JOURNAL OF BACTERIOLOGY, June 1990, p. 3509–3511
0021-9193/90/063509-03$02.00/0

Vol. 172, 1990

Transduction of a Plasmid Containing the Bacteriophage D3 *cos* Site in *Pseudomonas aeruginosa*

ROBERT SHARP, EVA GERTMAN, MARK A. FARINHA, AND ANDREW M. KROPINSKI*

Department of Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada K7L 3N6

Received 27 December 1989/Accepted 24 March 1990

Plasmids harboring the *cos* sequences of bacteriophage D3 can be transferred, by bacteriophage D3, into *Pseudomonas aeruginosa* by a mechanism which is insensitive to DNase. Transducing activity was separated from the plaque-forming particles by CsCl equilibrium gradient centrifugation. Restriction endonuclease digestion patterns suggest that the transducing particles contain plasmid concatemers.

D3 is a temperate, serotype-converting bacteriophage of *Pseudomonas aeruginosa* PAO (6). It has a 56.4-kilobase-pair linear double-stranded DNA genome with cohesive ends (5). This phage shares a number of features with coliphage lambda, including morphology (H.-W. Ackermann, personal communication), moles percent of GC, and genomic organization (M. A. Farinha, B. J. Allan, S. L. Ronald, and A. M. Kropinski, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, H-114, p. 188). Furthermore, Miller and Kokjohn (8) have cloned the D3 repressor (*c1*) gene in *Escherichia coli* and have shown that its expression results in repression of lambda development.

Coliphage lambda has provided the basis for a number of useful cloning techniques, which depend either on the use of the phage as a vector or on the ability of "packaging extracts" to encapsidate plasmids containing the lambda *cos* sequences (cosmids) (3). The resulting transducing particles (TPs) can then be used to transduce recipient cells. Since vectors based on phage are not yet available for *P. aeruginosa*, we are interested in the potential for utilizing phage D3 as a cloning vehicle for this bacterium.

*Sal*I and *Pvu*I-*Pst*I digestion fragments of D3 genomic DNA were cloned into the broad-host-range tetracycline and carbenicillin resistance plasmid, pRO1614 (10). The constructs were then transformed into a restrictionless mutant of *P.aeruginosa* PAO, OT684 (*leu-1 lys-1 res-4* [12]) by using the procedures of Berry and Kropinski (2). Phage D3 was propagated on this strain, and transducing activity was measured by mixing portions of diluted phage lysates with OT684. Following incubation at 37°C for 30 min, samples were plated in triplicate on tryptic soy broth (Difco Laboratories, Detroit, Mich.) supplemented with agar (15 g/liter) and tetracycline (250 μg/ml). The phage titer (in PFU) was measured by the standard overlay technique (1). Plasmids containing the *cos* site (pAK1560) were transduced, while plasmids with a similarly sized non-*cos* fragment (pAK1561) or with no cloned D3 fragment (pQF10) were not transduced (Table 1). For lysates derived from OT684 (pAK1650), the number of TPs was approximately 1% of the number of PFU.

The kinetics of gene transfer and expression of tetracycline resistance were studied by incubating pAK1560-derived lysates for 10 to 120 min with OT684 target cells and then plating them as described above. The number of transductants appeared to level off after about 2 h of incubation (Fig. 1).

* Corresponding author.

In order to gain insight into the nature of this genetic transfer system, lysates derived from cells carrying pAK1560 were incubated with DNase I (0.1 mg/ml; Boehringer Mannheim Canada, Dorval, Quebec, Canada) for 2 h, with *Streptomyces griseus* protease (0.1 mg/ml; Sigma Chemical Co., St. Louis, Mo.) for 2 h, and with 5 to 25 mM sodium deoxycholate for 1 h. These samples were then tested for transducing activity and PFU. None of these treatments significantly affected plaque-forming or transducing activity of the lysates.

