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Properties of the Streptomycete Temperate Bacteriophage FP43

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FP43 is a temperate bacteriophage for *Streptomyces griseofuscus* that forms plaques on many *Streptomyces* species. FP43 virions contain 56 kb of double-strand DNA that is circularly permuted and terminally redundant, and contains 65% G+C. A physical map of the FP43 genome was constructed, and the origin for headful packaging (*pac*) was localized to an 8.8-kb region of the genome (*hft*) that mediates high-frequency transduction by FP43 of plasmid pRHB101. The phage attachment site (*attP*), a replication origin (*rep*), a region that inhibits plaque formation (*pin*), and a 3-kb deletion (*rpt*) that caused a 100-fold reduction in plasmid transduction were mapped.

Bacteriophage FP43 has broad host specificity for *Streptomyces* spp. (8, 22, 23, 25–27) and has been used in conjunction with several other bacteriophages to explore restriction and modification in streptomycetes (8, 22, 23). FP43 has also been used as a vector to transduce plasmid pRHB101 into species of *Streptomyces*, *Chainia*, *Streptoverticillium*, and *Saccharopolyspora*, including strains that restrict FP43 plaque formation (4, 25–27). pRHB101 contains an 7.7-kb segment of FP43 DNA (designated *hft*) cloned into the *Sph*I site of plasmid pIJ702 (26). The transducing particles contained linear concatemers of pRHB101, which formed circular monomers in the transductants. Since this transduction system can be used to circumvent certain restriction barriers (25–27) and might be developed to transfer cloned genes into many streptomycete hosts (4), we were interested in further characterizing FP43 and the mechanism of plasmid transduction. We report here that FP43 is a temperate bacteriophage that packages its DNA by a headful mechanism. We constructed a physical and partial functional map of the FP43 genome and determined that the fragment of DNA that mediates high-frequency plasmid transduction (*hft*) contains a packaging origin or *pac* site.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophages. The *Streptomyces griseofuscus* strains and plasmids used in this study are described in Table 1. The bacteriophages used included FP4, FP43, FP46, FP50, FP55, FP60, FP61 (8), FP22 (8, 13), VP11 (11), and R4 (7).

Media and growth conditions. *Streptomyces* strains were grown in Trypticase soy (TS) broth supplemented with the appropriate antibiotics as described (3, 25). Bacteriophage lysates were prepared on *S. griseofuscus* on NC [nutrient broth plus 4 mM $Ca(NO_3)_2$] agar plates as described previously (8); NC broth was as previously described (8).

Protoplast formation, regeneration, and transformation. Formation of protoplasts from *S. griseofuscus* cells and regeneration of cells from protoplasts was carried out as previously described (3). Transformation of protoplasts by plasmid DNA was as previously described (26).

Plasmid transduction. Transduction of plasmid DNA was carried out as previously described (26).

Molecular techniques. Bacteriophage DNA was isolated by the method of Hopwood et al. (15). Phage DNA was cleaved with restriction enzymes according to the specifications of the suppliers (Boehringer Mannheim Biochemicals, Bethesda Research Laboratories, Inc., and New England BioLabs Inc.). FP43 DNA was cut slowly by some enzymes (e.g., *Eco*RI, *Bsp*HI, *Bal*I, *Aat*II, *Sph*I); therefore, additional amounts of enzyme and long incubation times were used to obtain complete digestion. DNA samples were run on 0.6 to 0.8% agarose gels in Tris phosphate buffer (30). DNA fragments were isolated from agarose gels by electrophoresis onto dialysis membranes (30). Total DNA of *S. griseofuscus* was isolated by the method of Fishman and Hershberger (12). FP43 DNA was labeled with [^{32}P]dCTP by using a random primed DNA labeling kit (Boehringer Mannheim Biochemicals), and Southern hybridizations were carried out as described by McHenney and Baltz (26). Pulsed-field gel electrophoresis was carried out as previously described (31).

