DETECTION OF GIANT LINEAR PLASMIDS IN ANTIBIOTIC PRODUCING STRAINS OF *STREPTOMYCES* BY THE OFAGE TECHNIQUE

Sir:

Pulsed field gel electrophoresis (PFG) or orthogonal-field-alternation gel electrophoresis (OFAGE) were recently developed by SCHWARTZ and CANTOR[1], and CARLE and OLSON[2] respectively, in order to separate large linear DNA molecules such as yeast chromosomes. Our first application of OFAGE to *Streptomyces* DNA in combination with a mild DNA preparation method enabled detection of a giant linear plasmid of 520 kilobase (kb) designated pKSL[3] in *Streptomyces lasaliensis* NRRL3382R, which produces lasalocid A[4], a polyether antibiotic, and echinomycin[5], a quinoxaline antibiotic. A parallel relationship between the presence of pKSL and antibiotic production in various producers, fusants and nonproducers of *S. lasaliensis* suggested that pKSL was involved in the production of lasalocid A and/or echinomycin[3].

Thus far, separation of large linear DNA plasmid such as pKSL from chromosomal DNA in streptomycetes has been unsuccessful for the following reasons; 1) the bacterial chromosome degrades during the usual extraction procedure to form linear DNA fragments. 2) Large linear DNAs of more than 30 kb comigrate in conventional agarose gel electrophoresis and are not separable.

The finding of pKSL suggested the involvement of large linear plasmids when no plasmid could be detected in spite of genetic data supporting the existence of a plasmid. Thus, we have examined for the presence of giant linear plasmids in *Streptomyces* in which antibiotic production had been postulated to be plasmid-determined, using the OFAGE technique.

Among 11 antibiotic producing strains of

Fig. 1. Orthogonal-field-alternation gel electrophoresis (OFAGE) of antibiotic producing strains of *Streptomyces* with a giant linear plasmid(s).

Streptomyces DNAs and yeast DNA were prepared as described by us[3], and CARLE and OLSON[2], respectively. OFAGE was conducted following the method of CARLE and OLSON[2] with 1.5% agarose in 0.5× TBE at 300 V. A switching interval and an operation time were 30 seconds and 24 hours (lanes 1~6), and 20 seconds and 18 hours (lanes 7~11), respectively. *Saccharomyces cerevisiae* YNN27 chromosomes, λDNA and λ/*Hin*d III were used as size standards.

1, 6, 7: *S. cerevisiae* YNN27; 2: *Streptomyces lasaliensis* NRRL3382R; 3: *S. violaceoruber* JCM 4979 (*S. coelicolor* A3(2)); 4: *S. fradiae* C373.1; 5: *S. parvulus* JCM 4068; 8: *S. venezuelae* JCM 4526; 9: λDNA; 10: *S. rochei* 7434-AN4; 11: λ/*Hin*d III.

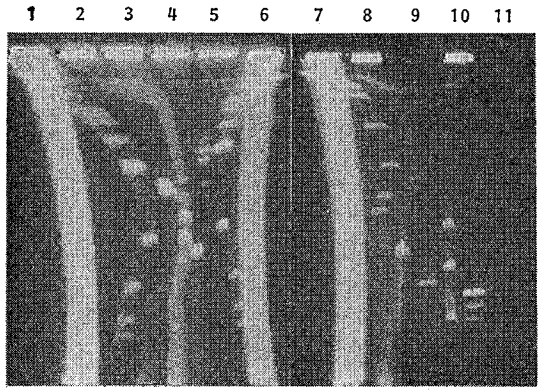

Fig. 2. Conventional agarose gel electrophoresis of antibiotic producing strains of *Streptomyces* with a giant linear plasmid(s).

Electrophoresis was carried out with 0.7% agarose in 1× TBE at 40 V for 15 hours. The same sample gels as in Fig. 1 were used. All DNA preparations gave only "chromosomal DNA band" except *Streptomyces rochei* 7434-AN4, which in addition showed a band of pSLA2 (lane 7). "Chromosomal DNA band" comprised of degraded chromosomal DNA fragments and giant linear plasmids.

1: λ/*Hin*d III; 2: *S. lasaliensis* NRRL3382R; 3: *S. violaceoruber* JCM 4979; 4: *S. fradiae* C373.1; 5: *S. parvulus* JCM 4068; 6: *S. venezuelae* JCM 4526; 7: *S. rochei* 7434-AN4.

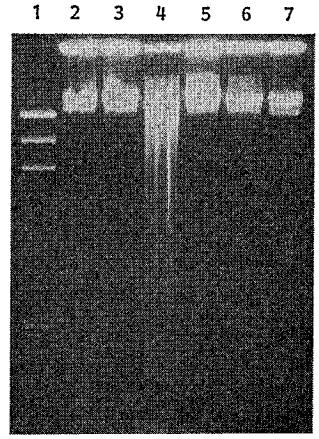

Table 1. Giant linear plasmids in antibiotic producing strains of *Streptomyces*.

