JOURNAL OF BACTERIOLOGY, July 1977, p. 251-258
Vol. 131, No. 1
Copyright © 1977 American Society for Microbiology
Printed in U.S.A.

Characterization of a Plasmid from *Streptomyces coelicolor* A3(2)

HILDGUND SCHREMPF* AND WERNER GOEBEL

Institut für Genetik und Mikrobiologie, der Universität, 8700 Würzburg, Germany

Received for publication 25 January 1977

Covalently closed circular deoxyribonucleic acid (DNA) with a molecular weight of 20×10^6 was identified in strains of *Streptomyces coelicolor* A3(2) of various fertility types. Hybridization studies and digestion by various restriction endonucleases indicated that the circular DNAs (pSH1) were identical regardless of the fertility type (UF, IF, or NF) of the strain from which it was isolated. The pSH1 DNA was cleaved to many fragments by the endonucleases *Hin*cII, *Sma*I, and *Sal*I and to three or four fragments by *Bam*HI and *Pst*I. Plasmid pSH1 carries single sites for each of the two restriction enzymes, *Eco*RI and *Hin*dIII. These sites are 7.6×10^6 daltons apart. Attempts to isolate the fertility factor SCP1 as covalently closed circular DNA were unsuccessful. These data suggest that the biochemically isolated plasmid pSH1 is not identical to the genetically characterized fertility factor SCP1, which has been identified in an autonomous state in IF-type strains and in an integrated state in NF-type strains.

Previous genetic studies provided evidence that IF strains of *Streptomyces coelicolor* A3(2) contain a plasmid designated SCP1 (8). This plasmid acts as a fertility factor and carries genes for the antibiotic methylenomycin (18), which inhibits sporulation of UF strains of *S. coelicolor* lacking SCP1. The ability to synthesize methylenomycin can be efficiently transferred by conjugation to UF strains (16) or to strains of *S. lividans* (9). The SCP1 plasmid may be integrated into the chromosome of *S. coelicolor* strains of the NF fertility type (8). By mating IF strains with UF strains it has been also possible to construct *S. coelicolor* strains harboring derivatives of SCP1 in which various chromosomal segments of the donor strain have been inserted (9).

Our previous studies have shown that covalently closed circular (CCC) plasmid deoxyribonucleic acid (DNA) of about 20×10^6 daltons can be isolated from *S. coelicolor* A3(2) (12); in this report we describe the isolation of identical extrachromosomal DNAs from several strains of *S. coelicolor* with different fertility properties (IF, NF, or UF). No other CCC DNA that corresponded to the genetically described SCP1 plasmid could be detected by the biochemical procedures employed.

MATERIALS AND METHODS

Bacterial strains. *S. coelicolor* strains A3(2) (IF type), A332 *pheA1* (NF type), 1098 *pheA1* (UF type), 1190 *hisA1*, *uraA1 strA1* (UF type), and 1984 *hisA3 cysB*(SCP1′ *cysB*$^+$) and *S. lividans* strains 1326 (wild type), 1671 (SCP1), and 1923 *cys-3* (SCP1′ *cysB*$^+$) were kindly provided by D. A. Hopwood.

Reagents. [*methyl*-^{3}H]thymidine (specific activity, 24 Ci/mmol) was obtained from Radiochemical Centre, Amersham, England. Lysozyme, proteinase K and cesium chloride were purchased from Merck (Germany). Ethidium bromide was obtained from (Serva) Germany; agarose was from Seakem (USA).

Media. Minimal salt medium, complete medium (7), and sucrose-Casamino Acids-glycine medium (12) were used.

Growth of strains. Strains of *S. coelicolor* were grown on minimal salt-agar slants until sporulation was completed. Spore suspensions in water were used to inoculate liquid medium.

Isolation of extrachromosomal DNA. Plasmid DNA was isolated from mycelium grown under vigorous shaking at 30°C for 40 h in 300 ml of sucrose-Casamino Acids-glycine medium that had been inoculated with about 10^6 spores per ml.

