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Physical Map of the *Streptomyces lividans* 66 Genome and Comparison with That of the Related Strain *Streptomyces coelicolor* A3(2)

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A physical map of the chromosome of *Streptomyces lividans* 66 ZX7 was constructed by ordering the macrorestriction fragments generated from the genomic DNA with the restriction enzymes *AseI* and *DraI*. *AseI* and *DraI* linking cosmids (i.e., recombinant cosmids including either *AseI* or *DraI* sites) were isolated from a gene bank and used as hybridization probes against Southern transfers of pulsed-field gel electrophoresis (PFGE) restriction patterns. The *DraI* sites were precisely mapped by PFGE analyses of *AseI-DraI* double digests and hybridization with the *AseI* junctions. The 16 *AseI* and 7 *DraI* fragments were aligned as a single chromosome of about 8,000 kb. The data supported the interpretation that the chromosome is a linear structure. The related strain *Streptomyces coelicolor* A3(2) M145, recently mapped by H. Kieser, T. Kieser, and D. A. Hopwood (J. Bacteriol. 174:5496-5507, 1992), was compared with *S. lividans* at the level of the genomic structure by hybridizing the linking cosmids to Southern transfers of PFGE patterns. In spite of little apparent similarity in their restriction patterns, the comparison of the physical maps revealed a common structure with an identical ordering of the cosmid sequences. This conservation of the map order was further confirmed by assigning genetic markers (i.e., cloned genes and DNA elements relevant to the unstable region) to the *AseI* fragments.

The genus *Streptomyces* comprises gram-positive filamentous soil bacteria with a complex life cycle: spores germinate to form a branched, vegetative mycelium which produces an aerial mycelium and, subsequently, spores. The chromosome is present in multiple copies in the vegetative and aerial mycelia but is present as only a single copy in the spores. In addition to this morphological differentiation, the remarkable features of the *Streptomyces* genus include the extensive secondary metabolism directing the production of various molecules of industrial importance such as hydrolytic enzymes, enzyme inhibitors, and over 60% of naturally occurring antibiotics (3) and the high G+C content of the genomic DNA (70 to 74%) (8). The genome is also characterized by the presence of giant linear plasmids in numerous species (30), multiple insertion sequences (7), and repetitive sequences (43). A remarkable trait of *Streptomyces* species is the genomic plasticity associated with genetic instability, which is well characterized for *Streptomyces lividans* (11, 14, 38) as well as several other *Streptomyces* species (4, 31, 32). This plasticity consists of large deletions and extensive amplifications. Thus, mapping the genome would provide a new approach to the investigation of genetic instability at the level of the whole genome.

Since the advent of pulsed-field gel electrophoresis (PFGE), the physical mapping of many bacterial genomes has been reported (29 and references therein). As part of our study of genetic instability in *S. lividans* 66, we wanted to construct the physical map of the chromosome. However, most derivatives of *S. lividans* 66 are not amenable to PFGE because of a double-strand break activity during electrophoresis (47). We therefore used *S. lividans* ZX7, which carries a mutation that abolishes this activity.

Very recently, Kieser et al. (29) reported a combined genetic and physical map of the well-characterized strain *Streptomyces coelicolor* A3(2). They also showed that the *AseI* restriction pattern of *S. lividans* ZX7 was significantly different from that of *S. coelicolor* A3(2) (7 conserved sites of the 17 observed). *S. lividans* 66 and *S. coelicolor* A3(2) are very closely related and belong to taxonomic cluster 21 (45, 46). Thus, a comparison of the restriction maps of the two strains could provide information about the genomic organization divergence of species within a taxonomic group.

Here, we report the physical mapping of the *S. lividans* 66 ZX7 genome using the strategy of isolating clones carrying sites for restriction enzymes and subsequent hybridization to Southern transfers of PFGE restriction patterns. The map was constructed for two endonucleases, *AseI* and *DraI*. Comparisons of the genome structures and genetic organizations of the closely related strains *S. lividans* 66 ZX7 and *S. coelicolor* A3(2) M145 are presented.