Strain	Antibiotic	Size of plasmid (kb)	Reference No.
Streptomyces lasaliensis NRRL3382R	Lasalocid A, echinomycin	520	3
S. violaceoruber JCM 4979[a] (=*S. coelicolor* A3(2))	Methylenomycin	410, 440, 470, 500, 530, 560, 590	7, 8, 9
S. fradiae C373.1	Tylosin	420	13
S. parvulus JCM 4068 (ATCC 12434)	Actinomycin D	520, 560, 580	14
S. antibioticus JCM 4620 (ATCC 8863)	Actinomycin D	—	14
S. venezuelae JCM 4526 (ATCC 10712)	Chloramphenicol	130	15, 16
S. rochei 7434-AN4	Lankacidins	7, 90, 180	10, 17
S. kasugaensis IFO 13851[b] (ATCC 15714)	Kasugamycin, aureothricin	—	11
S. rimosus subsp. *rimosus* JCM 4073 (ATCC 10970)	Oxytetracycline	—	18
S. bikiniensis subsp. *bikiniensis* JCM 4945 (ATCC 11062)	Streptomycin	—	19
S. kanamyceticus JCM 4433 (ATCC 12853)	Kanamycin	—	20
S. fradiae JCM 4579 (ATCC 10745)	Neomycin	—	21

[a] Japan Collection of Microorganisms, Wako, Saitama.
[b] Institute for Fermentation, Osaka.

Streptomyces tested, a giant linear plasmid(s) was found in the following strains as shown in Fig. 1; *Streptomyces violaceoruber* JCM 4979 (=*Streptomyces coelicolor* A3(2), methylenomycin), *Streptomyces fradiae* C373.1 (tylosin), *Streptomyces parvulus* JCM 4068 (actinomycin D), *Streptomyces venezuelae* JCM 4526 (chloramphenicol) and *Streptomyces rochei* 7434-AN4 (lankacidins). All plasmids were shown to have a linear DNA structure from the following results; 1) circular DNA moves very slowly compared to linear DNA on the OFAGE gel (see ref 6 concerning the moving properties of linear and circular DNAs on the OFAGE gel). No small circular plasmid was detected in conventional agarose gel electrophoresis of the same samples as shown in Fig. 2, indicating that plasmids in Fig. 1 were not circular. 2) All plasmids maintained their positions on the gel relative to yeast linear chromosomes under different switching conditions, which clearly shows that they moved as linear DNAs (data are not shown). Sizes of plasmids were determined on the OFAGE gel by comparing their positions with those of size standards and are listed in Table 1.

Genetic studies have shown that antibiotic production in the strains containing a giant linear plasmid(s) is plasmid-determined except lankacidins in *S. rochei* (see ref in Table 1). In particular, *S. violaceoruber* JCM 4979 contains at least seven giant linear plasmids (410, 440, 470, 500, 530, 560 and 590 kb), which formed a ladder of plasmid bands with a size difference of 30 kb (lane 3). This strain was obtained originally from G. SERMONTI as *S. violaceoruber* A3(2), is therefore identical to *S. coelicolor* A3(2). Methylenomycin production in *S. coelicolor* A3(2) is well known to be controlled by an unisolatable plasmid SCP1[7,8], which contains a cluster of the biosynthetic (*mmy*) and resistant (*mmr*) genes[9]. Accumulated data on SCP1, in particular difficulties in its detection by conventional methods suggest that giant linear plasmids in *S. violaceoruber* JCM 4979 may correspond to SCP1.

Three large linear plasmids were detected in *S. rochei* 7434-AN4, a producer of the lankacidin group of antibiotics. The sizes of these plasmids were determined as 7, 90 and 180 kb, respectively, on the OFAGE gel (lane 10). The small-

est linear plasmid was identified with pSLA2, which was isolated by HAYAKAWA *et al.* from the same strain[10].

On the other hand, no giant linear plasmid was found in the following antibiotic producers: *Streptomyces kasugaensis* IFO 13851 (kasugamycin and aureothricin), *Streptomyces rimosus* subsp. *rimosus* JCM 4073 (oxytetracycline), *Streptomyces bikiniensis* subsp. *bikiniensis* JCM 4945 (streptomycin), *Streptomyces kanamyceticus* JCM 4433 (kanamycin) and *Streptomyces fradiae* JCM 4579 (neomycin). Plasmid involvement in the production of these antibiotics was suggested by "curing" studies without supporting genetic evidence (see ref in Table 1). These results demonstrate that "curing" studies are unreliable as an indication of plasmid involvement, although more detailed OFAGE analysis is necessary.

Since the first suggestion by OKANISHI *et al.*[11] for the possible involvement of a plasmid in antibiotic production, much effort to find plasmids in antibiotic producing streptomycetes has been carried out using conventional agarose gel electrophoresis, CsCl-EtBr ultracentrifugation and electron microscopy. However, no plasmid containing structural and/or regulatory genes for antibiotic production has been isolated physically except pSV1 in *Streptomyces violaceus-ruber* SANK 95570[12]. It was thought that the ccc DNA plasmids involved were too large to be isolated in their intact forms. The involvement of a large linear DNA plasmid in antibiotic production was not considered, because its separation from chromosomal DNA had been, until recently, impossible by conventional techniques.

However, application of the OFAGE technique in combination with gentle DNA preparation enabled us to detect many giant linear plasmids in antibiotic producing strains of *Streptomyces*. Thus this method opened a powerful way of detecting giant linear plasmids. Furthermore, the correlation between the presence of a giant linear plasmid(s) and genetic evidence suggests that these plasmids are involved in antibiotic production in *Streptomyces*. Studies are in progress to attempt to confirm this hypothesis.

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HARUYASU KINASHI
MIYUKI SHIMAJI

Mitsubishi-Kasei Institute of Life Sciences,
Minamiooya, Machida-shi,
Tokyo 194, Japan

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