Radioactively labeled extrachromosomal DNA was isolated after growth in sucrose-Casamino Acids-glycine medium containing 50 μCi of [*methyl*-^{3}H]thymidine per ml (12). Mycelium was harvested by centrifugation and washed twice in TE buffer [0.01 M tris(hydroxymethyl)aminomethane (Tris), 0.005 M ethylenediaminetetraacetic acid (EDTA), pH 7.5, containing 34% sucrose]. A 2-g amount of mycelium was resuspended in 20 ml of the same buffer; 5 ml of 0.25 M EDTA (pH 8.0) and 60 mg lysozyme in 2 ml of TE buffer were added, and the mixture was incubated at 30°C. Formation of protoplasts, which was monitered by microscopy, occurred after 5 to 7 min. Sodium dodecyl sulfate, to a

concentration of 0.5%, and 200 μg of proteinase K per ml were added, and the mixture was kept for a further 30 min at 30°C. The concentration of sodium dodecyl sulfate was then raised to 1%, and the suspension was incubated for another 15 to 30 min at 30°C. Solid cesium chloride was then added to a final concentration of 1 M. The suspension was cleared by centrifugation as described previously (12).

Dye-buoyant centrifugation. A 14-ml amount of the cleared lysate, 0.5 ml of 0.25 M EDTA (pH 8.0), 0.5 ml of ethidium bromide (10 mg/ml), and 11.6 g of CsCl were mixed in a nitrocellulose tube of a Ti60-type rotor. Centrifugation was carried out at 20°C and 44,000 rpm for 40 h in a Beckman L5-65 centrifuge. Bands containing CCC DNA from three Ti60 gradients were pooled and rerun in a Ti50 gradient to equilibrium. Thus, the CCC DNA was concentrated to 50 to 100 μg/ml. From a lysate of 2 g of mycelium, 20 μg of CCC DNA could be obtained. After centrifugation in a cesium chloride gradient, the chromosomal DNA and CCC DNA appeared at the same density: 1.74 g/cm^3.

Fractions containing CCC DNA were pooled, extracted with isopropanol to remove ethidium bromide, and dialyzed against cold 0.1 × SSC (0.015 M NaCl, 0.0015 M sodium citrate [pH 7.0], and 0.0025 M EDTA). When required, CCC plasmid DNA was further purified by centrifugation on a linear 5 to 20% sucrose gradient in TES buffer (0.03 M Tris-hydrochloride, 0.005 M EDTA, and 0.05 M NaCl, pH 8.0). Centrifugation was performed at 20°C and 39,000 rpm for 80 min in an SW40 rotor.

Electron microscopy. Samples of 50 to 100 μl containing circular DNA were prepared for electron microscopy by the droplet method (1, 10).

Hybridization studies. DNA-DNA hybridization was performed by the membrane filter method (3) and as described earlier (4).

Restriction endonucleases. *Eco*RI and *Pst*I were kindly provided by H. Mayer. *Hin*dIII was a gift from D. Blohm. *Hin*cII was obtained from H. Luibrand. *Sma*I was isolated from *Serratia marcescens* (15), and *Sal*I was purified from *S. albus* as described by J. Groneberg. *Bam*HI was isolated from *Bacillus amyloliquefaciens* H (17).

Assays for digestion of pSH1 DNA with restriction enzymes. Cleavage of plasmid DNA with endonuclease *Eco*RI was performed in 25 mM Tris-hydrochloride (pH 7.5)-20 mM NaCl- 10 mM $MgCl_2$; with *Hin*dIII, in 10 mM Tris-hydrochloride (pH 7.4)-10 mM $MgCl_2$- 25 mM NaCl; with *Hin*cII and *Pst*I, in 10 mM Tris-hydrochloride (pH 7.5)-10 mM $MgCl_2$; with *Bam*HI, in 6 mM Tris-hydrochloride (pH 7.4)-100 mM NaCl-6 mM $MgCl_2$-6 mM 2 mercaptoethanol; with *Sma*I, in 15 mM Tris-hydrochloride (pH 9.0)-8 mM $MgCl_2$-15 mM KCl; and with *Sal*I, in 50 mM Tris-hydrochloride (pH 7.4)-40 mM NaCl-10 mM $MgCl_2$. Incubations were carried out in 50 to 100 μl at 37°C for 30 min to 4 h depending on the endonucleases. The reactions were terminated by the addition of sodium dodecyl sulfate and urea to final concentrations of 1% and 0.5 M, respectively.

Agarose gel electrophoresis. Electrophoresis was performed in a slab gel apparatus constructed by D. Blohm. Gels contained 0.7 to 1% agarose in TEA buffer (0.04 M Tris, 0.02 M sodium acetate, 2×10^{-3} M disodium EDTA, and 0.018 M NaCl, adjusted with acetic acid to pH 8.0) (7). The samples were adjusted to 0.002% bromophenol blue and 12% sucrose and applied to the gel. Electrophoresis was performed at 2 to 6 V/cm at 4°C for 6 to 15 h. The gels were stained for 20 min in TEA buffer containing 4 μg of ethidium bromide per ml. The bands were visualized by fluorescence under ultraviolet light. Gels were photographed with an orange filter and HP_4 film.

