ISOLATION AND CHARACTERIZATION OF PLASMIDS FROM *STREPTOMYCES*

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In view of the importance of actinomycetes in the production of antibiotics, it would be useful to find plasmids in these organisms in order to construct molecular breeding systems. Since the first discovery of plasmid involvement in antibiotic production[1], several plasmids associated with the production of antibiotics have been found in *Streptomyces* (references are listed in AKAGAWA *et al.*[2]). For the study of gene cloning, plasmids with smaller molecular weights are preferable for use as cloning vehicle. In order to find candidates for vector plasmids and to extend the study of plasmid involvement in antibiotic production, we examined thirty-four strains of actinomycetes for plasmids (Table 1). Among them, we found a linear plasmid-like DNA from a lankacidin producer, *Streptomyces* sp. 7434-AN$_4$[3]. In this communication, we report the isolation and characterization of covalently closed circular (CCC) DNA molecules from four strains among the strains we tested.

Cells of *Streptomyces puniceus* KCC-S-0406, *Streptomyces* sp. 2217-G$_1$, *Streptomyces* sp. 7068-CC$_1$ and *Streptomyces hygroscopicus* 434 were grown at 27°C in a medium which contained (in grams per liter): glucose, 3; peptone, 3; yeast extract, 4; pH 7.0. The methods for isolation of CCC DNA and the preparation of [^{3}H]thymidine-labeled DNA were as described previously for the isolation of plasmids from *Bacillus subtilis*[4]. [^{3}H]Labeled CCC DNAs obtained from CsCl-ethidium bromide (EtBr) density gradients were subjected to neutral 5 to 20% (w/v) linear sucrose gradient centrifugation with [^{14}C]labeled λ DNA added as an internal standard (33.6S) for determination of molecular weights.

Fig. 1. Neutral sucrose gradient centrifugation (5 to 20%, w/v) of [^{3}H]thymidine-labeled CCC DNA from: (a) *S. puniceus* KCC-S-0406; (b) *Streptomyces* sp. 2217-G$_1$; (c) *Streptomyces* sp. 7068-CC$_1$; (d) *S. hygroscopicus* 434. Arrows indicate the position of [^{14}C]thymidine-labeled λ DNA cosedimented as a molecular weight marker. The centrifugation for the samples (a), (c) and (d) was for 40 minutes and for the sample (b) for 60 minutes at 50,000 rpm in a Hitachi RPS65 rotor.

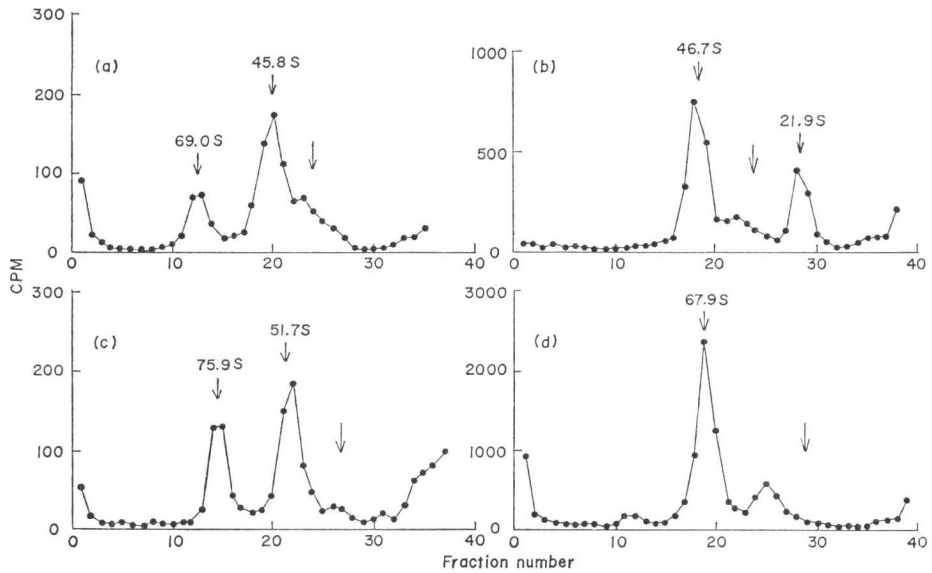

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Table 1. Actinomycetes strains tested for the presence of plasmid.

