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Physical mapping shows that the unstable oxytetracycline gene cluster of *Streptomyces rimosus* lies close to one end of the linear chromosome

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A restriction map of the 8 Mb linear chromosome of *Streptomyces rimosus* R6-501 was constructed for the enzymes *Ase*I (13 fragments) and *Dra*I (7 fragments). Linking clones for all 12 *Ase*I sites and 5 of the 6 *Dra*I sites were isolated. The chromosome has terminal inverted repeats of 550 kb, which are the longest yet reported for a *Streptomyces* species. The oxytetracycline gene cluster lies about 600 kb from one end, which might account for its frequent spontaneous amplification and deletion. Several other markers were localized on the chromosome (*dnaA* and *recA*, the *rrn* operons, the attachment site for pSAM2 and prophages RP2 and RP3). Comparison of the conserved markers with the map of *Streptomyces coelicolor* A3(2) suggested there are differences in genome organization between the two species.

Keywords: *Streptomyces rimosus*, physical map, linear chromosome, terminal repeats, oxytetracycline

INTRODUCTION

The genes for the biosynthesis of the commercially important antibiotic oxytetracycline (OTC) in *Streptomyces rimosus* lie in a cluster of about 30 kb in size flanked by two resistance genes. This pattern is seen both in the 'Pfizer strain' (*S. rimosus* M4018 lineage; Butler *et al.*, 1989) and in the 'Zagreb strain' (*S. rimosus* R6 lineage; Perić, 1995). OTC production in *S. rimosus* R6 is genetically unstable and some spontaneous mutants carry large-scale DNA rearrangements involving the OTC-cluster (Gravius *et al.*, 1993). Class II mutants showed large (> 450 kb) deletions that remove the whole cluster, whereas class III mutants showed reiteration of the cluster resulting in higher production of, and resistance to, OTC.

Investigations in *Streptomyces lividans* 66 (Redenbach *et al.*, 1993) and *Streptomyces ambofaciens* (Leblond *et al.*, 1996) showed that unstable regions subject to deletion and amplification are located close to the ends of the linear chromosomes in these species. These two species have inverted repeats of 30 kb and 210 kb, respectively, at the ends of their chromosomes, which are about 8 Mb in size. These observations suggested that the OTC-cluster might also lie near one end of the chromosome in *S. rimosus*. This would also have consequences for the formation of plasmid primes: it has been suggested that the plasmid pPZG103 which carries the OTC-cluster might have been formed by a single cross-over between the linear plasmid pPZG101 and the chromosome of *S. rimosus* (Gravius *et al.*, 1994b; Hranueli *et al.*, 1995).

In this paper, we report the establishment of a physical map of the chromosome of *S. rimosus* R6 and the mapping of several loci, including the OTC-cluster. This allows comparison with the map of *Streptomyces coelicolor* A3(2) (Redenbach *et al.*, 1996), which is interesting because the two species are not closely related [*S. coelicolor* A3(2) belongs to the *S. griseoruber* cluster of Williams *et al.*, 1983; E. M. H. Wellington, personal communication].

METHODS

Bacterial strains, phages and plasmids. *S. rimosus* R6 strains 500 and 501 and phages RP2 and RP3 are described by Hranueli *et al.* (1979) and Rausch *et al.* (1993). The linear

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Abbreviation: OTC, oxytetracycline.

chromosome map of *S. lividans* 66 strain ZX7 and the derivative with a circular chromosome, strain MR02 are described by Redenbach *et al.* (1993). *S. coelicolor* A3(2) strain 1147 is described by Hopwood *et al.* (1985). The construction of the cosmid gene bank of strain *S. rimosus* R6-501 is described by Rausch *et al.* (1993); the same methods were used to construct the cosmid gene bank of the *Ase*I-J band. The vector used (sCos-1; Evans *et al.*, 1989) has T3 and T7 promoter sequences flanking the insert and the insert is also flanked by *Eco*RI sites. pBR328 (Bolivar *et al.*, 1977) was used to construct the *Ase*I-linking library in *Escherichia coli* strain XL1-Blue (Bullock *et al.*, 1987).

The following plasmids were used to localize loci on the physical map: pTS55 (Smokvina *et al.*, 1991), used to localize *att*-pSAM2; pIM (Pujić, 1992), containing an 8·8 kb *Bam*HI fragment carrying the whole copy of the *rrn* operon of *S. rimosus* R7; pFF911 and pFF914 (Musialowski *et al.*, 1994), carrying the *dnaA–oriC* region of *S. coelicolor* A3(2); pBN104 (Nußbaumer & Wohlleben, 1994), carrying the *recA* gene of *S. lividans* 66; and pMT2005 (Ali-Dunkrah *et al.*, 1990), carrying the *gal* operon of *S. lividans* 66.

Molecular genetic techniques. Media and growth conditions, total DNA preparation, plasmid DNA preparation, restriction digests, agarose gel electrophoresis, Southern blots and DNA labelling were done as described by Gravius *et al.* (1993). Exonuclease III digestions were carried out similarly to restriction digests using the buffer recommended by the manufacturer (Boehringer Mannheim). Digoxigenin-labelled RNA using T3 or T7 RNA polymerase was done according to the manufacturer's instructions (Boehringer Mannheim). Before T3 or T7 labelling clones were doubly digested with *Eco*RI and *Sal*I (which cuts frequently in *Streptomyces* DNA) so as to achieve specific labelling of the ends of the inserts.