Molecular weight determination. Molecular weights of the fragments were determined by coelectrophoresis of an *Eco*RI digest of λ DNA. The molecular weights of the *Eco*RI fragments of lambda were established previously (6).

RESULTS

Isolation of plasmid DNA from *S. coelicolor* strains having different fertility properties. CCC DNA could be isolated by cesium chloride-ethidium bromide centrifugation of cleared lysates prepared from UF, IF (12), and NF strains of *S. coelicolor* A3(2). The copy number of this extrachromosomal DNA is three to four per chromosome. The circular molecules represent a homogenous population of molecules with a contour length of 9.8 μm, independent of the strain from which the DNA was isolated (i.e., from a UF, IF, or NF strain). *S. coelicolor* strain 1984, which harbors a plasmid derived from SCP1 carrying the *cysB* region of the chromosome, was also analyzed for the presence of CCC DNA. Again, only a homogenous population of circular DNA of 20×10^6 daltons or its multimers could be isolated (Table 1; Fig. 1). The *S. coelicolor* A3(2) strain 1984 produces the

TABLE 1. *Physical properties of circular DNA molecules*

Strain	$s_{20,w}$ of supercoiled DNA	Copy no. per chromosome	Contour length (μm)[a]	
			Monomer	Dimer
1098 (UF type)	42–43	3–4	9.89 ± 0.2	18.70 ± 0.35
A3(2) (IF type)	42–43	3–4	9.78 ± 0.2	18.80 ± 0.40
1984 (SCP1′ *cysB*$^+$ type)	42–43	3–4	9.90 ± 0.2	18.85 ± 0.35
A 332 (NF type)	42–43	3–4	NM[b]	NM

[a] ColE1 DNA (2.04 μm) was used as an internal standard.
[b] NM, Not measured.

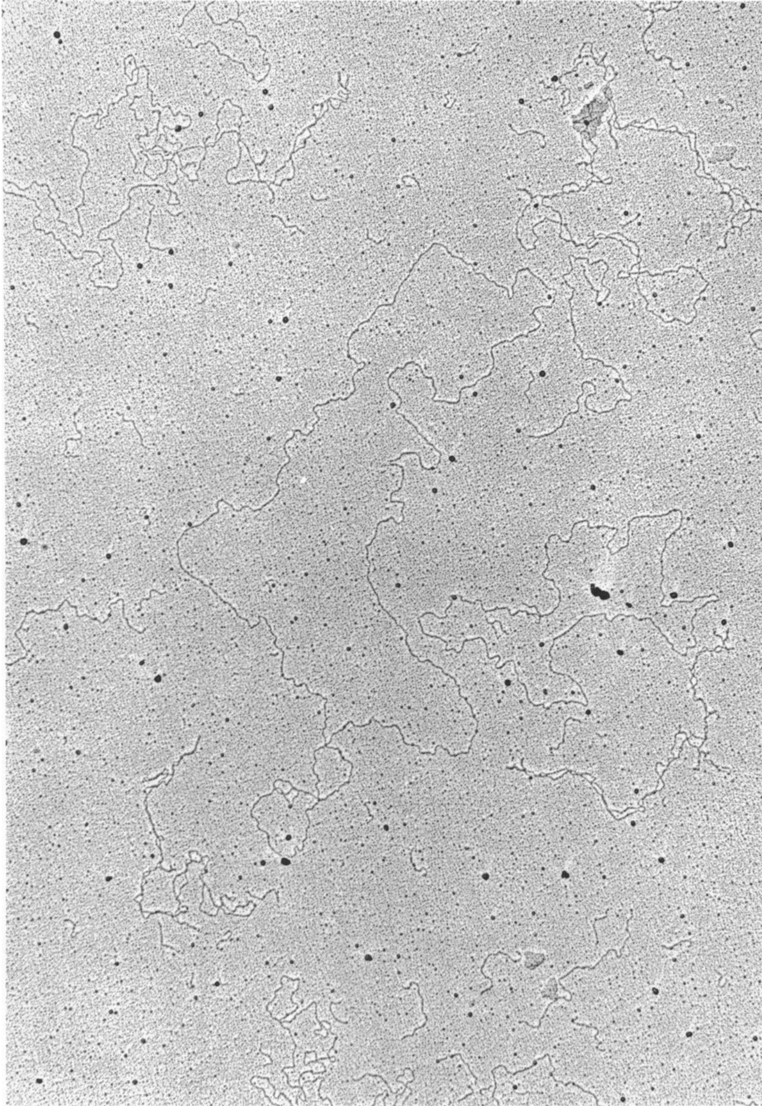

FIG. 1. *Electron microscopy of circular DNA molecules (monomers and multimers) isolated from S. coelicolor A3(2). Magnification, ×16,500.*