Antibiotics or β-lactamase produced	Strain	Antibiotics or β-lactamase produced	Strain
Blasticidin S	*Streptomyces griseochromogenes* 2A-327	Streptothricin	*Streptomyces lavendulae* NIHJ-E-2
			Streptomyces microflavus NIHJ-13-A
Lankacidin	*Streptomyces* sp. 6642-GC_1	Chlortetracycline	*Streptomyces aureofaciens* NRRL-B-2209
	Streptomyces sp. 7434-AN_4		
Lonomycin	*Streptomyces ribosidificus* TM-481	Polyoxin	*Streptomyces cacaoi* var. *asoensis* POM-23-1
Viomycin	*Streptomyces diastatochromogenes* KCC-S-0119	Polyoxin + lysocellin	*Streptomyces cacaoi* var. *asoensis* POM-23-1 mutant
	Streptomyces vinaceus ICC-S-0091		
	Streptomyces californicus KCC-S-0143	Methylsalinomycin	*Streptomyces albus* ATCC-21838
	Streptomyces puniceus KCC-S-0406	Xanthomycin	*Streptomyces* sp. 1134-7
	Streptomyces sp. KCC-U-0197	Geldanamycin	*Streptomyces hygroscopicus* 434
	Streptoverticillium olivoreticuli KCC-S-0197	Cephamycin	*Streptomyces clavuligerus* KCC-X-0710
Mikamycin	*Streptomyces mitakaensis* 74-4		*Streptomyces clavuligerus* IFO 13307
Cycloheximide	*Streptomyces* sp. 2217-G_1		*Streptomyces lipmanii* KCC-S-0711
	Streptomyces sp. 8465-MC_1		*Streptomyces lipmanii* IFO 13306
	Streptomyces sp. 8482-CC_1		*Streptomyces rochei* IFO 12908
Neomycin	*Streptomyces albogriseolus* NRRL-B-1305		*Streptomyces fimbriatus* KCC-S-0190
		Gentamicin	*Micromonospora echinospora* NRRL-2985
	Streptomyces sp. 7068-CC_1		
	Streptomyces sp. 8104-MC_2	β-Lactamase	*Streptomyces* sp. E-750-3
			Streptomyces sp. E-756-1

Table 2. Summary of plasmids from four strains of *Streptomyces*.

Strain	Antibiotics produced	Plasmid	S-value	Mol.Wt. ($\times 10^{-6}$)
S. puniceus KCC-S-0406	viomycin	pSPU1	45.8	17.3
		pSPU2	69.0	39.1
Streptomyces sp. 2217-G_1	cycloheximide	pSCY1	21.9	3.2
		pSCY2	46.7	18.0
Streptomyces sp. 7068-CC_1	neomycin	pSNE1	51.7	22.2
		pSNE2	75.9	47.0
S. hygroscopicus 434	geldanamycin	pSHY1	67.9	37.9

Streptomyces puniceus KCC-S-0406, *Streptomyces* sp. 2217-G_1 and *Streptomyces* sp. 7068-CC_1 harbored at least two kinds of plasmids, respectively, as revealed by the number of peaks shown in Fig. 1 and by two fluorescent bands seen in CsCl-EtBr gradients (data not shown). *Streptomyces hygroscopicus* 434 seemed to carry a single plasmid, since the peak at the fraction 25 corresponded to the position at which the open circular form of the plasmid is expected to appear, as calculated by the equations of HUDSON *et al.*[5] Molecular weights of these plasmids calculated by the equation of HUDSON *et al.* are presented in Table 2. Also shown in Table 2 are the antibiotics which these strains produce. Among the four strains harboring plasmids, viomycin-producing ability of *Streptomyces puniceus* KCC-S-0406 was lost at a frequency of 4.4% after treatment with ethidium bromide. The relation ship between viomycin production and the presence of the plasmids remains to be elucidated.

pSCY1 was insensitive to *Eco*RI and *Hind*III, but was cleaved by *Bam*NI and *Sma*I into one and several fragments, respectively (data not shown). Since pSCY1 is relatively small in size and cleaved once with *Bam*NI, the plasmid would be a useful cloning vector in *Streptomyces*.

References

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