DNA was prepared in agarose blocks, digested with restriction enzymes, separated on a Bio-Rad CHEF DRII apparatus and transferred to membranes by Southern blotting as described by Gravius *et al.* (1993). The pulse programme for separating intact chromosomes was 50 V, 192 h, with a 1 h constant pulse time; other programmes are indicated in the figure legends. Bands were eluted from PFGE gels as described by Gravius *et al.* (1994b).

To construct the *Ase*I-linking libraries, total DNA of *S. rimosus* R6-501 was digested with *Sal*I or *Pst*I and the digested DNA ligated at a low DNA concentration ($<$ 1 μg ml^{-1}) to promote intramolecular circularization. The religated DNA was digested with *Ase*I and ligated together with alkaline-phosphatase-treated *Ase*I-restricted DNA of pBR328. The ligation mixtures were introduced into *E. coli* XL1-Blue by electroporation using a BioRad GENEPULSER apparatus and conditions recommended by the manufacturer (voltage 2500 V, resistance 200 Ω, capacitance 25 μF which gave a time constant, τ, of 4·5–4·8 ms). Transformants were selected on chloramphenicol-containing medium (50 μg ml^{-1}) and tested for ampicillin (100 μg ml^{-1}) resistance by replica plating.

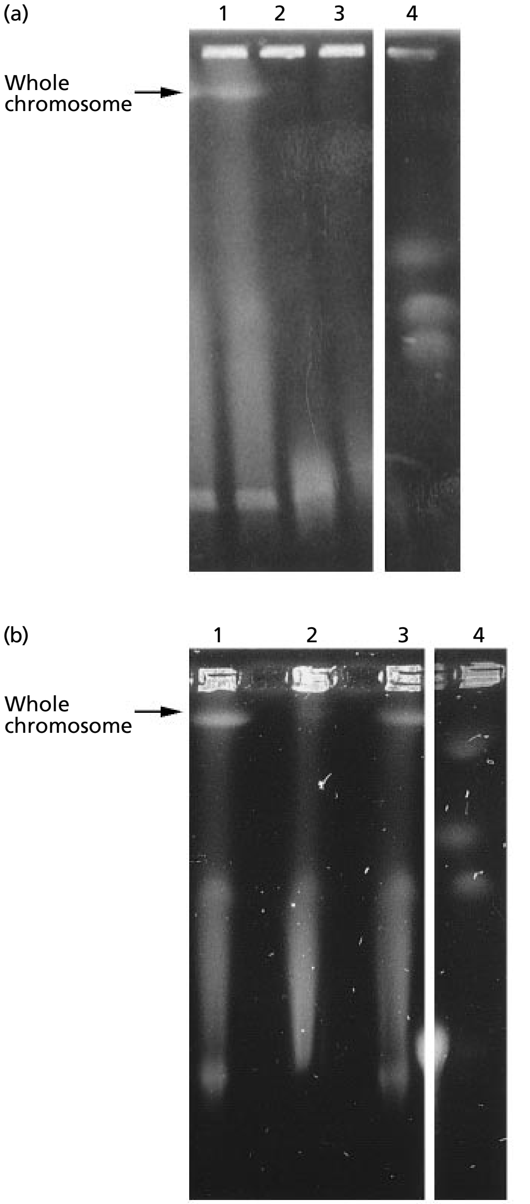

Fig. 1. PFGE of undigested DNA of *Streptomyces* species. Pulse programme: 50 V, 192 h, 1 h constant pulse time. The bands corresponding to whole chromosomes are marked. (a) Tracks: 1, *S. rimosus* R6-501; 2–3, *S. rimosus* R6-501 treated with 100 U and 200 U, respectively, exonuclease III; 4, chromosomes of *Schiz. pombe.* (b) Tracks: 1, *S. coelicolor* A3(2) strain 1147; 2, *S. lividans* 66 strain MR02; 3, *S. lividans* 66, strain ZX7; 4, chromosomes of *Schiz. pombe*.

RESULTS

Size of the chromosome

Undigested DNA from *S. rimosus* R6-501 was subjected to PFGE using a pulse programme developed to allow visualization of the linear chromosome of *S. lividans* 66 (Lin *et al.*, 1993). A band was produced that migrated slower than the largest chromosome of *Schizosaccharomyces pombe* (Fig. 1a, track 4). Similar results were obtained with *S. coelicolor* A3(2) and *S. lividans* 66 (Fig. 1b, tracks 1 and 3), which have linear chromosomes (Lin *et al.*, 1993), whereas a mutant (MR02) of *S. lividans* that possesses a circular chromosome (Redenbach *et al.*, 1993) does not produce a high-molecular-mass band (Fig. 1b, track 2). Treatment of the DNA of *S. rimosus*