antibiotic methylenomycin. Individual colonies can be tested for this antibiotic production in a plate test involving inhibition of growth of the *S. coelicolor* UF strain 1190, which is initially present as a lawn of spores (9). Four isolates had spontaneously lost the ability to produce methylenomycin. These were also found to have lost the ability to grow without cystine. Both of these results indicated that the SCP1′ *cysB*$^+$ plasmid had been lost. These segregants, obtained with a frequency of about 1%, showed no detectible loss of the 20×10^6-dalton plasmid (Table 2).

Strains of *S. coelicolor* A3(2) produce at least two bacteriocins that kill *S. griseus* 1157 and *Streptomyces* 1158 (D. A. Hopwood, personal communication). One UF strain, 2169, was isolated which had spontaneously lost the ability to produce the bacteriocin that kills *S. griseus* 1157. This strain had also retained the 20×10^6-dalton plasmid. In addition, crosses between the UF strain 2169 and the NF strain were performed. Analysis of the recombinants revealed that the ability to produce this bacteriocin is located on the chromosome next to the *cysC,D* locus (H. Schrempf, unpublished data).

TABLE 2. *Plasmids in strains of S. coelicolor A3(2) and S. lividans*

Strain	Plasmid present	
	pSH1	SCP1
S. coelicolor A3(2)		
UF	+	−
IF	+	+ (autonomous)
UF (bacteriocin⁻)	+	−
IF (bacteriocin⁻)	+	+ (autonomous)
UF (ϕC31⁻)	+	−
IF (ϕC31⁻)	+	+ (autonomous)
IF (SCP1′ *cysB*⁺)	+	+ (autonomous)
UF (spontaneous, loss of SCP1′ *cysB*⁺)	+	−
NF	+	+ (integrated)
S. lividans		
Wild type	−	−
Transconjugant SCP1⁺ after cross with *S. coelicolor* IF	−	+

Strains of *S. coelicolor* A3(2) normally contain a defective lysogenic prophage, ϕC31 (11). However, the 20 × 10^6-dalton plasmid is apparently not identical to the defective prophage since strains of *S. coelicolor* that had lost this prophage (UF 1889, ϕC31⁻; and IF 1890, ϕC31⁻) showed no detectable loss of CCC DNA. (Table 2)

Previous genetic studies have shown that SCP1 and SCP1′ *cysB*⁺ plasmids could be transferred from *S. coelicolor* A3(2) to strains of *S. lividans* (9). The *S. lividans* strains thus obtained, 1671 and 1923, also synthesize the antibiotic methylenomycin as shown by their ability to inhibit the growth of the UF tester strain of *S. coelicolor* A3(2), 1190, or of the *S. lividans* wild type, 1326. In addition, a *S. lividans cys-3* mutant regained the ability to grow in the absence of cystine when the SCP1′ *cysB*⁺ plasmid of *S. coelicolor* 1984 was transferred into this mutant.

An attempt was made to isolate CCC DNA from these *S. lividans* strains by the same procedure as described before for *S. coelicolor*. However, no CCC DNA could be detected in the *S. lividans* wild type or in any of the transconjugants containing the SCP1′ *cysB*⁺ plasmid.

In summary, these results indicate that with this technique it is not possible to isolate plasmid DNA that corresponds to the SCP1 plasmid from either *S. coelicolor* or *S. lividans* strains. However, plasmid DNA with a molecular weight of 20 × 10^6 can be isolated as covalently closed circles from all strains of *S. coelicolor*. The biochemical functions coded on this plasmid are still unknown. It does not seem to be involved in the determination of a bacteriocin active against *S. griseus* 1157, in methylenomycin production, or in the fertility exhibited by the *S. coelicolor* A3(2) wild-type strain.

Hybridization studies with the CCC DNA from various sources. Hybridization studies were performed to test whether plasmid DNA isolated from various strains of *S. coelicolor* A3(2) is indeed homologous. Various amounts of unlabeled circular plasmid DNA isolated from UF or IF strains were fixed on nitrocellulose filters as described previously (3, 4). The filters were incubated with ^{3}H-labeled plasmid DNA of UF strain 1098. This DNA was degraded to fragments of about 8S by sonic oscillation and then denatured by heat. Figure 2 shows the saturation curves obtained. The amount of ^{3}H-labeled DNA that bound to the corresponding unlabeled DNA of UF strain 1098 was taken as 100% homology. The hybridization of the labeled DNA with the DNA isolated from the other strains was related to this value.