MATERIALS AND METHODS

Bacterial strains and cosmids. The strain used in this study was *S. lividans* 66 ZX7 (29, 47) and was provided by Tobias Kieser. *S. coelicolor* M145 (25) was a gift of David Hopwood. Total DNA was isolated from *S. lividans*, cultured in YEME medium as described by Hopwood et al. (25). The gene bank was constructed in the cosmid Supercos1 (15) by using the Packagene kit (Promega, Madison, Wis.) and amplified in *Escherichia coli* Sure (Stratagene).

DNA preparation, restriction, and PFGE analyses. PFGE DNA was prepared by a method adapted from that of Häusler et al. (22). Mycelium was grown for 4 days in YEME medium (25). Cells were centrifuged and resuspended in SucTE (0.3 M sucrose, 25 mM Tris-HCl [pH 8.0], 25 mM EDTA). The optical density at 600 nm of the suspension was adjusted to 1.8 to 2.0, and the suspension was mixed with 2 volumes of molten 1.5% InCert agarose (FMC, Philadelphia,

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Pa.) to give a final agarose concentration of 1%. Agarose blocks were incubated at 37°C for 12 h in lysozyme solution (2 mg/ml in TE [10 mM Tris-HCl, 1 mM EDTA, pH 8.0]) and then treated twice for 24 h each time at 50°C with NDS (1% *N*-lauroylsarcosine, 2 mg of proteinase K [Boehringer GmbH, Mannheim, Germany] per ml, 0.5 M EDTA, pH 8.0). Proteinase K activity was then inhibited by rinsing the blocks twice for 4 h each time at 4°C in phenylmethylsulfonyl fluoride (40 µU/ml) (Sigma, St. Louis, Mo.). The blocks were stored at 4°C in TE.

For restriction analysis, slices of the agarose plugs containing the DNA were incubated in the buffer recommended by the supplier (*AseI*, *DraI*, and *HindIII* were purchased from Biolabs and *BfrI* was purchased from Boehringer) with 50 U of a restriction enzyme at 37°C for 12 h. In the double-digest experiments, the digest needing the higher salt concentration was performed first; then, the buffer was removed and the second digest was performed in the appropriate buffer.

All the PFGE experiments were performed in a contour-clamped homogeneous electric field system (10) supplied by Bio-Rad (Richmond, Calif.). In all experiments, the electrophoresis buffer was 50 mM Tris-borate buffer (pH 8.0)–0.1 mM EDTA (0.5× TBE) and the gels were 1% agarose. Pulse times were optimized with respect to the length of DNA to be separated. Lambda concatemeric DNA and *Saccharomyces cerevisiae* yeast chromosomes (Bio-Rad) were used as size standards for high-molecular-weight DNA fragments. Phage lambda digested with *HindIII* was embedded in 1.5% InCert agarose (FMC) and used as a size standard for low-molecular-weight DNA fragments in PFGE experiments.

The 30-kb *DraI* fragment of *S. lividans* was eluted from the gel, purified with phenol-chloroform extractions, and used as a hybridization probe for further colony hybridization experiments (39).

Hybridization experiments. Cosmid DNA was isolated by the alkaline lysis method (5).

Cosmid DNA and DNA fragments used as probes were labelled with digoxigenin-labelled dUTP (Boehringer). After electrophoresis, DNA fragments were transferred to a Hybond-N nylon membrane (Amersham) as described by Sambrook et al. (39). Southern analyses were carried out by using a nonradioactive DNA labelling and detection kit (Boehringer). Colony hybridizations were performed as described by Sambrook et al. (39).

Isolation of *AseI* DNA fragments for *BfrI* restriction digest analysis. *AseI* digests were electrophoresed in a 1% low-melting-point agarose (Sigma) gel by using a ramp of pulse times from 5 to 90 s over 24 h. After electrophoresis, but before ethidium bromide staining, the central lanes of the gel were removed and only the outer lanes were stained. After the restriction fragments to be analyzed were located, the appropriate region was excised from the unstained gel portion. After incubation in the *BfrI* digestion buffer for 6 h at 4°C, digestion with *BfrI* was performed in a fresh buffer.