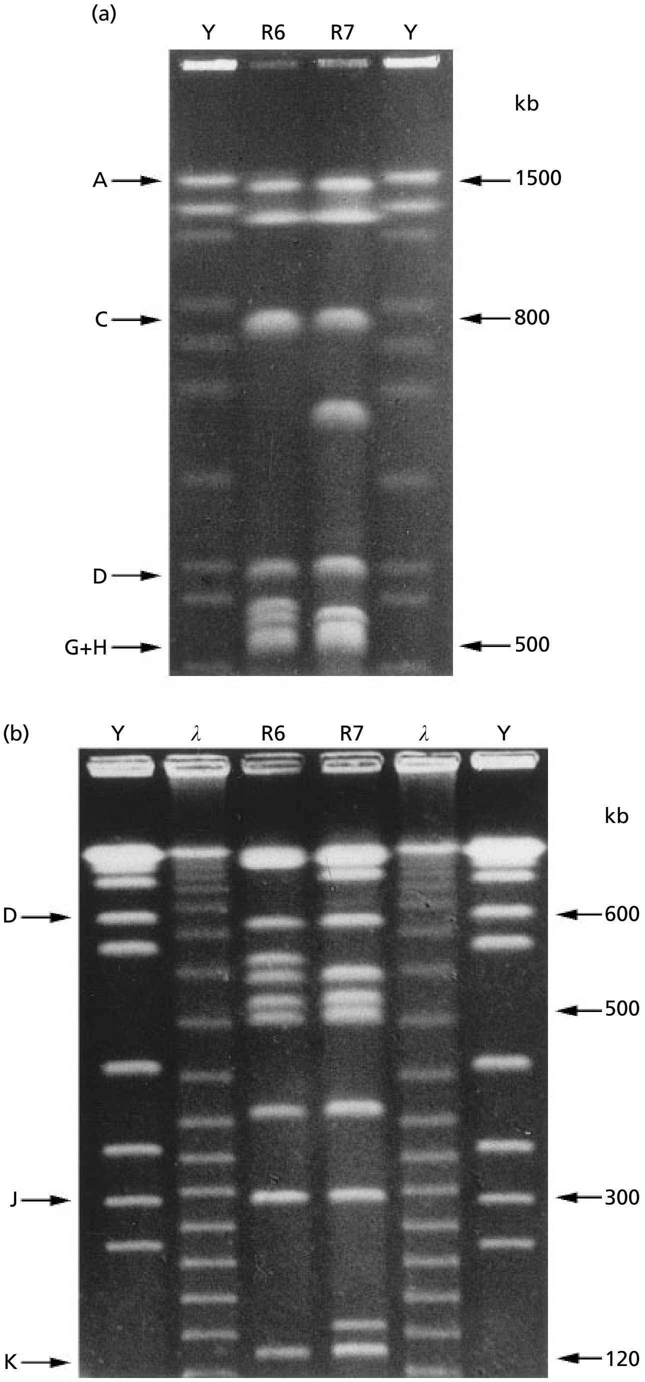

Fig. 2. Chromosomal DNA of *S. rimosus* strains R6-501 and R7 (ATCC 10970) digested with *Ase*I. Plasmid DNA was removed by a prerun. Tracks: R6, *S. rimosus* R6-501; R7, *S. rimosus* ATCC 10970; Y, chromosomes of *Sacch. cerevisiae*; λ, lambda ladders. (a) Pulse programme: 160 V, 60 h, ramp of pulse times 70–80 s. (b) Pulse programme: 160 V, 36 h, ramp of pulse times 40–50 s.

with exonuclease III (Fig. 1a, tracks 2 and 3) leads to the loss of the slowly migrating band, supporting the idea that it is a large linear molecule rather than a smaller circular molecule.

Total DNA preparations of *S. rimosus* in agarose blocks were subjected to a prerun to remove the DNA of the linear plasmid pPZG101 (Gravius *et al.*, 1994b). The resulting chromosomal DNA preparations were digested with the enzymes *Ase*I, *Dra*I, *Ssp*I and *Xba*I and separated by PFGE. Fig. 2 shows the *Ase*I digests run with two different pulse programmes to optimize separation in different parts of the molecular mass range; 11 fragments can be seen. The sizes of the larger fragments (>500 kb) were estimated (Table 1) by comparison with lambda ladders and the chromosomes of *Saccharomyces cerevisiae*. As smaller fragments of high G+C-content migrate faster than similar-sized fragments of lower G+C-content (Gravius *et al.*, 1994a), the fragment sizes were recalculated relative to high G+C-markers as described by Gravius *et al.* (1994b). The high G+C effect is particularly striking for the *Ase*I-K fragment of 120 kb (Table 1) which migrates faster than the 100 kb lambda-dimer (Fig. 2b, tracks labelled λ and R6). Scanning of gel photographs with a densitometer suggested that the C band and the J band consisted of double fragments (Table 1). The OTC-cluster lies on one of the C fragments and analysis of deletions affecting the cluster had already suggested that there were two distinct fragments of this size (Gravius *et al.*, 1993). The *Dra*I digest could be separated into seven distinct fragments (Table 1). Comparison of the restriction patterns with total DNA (without a prerun to remove pPZG101) showed an identical pattern except for the addition of the three known *Ase*I fragments and two known *Dra*I fragments (Gravius *et al.*, 1994b) of pPZG101. Table 1 also shows the sizes of the *Ssp*I and *Xba*I fragments, which because of their larger numbers (21 and 26 fragments, respectively) were not used for mapping of the entire chromosome. The sums of the fragment sizes for the four enzymes were consistent with each other, only varying between 7735 and 8090 kb. Thus, *S. rimosus* R6-501 has a chromosome size of about 8 Mb, similar to that of other *Streptomyces* species (Kieser *et al.*, 1992; Leblond *et al.*, 1993, 1996; Lezhava *et al.*, 1995). In addition, DNA from another *S. rimosus* strain (R7) was digested with the same enzymes (Fig. 2). The digestion patterns were clearly related, but there were also differences between the two strains.

