Physical-genetic map of the erythromycin-producing organism *Saccharopolyspora erythraea*

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A physical map of the chromosome of the erythromycin-producing actinomycete *Saccharopolyspora erythraea* NRRL 2338 has been constructed using the restriction enzymes *Asel* and *DraI*. The map was constructed by a variety of methods including linking clone analysis, cross-hybridizations using labelled macrorestriction fragments, gene probing, two-dimensional PFGE and restriction enzyme site generation. Analysis of the individual macrorestriction patterns of the 17 *Asel*-, 6 *DraI*- and 22 *Asel/DraI*-digested fragments indicated a chromosome size of about 8 Mb. Linking clones for five contiguous *Asel* fragments were obtained, covering 32% of the chromosome. The linkage of an additional eight *Asel* fragments was aided by the finding that the rRNA operons of *S. erythraea* contain an *Asel* site within the 16S (*rrs*) gene. Generation of *S. erythraea* strains that contain additional *DraI* sites within selected *Asel* fragments, followed by PFGE analysis and Southern hybridization to determine specific linkages, facilitated the completion of the *Asel* map. The entire *DraI* map was constructed by gene probing and cross-hybridizations. PFGE analysis of agarose-embedded DNA prepared in either the presence or absence of proteinase K suggested that the *S. erythraea* NRRL 2338 chromosome is linear. A total of 15 genes or gene clusters were mapped to specific *Asel* and *DraI* fragments, including the erythromycin-biosynthetic gene cluster and the rRNA operons.

Keywords: *Saccharopolyspora erythraea*, physical mapping, macrorestriction analysis, linear chromosome, erythromycin

INTRODUCTION

Saccharopolyspora erythraea, a mycelium-forming, Gram-positive actinomycete, is the major producer of the macrolide antibiotic erythromycin. Extensive biochemical and genetic studies aimed at identifying and characterizing the genes involved in erythromycin biosynthesis in *S. erythraea* have been reported (Weber *et al.*, 1990; Donadio & Katz, 1992). A 56 kb region of DNA encoding enzymes necessary for the production of erythromycin has been identified and sequenced. Functions for the majority of the predicted genes have been proposed (Donadio *et al.*, 1993; Summers *et al.*, 1997). Several additional genes thought to be involved in erythromycin biosynthesis have been identified and characterized (Hsieh & Kolattukudy, 1994; Linton *et*

al., 1995; Donadio *et al.*, 1996). However, the physical location of these additional genes on the *S. erythraea* chromosome is unknown.

With the recent development of high-resolution PFGE, physical mapping of large (>5 Mb) bacterial chromosomes has become feasible. Recently, the physical maps of the ~8 Mb chromosomes of several *Streptomyces* spp., which are closely related to *Saccharopolyspora*, have been reported (Kieser *et al.*, 1992; LeBlond *et al.*, 1993, 1996; Lezhava *et al.*, 1995; Pandza *et al.*, 1997). Besides the relatively large size of these chromosomes, several additional interesting features have been noted. These include a linear topology, the presence of terminal inverted-repeat (TIR) sequences at each chromosomal end and large genetically unstable regions of DNA.

Here, we present the chromosome map of the wild-type strain *S. erythraea* NRRL 2338. A variety of approaches were employed to construct the physical maps for the

Abbreviations: 2D-PFGE, two-dimensional PFGE; TIR, terminal inverted repeat.

restriction enzymes *AseI* and *DraI*, including the construction of an *AseI* linking clone library, cross-hybridizations using labelled *AseI* or *DraI* fragments, gene probing, two-dimensional PFGE (2D-PFGE) and restriction enzyme site generation. Also presented are the locations of 15 *S. erythraea* genes or gene clusters mapped to specific *AseI* and *DraI* fragments, including the erythromycin-biosynthetic gene cluster, the four rRNA operons and their orientations, and the insertion sites for the integrative plasmids pSE101 and pSE211. This work represents the first report of a physical-genetic map of a *Saccharopolyspora* species. Lastly, results of a comparative study of the PFGE profiles of *S. erythraea* NRRL 2338 and of *S. erythraea* CA340, an industrially improved erythromycin-producing strain, are presented.