RESULTS

PFGE resolution of restriction endonuclease digests of the wild-type DNA: genome size estimation of the *S. lividans* 66 genome. Because of the high G+C content of the *Streptomyces* DNA (70 to 74%), restriction enzymes with recognition sites containing only A and T nucleotides, such as *AseI* (ATTAAT) and *DraI* (TTTAAA), will usually generate rel-

TABLE 1. Sizes of the *AseI*, *DraI*, and *BfrI* restriction fragments and estimations of the size of the *S. lividans* ZX7 genome

<i>AseI</i>		<i>DraI</i>		<i>BfrI</i>	
Frag-ment	Size ^a (7,970)	Frag-ment	Size (7,980)	Frag-ment	Size (7,901)
A	2,600	I	2,600	1	1,050
B	975	II	2,500	2	900
C	710	III	1,700	3	650
D	615	IV	700	4	590
E ₁	525	V	300	5	470
E ₂	515	VI	150	6	390
E ₃	485	VII	30	7	360
F	355			8	330
G	270			9	300
H ₁	225			10	280
H ₂	225			11	245
I ₁	145			12	240
I ₂	145			13	220
J	70			14	220
K	25			15	205
L	15			16	195
				17	190
				18	160
				19	140
				20	110
				21	100
				22	90
				23	90
				24	82
				25	82
				26	80
				27	43
				28	33
				29	22
				30	18
				31	16

^a In kilobases. The total is given in parentheses.

atively few fragments and be suitable for PFGE experiments.

Resolution of all fragments requires optimal adjustment of the pulse time program. The *S. lividans* DNA genome has 16 *AseI* fragments, which vary in size from 2,600 to 15 kb (Table 1). A ramp of pulse times from 5 to 120 s over 24 h gave optimal separation of 9 of the 16 fragments (Fig. 1A1). A diffuse band of about 500 kb was revealed to be a triple band (E₁, E₂, and E₃ [525, 515, and 485 kb, respectively]) by using a ramp of pulse times from 50 to 55 s for 40 h (Fig. 1A2). In addition, the two bands H₁₋₂ (225 kb) and I₁₋₂ (145 kb) exhibited a relative intensity consistent with the presence of a double band. We suspected the presence of two comigrating restriction fragments, but attempts to separate them failed. We therefore excised the two bands from the gel and digested them further with *BfrI* (see Materials and Methods). The H₁₋₂ band gave rise to six *BfrI* fragments, of 185, 80, 65, 55, 25, and 20 kb (data not shown), whose sum of 430 kb corresponds to about double the size of the H₁₋₂ band. From these data, we concluded that H₁₋₂ consisted of two different fragments of the same size. This was supported by the mapping of an unstable region, which is subject to frequent deletions: a 225-kb *AseI* fragment was demonstrated to belong to the deletable region, but a fragment of this size was still visible in the *AseI* pattern of deletion mutants in this region generated by genetic instability (38). The I₁₋₂ band was not digested by *BfrI*, suggesting the absence of *BfrI* sites on both putative fragments constituting