Guanine-plus-cytosine (G+C) content was determined as described (10, 20) by using a Gilford spectrophotometer and thermoprogrammer with bacteriophage λ DNA as a control. Electron microscopy was carried out on negatively stained FP43 phage particles in 1% aqueous uranyl acetate.

Construction of a physical map of FP43. A preliminary restriction map of FP43 was constructed by agarose gel analysis of double and triple digests of FP43 DNA with *Xho*I, *Eco*RV, *Eco*RI, *Sna*BI and *Sph*I. Care was taken to note submolar and diffuse bands in these digests. This restriction map was confirmed and expanded by two methods. First, local regions were compared with restriction maps of cloned segments of FP43 DNA. Second, FP43 DNA cut with specific restriction enzymes was run on agarose gels, transferred to nylon membranes, and probed with ^{32}P-labeled DNA probes. DNA probes were either cloned segments of FP43 DNA or purified *Sph*I and *Xho*I restriction fragments of FP43 DNA.

Anti-FP43 antiserum. Anti-FP43 antiserum (26) (K, 3,000 min^{-1}) was diluted 10^4-fold into NC broth containing bacteriophages at about 10^8 PFU/ml and then incubated for 30 min at 37°C. Bacteriophages were diluted and assayed for plaque formation on *S. griseofuscus* C581. First-order inactivation rate constants (K) were calculated: $K = \ln (N/N_0)T^{-1}$, where N is the number of PFU surviving antiserum treatment, N_0 is the number of PFU before antiserum treatment, and T is time in minutes. Antiserum cross-reactivity was

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TABLE 1. Strains and plasmids

Strain or plasmid	Relevant characteristic[a]	Source or reference
S. griseofuscus strains		
C581	Nonrestricting host	ATCC 23916; 8
KC18	FP43 lysogen of C581	This study
BES2057	C581 containing plasmid pKC684 integrated into the chromosome	2a
MM10	C581 containing plasmid pRHB101	25
MM27	C581 containing plasmid pIJ702	This study
MM28	C581 containing plasmid pRHB121	This study
Plasmids		
pIJ702	Vector for actinomycetes; Tsr^r	15, 16, 18, 21, 22
pMT660	*ts* derivative of pIJ702	5
pRHB101[b]	pIJ702 containing 7.7-kb *Sph*I (*hft*) fragment from FP43	27a
pRHB106	pMT660 containing 8.8-kb *Sph*I C (*hft*) fragment from FP43	26, 27a
pRHB121	pIJ702 containing the 4.4-kb *Sph*I E (*pin*) fragment from FP43	This study
pKC684	Bifunctional for *E. coli* and streptomycetes; contains plasmid pSAM2 *att* and *int* functions, Am^r and 4.4-kb *pin* and *rep* from FP43	2b

[a] Tsr^r, thiostrepton resistance; Am^r, apramycin resistance.
[b] The 7.7-kb *Sph*I insert in pRHB101 contains the FP43 *Sph*I C fragment with a spontaneous 1.4-kb deletion and a 0.3-kb *Sph*I fragment.

determined by dividing the K values obtained for the different bacteriophages by the K value obtained for FP43.

RESULTS

Physical properties of FP43. FP43 phage particles contained double-stranded DNA (8) with a G+C content of 65% as determined by the melting temperature. Electron microscopic analyses indicated that FP43 had a hexagonal (probably icosahedral) head of 50 ± 3 nm maximum diameter and a tail of 223 ± 30 nm length, with a spike at the end (morphology type B1 [1]).

Restriction endonuclease cleavage analysis and restriction map of FP43 DNA. Since restriction is a major barrier of plaque formation for broad-host-range streptomycete bacteriophages (6, 8, 22, 23), we examined the restriction endonuclease cleavage profile of FP43 DNA, with particular emphasis on enzymes or isoschizomers of enzymes produced by streptomycetes. Twenty-four of 50 restriction enzymes with six-base palindromic recognition sequences failed to cleave FP43 DNA (Table 2). FP43 DNA was not cut by 14 of 20 enzymes that recognize A+T-rich sequences, possibly because of the high G+C content of FP43 DNA. We examined 14 restriction enzymes or isoschizomers identified in *Streptomyces* spp. (29); nine cut FP43 DNA and five did not.