Isolation of linking clones

We used a cosmid gene bank of *S. rimosus* R6-501 (Rausch *et al.*, 1993) in the vector sCos-1 (Evans *et al.*, 1989). Cosmid DNA was prepared from 1600 clones and digested with the enzyme *Ase*I. The vector contains two *Ase*I sites, so if no *Ase*I site is present in the insert, two bands of 1·73 kb and 45–50 kb are seen. If the insert contains one *Ase*I site then three bands are seen. Using this method 47 cosmids containing 7 different *Ase*I sites were isolated. Similarly, 24 clones containing 5 different *Dra*I sites were isolated.

Another strategy to isolate *Ase*I-linking clones used a method adapted from Poustka & Lehrach (1986). 288 potential *Ase*I-linking clones were isolated. These were

Table 1. Sizes of the *Dra*I, *Ase*I, *Ssp*I and *Xba*I restriction fragments of the *S. rimosus* R6-501 chromosome

The sum of the fragment sizes is given in parentheses at the bottom of the respective columns.

***Dra*I**		***Ase*I**		***Ssp*I**	***Xba*I**
Fragment	**Size (kb)**	**Fragment**	**Size (kb)**	**Size (kb)**	**Size (kb)**
I	2400	A	1540	1400	1120
II	1670	B	1120	1000	800
III	1230	C1	795	745	680
IV	1180	C2	795	745	610
V	850	D	600	710	510
VI	290	E	550	500	480
VII	270	F	525	500	415
	(8070)	G	510	445	350
		H	500	415	320
		I	415	280	280
		J	300	280	230
		J	300	270	230
		K	120	205	200
			(7890)	160	200
				95	195
				80	185
				65	160
				60	155
				52	145
				45	115
				38	90
				(8090)	85
					80
					40
					31
					29
					(7735)

used for colony hybridization against a pool of the existing *Ase*I-linking cosmids to exclude duplicates. This yielded four new classes of *Ase*I-linking clones.

Construction of the restriction map of the chromosome

Representative *Ase*I- and *Dra*I-linking clones were used as hybridization probes against Southern blots of PFGE gels of *Ase*I and *Dra*I single digests and double digests of chromosomal DNA. In most cases the linking clones for a particular enzyme hybridized to two different fragments obtained after digestion with that enzyme (e.g. see Fig. 3, tracks 1–2). Thus, C-A11 hybridized to *Ase*I-A and K, C-A41 to *Ase*I-G and H, C-A5 to *Ase*I-H and I, C-AF2 to *Ase*I-B and F, C-A23 to *Ase*I-D and F, and C-A21 to *Ase*I-D and E. Each of the *Dra*I-linking clones shown in the map (Fig. 4) hybridized only to the corresponding two *Dra*I fragments. In the case of the double band *Ase*I-C, C-AE8 hybridized to *Ase*I-C and K, C-AB4 to *Ase*I-A and C, and C-A9 to *Ase*I-C and G; the two *Ase*I-C fragments were distinguished by hybridizing to digestions of the class II mutant MV7 (Gravius *et al.*, 1993), which carries a deletion affecting the *Ase*I-C1 fragment that carries the OTC-cluster; this assigned C-AB4 and C-A9 to the *Ase*I-C2 fragment, and C-AE8 to the *Ase*I-C1 fragment. The *Ase*I-linking clone C-AE6 hybridized with the *Ase*I-A, B, C and I bands and also gave very weak hybridization with the *Ase*I-H band (data not shown). DNA from this clone was doubly digested with *Ase*I and *Sal*I and the two *Ase*I–*Sal*I fragments of the insert eluted from an agarose gel. When one of these fragments was hybridized with a Southern blot of a PFGE gel, only the *Ase*I-I band hybridized, whereas the other fragment hybridized to the other four *Ase*I bands (A, B, C and H). This shows that sequences at one end of the insert in C-AE6 are derived from *Ase*I-I and suggests that there is a repeated sequence on the other side of the *Ase*I site in C-AE6. As both linking clones at the ends of the *Ase*I-A, C2 and H fragments were already known and further analysis (see below)

