliquots were directly spread onto tryptose blood agar base containing 10 μg of neomycin sulfate per ml.

were induced with mitomycin C. The resulting lysates showed no transducing activity for Chr^r regardless of whether the recipient was BR151, BR151(SP02), or BR151(ϕ105). In addition a strain of *B. subtilis* cured of SPβ(1A1000) and one cured of SPβ and noninducible for PBSX(1A304) were each lysogenized separately with ϕ105 and SP02. Plasmids pPL1005 and pPL1011 were transformed into each lysogenic derivative, and all were induced with mitomycin C. The transducing activity of these lysates was comparable to that obtained with donor cells that are lysogenic for ϕ105 or SP02 in addition to PBSX and SPβ (data not shown). Thus, there appears to be no relationship between SP02 and ϕ105 transduction and the presence of PBSX or SPβ.

To test the specificity of the transductions, we determined whether one plasmid containing a phage DNA insert would render other plasmids in the same cell (which do not contain phage DNA inserts) susceptible to transduction. Derivatives of BR151 were constructed to contain the compatible plasmids pCM194 and pUB110, and various combinations of both plasmids in which one contained a phage DNA insert and the other did not, or both plasmids contained inserts but one insert was a ϕ105 DNA fragment and the other insert was an SP02 fragment. Each derivative was separately lysogenized with SP02 or ϕ105 and the lysogens were induced. The presence of a chimeric derivative of pUB110 or pCM194 containing a ϕ105 insert did not render other plasmids in the cell susceptible to ϕ105 transduction (Table 4). Comparable results were obtained with SP02. For example, ϕ105 propagated on BR151(pPL1003, pPL1010) generated

TABLE 4. *Specificity of ϕ105-mediated transduction*[a]

Plasmids in donor cell	Transductants per plaque-forming unit (10^{-7})[b]	
	Neo^r	Chr^r
pCM194	<0.01	<0.01
pUB110	<0.01	<0.01
pCM194 pUB110	<0.01	<0.01
pPL1001	10	<0.01
pPL1003	<0.01	400
pPL1001 pCM194	20	<0.01
pPL1003 pUB110	<0.01	200
pPL1001 pPL1003	10[c]	100[c]

[a] ϕ105 was induced from each donor cell. BR151(ϕ105) was the recipient for transductions.

[b] Transduction frequency of <0.01 indicates that no transductants were detected.

[c] Of the 3,200 Neo^r transductants, 4% were also Chr^r. Two percent of the 31,000 Chr^r transductants were also Neo^r.

<0.01 Neo^r and 60 Chr^r transformants per 10^7 plaque-forming units, whereas SP02 grown on the same strain generated 200,000 Neo^r and <0.01 Chr^r transformants per 10^7 plaque-forming units. Accordingly, the transduction process is highly selective for plasmids containing the proper phage DNA insert.

Properties of lysogenic and nonlysogenic recipients. Plasmid DNA isolated from several transductants resulting from at least three successive transductions of pPL1000 and pPL1003 (with ϕ105 as vector) and pPL1010 (with SP02 as vector) retained the same size and sensitivity to *Eco*RI or *Hin*dIII as the original plasmid isolates. Accordingly, the transductions appeared not to alter plasmid structure. In these experiments the recipients were made lysogenic for the phage to be used for transduction before performing the transductions. We were interested in determining the structure of transduced plasmids in cells that were not lysogens. This was directly accomplished only with SP02. SP02-generated, pPL1010- or pPL1012-containing transductants of BR151 (more than 200 examined) were not lysogenic for SP02 when the transductions were performed in Penassay broth and the transductants were selected on tryptose blood agar base containing the appropriate antibiotic (chloramphenicol or neomycin). The replication of SP02 is prevented when host cells are infected in these media (R. Yasbin, personal communiation). In contrast, when the transductions were performed in, and transductants were selected on, M medium, which permits SP02 replication, each of more than 100 transductants was an SP02 lysogen. The only method found which generated nonlysogenic ϕ105 transductants involved transduction of a plasmid, e.g., pPL1002, into BR151(pPL1004) with a clear-plaque variant of ϕ105. By using this approach, approximately one-third of the resulting transductants were nonlysogenic. In each case, the plasmid isolated from the nonlysogenic transductants was comparable in size and endonuclease sensitivity to the original plasmid isolates. Equally important, these data indicate that lysogeny is not necessary for transduction.

Properties of SP02(pPL1010) transducing particles. SP02 transduction of pPL1010 occurred at frequencies in the range of 10^{-2} to 10^{-3} transductants per plaque-forming unit. This frequency of transduction exceeds that of all other plasmids tested (Table 1). Examination of an SP02(pPL1010) transducing lysate after equilibrium centrifugation in CsCl demonstrated that the infectious particles had a buoyant density greater than the majority of the transducing particles (Fig. 2). In contrast, similar analysis of

a ϕ105 transducing lysate demonstrated that the transducing particles had a greater buoyant density than the infectious particles (Fig. 3).