METHODS

Bacterial strains and growth conditions. *Saccharopolyspora erythraea* NRRL 2338 was used in all mapping experiments. *S. erythraea* cultures were maintained on R3M agar plates (adapted from Hopwood *et al.*, 1985) and grown as liquid cultures in ABB17 medium (l^{-1} : soytone, 10 g; soluble starch, 10 g; yeast extract, 9 g; cerelose, 15 g; MOPS, 2.1 g). For the preparation of agarose-embedded DNA, 35 ml ABB17 medium was directly inoculated from R3M culture stocks and incubated at 33 °C for 1.5–2 d. *Escherichia coli* strains were grown on Luria–Bertani (LB) agar or in LB broth (Sambrook *et al.*, 1989) containing the appropriate antibiotic. The final concentrations of antibiotics used were: 100 µg ampicillin (Ap) ml^{-1} ; 10 µg tetracycline (Tc) ml^{-1} ; and 10 µg thiostrepton (Th) ml^{-1} .

Generation of an *AseI* linking clone library. To generate a linking clone library in pBR322, the procedure of Lezhava *et al.* (1995) was used, except that *NarI* was used in the chromosomal digests. Tc^r Ap^r transformants were further analysed by restriction digestion with *AseI* and *NarI*. Plasmids containing inserted DNA with two *AseI* sites and one *NarI* site internal to the *AseI* fragment were used in the linking clone analysis.

PFGE. Agarose-embedded DNA plugs for PFGE were generated by adapting the procedure of Kieser *et al.* (1992) with the following modifications. *S. erythraea* NRRL 2338 spores were inoculated into ABB17 medium and harvested after 2 d. Agarose-embedded DNA plugs were exposed to 1 mg lysozyme ml^{-1} for 5–6 h. Plugs were stored for up to 1 month in the wash buffer ($0.1 \times$) provided with the PFGE plug-making kit (Bio-Rad). Preparation of agarose-embedded DNA in the absence of proteinase K was performed using the procedure of Lin *et al.* (1993). 2D-PFGE was performed according to the procedure described by Birren & Lai (1993).

A Bio-Rad CHEF Mapper was used for all PFGE analysis. Standard run programmes were used in accordance with the manufacturer's instructions, except when resolution of specific bands was necessary. Alternative PFGE run programmes are described in the appropriate figure legends. All agarose gels were prepared in $0.5 \times$ TBE buffer (50 mM Tris, 45 mM borate, 0.5 mM EDTA, pH 8.0) and run at 14 °C, unless otherwise stated.

Agarose-embedded DNA was digested with *AseI* and *DraI* following the procedure described by Kieser *et al.* (1992).

Chromosomal and plasmid DNA used for probe generation were excised from agarose gels and purified using the GeneClean II kit (Bio101). DNA was labelled with digoxigenin-dUTP using the Genius kit (Boehringer Mannheim) according to the manufacturer's instructions.

Southern hybridization. PFGE-resolved DNA was transferred to nylon membranes either by capillary blotting (Sambrook *et al.*, 1989) or by vacuum blotting according to the procedure outlined in the CHEF Mapper manual (Bio-Rad). Hybridizations were performed overnight at 68 °C. Post-hybridization washes and the detection procedure were performed according to the instructions outlined in the Genius System Manual (Boehringer Mannheim).

Generation of additional restriction sites in the chromosome.