We examined the cleavage patterns of DNA from wild-type FP43 and a deletion mutant, FP43 *rpt-1* (see below), with several restriction endonucleases that cleaved FP43 DNA less than 10 times. Figure 1 shows that submolar or diffuse bands were observed after cleaving FP43 *rpt-1* with *Sph*I (lane 1B), *Eco*47III (lane 2D), *Sna*BI (lane 3F) and *Mse*I (lane 4H). The submolar bands and diffuse bands noted (by arrowheads) were not observed visually when wild-type FP43 DNA was cleaved by the same enzymes (lanes 1A, 2C, 3E, and 4G). However, Southern hybridization analysis, with the 2.6-kb *Sph*I fragment (see lane 1B, arrowhead) as a probe, demonstrated that the 2.6-kb fragment was present in FP43 DNA and migrated at 2.6 kb after cleavage with *Sph*I.

The presence of submolar or diffuse bands from FP43 *rpt-1* DNA suggested that FP43 packaged its DNA by a headful mechanism. The inability to observe the submolar bands without radiolabeling of wild-type FP43 DNA suggested that it may package many headfuls from linear concatemers after initiating packaging, thus diluting out the end fragments from the initial packaging event. By using P22 headful packaging as a model (17, 32), a map of FP43 DNA was constructed, oriented to the site of initiation of DNA packaging (*pac*) (Fig. 2). The average sum of restriction fragment sizes indicated that approximately 56 kb of DNA was packaged in each FP43 phage particle (Fig. 2 and 3).

The orientation of DNA packaging and the size of the terminal redundancy was determined by analyzing *Sph*I digests of FP43 DNA. In *Sph*I digests of FP43 DNA, a 2.6-kb substoichiometric band corresponded to the *pac* fragment. The *pac* fragment, which has one end at *pac* and one end at

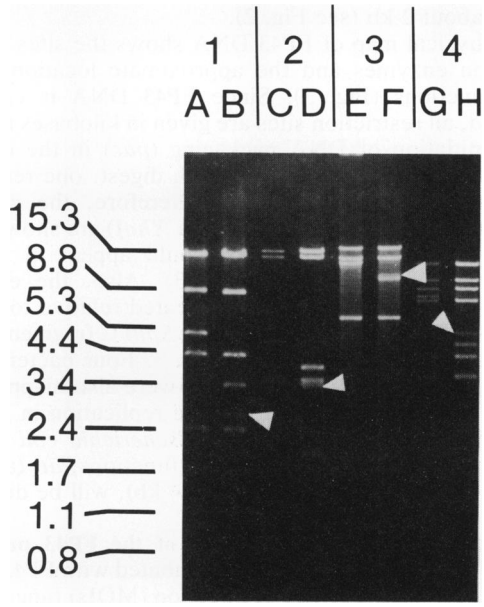

FIG. 1. Restriction digests of FP43 (A, C, E, G) and FP43 *rpt-1* (B, D, F, H) DNA. A and B, *Sph*I; C and D, *Eco*47III; E and F, *Sna*BI; G and H, *Mse*I. The molecular weight markers are in kilobases. Submolar or diffuse bands are marked with arrowheads.

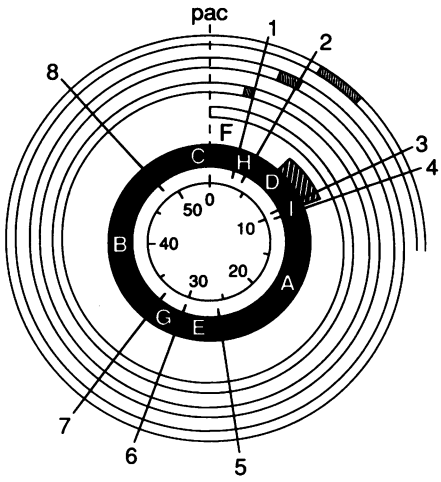

FIG. 2. Circular map of FP43 DNA and proposed model for headful packaging of FP43 DNA. The central scale is in kilobases from the proposed *pac* site (dashed line). *Sph*I fragments are lettered, and restriction sites are indicated by radial lines. DNA from an FP43 concatamer is presented as a spiral around the FP43 restriction map in the direction of proposed DNA packaging. ▨, sites of termination of successive headfuls and initiation of next headful. ▨, approximate extent of the deletion in FP43 *rpt-1*.