The labeled DNA annealed, to the same extent, to filter-fixed plasmid DNA independent of whether it was isolated from UF strain 1098,

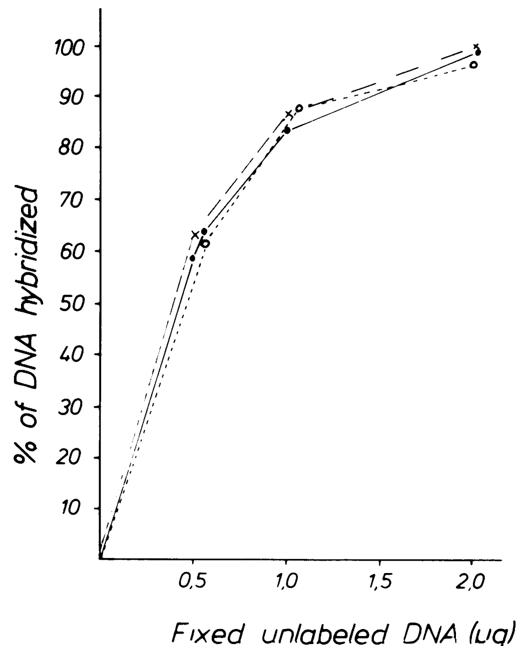

FIG. 2. *DNA-DNA hybridization of ^{3}H-labeled plasmid DNA extracted from S. coelicolor (1098, UF type) with unlabeled plasmid DNA isolated from S. coelicolor strains 1098 (UF type) (●), A3(2) (IF type) (○), and 1984 (IF [SCP1′ cysB⁺]type) (×).*

IF strain A3(2), or IF strain 1984, which contained the SCP1′ *cysB*$^+$ plasmid.

Digestion of CCC DNA with restriction enzymes. In addition to the hybridization studies, digestion of the CCC DNA isolated from various *S. coelicolor* strains with restriction endonucleases *Eco*RI (5), *Hin*dIII (14), *Bam*HI (17), *Pst*I (13), and *Hin*cII (2) was performed. Plasmid DNA from all sources carried a single site for *Eco*RI. This was shown by analyzing the *Eco*RI digestion products by agarose gel electrophoresis (Table 3). *Hin*dIII also cleaved CCC DNA from all three sources at a unique site: 7.6 $\times 10^6$ daltons from the *Eco*RI recognition site. The restriction enzyme from *Providencia stuartii*, *PSt*I, gave three indistinguishable fragments with each of the CCC DNAs after complete digestion: *Pst*-A (10.2 $\times 10^6$ daltons), *Pst*-B (5.7 $\times 10^6$ daltons), and *Pst*-C (4.1 $\times 10^6$ daltons). Restriction enzyme *Bam*HI cleaved these DNAs into four fragments (Table 3).

Digestion of the CCC DNAs with *Hin*cII yielded many fragments of small size. The largest fragment had a molecular weight of 2 $\times 10^6$. This enzyme is therefore useful for recognizing possible minor differences in the nucleotide sequence of the plasmid DNA. Although a perfect resolution of the *Hin*cII fragments of CCC DNA can hardly be achieved even on a 2% agarose gel, a comparison of the *Hin*cII patterns of CCC DNA from IF and UF strains suggests that both plasmid DNAs are identical (Fig. 3). Indistinguishable cleavage patterns of the plasmid DNA from different sources were also obtained with *Sal*I and *Sma*I, which also yielded several relatively small fragments after complete digestion (Fig. 4 and 5).

These results indicate that the isolated plasmid DNAs have indistinguishable properties regardless of their origins. The plasmid is therefore designated pSH1 to distinguish it from the fertility factor SCP1, which has been genetically identified in IF and NF strains of *S. coelicolor* A3(2) (7).