AseI-J and -K fragments were excised from a 1% agarose gel, purified and subjected to partial *Sau3AI* digestion. Partially digested fragments were subcloned into the *Bam*HI site of pWHM3, a 7.2 kb pUC19-based integration vector containing the thiostrepton-resistance (*tsr*) gene and a *Streptomyces* origin of replication (Vara *et al.*, 1989). This vector contains three *AseI* and three *DraI* sites within the pUC19 portion of the vector, which are all separated by 1 kb or less. Two *Sau3AI* clones designated pJ14 and pK5, containing a 1.1 kb fragment from *AseI*-J and a 0.52 kb fragment from *AseI*-K, respectively, were chosen for further analysis. Protoplast transformation of *S. erythraea* was performed according to an adapted procedure originally described by Hopwood *et al.* (1985). The Th^r *S. erythraea* strains containing integrated pJ14 and pK5 DNA were designated Ω J14 and Ω K5. PFGE analysis and Southern blotting were performed on these integrants as described above.

RESULTS

Macrorestriction analysis reveals a chromosome size of 8 Mb

Restriction analysis was performed on agarose-embedded *S. erythraea* DNA using the rare-cutting enzymes *AseI* (ATTAAT) and *DraI* (AAATTT). *S. erythraea* is known to have a high G+C content (72 mol%; Labeda, 1987). Fig. 1 shows the results of *DraI* and *AseI* digestions performed individually or in combination. *AseI* and *DraI* digestion yielded 17 and 6 fragments, respectively (Table 1). To determine accurately the size of the largest *DraI* fragment (about 3 Mb), the individual *AseI* and *AseI*-*DraI* fragments known to make up this band were summed. Summation of the single- and double-digestion restriction fragments indicated a chromosome size of approximately 8.0 ± 0.1 Mb (Table 1, Fig. 1). Analysis of the restriction pattern of the double-digested DNA using high-resolution 2D-PFGE (Fig. 2) and Southern hybridization showed that the *AseI*-A, -B, -C, -D₂ and -I₂ fragments contained *DraI* sites (Fig. 1e, Table 1).

Linking clone analysis

The initial approach taken to link the 17 *AseI* fragments was to construct an *AseI* linking clone library. Four different linking clones were isolated. The hybridization patterns of two of the linking clones (pLC1-18, pLC2-

Fig. 1. Macrorestriction analysis of the *S. erythraea* NRRL 2338 chromosome using the enzymes *DraI* (a, b), *Asel* (c, d) and *Asel/DraI* (e) separated by PFGE. All gels contained 1% agarose and were run in 0.5× TBE buffer at 14 °C. *Saccharomyces cerevisiae* (S.c.) chromosomes were used as the size markers in (a). The largest *DraI* fragment in (a) was resolved as an ~3 Mb fragment in (b) and is indicated by 'I' at the right of panels (a) and (b). *S. cerevisiae* and *Schizosaccharomyces pombe* (S.p.) were used as the size markers in (b). Lambda concatemers (L) were used as the size markers in (c) and (e). *Asel-DraI* fragments in (e) are shown in parentheses. Pulse programmes: (a) *DraI*-I to VI, 200 V, 24 h, ramp of pulse times 60–110 s; (b) *DraI*-I, 50 V, 68 h, constant pulse time of 27 min, included angle 106°. (c) *Asel*-A to N, 200 V, 22 h, ramp of pulse times 50–90 s; (d) *Asel*-A to D₂, 50 V, 41 h, ramp of pulse times 58–78 s; *Asel*-F to I₂, 50 V, 35 h, ramp of pulse times 24–35 s; *Asel*-J to O, same as for *Asel*-A to N (e) 200 V, 26 h, ramp of pulse times 50–90 s. S.e.; *S. erythraea* chromosomal digest.

12) are shown in Fig. 3. In three of the four cases, the two *Asel-NarI* restriction fragments obtained by double digestion of the isolated *Asel* linking clone were used separately as probes. In these cases, independent use of the *Asel-NarI* fragment allowed specific determination of each side of the linking clone on the *Asel* fragments. In total, the four linking plasmids linked five contiguous *Asel* fragments, C, I₁, H, D₁ and L (see Fig. 4), which accounted for 32% of the *Asel* chromosome map.