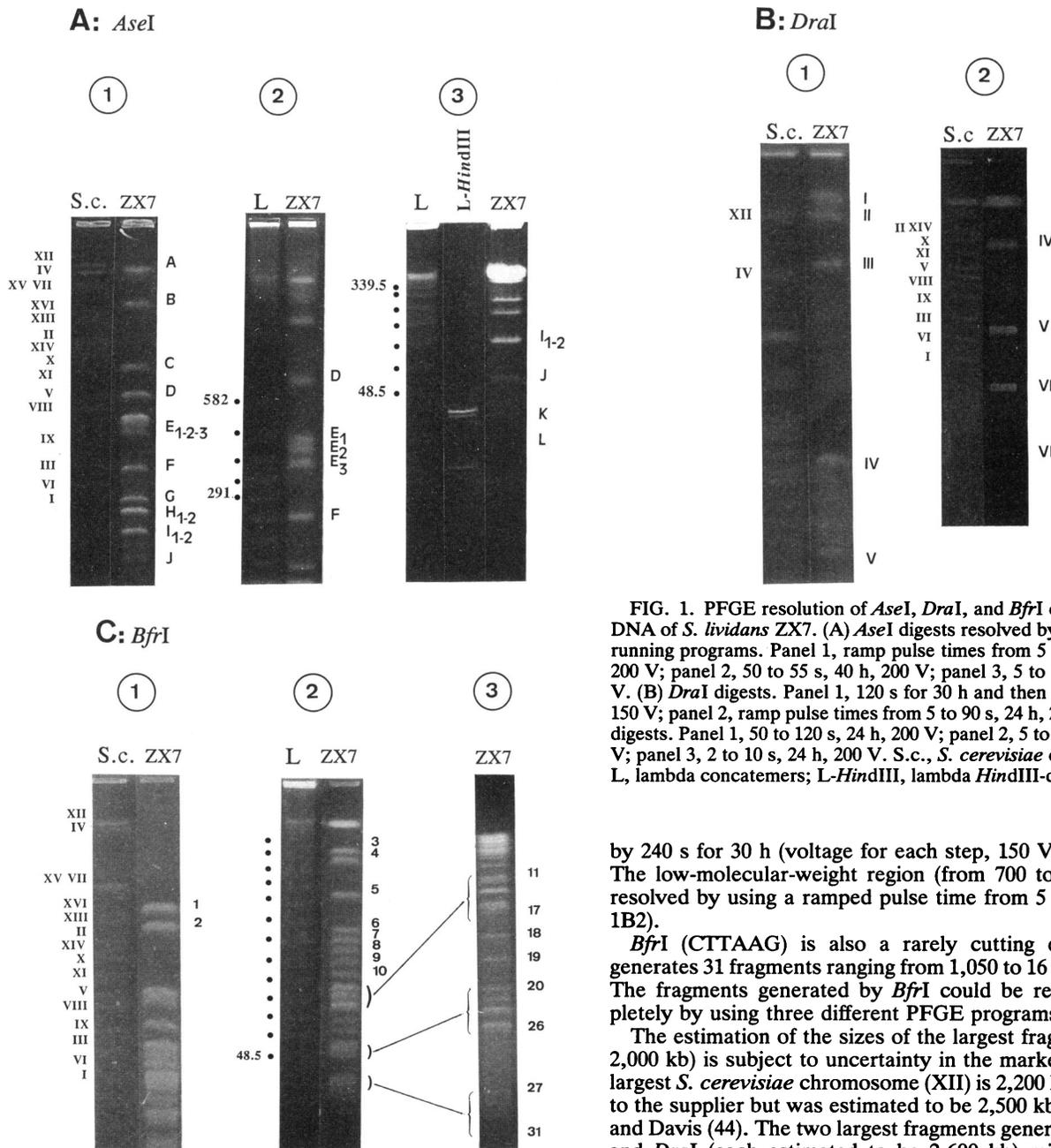


FIG. 1. PFGE resolution of *AseI*, *DraI*, and *BfrI* digests of total DNA of *S. lividans* ZX7. (A) *AseI* digests resolved by using several running programs. Panel 1, ramp pulse times from 5 to 120 s, 24 h, 200 V; panel 2, 50 to 55 s, 40 h, 200 V; panel 3, 5 to 30 s, 24 h, 200 V. (B) *DraI* digests. Panel 1, 120 s for 30 h and then 240 s for 30 h, 150 V; panel 2, ramp pulse times from 5 to 90 s, 24 h, 200 V. (C) *BfrI* digests. Panel 1, 50 to 120 s, 24 h, 200 V; panel 2, 5 to 90 s, 24 h, 200 V; panel 3, 2 to 10 s, 24 h, 200 V. S.c., *S. cerevisiae* chromosomes; L, lambda concatemers; L-HindIII, lambda *HindIII*-digested DNA.

by 240 s for 30 h (voltage for each step, 150 V) (Fig. 1B1). The low-molecular-weight region (from 700 to 30 kb) was resolved by using a ramped pulse time from 5 to 90 s (Fig. 1B2).

BfrI (CTTAAG) is also a rarely cutting enzyme that generates 31 fragments ranging from 1,050 to 16 kb (Table 1). The fragments generated by *BfrI* could be resolved completely by using three different PFGE programs (Fig. 1C).