DISCUSSION

Previous genetic studies of Hopwood et al. (8) indicated that a transmissible plasmid, SCP1, determines the fertility in *S. coelicolor*. According to these studies, IF strains seem to contain SCP1 in an autonomous state. In UF strains SCP1 is absent, and in NF strains it is integrated in the chromosome. Furthermore, SCP1 derivatives have been obtained by crosses between IF and UF strains, which carry, like the F′ plasmids in *Escherichia coli*, additional chromosomal segments. Fertility factor F of *E. coli*, as well as F′ factors, can be readily iso-

TABLE 3. *Molecular weights (×10^6) of fragments after digestion of CCC DNA by various endonucleases*

*Eco*RI	*Hin*dIII	*Eco*RI/ *Hin*dIII	*Pst*	*Pst*/ *Eco*RI	*Pst*/ *Hin*dIII	*Pst*/ *Bam*	*Bam*	*Sal*I	*Sma*	*Sma*/*Eco*RI	*Hin*cII	*Hin*cII/*Eco*RI
~20.0	~20.0	~12.4	~10.2	~9.9	~10.2	~10.2	~11.5	4.6	3.0	2.0	2.05	2.05
		7.6	5.7	5.7	5.7	4.5	6.0	1.1	1.6	1.6		
			4.1	4.1	3.5	3.0	2.0	0.95	1.0	1.0	1.2	0.8
				0.3			0.5	0.90	0.48	0.48		
					0.6	~1.3 (double band)		0.56	0.38	0.38	0.8	0.5
								0.44	0.33	0.33		
								0.40	0.26 and several smaller fragments	0.26 and several smaller fragments	0.49	0.35 and several smaller fragments
						0.5		0.18				
								0.17 and several fragments			0.35 and several smaller fragments	

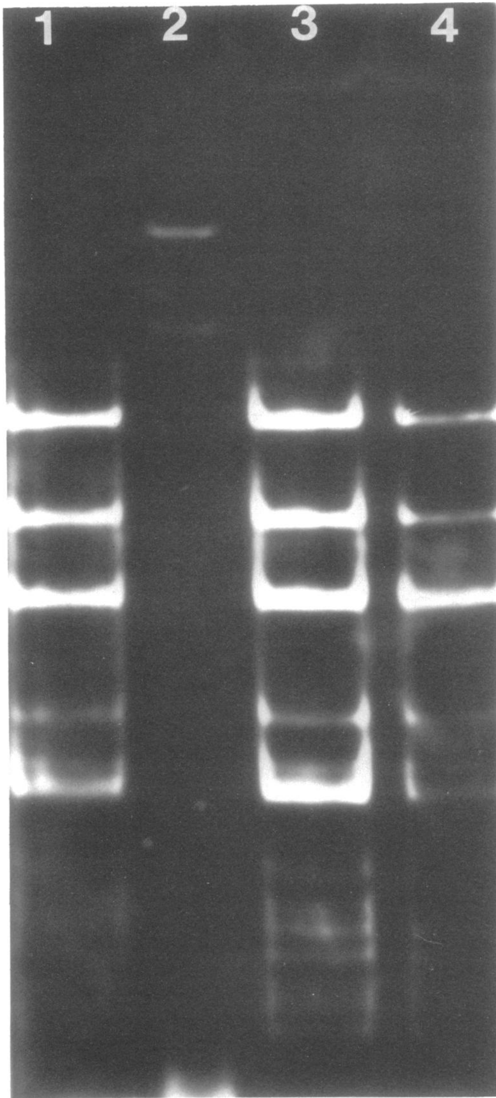

FIG. 3. *Agarose gel electrophoresis of plasmid DNA cut by HincII (1, 3, and 4). Circular DNA was isolated from S. coelicolor strains of the UF type (1), IF type (3), or IF (SCP1' cysB⁺) type (4). EcoRI λ fragments (2). Electrophoresis was performed as described in the text.*

lated as CCC DNA molecules by cesium chloride-ethidium bromide centrifugation of cleared lysates.

By the same technique, homogenous circular plasmid DNA of 2×10^7 daltons could be detected in several strains of *S. coelicolor* independent of their fertility type, i.e., IF, UF, or NF, which suggests that this plasmid is not related to SCP1. In addition, segregants of *S. coelicolor* SCP1' $cysB^+$ that had spontaneously lost the SCP1' $cysB^+$ plasmid still contained the 2×10^7-dalton plasmid. On the other hand, no CCC DNA could be isolated from *S. lividans* strains to which SCP1 or SCP1' $cysB^+$ plasmids had been transferred. It can be concluded, therefore, that the plasmid isolated is not related to the fertility of *S. coelicolor*. To distinguish it from the genetically defined plasmid SCP1, the biochemically isolated plasmid was designated pSH1. Functions of pSH1 have not yet been identified. It does not represent the genome of prophage ϕC31 isolated from strain A3(2) and does not seem to determine the production of the antibiotic methylenomycin or the bacteriocin active against *S. griseus* 1157, since strains from which these properties have been

