ISOLATION AND CHARACTERIZATION OF SMALL PLASMIDS FROM *STREPTOMYCES*

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In view of the importance of streptomycetes in the production of antibiotics and physiologically-active substances, it would be useful to find small plasmids in these organisms to establish molecular breeding systems in *Streptomyces*. Many plasmids have been isolated from antibiotic-producing streptomycetes (see reference listed in TOYAMA *et al.*¹⁾ and references 2, 3 and 4), but only few restriction enzyme cleavage maps have been determined: pSK1 and pSK2 from *Streptomyces* (*S.*) *kasugaensis*¹⁾, pS10147 from *S. coelicolor* ATCC10147⁴⁾, SCP2* from *S. coelicolor*⁵⁾, SLP1.2 from *S. lividans*⁵⁾ and pUC3 from *S. venezuelae*⁶⁾. Among them, SLP1.2 and SCP2* have already been used as cloning vectors in *Streptomyces*^{5,7)}. In this paper, we describe the isolation of five small plasmids with molecular weights of less than 10⁷ from various streptomycetes and the determination of their restriction enzyme maps.

Sixty-nine strains of streptomycetes species from the collection of this laboratory were used. The methods for the cultivation, preparation and purification of covalently closed circular DNA (ccc DNA) and the analysis of agarose gel electrophoresis were as described previously by NOJIRI *et al.*⁸⁾ or OKANISHI *et al.*⁹⁾. DNA samples were prepared for electron microscopy using the formamide technique¹⁰⁾ and examined in a JEOL JEM-100 electron microscope. For restriction enzyme digestion, DNA (0.5~1 µg) was digested with each restriction enzyme (3~5 units) at 37°C for 2 hours under the conditions according to the supplier's instructions. The method for partial digestion was described in legend of Fig. 5. *Hind*III digests of phage lambda DNA and ϕ X174 DNA served as molecular weights markers. The restriction enzymes *Eco*RI,

*Bam*HI, *Hind*III, *Bgl*II and *Pst*I were from Miles Laboratories, Ltd., *Xba*I and *Sst*I were from Bethesda Research Lab., and *Stu*I¹¹⁾ was kindly supplied by Mr. SHIMOZU.

Sixty-nine strains of *Streptomyces* were ex-

Table 1. Streptomycetes species harboring plasmids.

Host	Product	Plasmid
<i>S. hygroscopicus</i> SF619	Paromomycin	pSF619
<i>S. ribosidificus</i> SF733	Ribostamycin	pSF733
<i>S. fradiae</i> SF765	Neomycin	pSF765
<i>S. griseochromogenes</i> SF701	SF701 substance	pSF701-1 pSF701-2
<i>S. albobacillus</i> SF588	Oxytetracycline	pSF588
<i>S. platensis</i> SF689	Oxytetracycline	pSF689
<i>S. sp.</i> SF601	Tubercidin	pSF601
<i>S. sp.</i> SF1306	Nucleoside substance	pSF1306
<i>S. sp.</i> SF425	Nojirimycin	pSF425
<i>S. lavendulae</i> SF609	Cycloserine	pSF609-1 pSF609-2
<i>S. sp.</i> SF906	Aureothricin	pSF906-1 pSF906-2
<i>S. sp.</i> SF506	Azomycin	pSF506
<i>S. sp.</i> SF760	Oleandomycin	pSF760
<i>S. sp.</i> SF674	Rhizomycin	pSF674
<i>S. sp.</i> SF1223	SF1223 substance	pSF1223
<i>S. sp.</i> SF930	β -Glucuronidase	pSF930

Fig. 1. Agarose gel electrophoresis of pSF588 DNA digested with *Bgl*III.

Slots 1, 4: phage lambda DNA digested with *Hind*III, 2: pSF588 DNA undigested, 3: pSF588 DNA digested with *Bgl*III.

Table 2. Characterization of streptomycete plasmids.