a restriction site, is present only in the first headful, due to the circularly permuted DNA (17, 32). The *Sph*I C band was diffuse and ran as a doublet under some electrophoretic conditions (data not shown), indicating that termination took place close to one end of this fragment. Also, the radiolabeled 2.6-kb *Sph*I F band hybridized to the 8.8-kb *Sph*I C band of wild-type FP43 (not shown). Therefore, *pac* must map 2.6 kb from the *Sph*I site and reside on the *Sph*I C fragment (Fig. 2 and 3); the *Sph*I C fragment was shown previously to facilitate transduction of plasmid DNA (26). These data imply that the terminal redundancy in FP43 DNA may be about 2 kb (see Fig. 2).

The physical map of FP43 DNA shows the sites for nine restriction enzymes and the approximate locations of six phage functions (Fig. 3). Since FP43 DNA is circularly permuted, all restriction sites are given in kilobases from the site of initiation of DNA packaging (*pac*) in the order of expected DNA packaging. For each digest, one restriction fragment was bisected by *pac*; therefore, the complete *pac*-containing fragments (except for *Xho*I) are shown at the right end of the map as they would appear in a linear concatemer of FP43 DNA (Fig. 3). Also, the expected location of headful cleavage is indicated relative to restriction sites. The 8.8-kb *pac*-containing *Sph*I C fragment (*hft*) is indicated at the bottom right in Fig. 3. Four bacteriophage markers, in addition to *pac* and *hft*, were also mapped. The *rep* function, which allowed plasmid replication in *Streptomyces* spp. when cloned into an *Escherichia coli* plasmid (2b), mapped at 29 kb. Three other functions, *att* (at 2 kb), *pin* (at 28 kb), and *rpt* (at 5.2 to 10.4 kb), will be discussed below.

Lysogeny and physical structure of the FP43 prophage. When *S. griseofuscus* cells were incubated with FP43 on NC agar plates at multiplicities of infection (MOIs) ranging from about 0.1 to 10, confluent lysis was observed, followed by growth of discrete colonies. The frequency of FP43-resistant colonies per PFU was highest at low MOIs ($\sim 5 \times 10^{-4}$) and was about 2×10^{-5} at MOI ~10. The frequency of FP43-resistant colonies per CFU increased from $\sim 4 \times 10^{-5}$ to 2×10^{-4} as the MOI was increased from 0.06 to 11.

Eighteen FP43-resistant colonies were streaked for isolation, and cells from isolated colonies were patched on NC agar and overlaid with wild-type *S. griseofuscus* at 48 h. Seventeen patches yielded PFU after overnight incubation. Well-isolated colonies from nine of the original FP43-resistant clones were grown in NC broth for 24 h, and filtrates from eight contained PFU. Also, none of the eight strains were hosts for FP43 plaque formation (efficiency of plating, $< 10^{-7}$).

One of the putative lysogens, *S. griseofuscus* KC18, was tested for plaque formation by several other streptomycete bacteriophages (FP4, FP22, FP46, FP50, FP55, FP60, FP61, VP11, and R4), and all nine phages formed plaques. Even though KC18 blocked plaque formation by FP43, it was a recipient for FP43-mediated transduction of plasmid pRHB101, indicating that it blocked FP43 plaque formation at a stage beyond attachment and injection of DNA. Putative FP43 lysogens were readily cured of prophage (>10%) by converting cells to protoplasts and then regenerating cells from protoplasts.