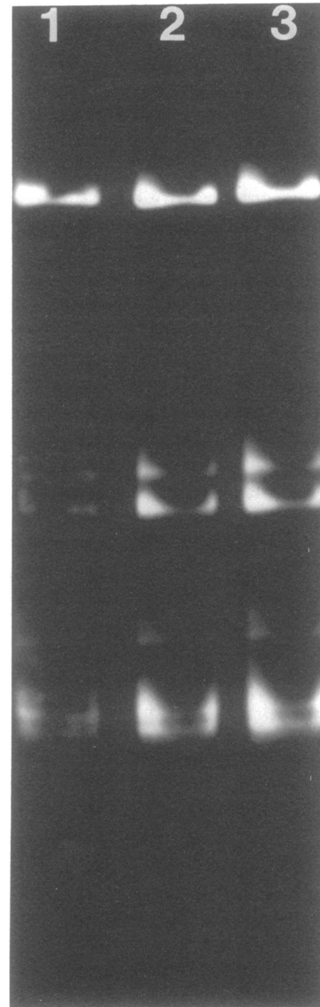

FIG. 4. *SalI digestion patterns of plasmid DNA isolated from S. coelicolor strains of the UF type (1), the IF type (2), and the IF (SCP1' cysB⁺) type (3).*

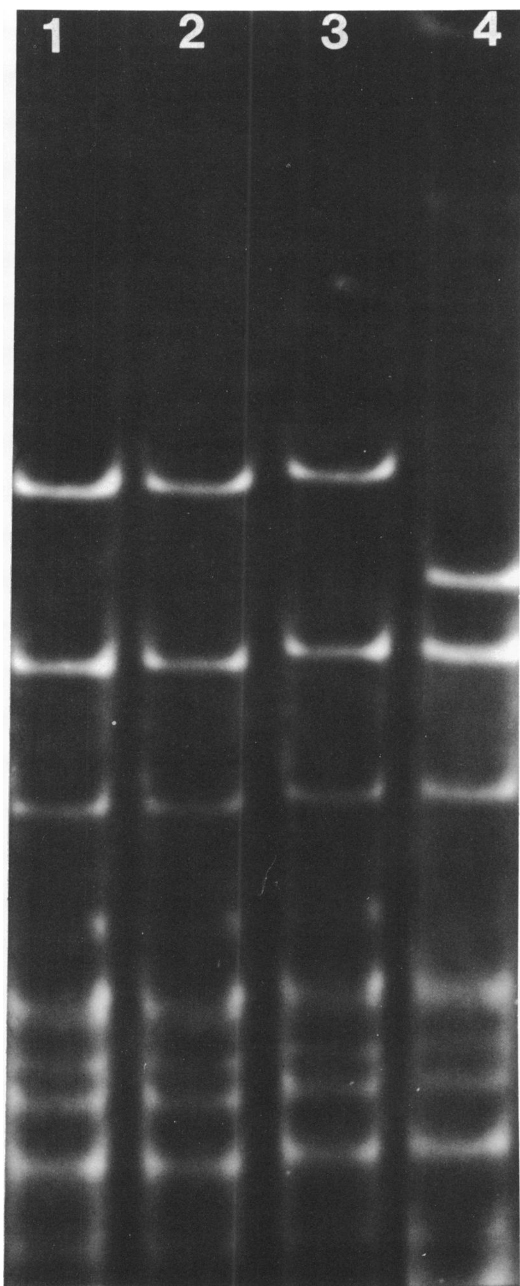

FIG. 5. *Sma (1, 2, and 3) and Sma/EcoRI (4) cleavage patterns of extrachromosomal DNA isolated from S. coelicolor strains of the UF type (1, 4), the IF type (2), and the IF (SCP1' cysB⁺) type (3).*

eliminated still retain pSH1.

The failure to isolate SCP1 may be caused by the physical properties of this plasmid; i.e., SCP1 may represent an extremely large plasmid that cannot be isolated by the procedure employed, or may be a DNA molecule with a conformation other than the CCC type that is required for the isolation by the dye-buoyant density centrifugation technique.

DNA hybridization studies and digestion with restriction enzymes *Hin*cII, *Sma*I, and *Sal*I suggest that plasmid pSH1 has the same nucleotide sequence regardless of its origin, which, furthermore, rules out the possibility that a plasmid of the same size but with different nucleotide sequences, and therefore different functions, may be present in *S. coelicolor* strains with different fertility properties.

Digestion of pSH1 with *Eco*RI and *Hin*dIII show that this plasmid has single sites for both enzymes, which are 7.6×10^6 daltons apart. It may be used, therefore, like RP4 in *Pseudomonas*, as a vehicle for studying transformation in *Streptomyces*. Although the location of the replication origin on the genome of pSH1 has not yet been determined, it appears quite possible, considering the *Bam*HI or *Pst*I physical map of pSH1, to construct from *Bam*HI or *Pst*I restriction fragments smaller plasmids that may be even more suitable as transformation vectors.