Analysis of the DNA sequence of a cloned rRNA operon (M. J. Staver & R. G. Summers, unpublished results) of *S. erythraea* NRRL 2338 revealed an *Asel* site near the middle of the 16S (*rrs*) gene. Results of Southern hybridization of chromosomal digests using the frequently cutting enzymes *NcoI* and *PstI* indicated that the *S. erythraea* chromosome contains four rRNA operons. Four fragments were also observed when the *rrs* probe or the 23S (*rrl*) probe was hybridized to the *Asel*- and

Asel/DraI-digested DNA. In particular, the *rrs* probe hybridized to *Asel*-G, -E, -M and -I₂ and the *rml* probe hybridized to *Asel*-A, -K, -F and -I₂. *Asel*-I₂ hybridized to both probes, the *rrs* probe hybridized to the 130 kb *Asel-DraI* fragment and the *rml* probe hybridized to the 225 kb *Asel-DraI* fragment. This suggested that *Asel*-I₂ contains a *rrs* and a *rml* portion of two rRNA operons. When the *rrs* and *rml* probes were hybridized to membranes containing *DraI*-digested DNA, *DraI* fragments I–IV were detected in both cases (data not shown), indicating that there is one rRNA operon on each of the fragments.

Plasmids pSE101 (Brown *et al.*, 1988) and pSE211 (Brown *et al.*, 1990) are both known to replicate autonomously and to integrate into the *S. erythraea* chromosome at low frequency at different, but specific, attachment (*attB*) sites. Each plasmid contains the genes encoding an integrase (*int*), excisionase (*xis*) and the *attP*

Table 1. Estimated sizes of *AseI* and *DraI* macrorestriction fragments

<i>DraI</i>		<i>AseI</i>		<i>AseI-DraI</i>	
Fragment	Size (kb)	Fragment	Size (kb)	Fragment	Size (kb)
I	2935	A	1114	D ₁	782
II	1265	B	970	(B)	684
				(A)	668
III	1211	C	824	(D ₂)	620
IV	1112	D ₁	782	E	572
V	1020	D ₂	734	F	510
VI	535	E	572	(A)	476
		F	510	(C)	476
		G	441	G	441
		H	401	H	401
		I ₁	357	I ₁	357
		I ₂	348	(C)	348
		J	243	(B)	283
		K	202	J	243
		L	182	(I ₂)	218
		M	162	K	202
		N	48	L	182
		O	10	M	162
				(I ₂)	127
				(D ₂)	114
				N	48
				O	10
Total	6	8078	17	7930	22

site (Brown *et al.*, 1988). When a labelled fragment containing the *int* gene and the *attP* site from pSE101 (which was expected to hybridize to both integrated pSE101 and pSE211, since the *int* genes are highly homologous) was used to probe *AseI*- and *AseI/DraI*-digested DNA, it was found that the two different *attB* sites were located on different *AseI-DraI* fragments of *AseI*-B (Fig. 1e). Southern hybridization of *DraI*-digested DNA revealed that the *DraI*-II and -VI fragments hybridized to the probe and are therefore adjacent to each other on the chromosome (Fig. 4).

Assignment of *AseI* fragments on the *DraI* map

Labelled *AseI* fragments were used to probe *DraI*-digested *S. erythraea* DNA to determine the positions of the *AseI* fragments relative to the *DraI* fragments. *AseI*-A hybridized to *DraI*-III and -IV, *AseI*-C hybridized to *DraI*-I and -V, and *AseI*-D₂ hybridized to *DraI*-III and -V, indicating that these *DraI* fragments are linked on the chromosome. When the 225 and 130 kb portions of *AseI*-I₂ were purified, labelled and hybridized to *DraI*-digested DNA, fragments *DraI*-I and -II, respectively, were detected. Fragments *DraI*-III and -IV, -III and -V, -I and -V, and -I and -II were shown to be adjacent to each other by this method. Along with the previously determined linkage of *DraI*-II and -VI, the complete *DraI* physical map was obtained by gene probing and cross-hybridization. Many *AseI* fragments hybridized to individual *DraI* fragments: *AseI*-G to *DraI*-II; *AseI*-D₁