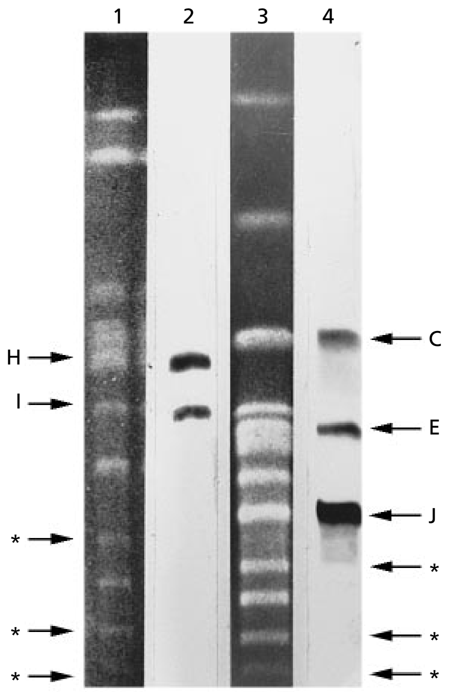

Fig. 3. Hybridization with linking clones. Total DNA of *S. rimosus* R6-501 was digested with *Ase*I and separated by PFGE (pulse programmes: track 1, 200 V, 28 h, ramp of pulse times 60–130 s; track 3, 200 V, 30 h, ramp of pulse times 80–130 s). The bands marked with an asterisk are derived from the linear plasmid pPZG101. Southern blots of the digests in tracks 1 and 3 were hybridized with digoxigenin-labelled DNA from the linking clone C-A5 (track 2) and C-A4 (track 4).

defined the second linking clone for *Ase*I-C1 (C-A4) it was concluded that C-AE6 was the linking clone between the *Ase*I-B and I fragments. The insert in C-AE6 did not cross-hybridize with any of the other linking clones and hybridization experiments with a plasmid pIM (Pujić, 1992) carrying an rRNA operon of *S. rimosus* showed that the repeated sequence was not part of an rRNA operon.

A second *Ase*I-linking clone that hybridized to more than two fragments (Fig. 3, tracks 3–4) was clone C-A4, which hybridized to the *Ase*I-C, E and J fragments. Since the densitometer results had suggested that *Ase*I-J was a double band and there were no other *Ase*I-linking cosmids that hybridized with the *Ase*I-J band, it was suspected that the *Ase*I-J fragment might be contained within a large duplication. When a map of the chromosome was constructed according to this hypothesis, a linear map resulted with the two copies of the *Ase*I-J fragment at the ends (Fig. 4). The linear map is supported by the failure to isolate linking clones connecting the presumed end fragments (the two *Ase*I-J fragments and the *Dra*I-A and B fragments) and a more detailed study of the terminal inverted repeats below. As expected, the linking clone C-A4 hybridized to both the *Dra*I-I and II fragments. This means that all 12 of the *Ase*I-linking clones and 5 out of 6 of the *Dra*I-linking clones had been isolated.

Analysis of the inverted repeats with cosmid clones

The agarose containing the 300 kb *Ase*I-J band was excised from a gel. DNA was eluted, partially digested with *Mbo*I and used to construct a cosmid bank in sCos-1. Forty clones were obtained and were ordered by cross-hybridization. This yielded a contig in fragment *Ase*I-J which was spanned by 9 cosmids starting with the linking clone C-A4 (Fig. 5). One of the clones (J-39) contained a *Bfr*I site which lies 180 kb from the chromosome end. This means that the most distal cosmid (J-28) is still 50–100 kb away from the chromosome end.

The *Ase*I-linking clone C-A4 contains an *Xba*I restriction site about 10 kb distant from, and proximal to, the *Ase*I site. When the clone was used as a hybridization probe against *Xba*I digests of chromosomal DNA, fragments of 415 kb and 300 kb hybridized. The 415 kb fragment is the fragment that carries the OTC-cluster (Gravius *et al*., 1993). This fragment was isolated from a PFGE gel, labelled with digoxigenin and used as a probe for colony hybridizations of the *S. rimosus* gene bank (S. Pandza and others, unpublished results). It was possible to construct a contig of 10 overlapping cosmid clones starting from the *Ase*I-linking clone C-A4 up to cosmid C-136 (Fig. 5). These clones were used as hybridization probes against Southern blots of *Ase*I digests (data not shown) and hybridize to both the *Ase*I-C and E bands. A complication arose when four cosmids that cross-hybridized with C-136 were examined. When Southern blots of *Eco*RI digests of three of the cosmids (C-19, C-86 and C-88) were hybridized with a C-136 probe, there was only one hybridizing fragment of 5 kb in size (data not shown). A non-hybridizing band from each cosmid was used as a hybridization probe against Southern blots of an *Ase*I digest of chromosomal DNA. In each of the three cases, only the *Ase*I-A fragment hybridized, which suggests the presence of a repeated element in cosmid C-136, which is also present in the AseI-A fragment. The fourth cosmid (C-61) showed a longer homology with C-136, with only two *Eco*RI fragments of 9 kb and 5 kb not hybridizing. C-61 hybridized to the *Ase*I-C band alone which means that it lies outside the inverted repeat in the *Ase*I-C1 fragment. In order to find a cosmid that carries the end of the inverted repeat in the *Ase*I-E fragment, C-136 was used to isolate a further four cosmids from the gene bank which did not hybridize with C-62, the distal cosmid overlapping C-136. Restriction fragments which did not hybridize with C-136 were identified in each cosmid and used as hybridization probes against Southern blots of *Ase*I digests of total DNA. One of these cosmids (C-123) contained a 6 kb *Eco*RI fragment that hybridized only with the *Ase*I-E fragment. Thus, the ends of the inverted repeat in the *Ase*I-C1 and *Ase*I-E fragments lie within C-136 and C-123, respectively. The inverted repeat is about 550 kb long (Fig. 5).