If properly handled, the described isolation procedure yields reasonable amounts of pSH1 DNA from *Streptomyces* strains for biochemical studies. However, we recently succeeded in joining pSH1 DNA to RSF2124(ColE1 Ap) and cloning this hybrid in *E. coli* (to be published). This interesting *E. coli-Streptomyces* hybrid plasmid can be amplified in *E. coli* by chloramphenicol to large amounts. The pSH1 part can then readily be cut out by *Eco*RI and separated from the ColE1 Ap part.

ACKNOWLEDGMENTS

We thank H. Bujard (Heidelberg), A. Pühler, and H. J. Burckardt (Erlangen) for performing electron microscopy and C. Bonnet for skillful technical assistance.

LITERATURE CITED

1. **Bujard, H.** 1970. Electron microscopy of single stranded DNA. J. Mol. Biol. 49:125-137.
2. **De Filippes, F. M.** 1974. A new method for isolation of a restriction enzyme from *Hemophilus parainfluenzae*. Biochem. Biophys. Res. Commun. 58:586-596.
3. **Denhardt, D. T.** 1966. A membrane filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. 23:641-646.
4. **Goebel, W., and H. Schrempf.** 1972. Isolation of minicircular deoxyribonucleic acids from wild strains of *Escherichia coli* and their relationship to other bacterial plasmids. J. Bacteriol. 111:696-704.
5. **Hedgpeth, J., H. M. Goodman, and H. W. Boyer.** 1972. DNA nucleotide sequence restricted by the RI endonuclease. Proc. Natl. Acad. Sci. U.S.A. 69:3488-3452.
6. **Helling, R. B., H. M. Goodman, and H. W. Boyer.** 1974. Analysis of endonuclease R·EcoRI fragments of DNA from lambdoid bacteriophages and other viruses by agarose-gel electrophoresis. J. Virol. 14:1235-1244.

7. **Hopwood, D. A.** 1967. Genetic analysis and genome structure in *Streptomyces coelicolor*. Bacteriol. Rev. 31:378-403.
8. **Hopwood, D. A., K. F. Chater, J. E. Dowding, and A. Vivian.** 1973. Advances in *Streptomyces coelicolor* genetics. Bacteriol. Rev. 37:371-405.
9. **Hopwood, D. A., and H. M. Wright.** 1973. A plasmid of *Streptomyces coelicolor* carrying a chromosomal locus and its interspecific transfer. J. Gen. Microbiol. 79:331-342.
10. **Lang, D., and M. Mitani.** 1970. Simplified quantitative electron microscopy of biopolymers. Biopolymers 9:373-379.
11. **Lomovskaya, N. D., N. M. Mkrtumian, N. L. Gostimskaya, and V. N. Danilenko.** 1972. Characterization of temperate actinophage ϕC31 isolated from *Streptomyces coelicolor* A3(2). J. Virol. 9:258-262.
12. **Schrempf, H., H. Bujard, D. A. Hopwood, and W. Goebel.** 1975. Isolation of covalently closed circular deoxyribonucleic acid from *Streptomyces coelicolor* A3(2). J. Bacteriol. 121:416-421.
13. **Smith, D. J., F. R. Blattner, and J. Davies.** 1976. The isolation and partial characterization of a new restriction endonuclease from *Providencia stuartii*. Nucleic Acids Res. 3:343-353.
14. **Smith, H. O., and K. W. Wilcox.** 1970. A restriction enzyme from *Hemophilus influenzae*. I. Purification and general properties. J. Mol. Biol. 51:379-392.
15. **Tanaka, T., and B. Weisblum.** 1975. Construction of a colicin E1-R factor composite plasmid in vitro: means of amplification of deoxyribonucleic acid. J. Bacteriol. 121:354-362.
16. **Vivian, A.** 1971. Genetic control of fertility in *Streptomyces coelicolor* A3(2): plasmid involvement in the interconversion of UF and IF strains. J. Gen. Microbiol. 69:353-364.
17. **Wilson, G. A., and F. E. Young.** 1975. Isolation of a sequence specific endonuclease (BamI) from *Bacillus amyloliquefaciens* H. J. Mol. Biol. 97:123-125.
18. **Wright, L. F., and D. A. Hopwood.** 1976. Identification of the antibiotic determined by the SCP1 plasmid of *Streptomyces coelicolor* A3(2). J. Gen. Microbiol. 95:96-106.