To determine the physical structure of the FP43 prophage, DNA from KC18 and wild-type *S. griseofuscus* C581 was probed with FP43 DNA labeled with [α-^{32}P]dCTP. FP43

TABLE 2. Cleavage of FP43 DNA by restriction endonucleases that recognize hexanucleotide palindromes

No. of G-C base pairs in restriction site	Enzyme[a]	No. of sites in FP43 DNA[b]
6	*Sma*I	0
	**Sac*II	~15
	*Eag*I	>30
	**Nae*I	0
	*Bss*HII	>10
	*Nar*I	16
	*Apa*I	>20
4	*Mlu*I	10
	*Eco*47III	9
	**Stu*I	0
	**Pvu*II	~20
	*Nco*I	11
	*Pvu*I	0
	*Avr*II	0
	*Spl*I	>6
	**Xho*I	1
	**Pst*I	0
	*Aat*II	+
	*Pml*I	>10
	**Sac*I	0
	**Sph*I	8
	*Bam*HI	0
	*Nhe*I	0
	*Kpn*I	7
	**Sal*I	>30
	**Apa*LI	10
	*Bsp*MII	0
	**Nru*I	16
	*Fsp*I (*Avi*II)	13
	*Bal*I	+

[a] Asterisks indicate restriction enzyme specificities found in the streptomycetes (29).

[b] Six of twenty enzymes that recognize A+T-rich hexanucleotide palindromes cut FP43 DNA the following number of times: *Bsp*HI, 8; **Bcl*I, 13; *Eco*RI, 4; *Eco*RV, 2; *Sna*BI, 4; **Asu*II, 10. Those that failed to cut were: *Afl*II, *Ase*I, *Bgl*II, *Cla*I, *Dra*I, *Hin*dIII, *Hpa*I, *Nde*I, *Nsi*I, **Sca*I, *Spe*I, *Ssp*I, *Xba*I, and *Xca*I. + indicates failure to generate reproducible complete digests.

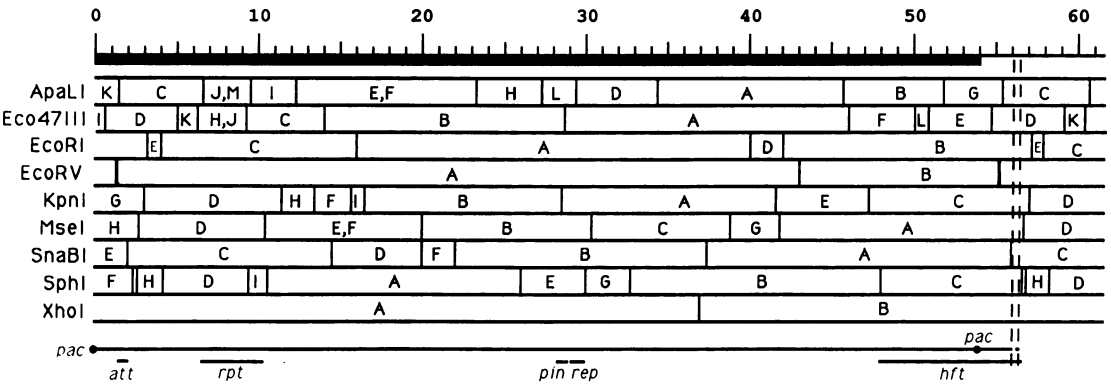

FIG. 3. Restriction map of FP43 DNA. The *pac* site was used as the left end of the linear FP43 map. The scale at the top of the figure is in kilobases, and the bold part of the line indicates the FP43 genome. The line at the bottom indicates the length of DNA from the first headful packaged, with cleavage taking place within the dashed vertical lines. The approximate locations of FP43 functions are indicated at the bottom of the figure. Substoichiometric *pac*-containing fragments were evident in most digestions of FP43 *rpt-1* (i.e., ApaLI K; *Eco*47III I; *Kpn*I G; *Mse*I H; *Sna*BI E; and *Sph*I F; see Fig. 1).