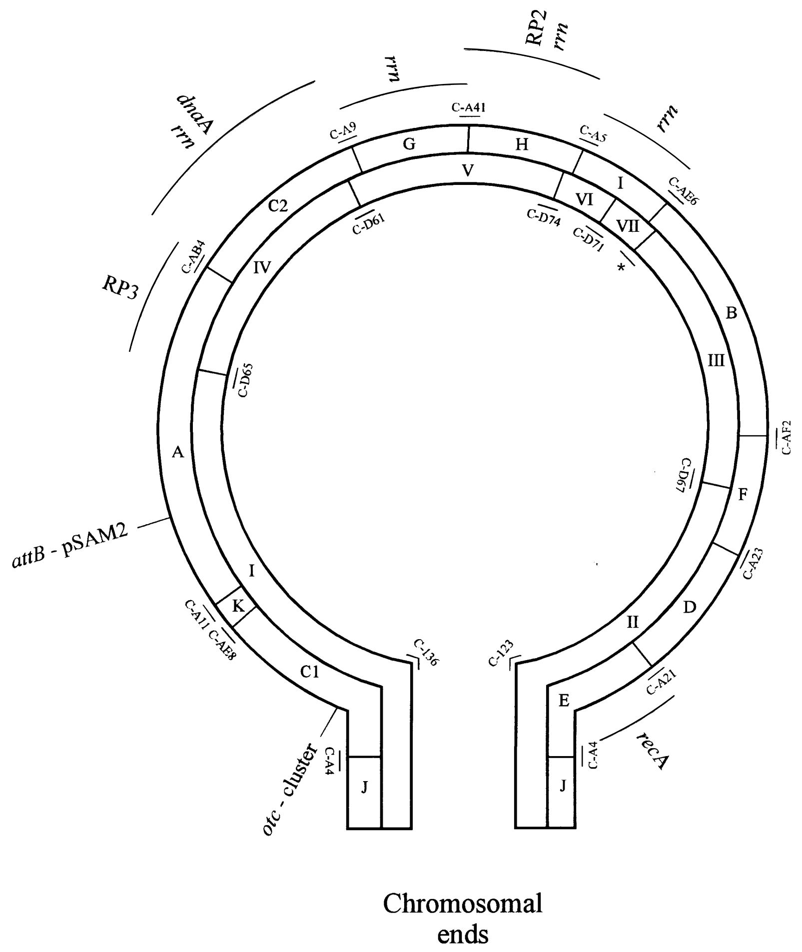

Fig. 4. Restriction map of the chromosome of *S. rimosus* R6-501 for the enzymes *Ase*I (outer arc) and *Dra*I (inner arc). The terminal inverted repeats are drawn as a stem structure. The numbers of the linking clones are indicated adjacent to the corresponding restriction sites; the missing *Dra*I linking clone is indicated by an asterisk. The cosmid clones carrying the ends of the terminal inverted repeats (C-136 and C-123) are also indicated. The OTC-cluster and *attB*-pSAM2 have been precisely localized. The other markers have only been localized to particular *Ase*I and *Dra*I fragments.

Mapping of genetic loci

Previous work (Gravius *et al.*, 1993) had shown that genes of the OTC-cluster hybridize only with the 415 kb *Xba*I fragment and the 795 kb *Ase*I-C1 fragment. As the genes do not hybridize with the *Ase*I-E fragment at the other chromosome end or with the cosmids from the terminal inverted repeat (data not shown), the cluster must lie between the end of the terminal inverted repeat and the *Xba*I site (i.e. 550–720 kb from the chromosome end; Fig. 5). In a *Bfr*I digest the cluster was localized to a 490 kb *Bfr*I fragment (data not shown), which overlaps with the 415 kb *Xba*I fragment. Thus, the 30 kb long OTC-cluster lies in the 120 kb region between the end of the terminal inverted repeat and the *Bfr*I site (i.e. 550–670 kb from the chromosome end; Fig. 5).