We analyzed the DNA present in particles in a transducing lysate in the following manner. SP02(pPL1010) was centrifuged to equilibrium in CsCl. Fractions containing both the infectious particles (about 10^{12}) and the transducing particles (about 10^{10}) were combined. DNA was extracted from the phage and subjected to agarose gel electrophoresis. Standards run in parallel included intact pPL1010 extracted from a recent transductant and pPL1010 converted to a linear form by cleavage with *Bam*HI endonuclease (Fig. 4).

The resolved DNA molecules were transferred to nitrocellulose paper and hybridized with nick-translated pUB110 (pUB110 is the vector portion of pPL1010). Inspection of both the ethidium bromide gel and the autoradiograms demonstrated that the SP02 transducing particles contain no detectable monomeric pPL1010 (Fig. 4). Thus, pPL1010 is carried by transducing particles in a higher-molecular-weight form than the monomer. The autoradiograms demonstrate that pUB110 homologous sequences present in the transducing lysate DNA occur at two discrete positions in the gel (A and B). We suspect that bands A and B represent a multimer of pPL1010 in the linear and open circular forms, respectively. Although this idea remains to be proven, the transducing activity present in an SP02(pPL1010) transducing lysate is more resistant to inactivation by UV irradiation than plaque-forming activity (data not shown). This would be the case if each transducing particle carried a multimer of pPL1010.

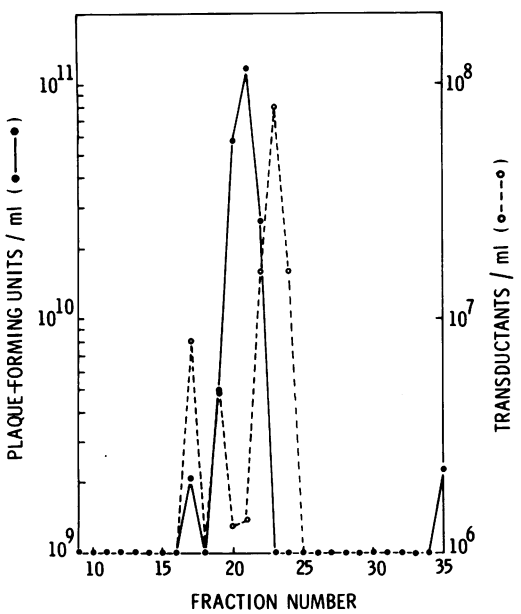

FIG. 2. *Equilibrium centrifugation in CsCl of an SP02(pPL1010) transducing lysate. One milliliter of an SP02(pPL1010) lysate in M broth (ca. 10^{11} plaque-forming units per ml and 10^{9} transducing particles per ml) was mixed with 4 ml of 10 mM Tris buffer, pH 7.2. Solid CsCl was added to achieve a final buoyant density of ca. 1.5 g/cm^3. Five milliliters of the solution was centrifuged at 45,000 rpm for 48 h in an SW50.1 rotor. The tube bottom was punctured, and 51 fractions were collected. Each was dialyzed against 10 mM Tris buffer, pH 7.2, and assayed for plaque-forming units and transducing activity on BR151. Density increases from right to left.*

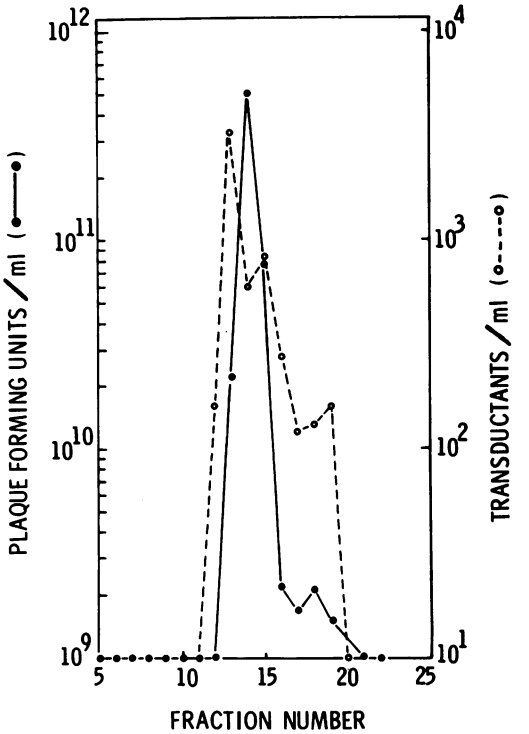

FIG. 3. *Equilibrium centrifugation in CsCl of a ϕ105(pPL1001) transducing lysate. The method used is described in the legend for Fig. 2. The ϕ105 (pPL1001) lysate contained ca. 10^{10} plaque-forming units per ml and 10^{6} transducing particles per ml.*