Phage was precipitated from a 2-liter lysate by using 10% polyethylene glycol 6000 (13), and the pellet was suspended in 10 mM Tris hydrochloride (pH 8.0)–100 mM NaCl–10 mM $MgCl_2$ buffer. This suspension was sequentially treated at 37°C for 30 min with DNase I (2 μg/ml), for 30 min with *S. griseus* protease (100 μg/ml), and for 1 h with 10 mM sodium deoxycholate. The suspension was clarified by 10 min of centrifugation at 10,000 × *g*, and the supernatant was adjusted to a density of 1.3 g/cm^3 by the addition of solid CsCl. This adjusted supernatant was layered onto a CsCl step gradient (1.45, 1.52, and 1.6 g/cm^3) and centrifuged at 45,000 × *g* for 4 h. The phage bands were removed with a Pasteur pipette and then combined and centrifuged to equilibrium (160,000 × *g*, 20 h, 10°C). Three blue bands, corresponding to phage, TPs, and empty phage heads, were visible on the gradient. The gradient was fractionated from the top, and samples were tested for PFU, transducing activity, and refractive index (Fig. 2). The D3 PFU band had an average density of 1.528 g/cm^3, while TPs banded at densities of 1.498 and 1.481 g/cm^3. In one case, an additional band was observed at 1.523 g/cm^3. If it is assumed that pAK1560 has a base composition similar to that of D3, then the density of TPs should be dependent on the number of plasmids in the packaged concatemer. The gradient results suggest that two or three different concatemers slightly shorter than the D3 genome are packaged. A 50-fold purification of TPs was achieved in the fraction showing the maximum transducing activity.

In order to analyze the nature of the packaged plasmid DNA, DNA from the plaque-forming and transducing particles was isolated by using the following procedure. Phage (1 volume) was mixed with 1/10 volume of 2 M Tris hydrochloride–0.2 M EDTA (pH 8.5). Phage was lysed by adding 1 volume of formamide and 1 volume of water. DNA was precipitated with 2 volumes of 95% ethanol, washed with 70% ethanol, dried in vacuo, and finally dissolved in 10 mM Tris hydrochloride–1 mM EDTA (pH 8.0).

Phage D3 (5), pQF10 (4), and pAK1560 DNAs each

TABLE 1. Transduction of plasmids in *P. aeruginosa* by phage D3

Host strain	Phage titer in lysate (10^{10} PFU/ml)	Transducing activity in lysate (CFU/ml)[a]	% TP in phage lysate
OT684	1.6	<10	$<6.2 \times 10^{-8}$
OT684(pAK1560)	4.5	4.5×10^{8}	1.0
OT684(pAK1561)	4.1	<10	$<2.4 \times 10^{-8}$
OT684(pQF10)	5.4	<10	$<1.9 \times 10^{-8}$

[a] Transducing activity was measured with OT684 recipient cells and scoring for Tet^r colonies after 48 h of incubation at 37°C.

contain a single *Bam*HI-sensitive site. *Bam*HI-restricted and undigested DNA were electrophoretically resolved on 0.5% agarose gels. The DNA was then transferred to Biotrace NT membranes (Gelman Sciences, Inc., Ann Arbor, Mich.) by using the protocols suggested by the manufacturer and was probed with D3 and pQF10 DNAs labeled with [α-^{32}P]dCTP (Du Pont Canada, Inc., Mississauga, Ontario, Canada) by means of a nick translation kit (GIBCO/BRL Canada, Burlington, Ontario, Canada).

As expected, the *Bam*HI-digested DNA from the plaque-forming-particle preparation exhibited two bands when probed with ^{32}P-labeled D3c DNA (Fig. 3). *Bam*HI-digested TP DNA contained four hybridizing bands, two of which corresponded in size to the two phage DNA-derived fragments; the third corresponded to a fragment the size of pAK1560. In addition, a fourth band migrating slightly slower than D3 *Bam*HI-B was observed. The origin of this fragment, which hybridizes only to D3 DNA, is unknown but may be defective phage particles. The phage-derived bands in the TP DNA preparation are due to the incomplete purification of TPs. The undigested DNA from the TP preparation showed several hybridizing bands, probably corresponding to linear and open circular forms of phage and polymeric plasmid DNAs. Since no DNA the size of pAK1560 was detected, we conclude that a polymerized form of the plasmid was packaged. This conclusion was enhanced by the results with the pQF10 probe, which only hybridized to a low-molecular-weight band in the TP DNA