The estimation of the sizes of the largest fragments (over 2,000 kb) is subject to uncertainty in the marker sizes. The largest *S. cerevisiae* chromosome (XII) is 2,200 kb according to the supplier but was estimated to be 2,500 kb by Vollrath and Davis (44). The two largest fragments generated by *AseI* and *DraI* (each estimated to be 2,600 kb) migrated more slowly than this chromosome when a pulse time optimal for the resolution of the high-molecular-weight DNA fragments was used (data not shown [*AseI*] and Fig. 1B1 [*DraI*]). In addition to this size marker problem, there could be a systematic error due to the G+C content effect (6).

The total genome size of *S. lividans* could be estimated by adding up the sizes of restriction fragments to give 7,970, 7,980, and 7,901 kb with *AseI*, *DraI*, and *BfrI*, respectively (Table 1). *BfrI* provided the most reliable estimation, since all 31 fragments could be estimated relative to λ DNA (G+C, around 50% [40]). This estimation is highly consistent with the *AseI* and *DraI* estimations, supporting the relative accuracy of the high-molecular-weight fragment size estimation.

Construction of the *AseI* and *DraI* physical maps. A gene bank was constructed from the *S. lividans* ZX7 strain in the cosmid Supercos1 by using *E. coli* Sure as the host (see

the I₁₋₂ band (data not shown). However, these two fragments could be distinguished on the basis of the *AseI* linking-clone analyses (see below).

The low-molecular-weight fragments were resolved by using ramped pulse times from 5 to 30 s over 24 h (Fig. 1A3). No fragment of less than 15 kb was observed with PFGE analysis or classical nonpulsed electrophoresis even after hybridization of a Southern blot of total DNA digested with *AseI* with labelled total DNA (data not shown).

DraI digested the *S. lividans* chromosome into seven fragments of sizes ranging from 2,600 to 30 kb (Table 1). Neither fragments under 30 kb nor double bands could be detected. High-molecular-weight fragments were separated by using a two-step running program: 120 s for 30 h followed

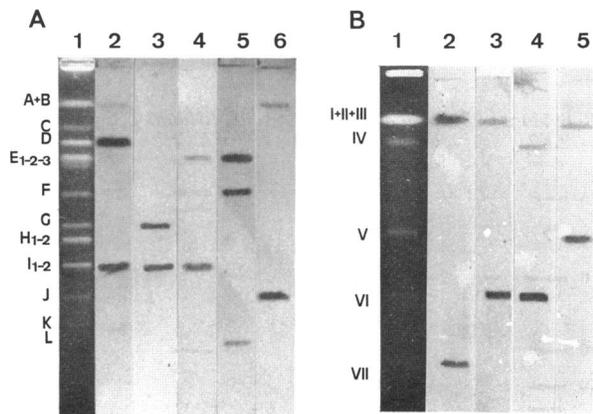


FIG. 2. Hybridization of linking clones to Southern blots of PFGE restriction patterns of *S. lividans* ZX7. (A) Hybridization of *AseI* linking clones to *AseI* PFGE Southern blots. Lane 1, *AseI* PFGE resolution; ramp pulse times from 5 to 90 s, 24 h, 200 V; lane 2, C-17; lane 3, C-107; lane 4, C-44; lane 5, C-6; lane 6, C-4. (B) Hybridization of *DraI* linking clones to *DraI* PFGE Southern blots. Lane 1, *DraI* PFGE resolution, 5 to 90 s, 24 h, 200 V; lane 2, C-68; lane 3, C-213; lane 4, C-234; lane 5, C-204.