DISCUSSION

ϕ105 and SP02 are incapable of transduction of two small drug resistance plasmids, pCM194 and pUB110. Cloning segments of the phage genomes into either plasmid renders the constructed derivatives susceptible to transduction specifically by the phage whose DNA is present in the construct. The properties of the transduc-

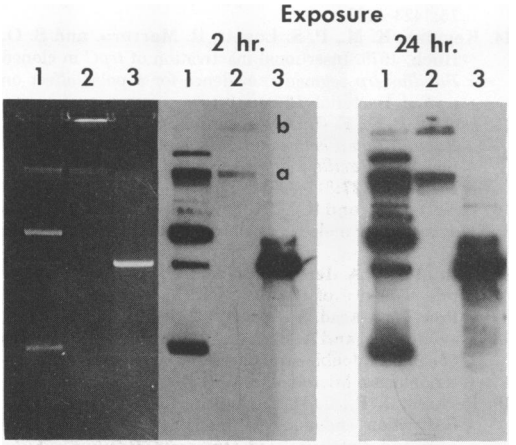

FIG. 4. *Analysis of DNA from an SP02(pPL1010) transducing lysate by agarose gel electrophoresis and hybridization with nick-translated pUB110. Well 1 contained 2 μg of pPL1010 isolated from a recent SP02-generated transductant. Well 3 contained pPL1010 converted to a linear form by cleavage with BamHI endonuclease. Well 2 contained about 10 μg of phenol-extracted (4) DNA from a CsCl gradient-purified preparation of an SP02(pPL1010) transducing lysate (10^{12} plaque-forming units per ml and 10^{10} transducing particles per ml). Autoradiograms were prepared by exposure of the hybrids to X-ray film for 2 and 24 h. The visible bands in well 1 of the autoradiograms represent (from bottom to top) the covalently closed, linear, open circular, and multimeric forms of pPL1010. All these forms are not visible in the ethidium bromide-stained gel.*

tion system thus far studied demonstrate that the process should not be classed as typical specialized transduction, although certain properties characteristic of specialized transduction are evident. A plasmid is susceptible to transduction only when a segment of phage DNA is covalently attached to the plasmid. Moreover, the susceptibility to transduction conferred on a plasmid by inserting a phage DNA segment does not exert a *trans* effect; plasmids lacking a phage DNA insert are not rendered susceptible to transduction when present in a cell with plasmids containing inserts.

The term selective plasmid transduction is used in this paper to describe what we feel are possibly two different transduction mechanisms. The transduction of plasmids containing fragments that appear to be internal within the genome of both φ105 and SP02 shows a low frequency of plasmid transfer (possibly due to a low frequency of plasmid packaging), and recovery of transductants was enhanced when the recipients were lysogenic. In the φ105 system, this enhancement is possibly due to protection of transductants against killing by infectious phage resulting from replication during the transduction process, or to the occurrence of a viral genome in the transducing particles, or both. In the SP02 system, the medium used during the transduction process suppresses replication of SP02, yet the enhancement effect was seen. This would be the case if each SP02 transducing particle (carrying a plasmid other than pPL1010) also carried a viral genome that had a probability of killing a host cell even when replication was suppressed by the growth media.

Phage packaging of hybrid plasmids containing internal segments of the φ105 or SP02 genomes may indicate that, in addition to the cohesive ends of the viral genomes (8, 18), other sites within the phage genome play a role in selective DNA packaging by the viruses. Alternatively, transient recombination between the hybrid plasmids and the replicating viral genome could permit the specific packaging of the hybrid plasmids. Unfortunately, recombination-deficient mutants of φ105 or SP02 have not been reported, nor is information available on the assembly of the two phages. Thus, rigorous testing of the above models is not possible at present.

Transduction of a plasmid, pPL1010, containing the cohesive ends of SP02 may be explained by using the information developed on the assembly of λ. The cohesive ends serve as the site of initiation for packaging DNA into the phage head (8, 18). It is probable that the incorporation of pPL1010 follows this basic pattern. The molecular mass of pPL1010 (~4.6 Md) is smaller than the SP02 genome (23 Md [25]). The form of pPL1010 detected in transducing particles is of higher molecular weight than the monomer of pPL1010. At present, we feel it likely that the transducing particles carry a multimer of pPL1010 of sufficient molecular weight to be packaged, but of lower molecular weight than the viral genome on the basis of the reduced buoyant density of the transducing particles.

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