DNA hybridized strongly to DNA from KC18 (Fig. 4) but did not hybridize to DNA from *S. griseofuscus* C581 (data not shown). The FP43 prophage was integrated, since the 8.8-kb *Sph*I C fragment was not observed in KC18, but two new junction fragments were observed (Fig. 4, lane 1). The 13.4-kb *Sna*BI band was also not detected in KC18, but at least one new high-molecular-weight *Sna*BI band was observed. These results indicated that the *att* site must be located on the ~0.3-kb segment common to the *Sph*I C and the *Sna*BI C fragments.

Uncut total DNA of KC18 was analyzed by pulsed-field gel electrophoresis under conditions that give good separation of 60-kb and 200-kb linear plasmids (pSGF3 and pSGF2) from *S. griseofuscus* chromosomal DNA (31). KC18 had two linear plasmids identical in size to native pSGF2 and pSGF3, indicating that FP43 did not insert into either plasmid.

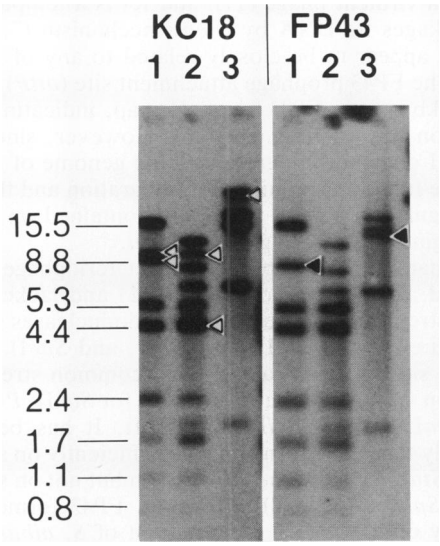

FIG. 4. Analysis of the FP43 prophage by Southern hybridization. The Southern blot of KC18 (a FP43 lysogen) and FP43 DNA was hybridized with random primer-labeled ([^{32}P]dCTP) FP43 DNA. ◀, FP43 bands containing *attP*; ◁, junction fragments. The scale at left is in kilobases. Lane 1, *Sph*I digest; lane 2, *Sph*I plus *Sna*BI digest; lane 3, *Sna*BI digest.

A 4.4-kb *Sph*I E fragment of FP43 DNA was cloned into the *Sph*I site of pIJ702. *S. griseofuscus* strains carrying the resulting plasmid (pRHB121) did not support FP43 plaque formation. The FP43 4.4-kb segment associated with plaque inhibition (*pin*) was subcloned into a plasmid which contains the pSAM2 attachment site and integrase (19). The resulting plasmid, pKC684, integrated into the chromosome of *S. griseofuscus* in a single copy after transformation (2a). *S. griseofuscus* BES2057, containing the integrated *pin* gene, conferred resistance to FP43 but retained sensitivity to bacteriophages FP4 and FP22. The *pin* gene mapped to 28 kb on the FP43 physical map, adjacent to the *rep* function.

Anti-FP43 antiserum cross-reactivity with other streptomycete bacteriophages. Antiserum raised against FP43 particles was incubated with FP43 and with nine other streptomycete bacteriophages. The temperate phage R4 and virulent phage VP11 showed the highest cross-reactivity, 25 and 30%, respectively. The remaining phages showed cross-reactivity ranging from 0 to 11% (data not shown).

Effects of lysogeny and the *pin* gene on transduction of plasmid DNA. It was shown previously that FP43-mediated transduction of plasmid pRHB101 was inhibited at phage concentrations higher than 10^5 PFU per plate. When anti-FP43 antiserum was added to plates 3.5 h after the addition of phage and cells, the number of transductants increased with increasing PFU up to about 10^6 per plate and then declined, presumably because of lysis of transductants (26). We wanted to know if resistance to FP43 infection could block lysis on plates and allow transduction to occur at higher MOIs. *S. griseofuscus* strains KC18 (carrying the FP43 prophage) and BES2057 (*pin*) were used as hosts for FP43-mediated transduction of pRHB101. When KC18 was used as a recipient, the number of transductants increased proportionally with increasing PFU up to 10^8 PFU per plate, and semiconfluent to confluent lawns of transductants were obtained from higher concentrations of PFU. Similar results were obtained with BES2057, which contained the *pin* gene inserted into the chromosome. BES2057 is a more convenient host, since it grows like wild-type *S. griseofuscus*, whereas KC18 appears to undergo some lysis at high cell densities.