to *DraI*-I; *AseI*-F to *DraI*-I; *AseI*-M to *DraI*-III; *AseI*-L to *DraI*-I; *AseI*-N to *DraI*-IV; and *AseI*-O to *DraI*-VI (Fig. 4). The location of *AseI*-G was determined to be between *AseI*-B and *AseI*-I₂ (on *DraI*-II), since *DraI*-II contains a 700 kb *AseI-DraI* portion of *AseI*-B and a 130 kb *AseI-DraI* (rRNA operon) portion of *AseI*-I₂. Similarly, the location of *AseI*-N was deduced from the rRNA-probe-based linkage of *AseI*-A and *AseI*-E on *DraI*-IV and the cross-hybridization of *AseI*-N with *DraI*-IV. To confirm many of the fragment assignments made by the cross-hybridization analysis, 2D-PFGE was performed. Fig. 2 shows the resulting *AseI* and *AseI/DraI* fragment assignments for *DraI* fragments II-VI. Only the *AseI* and *AseI-DraI* fragments contained on *DraI*-I did not appear in the 2-dimensional region, except for *AseI*-O (on *DraI*-VI), which was too small to be visualized by this pulse program. All fragment assignments determined by 2D-PFGE were in agreement with the cross-hybridization data. In total, the linkage of 14 *AseI* fragments (making up more than 90% of the chromosome) was obtained by the methods described above.

Completion of the *AseI* physical map by generating additional chromosomal *DraI* sites in *AseI*-J and -K

The final *AseI* map was determined by generating additional *DraI* sites in *AseI*-J and -K. The location of the *AseI*-O fragment was determined by using an *AseI*-

Fig. 2. Two-dimensional PFGE of fragments generated by *DraI* and *AseI* digestion of *S. erythraea* NRRL 2338 chromosomal DNA. (a) Banding pattern of *AseI/DraI*-digested *S. erythraea* DNA resolved in the first dimension (lane A/D) and the second dimension (lanes II–VI). (b) A schematic representation of the 2D-PFGE gel. *AseI* and *AseI–DraI* fragments resolved in the second dimension are indicated in (b) as circles with the corresponding fragment name. Lambda concatemers (L) served as the size marker. * indicates that there is a slight offset in the migration distance of the one-dimensionally and two-dimensionally resolved DNA; this was due to the initial positioning of the DNA plugs (L and A/D lanes) and excised lanes (*DraI/AseI*-digested DNA) at the start of the second dimension run. Pulse programme (both dimensions): 200 V, 22 h, ramp of pulse times 50–90 s.

O-specific probe against *DraI*-digested DNA from *S. erythraea* strain Ω J14. Similarly, the order of the *AseI*-M and *AseI*-K fragments was determined by hybridizing an *AseI*-M-specific probe to DNA obtained from *S. erythraea* strain Ω K5. The insertion in *AseI*-J occurred about 50 kb from one end, yielding two fragments of about 200 kb and 50 kb. *DraI* digestion of Ω J14 cleaves *DraI*-VI, which contains part of *AseI*-B and all of *AseI*-J and -O, into two fragments of about 475 and 60 kb. The size difference between the 60 kb *DraI* fragment and the 50 kb *AseI* fragment is due to the presence of *AseI*-O, which is about 10 kb (the integrated plasmid DNA would only account for between 400 bp and 1 kb of the 10 kb fragment, depending on the orientation of the plasmid in the chromosome). The *AseI*-O-specific probe hybridized to the 60 kb *DraI* fragment, indicating that *AseI*-O is at the end of the chromosome (Fig. 4). *DraI* digestion of Ω K5 gave two new fragments of about 900 and 300 kb, which when added together make the size of *DraI*-III (Fig. 1a, Table 1). PFGE analysis of *AseI*-digested Ω K5 indicated that the insertion had occurred about 25 kb from one end of *AseI*-K. Hybridization using an *AseI*-M probe showed that it is contained on the larger fragment, which also contains 700 kb of *AseI*-A. In addition, an *AseI*-K-specific probe hybridized much more strongly to the smaller fragment, which would be expected if this fragment contains most of *AseI*-K. Further, an *AseI*-D₂ probe hybridized to the