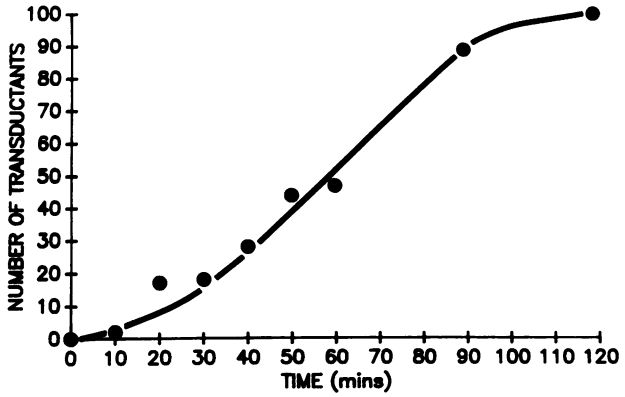

FIG. 1. Samples of phage D3 lysate (2.7×10^3 PFU), prepared by infecting OT684(pAK1560), were mixed with 1-ml cultures of *P. aeruginosa* OT684 grown in tryptic soy broth to an optical density at 650 nm of 0.4. The cultures were incubated at 37°C, and at the times indicated, 0.1-ml portions were plated in triplicate on tryptic soy agar plates supplemented with tetracycline (250 μg/ml). Colonies were counted after 48 h of incubation.

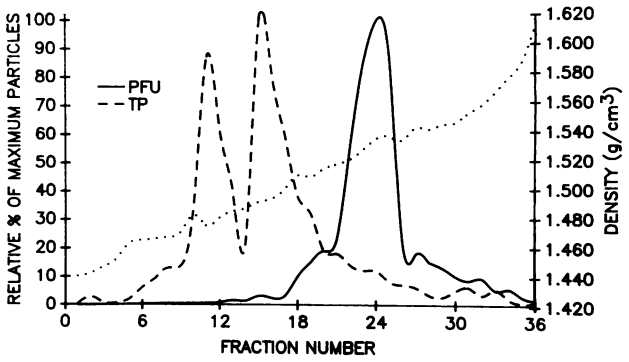

FIG. 2. Phage particles (1.7×10^{13} PFU) purified from a lysate of OT684(pAK1560) by sedimentation through a CsCl step gradient were centrifuged to equilibrium in CsCl (initial density, 1.5 g/cm^3). The gradient was fractionated from the top by displacement with dense CsCl (1.8 g/cm^3). The PFU (—) and transducing activity (TP) (- - - - -) of each fraction were measured. In addition, the density (. . . . .) of each fraction was calculated from refractometric measurements by using the following relationship: $\rho^{25} = 10.8601\ \eta_D{}^{25} - 13.4974$, where ρ is density and η_D is the refractive index. The titers of the phage particles (PFU per milliliter) and TPs (TP per milliliter) for the peak fractions were as follows: fraction 25, 1.4×10^{13} PFU/ml and 2.0×10^9 TP/ml; fraction 15, 4.4×10^{11} PFU/ml and 1.6×10^{10} TP/ml; and fraction 11, 8.2×10^{10} PFU/ml and 1.4×10^{10} TP/ml.

preparation after restriction endonuclease digestion. The *Pvu*I digest of D3c (a clear-plaque mutant of D3) DNA was included as a molecular weight marker.

These results suggest that plasmids containing *cos*-like sequences from phage D3 can act in the same manner in *P. aeruginosa* are cosmids do in *E. coli*. Transfer of these "cosmids" between *P. aeruginosa* strains cannot be due to conjugation or transformation, since donor cells were lysed with chloroform and DNase treatment of the lysates did not affect the transfer. This means that transfer must have occurred by transduction of the plasmid DNA inside phage particles. Since efficient transduction does not occur with a

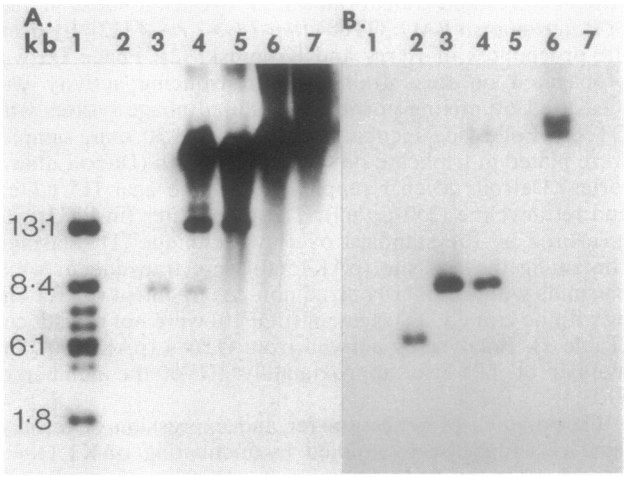

FIG. 3. Southern blots of DNA from CsCl-purified phage and TPs probed with ^{32}P-labeled D3c DNA (A) and ^{32}P-labeled pQF10 DNA (B). Lane 1, Molecular weight markers (*Pvu*I-cut D3c DNA); lane 2, *Bam*HI-cut pQF10; lane 3, *Bam*HI-cut pAK1560; lane 4, *Bam*HI-cut D3 TP DNA; lane 5, *Bam*HI-cut D3 phage DNA; lane 6, uncut TP DNA; lane 7, uncut D3 phage DNA. kb, Kilobase.