The ability to block FP43-mediated lysis of potential transductants by inserting the *pin* gene into the chromosome should allow much more accurate estimates of low-frequency transduction events. We were interested in knowing the magnitude of *hft*-mediated transduction over the frequency of transduction of plasmid pIJ702 containing no FP43 DNA insert. Lysates of FP43 were prepared on *S. griseofuscus* strains containing pIJ702, pRHB101, and pRHB106 and used to transduce *S. griseofuscus* BES2057. Both of the plasmids containing *hft* (pRHB101 and pRHB106) were transduced into BES2057 at frequencies of $\sim 10^{-5}$ per PFU, whereas pIJ702 was not transduced at detectable levels ($<10^{-9}$).

Effects of the 3-kb *rpt-1* deletion of FP43 DNA on headful packaging and plasmid transduction. After repeated passage on *S. griseofuscus*, a lysate of FP43 transduced pRHB101 at a 100-fold lower efficiency than originally observed. Restriction digests of DNA from this lysate indicated that more than 90% of the DNA from phage particles of this lysate contained a specific 3.0-kb deletion (Fig. 1). This deletion mapped between the *Eco*47III site at 5.2 kb and the *Mse*I site at 10.4 kb (Fig. 3) and was called *rpt* for reduced plasmid transduction. The plaques of the plaque-purified mutant (FP43 *rpt-1*) were indistinguishable from the wild type; however, the mutant had three measurable phenotypes. First, FP43 *rpt-1* transduced pRHB101 at frequencies averaging about 3×10^{-6} per PFU, whereas the plaque-purified wild-type FP43 transduced at an average frequency of 9×10^{-4} per PFU. Second, the *pac* fragment(s) in restriction enzyme digests of FP43 *rpt-1* DNA showed a greater intensity relative to neighboring bands than wild-type FP43 *pac* fragments (Fig. 1). Third, the yield of PFU per plaque for FP43 *rpt-1* was about 4×10^5 on plates containing less than 300 PFU or more than 10,000 PFU (and yielding confluent lysis). The yield of PFU per plaque for the wild-type FP43 containing less than 300 plaques per plate was 2.5×10^6 PFU per plaque, whereas plates exhibiting nearly confluent lysis yielded only 3×10^4 to 15×10^4 PFU per plaque.

DISCUSSION

We have been interested in bacteriophage FP43 because of its ability to transduce pIJ702-based plasmids containing a segment of FP43 DNA, *hft*. We showed previously that FP43 transducing particles contained linear concatemers of plasmid DNA (26). This suggested that the *hft* region might be involved in facilitating rolling-circle replication, packaging of plasmid DNA in phage heads, or both. We have shown here that FP43 packages its DNA by a headful mechanism and that virions contain circularly permuted DNA with about 4% terminal redundancy. We have mapped several functions to the ~54-kb genome and have shown that the *hft* region contains a packaging origin or *pac* site. The headful packaging mechanism and the presence of the *pac* site on *hft* may explain, at least in part, the very efficient transduction of plasmid pRHB101, which contains *hft*. Transduction studies reported here with *S. griseofuscus* BES2057, which contains the *pin* gene inserted in the chromosome, suggest that *hft* stimulates transduction of pIJ702 by at least 10^4-fold. It is not yet known if *pac* alone is sufficient to mediate high-frequency transduction, however. Since *S. griseofuscus* BES2057 was not lysed by FP43 at high MOIs and was transducible by pRHB101 but not by pIJ702, this strain should provide a means to accurately measure the effects on transduction of deleting parts of the *hft* fragment.