Plasmid	Molecular weight ($\times 10^6$)		Number of restriction sites								
	AGE* ¹	EM* ²	<i>Bgl</i> II	<i>Eco</i> RI	<i>Hind</i> III	<i>Bam</i> HI	<i>Sst</i> I	<i>Sal</i> I	<i>Pst</i> I	<i>Xba</i> I	<i>Stu</i> I
pSF588	3.0	3.0	1	0	0	0	0	0	0	0	0
pSF619	6.2	5.9	0	0	0	3	1	2	3	1	0
pSF689	9.1	9.2	1	0	0	1	1	5	1	0	0
pSF701-1	4.7	4.6	0	1	0	3	2	2	2	1	2
pSF765	4.8	4.4	0	0	0	0	0	6	1	0	1
pSF609-1	(5.1~6.0)* ³	—* ⁴	(1)	(0)	(0)	(2)	(>5)	(>3)	(0)	—	—
pSF906-1	11.5	—	2	1	2	3	—	5	1	—	—
pSF601	(13~18)* ³	—	(1)	(1)	(0)	(5)	—	(4)	(2)	—	—
pSF506	20.0	—	—	2	1	4	—	—	—	—	—

*¹ AGE: Molecular weight obtained by the analysis of agarose gel electrophoresis.

*² EM: Molecular weight obtained on the basis of electron micrograph.

*³ (): Not examined sufficiently because plasmid DNAs were not prepared enough amount to test.

*⁴ —: Not investigated.

Fig. 2. Agarose gel electrophoresis of pSF701-1 DNA digested with various restriction enzymes.

Slots 1, 6, 10, 11: phage lambda DNA digested with *Hind*III, 2: pSF701-1 DNA undigested, 3: pSF701-1 DNA digested with *Bam*HI, 4, 12: pSF701-1 DNA digested with *Eco*RI, 5: pSF701-1 DNA digested with *Sal*I, 7: pSF701-1 DNA digested with *Sst*I, 8: pSF701-1 DNA digested with *Stu*I, 9: pSF701-1 DNA digested with *Xba*I, 13: pSF701-1 DNA digested with *Eco*RI and *Bam*HI, 14: pSF701-1 DNA digested with *Bam*HI and *Sal*I, 15: pSF701-1 DNA digested with *Eco*RI and *Sal*I, 16: pSF701-1 DNA digested with *Eco*RI and *Xba*I, 17: pSF701-1 DNA digested with *Xba*I and *Sal*I.

aminated and nineteen ccc DNA were found in sixteen species as shown in Table 1. The molecular weights of plasmid DNA were calculated from contour lengths of open circular forms by electron microscopy¹²⁾, or by analyzing the fragment(s) obtained from treating with restriction enzymes following by agarose gel electrophoresis. The characteristics of the plasmids with molecular weights of less than 2×10^7 are summarized in Table 2. Six plasmids (designated pSF588, pSF619, pSF701-1, pSF689, pSF765 and pSF609-

1) were found to be less than ten megadaltons. pSF588, the smallest plasmid, had a molecular weight of 3.0×10^6 and only one recognition site for *Bgl*II out of nine restriction enzymes tested (Fig. 1). The other plasmids had recognition site(s) for three to seven different restriction enzymes. We determined the relationships among site(s) for various restriction enzymes of the plasmids. Plasmid DNAs of pSF701-1, pSF619 and pSF689, respectively, were digested with restriction enzymes. Examples of the agarose

Fig. 3. Agarose gel electrophoresis of pSF619 DNA digested with restriction enzymes.

Slots 1, 8: phage lambda DNA digested with *Hind*III, 2: pSF619 DNA digested with *Xba*I, 3: pSF619 DNA digested with *Sst*I, 4: pSF619 DNA digested with *Sst*I and *Xba*I, 5: pSF619 DNA digested with *Sst*I and *Sal*I, 6: pSF619 DNA digested with *Xba*I and *Sal*I, 7: pSF619 DNA digested with *Sal*I.

Fig. 4. Agarose gel electrophoresis of pSF689 DNA digested with restriction enzymes.

Slots 1, 6, 9: phage lambda DNA digested with *Hind*III, 2: pSF689 DNA undigested, 3: pSF689 DNA digested with *Bam*HI, 4: pSF689 DNA digested with *Bgl*II and *Bam*HI, 5: pSF689 DNA digested with *Pst*I and *Bam*HI, 7: pSF689 DNA digested with *Bgl*II and *Pst*I, 8: pSF689 DNA digested with *Bgl*II, *Pst*I and *Bam*HI, 10: pSF689 DNA digested with *Sst*I, 11: pSF689 DNA digested with *Bam*HI and *Sst*I, 12: pSF689 DNA digested with *Bgl*II and *Sst*I, 13: pSF689 DNA digested with *Bgl*II and *Bam*HI.