smaller fragment, thus confirming its linkage with *AseI*-K and the overlaps of *DraI*-III and -V. Therefore, the order of these fragments on the chromosome is *AseI*-A, -M, -K, -D₂.

The *S. erythraea* NRRL 2338 chromosome is linear

Chromosome linearity appears to be a general characteristic in *Streptomyces* species. We examined whether the *Saccharopolyspora erythraea* chromosome might also be linear, since the two genera are closely related taxonomically (Labeda, 1987). The results of a procedure that was originally used for determining the linearity of the *Streptomyces lividans* 66 chromosome are shown in Fig. 5. The analysis is based on the differential migration pattern observed when agarose-embedded DNA is prepared in the presence or absence of proteinase K, followed by PFGE analysis using extremely long pulse times. Linear chromosomes which assume a circular conformation due to the association of covalently bound terminal proteins are rendered linear when treated with protease. Only linear molecules enter the gel matrix in PFGE, whereas closed-circular molecules remain in the slot (Lin *et al.*, 1993). A fragment migrating above the 5.7 Mb *Schizosaccharomyces pombe* standard was observed only when the DNA was treated with proteinase K (Fig. 5).

Thus far, all the *Streptomyces* chromosomes charac-

Fig. 3. Southern analysis of *Asel* linking fragments. *Asel* and *Asel-NarI* fragments were isolated and used to probe *Asel*-digested *S. erythraea* DNA. 1-18 and 2-12 refer to the linking clone. The *Asel*-linked fragments are given at the right of each panel. Lambda concatemers (L) were used as the size marker. Pulse programme: 200 V, 26 h, ramp of pulse times 50-90 s were the same for each lane.

terized have been shown to contain TIRs of varying length at each chromosomal end. When end fragments *Asel*-N and *Asel*-O, and *DraI*-IV and *DraI*-VI, were hybridized separately to total *Asel*- and *DraI*-digested *Saccharopolyspora erythraea* DNA, no hybridizing fragments were detected other than the fragment from which the probe was derived. This indicates that there were no obvious homologous repeat sequences located at each chromosomal end.

Mapping of genetic loci to specific *Asel* and *DraI* fragments in *S. erythraea* NRRL 2338 and comparison of its *Asel* restriction pattern with that of *S. erythraea* CA340

Table 2 lists the *S. erythraea* genes that have been mapped to specific *Asel* and *DraI* fragments in this study. The erythromycin-biosynthetic gene cluster mapped to the 700 kb *Asel*-*DraI* fragment *Asel*-B. In addition, several genes that may be involved in either

Fig. 5. The *S. erythraea* NRRL 2338 chromosome migrates as a linear molecule of 8 Mb. Agarose-embedded DNA was either exposed to proteinase K treatment during plug preparation (lane +) or not exposed (lane -) as described by Lin *et al.* (1993). Pulse programme: 50 V, 140 h, pulse time a constant 60 min. Size standard was *S. pombe* (S.p.) chromosomes. The arrow at the right indicates the full-length *S. erythraea* NRRL 2338 chromosome.

erythromycin biosynthesis (*gdh*, *kde*) or transport (*ertX*), are also contained on this *Asel*-*DraI* fragment. This fragment also contains the *attB* site for pSE101.