RP2 and RP3 are prophages that are integrated into the chromosome of *S. rimosus* R6 (Rausch *et al.*, 1993). Hybridization experiments with DNA from phages RP2 and RP3 localized the prophages to the *Ase*I-H and *Dra*I-V and to the *Ase*I-A and *Dra*I-IV fragments, respectively (Fig. 4). In strain R6-500 (Rausch *et al.*, 1993), which has been cured of the RP2 prophage, the *Ase*I-H band was missing and was replaced by a fragment about 65 kb smaller, as expected for a simple excision event. The vector pTS55, which is based on the integrating plasmid pSAM2 (Smokvina *et al.*, 1991), was introduced into *S. rimosus* (J. Pigac, personal communication). Digestion of DNA from a strain containing pTS55 showed that the *Ase*I-A band had disappeared and been replaced by two bands of about 1 Mb and 600 kb (data not shown); this is expected, because of the presence of an *Ase*I site in pTS55. Southern blots of the digests were hybridized with the two *Ase*I-A-linking clones, which showed that the 600 kb fragment was linked to *Ase*I-K. This localized *attB*-pSAM2 precisely on the map. The rRNA genes were localized to *Ase*I and *Dra*I fragments using plasmid pIM (Pujić, 1992) as a hybridization probe. Four hybridizing bands were seen with each enzyme (*Ase*I-C, G, H and I, *Dra*I-IV, V, VI and VII, respectively). This allows approximate localization of the *rrn* operons on the map. The two plasmids pFF911 and pFF914 (Musialowski *et al.*, 1994), which carry the *dnaA*–*oriC* region of *S. coelicolor* A3(2), were used as hybridization probes. Both showed hybridization to the *Ase*I-C and *Dra*I-IV bands, which allows the approximate localization of the region on the map (Fig. 4). Plasmid pBN104, which carries the *recA* gene of *S. lividans* 66 (Nußbaumer & Wohlleben, 1994), showed strong hybridization to the *Ase*I-E and *Dra*I-II fragments. A plasmid (pMT2005, Ali-Dunkrah *et al.*, 1990) carrying the *gal* operon of *S. lividans* 66 did not give any hybridization signals with *S. rimosus* DNA.

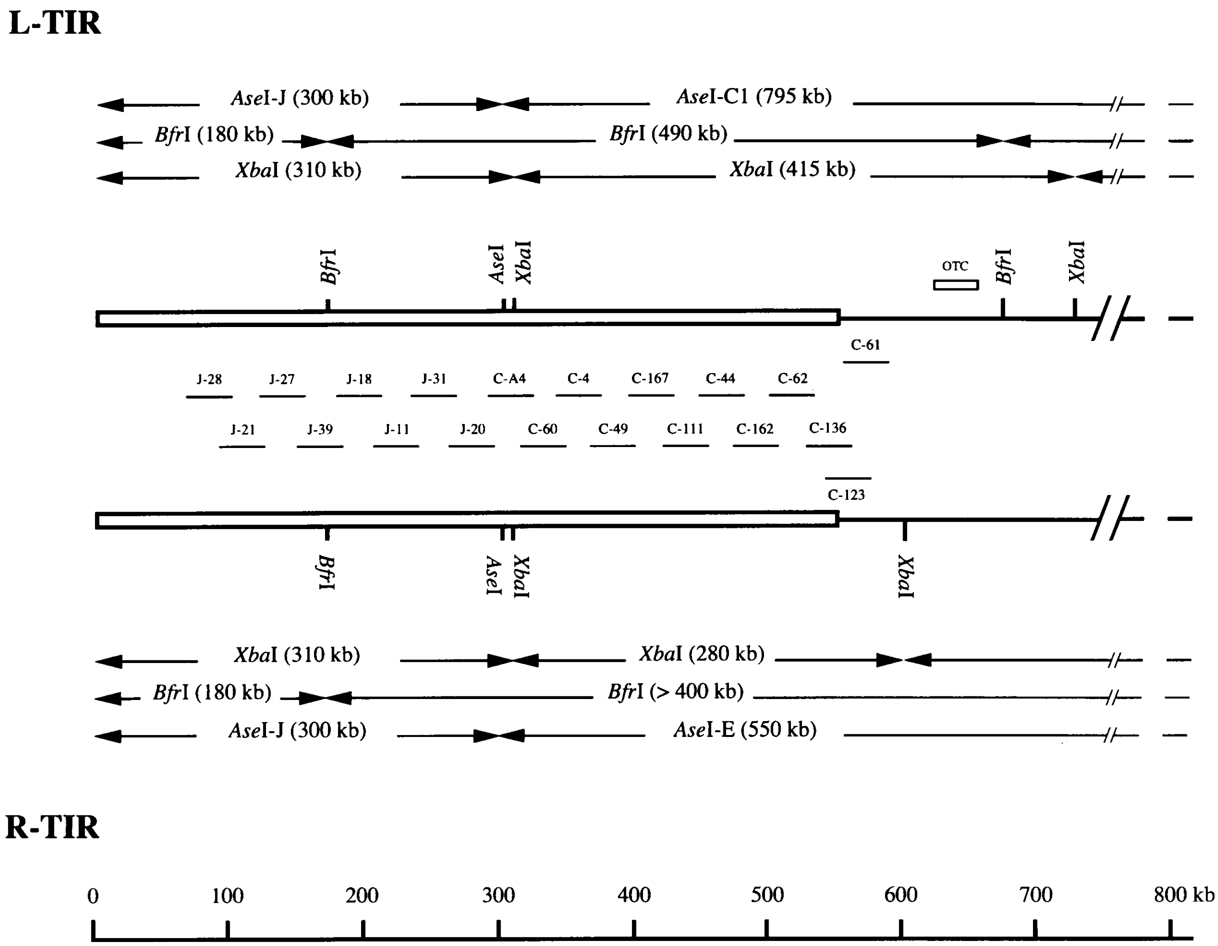

Fig. 5. Structure of the terminal inverted repeats. The cosmids from J-28 up to C-62 are in the inverted repeat and cannot be assigned to a particular chromosome end. C-136 and C-61 belong to the *Ase*I-C1 end, whereas C-123 carries the end of the inverted repeat at the *Ase*I-E end.