During the course of these studies, we identified a deletion mutant, FP43 *rpt-1*, that had an apparent selective growth advantage over wild-type FP43 in confluent lysates on NC agar plates, since it yielded more PFU per plaque than did the wild-type FP43 under these conditions. FP43 *rpt-1* was at least 100-fold less efficient than the wild-type FP43 in transducing pRHB101 in *S. griseofuscus*. Restriction endonuclease cleavage analysis indicated that FP43 *rpt-1* DNA had a higher molar ratio of the *pac* initiating fragment to other fragments than did the wild-type FP43. This indicated that FP43 *rpt-1* packaged fewer headfuls of DNA after cleavage at a given *pac* site. FP43 *rpt-1* may have initiated *pac* cleavage more frequently than FP43. Alternatively, FP43 *rpt-1* may have been less efficient at packaging subsequent headfuls after the initial (*pac*-initiated) event. More frequent initiation of *pac* cleavage could explain the reduced transduction of pRHB101, since this plasmid contains one *pac* site per 13 kb in linear concatemers, whereas FP43 DNA contains one *pac* site per 54 kb. More frequent cleavage at *pac* sites might also be associated with the high yields of PFU per plaque observed with FP43 *rpt-1* on NC agar plates containing nearly confluent plaques.

We demonstrated that FP43 is a temperate bacteriophage that forms lysogens of *S. griseofuscus* that release PFU during growth. *S. griseofuscus* lysogens were readily cured of FP43 by protoplast formation and regeneration. This procedure has been shown previously to be an efficient method to cure autonomously replicating plasmids from streptomycetes (16). However, our results indicated that FP43 DNA was not inserted into either of the large linear plasmids in *S. griseofuscus*. The insertion of FP43 into the *S. griseofuscus* genome also generated a large *Sna*BI junction fragment, indicating that FP43 DNA did not insert into the 6-kb circular plasmid sometimes observed in *S. griseofuscus* C581 (14). Therefore, it appears that protoplast formation and regeneration may facilitate the loss of prophage from a chromosomal insertion site. FP43 lysogens were resistant to FP43 superinfection but did not block plaque formation by nine other phages. Of these nine bacteriophages, only VP11 and R4 showed substantial antiserum cross-reactivity. Since VP11 is a virulent phage (11), and R4 is a temperate phage that packages its DNA by a *cos* mechanism (7, 28), FP43 does not appear to be closely related to any of the phages tested. The FP43 prophage attachment site (*attP*) mapped to about 2 kb on the FP43 physical map, indicating that it is located on the *hft* fragment (26). However, since plasmid pRHB101 does not integrate into the genome of streptomycetes, the functions required for integration and their appropriate regulation cannot be totally contained on the 8.8-kb *Sph*I fragment containing *pac* and *att*.

Like many other streptomycete bacteriophages (2, 6, 9), FP43 had a high G+C content (65%) and lacked sites for several streptomycete restriction endonucleases or isoschizomers (i.e., *Nae*I, *Stu*I, *Pst*I, *Sca*I, and *Sac*I). FP43 had only one site for *Xho*I, a relatively common streptomycete restriction system (29) but many sites for *Sac*II, *Pvu*II, *Sph*I, *Sal*I, *Apa*LI, *Nru*I, *Bcl*I, and *Asu*II. It has been shown previously that FP43 forms plaques efficiently on strains that express *Stu*I, *Pst*I, or *Xho*I restriction but not on strains that express *Sph*I or *Sal*I (8). However, FP43 formed plaques efficiently on a *Sal*I-defective mutant of *S. albus* G. Thus, FP43 may have evolved to eliminate sites for some but not all streptomycete restriction systems, thus allowing it to form plaques on about 50% of potential hosts (8, 22, 26, 27).

In summary, we have characterized several important properties of bacteriophage FP43, particularly as they relate to its mechanism of packaging DNA and transducing plasmid

DNA. This transduction system is being further characterized to determine its utility as a general mechanism to transfer cloned DNA between different species of *Streptomyces*. Since FP43 packages DNA by a headful mechanism, it is possible that it can be developed to transduce chromosomal DNA. The localization of the *pac* function described here might be further exploited by subcloning and inserting it into streptomycete chromosomes via integrating vectors or transposons to facilitate specialized transduction of chromosomal genes (33).

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