broad-host-range plasmid like pQF10, generalized transduction is not occurring. Also, the fragment of D3 DNA that was needed for transduction contained the D3 *cos* sequence, since a similar fragment without the *cos* sequence did not cause increased transduction. This rules out the possibility of transduction being mediated by recombination between the plasmid and helper phages at the phage DNA insert in the plasmid, as has been shown to occur in other transduction systems (7, 9, 11).

The results given above indicate that D3 encapsidation occurs in a *cos*-dependent manner similar to that of lambda encapsidation and that plasmid concatemers similar in length to the D3 genome are packaged in capsids at a frequency of 1% of phage genome packaging rates. This suggests that an efficient cosmid cloning system for *P. aeruginosa* could be developed from plasmids containing the D3 *cos* site. These plasmids would allow cloned DNA to be effectively introduced into *P. aeruginosa* after encapsidation by a packaging extract and would also allow the size selection of DNA fragments 40 to 50 kilobase pairs long.

This research was supported by grants from the Medical Research Council and the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

1. **Adams, M. H.** 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. **Berry, D., and A. M. Kropinski.** 1986. Effect of lipopolysaccharide mutations and temperature on plasmid transformation efficiency in *Pseudomonas aeruginosa*. Can. J. Microbiol. **32:** 436–438.
3. **Collins, J., and B. Hohn.** 1978. Cosmids: a type of plasmid gene-cloning vector that is packageable in vitro in bacteriophage lambda heads. Proc. Natl. Acad. Sci. USA **75:**4242–4246.
4. **Farinha, M. A., and A. M. Kropinski.** 1989. Construction of broad-host-range vectors for general cloning and promoter selection in *Pseudomonas aeruginosa* and *Escherichia coli*. Gene **77:**205–210.
5. **Gertman, E., D. Berry, and A. M. Kropinski.** 1987. Serotype-converting bacteriophage D3 of *Pseudomonas aeruginosa*: vegetative and prophage restriction maps. Gene **52:**51–57.
6. **Kuzio, J., and A. M. Kropinski.** 1983. O-antigen conversion in *Pseudomonas aeruginosa* PAO1 by bacteriophage D3. J. Bacteriol. **155:**203–212.
7. **Marrero, R., and P. S. Lovett.** 1980. Transductional selection of cloned bacteriophage ϕ105 and SP02 deoxyribonucleic acids in *Bacillus subtilis*. J. Bacteriol. **43:**879–886.
8. **Miller, R. V., and T. A. Kokjohn.** 1987. Cloning and characterization of the *c1* repressor of *Pseudomonas aeruginosa* bacteriophage D3: a functional analog of phage lambda *c*I protein. J. Bacteriol. **169:**1847–1852.
9. **Novick, R. P., I. Edelman, and S. Lofdahl.** 1986. Small *Staphylococcus aureus* plasmids are transduced as linear multimers that are formed and resolved by replicative processes. J. Mol. Biol. **192:**209–220.
10. **Olsen, R. H., G. DeBusscher, and W. R. McCombie.** 1982. Development of broad-host-range vectors and gene banks: self-cloning of *Pseudomonas aeruginosa* PAO chromosome. J. Bacteriol. **150:**60–69.
11. **Orbach, M. J., and E. N. Jackson.** 1982. Transfer of chimeric plasmids among *Salmonella typhimurium* strains by P22 transduction. J. Bacteriol. **149:**985–994.
12. **Potter, A. A., and J. S. Loutit.** 1982. Exonuclease activity from *Pseudomonas aeruginosa* which is missing in phenotypically restrictionless mutants. J. Bacteriol. **151:**1204–1209.
13. **Yamamoto, K. R., P. M. Alberts, R. Benzinger, L. Lawhorne, and B. Treiber.** 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology **40:**734–744.