The *Asel*, *DraI* and *Asel/DraI* restriction patterns of strains 2338 and CA340 were compared by PFGE. Both the single- and double-digestion restriction patterns appeared identical except for the loss of *Asel*-N and the appearance of a ~75 kb fragment in CA340 (Fig. 6). No apparent differences were observed in the *DraI* profiles.

DISCUSSION

An *Asel* and *DraI* physical map of the 8 Mb *S. erythraea* NRRL 2338 chromosome has been completed. The size of this chromosome is comparable to previously characterized *Streptomyces* chromosomes. This finding was not surprising considering that *Saccharopolyspora* species are taxonomically closely related to the *Streptomyces* and were once classified as such (Labeda, 1987). Among bacteria, these chromosomes are only smaller

Fig. 4. Physical map of the *S. erythraea* NRRL 2338 chromosome. The arrowheads indicate the orientation of the rRNA (*rrn*) operons. *ery*, erythromycin-biosynthetic gene cluster.

Table 2. Localization of *S. erythraea* NRRL 2338 genes to specific *AseI* and *DraI* fragments

Gene	Cellular function	Location		Reference
		<i>AseI</i> band	<i>DraI</i> band	
<i>bpl1</i>	Biotin protein locus-1	E	IV	Donadio <i>et al.</i> (1996)
<i>bpl2</i>	Biotin protein locus-2	I ₁	I	Donadio <i>et al.</i> (1996)
<i>ertX</i>	Putative ABC transporter	B	II	O'Neill <i>et al.</i> (1995)
<i>ery*</i>	Erythromycin biosynthesis	B	II	Donadio <i>et al.</i> (1993)
<i>gdh</i> †	Thymidine diphosphoglucose 4,6-dehydratase	B	II	Linton <i>et al.</i> (1995)
<i>gtt</i>	Thymidine diphosphoglucose synthase	E	IV	S. Zotslav (unpublished)
<i>ilvD</i>	Isoleucine/valine dehydratase	I ₂	II	This study
<i>int</i> ‡	Integrase	B	II, VI	Brown <i>et al.</i> (1990)
<i>kde</i> †	Putative thymidine diphospho-4-keto-6-deoxyglucose 3,5-epimerase	B	II	Linton <i>et al.</i> (1995)
<i>recA</i> §	Recombination protein	F	I	Nussbaumer & Wohlleben (1994)
<i>rrnA</i>	rRNA locus-A	E-A	IV	M. J. Staver & R. G. Summers (unpublished)
<i>rrnB</i>	rRNA locus-B	M-K	III	M. J. Staver & R. G. Summers (unpublished)
<i>rrnC</i>	rRNA locus-C	I ₂ -F	I	M. J. Staver & R. G. Summers (unpublished)
<i>rrnD</i>	rRNA locus-D	G-I ₂	II	M. J. Staver & R. G. Summers (unpublished)
<i>xis</i>	Excisionase	B	II, VI	Brown <i>et al.</i> (1990)

* Includes the entire ~ 56 kb erythromycin-biosynthetic gene cluster.

† *gdh* and *kde* are adjacent to one another on the chromosome (Linton *et al.*, 1995).

‡ pSE101 and pSE211 integrate into the *S. erythraea* NRRL 2338 chromosome at two different *attB* sites on *AseI*-B and *DraI*-II and VI.

§ Localized by using the *S. lividans* TK24 *recA* gene as a probe.

|| All the rRNA operons of *S. erythraea* NRRL 2338 span two adjacent *AseI* fragments, since there is an internal *AseI* site.

than the reported sizes of the chromosomes of *Mycococcus xanthus* and *Bradyrhizobium japonicum* (Cole & Saint Girons, 1994). The *S. erythraea* chromosome is linear, based on comparing the PFGE migration pattern of proteinase K-treated and -untreated DNA. This adds to the growing list of bacterial genera that contain members with linear chromosomes, which at present include *Streptomyces* (Kieser *et al.*, 1992; LeBlond *et al.*, 1993, 1995; Lezhava *et al.*, 1995; Pandza *et al.*, 1997), *Borrelia* (Baril *et al.*, 1989), *Agrobacterium* (Allardet-Servent *et al.*, 1993), *Rhodococcus* (Crespi *et al.*, 1992) and *Coxiella* (Willems, 1997).