Fig. 5. Agarose gel electrophoresis of pSF765 DNA digested with restriction enzymes.

a. Complete digestion, 1: phage lambda DNA digested with *Hind*III, 2: pSF765 DNA digested with *Stu*I and *Pst*I, 3: pSF765 DNA digested with *Pst*I, 4: pSF765 DNA digested with *Stu*I, 5: pSF765 DNA digested with *Stu*I, *Pst*I and *Sal*I, 6: pSF765 DNA digested with *Pst*I and *Sal*I, 7: pSF765 DNA digested with *Stu*I and *Sal*I, 8: pSF765 DNA digested with *Sal*I, 9: ϕ X174 DNA digested with *Hae*III.

b. Partial digestion (p. d.), 1: phage lambda DNA digested with *Hind*III, 2: pSF765 DNA digested with *Pst*I and *Sal*I (p. d.), 3: pSF765 DNA digested with *Stu*I and *Sal*I (p. d.), 4: pSF765 DNA digested with *Pst*I, *Stu*I and *Sal*I (p. d.), 5: pSF765 DNA digested with *Sal*I (p. d.), 6: pSF765 DNA digested with *Sal*I, 7: ϕ X174 DNA digested with *Hae*III.

The method of partial *Sal*I digestion: after pSF765 DNA (2 μ g) was digested completely with *Pst*I or (and) *Stu*I, the mixture was treated sequentially with *Sal*I (1 unit) at 37°C for 45 minutes. pSF765 DNA undigested was also treated with *Sal*I under the same condition.

Fig. 6. Cleavage maps of pSF588, pSF701-1, pSF619, pSF689 and pSF765.

gel electrophoresis patterns are shown in Figs. 2, 3 and 4. Molecular weights of the fragments yielded were analyzed and cleavage maps were constructed: pSF701-1 for *Bam*HI, *Eco*RI, *Sal*I, *Sst*I, *Stu*I and *Xba*I, pSF619 for *Bam*HI, *Sal*I, *Pst*I, *Xba*I and *Sst*I, and pSF689 for *Bam*HI, *Bgl*II, *Pst*I and *Sst*I (Fig. 6). For another plasmid pSF765: one of six *Sal*I recognition sites, the *Pst*I site and *Stu*I site were clustered within 0.3 megadaltons (Fig. 5. a). Therefore, the cleavage map of pSF765 was constructed by analyzing the fragments obtained by complete or partial digestion with *Sal*I after complete digestion with *Stu*I or *Pst*I (Figs. 5. b and 6).

There are several basic requirements for the use of plasmids as cloning vehicles in recombinant DNA experiments: the plasmid vector should (1) be extracted easily from host cells, (2) have a low molecular weight, (3) contain a limited number of (one if possible) recognition site(s) for various restriction enzymes, (4) have a characterized restriction map, (5) have one or more selective markers, and (6) have wide host range. We have found five small plasmids (pSF588, pSF701-1, pSF619, pSF765 and pSF689) in several species of *Streptomyces*. These plasmids satisfy the requirements except (5) and (6), and should be use-

ful as vectors for streptomycete recombinant DNA experiments. For example, these plasmids are extracted easily and in good yield of 5~10 μ g from 1 g wet mycelia. Their sizes were less than ten megadaltons (Md): pSF588, 3.0 Md; pSF765, 4.8 Md; pSF701-1, 4.7 Md; pSF619, 6.2 Md and pSF689, 9.1 Md and have single recognition sites for several restriction enzymes: pSF588, *Bgl*II; pSF765, *Pst*I and *Stu*I; pSF701-1, *Eco*RI and *Xba*I; pSF619, *Sst*I and *Xba*I; and pSF689, *Pst*I, *Sst*I, *Bam*HI and *Bgl*II. The relative map positions of these restriction sites for each plasmid were determined as shown in Fig. 6. Hitherto, only a few plasmids (pSK1, 6.8 Md¹¹; pSK2, 6.8 Md¹¹; pS10147, 5.9 Md¹¹; pSLP1.2, 8.2 Md¹²; pSCY1, 3.8 Md¹²) had been reported as plasmids less than ten megadaltons in *Streptomyces* and the restriction maps of pSK1, pSK2, pS10147 and pSLP1.2 were determined. The question of whether these plasmids have wide host range and can function, for example, as antibiotic resistance factors in the host cell or can form "pocks" as shown for SCP2* and SLP1.2, remains to be determined. About eight different restriction systems have been known in streptomycete strains¹⁴, and to overcome restriction it will be necessary to isolate restriction-deficient mutants¹⁵

from the host strains or to select plasmid(s) which are resistant to the restriction system of host cells to be used. Five plasmids reported in this paper may be useful as candidates for streptomycete cloning vectors.

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