DISCUSSION

The chromosome of *S. rimosus* R6 is about 8 Mb in size, which is similar to the values obtained with *S. coelicolor* A3(2) (Kieser *et al.*, 1992), *S. lividans* 66 (Leblond *et al.*, 1993), *S. griseus* (Lezhava *et al.*, 1995) and *S. ambofaciens* (Leblond *et al.*, 1996). The ends of the chromosome are inverted repeats of about 550 kb in size, which is considerably longer than those reported in other *Streptomyces* species (22–210 kb). The lengths of the inverted repeats of linear plasmids also vary markedly and it has been speculated that recombination events lead to evolution in the length of the repeats (Kalkus *et al.*, 1993; Gravius *et al.*, 1994b; Hranueli *et al.*, 1995). It is interesting to note that the cosmid clone carrying one end of the inverted repeat (C-136) cross-hybridized with cosmids from the *Ase*I-A fragment. This might indicate the presence of a transposable element, which could be involved in the formation of the extremely long inverted repeat structure.

The OTC-cluster lies 550–670 kb from one end of the chromosome (Figs 4 and 5), which probably accounts for the frequent DNA rearrangements affecting this region (Gravius *et al.*, 1993). More detailed restriction analysis of the cluster (N. Perić & D. Hranueli, unpublished results) showed that the *otrB* resistance gene was closest to the chromosome end. The increased copy number of the OTC-region seen in class III mutants might result from amplification events similar to those which affect sequences near the chromosome ends in other species (Redenbach *et al.*, 1993; Leblond *et al.*, 1996). Deletion of the OTC-cluster in class II mutants could either involve an internal deletion not affecting the chromosome end as observed in *S. ambofaciens* (Leblond *et al.*, 1996), or loss of one chromosome end as observed in *S. lividans* (Rauland *et al.*, 1995). Deletions arising from circularization of the chromosome with loss of both ends (as observed in both *S. ambofaciens* and *S. lividans*) is unlikely, because the deletion mutants retain the *Ase*I-J band (Gravius *et al.*, 1993). However, in some auxotrophic mutants (strains 605, 609 and 615; Gravius *et al.*, 1994b) the *Ase*I-J band is missing so it is possible that these strains have circular chromosomes.

Gravius *et al.* (1994b) reported that the integration of linear plasmids into the chromosome of *S. rimosus* can preserve at least one free plasmid end and a linear plasmid prime carrying the OTC-region was also observed. It was speculated that such events involved single cross-overs between linear plasmids and the linear chromosome. The location of the OTC-cluster near one end of the chromosome makes this more plausible and further results supporting this idea will be presented in a future paper.

S. ambofaciens and *S. coelicolor* A3(2) show a very similar location of genes on the physical map (Leblond *et al.*, 1996), which is not surprising given their relatively close taxonomic relationship. The recent localization of many genes to an ordered cosmid gene bank of *S. coelicolor* A3(2) (Redenbach *et al.*, 1996) provides precise locations, which can be compared with the *S. rimosus* results. Whereas the *oriC–dnaA* region is located almost exactly in the centre of the *S. coelicolor* A3(2) chromosome, it is asymmetrically placed in *S. rimosus* (from 34 to 44 % of the chromosome from the end depending on the exact location within the *Ase*I-C2 restriction fragment). The *recA* gene of *S. rimosus* is close to one end of the chromosome (550–850 kb away), whereas in *S. coelicolor* A3(2) it is 2 Mb away from the closer end. In *S. rimosus*, the *rrn* operons are in the central region of the chromosome, with no operon being within 2755 kb and 3095 kb of the respective ends. This contrasts with *S. coelicolor* A3(2) where the *rrnC* operon is about 1·4 Mb from one end and the *rrnE* operon about 2·1 Mb from the other end. The *rrnE* operon is close to the *recA* gene, whereas there is no *rrn* operon close to the *recA* gene in *S. rimosus* (Fig. 4). In *S. rimosus*, *attB*-pSAM2 (which is the gene for a tRNA^{pro}; Mazodier *et al.*, 1990) is about 1·8 Mb from the chromosome end whereas in *S. coelicolor* A3(2) it is near the centre of the chromosome. These comparisons suggest that the genetic organization of *S. rimosus* may differ significantly from that of *S. coelicolor* A3(2). This is seemingly in contradiction with results from the comparison of the genetic maps, which suggested similar organization (Pigac & Alačević, 1979). However, it must be remembered that the auxotrophic markers used were not characterized biochemically in either species, so it is not clear if every marker used was homologous between the two species. Resolution of this question awaits physical characterization of more markers in *S. rimosus*.

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