At present, all the *Streptomyces* chromosomes that have been characterized contain TIRs. These chromosomal elements vary widely in length, from 24 kb in *Streptomyces griseus* (Lezhava *et al.*, 1995) to 550 kb in *Streptomyces rimosus* (Pandza *et al.*, 1997). We were unable to detect putative TIRs using end-fragments as probes against blots containing either *AseI*- or *DraI*-digested DNA. It is possible that *S. erythraea* does not contain TIR sequences or that the sequences are short, and therefore would not be detected using much larger fragments as probes, as has been shown with the linear plasmid SLP2 (Chen *et al.*, 1993). Recently, Fischer *et al.* (1997) described significant TIR size heterogeneity within strains of *Streptomyces ambofaciens*.

In the industrially improved strain *S. erythraea* CA340, which produces tenfold more erythromycin than strain

NRRL 2338, it has been found by PFGE analysis that the size of the chromosome and its *AseI*, *DraI* and *AseI/DraI* profiles are the same as in the wild-type strain NRRL 2338, except for a single polymorphism in *AseI*-N. The precise nature of the change(s) observed in the *AseI* profile of CA340 remain to be elucidated. This polymorphism might be due to AUD-like sequences as found in *Streptomyces* spp. (Altenbuchner & Cullum, 1985).

A search of the GenBank database of all the sequenced *rrs* genes from *Saccharopolyspora* spp. identified an *AseI* site near the middle of the gene; no *AseI* site has so far been identified in a *Streptomyces rrs* gene. This is consistent with the reclassification of *S. erythraea* from a *Streptomyces* to a *Saccharopolyspora* species.

The erythromycin-biosynthetic gene cluster was found to be located from 550 kb to 1.25 Mb from one chromosomal end, depending on where it lies within the 700 kb *AseI-DraI* fragment of *AseI*-B. This places the erythromycin-biosynthesis cluster relatively close to one end of the chromosome, as has been found with several other gene clusters encoding antibiotic-biosynthetic pathways (Pandza *et al.*, 1997). Several other genes possibly involved in erythromycin production, including *ertX*, a putative ABC-type transporter, *gdh*, a thymidine diphosphoglucose 4,6-dehydratase and *kde*, a putative thymidine diphospho-4-keto-6-deoxyglucose 3,5-epimerase, lie within the same 700 kb region of the chromosome.

Fig. 6. Comparison of the *Asel* profiles of *S. erythraea* NRRL 2338 and *S. erythraea* CA340. Agarose-embedded DNA from the two *S. erythraea* strains were identically prepared and the *Asel* profiles determined. The arrow indicates the appearance in strain CA340 of a ~75 kb fragment. Pulse programme: 200 V, 26 h, ramp of pulse times 50–90 s. Lambda concatemers (L) were used as the size standard.

In conclusion, the *Saccharopolyspora erythraea* chromosome is a linear molecule having several features in common with the previously characterized *Streptomyces* chromosomes. These include a chromosome size of about 8 Mb, a linear topology and the location of genes encoding secondary metabolism biosynthetic pathways near one end of the chromosome. In contrast, two notable differences in the chromosome composition were observed: (1) no readily detectable TIR sequences were seen using the predicted end fragments as probes; and (2) all *rrs* genes from *Saccharopolyspora* spp. contain an *Asel* site, whereas none have been observed thus far in *Streptomyces* spp. Finally, a total of 15 genes or gene clusters were mapped to specific *Asel* and *DraI* fragments.

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