Materials and Methods). The cosmid vector includes two *AseI* and five *DraI* sites, enabling easy detection of *AseI* and *DraI* sites in the cloned DNA. The vector DNA yields a small restriction fragment(s), and the cloned DNA is contained in a high-molecular-weight DNA fragment (>40 kb). The presence of a site for *AseI* or *DraI* was detected when two or three fragments (presence of one or two sites, respectively) were present instead of the single high-molecular-weight fragment. We screened about 2,000 cosmids (corresponding to two to three genomes of cloned DNA) for the presence of *AseI* sites. Forty-seven cosmids carrying *AseI* sites in the inserts were isolated. These cosmids were assigned to groups by cross-hybridization experiments (not shown). One representative cosmid from each group was selected for further work and designated with the letter "C" and a number. Two cosmids (C-6 and C-27) were shown to include two *AseI* restriction sites; i.e., each includes an entire *AseI* fragment (L [15 kb] for C-6 and K [25 kb] for C-27). Each representative cosmid was labelled and hybridized to Southern blots of *AseI* digests of *S. lividans*. Figure 2 shows some examples of the hybridization patterns. For each cosmid including one *AseI* site, two fragments were revealed, demonstrating the alignment of fragments. Thus, C-17 proved to align fragments D and I₂, C-107 aligned fragments G and I₁, C-44 aligned fragments I₁ and E₁, and C-4 aligned fragments A and J (Fig. 2A, lanes 2 to 4 and 6, respectively). Faint signals corresponding to weak homologies could be detected when long detection times were used (e.g., C-17). When two sites were present, the order of the three fragments was obvious (e.g., C-6; Fig. 2A, lane 5). Thus, only 13 cosmids were necessary to map all of the 16 *AseI* fragments generated from the genome to a linear map (Fig. 3, outer circle).

With the same approach, *DraI* linking clones could be isolated by analyzing 1,000 recombinant cosmids (18 *DraI* site-containing cosmids were isolated). The linking clone spanning fragments IV and V is missing, but the other five sites were present in the five linking clones. Figure 2B shows the patterns of hybridization of *DraI* linking clones to Southern blots of *DraI* PFGE patterns of the wild-type

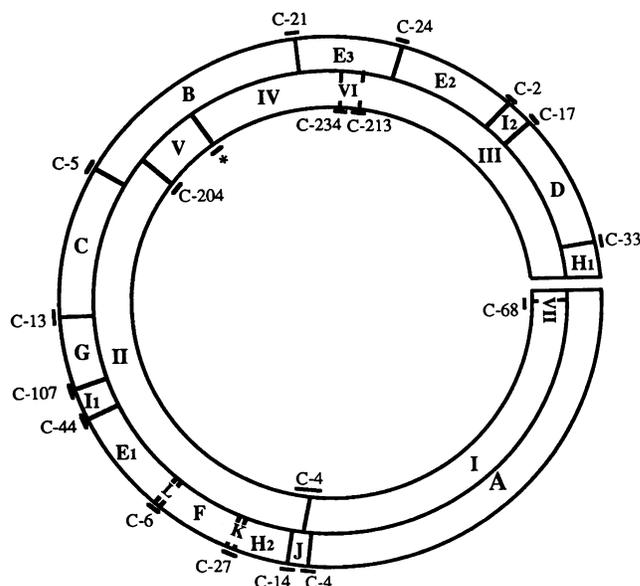


FIG. 3. Combined physical *AseI* (outer circle) and *DraI* (inner circle) maps of *S. lividans* ZX7. The relative sizes are derived from the sizes in Table 1. All sites except the site marked with an asterisk were cloned. The linking clones are designated with the letter "C" and the number of the cosmid.

strain. The alignment deduced from these experiments is presented in the inner circle in Fig. 3.

Positioning of the *DraI* map relative to the *AseI* map. The *DraI* sites were localized more precisely with the help of PFGE analysis of *AseI*-*DraI* double digests of *S. lividans*. The *DraI* sites were first assigned to the *AseI* fragments by noting the missing fragments in the double digest compared with the single *AseI* digest (Fig. 4). The localization of a *DraI* site relative to the *AseI* alignment was made easier by the finding of a *DraI* site in the *AseI* linking cosmid C-4. The

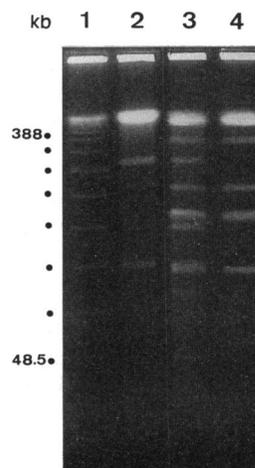


FIG. 4. PFGE analysis of single and double digests of the *S. lividans* ZX7 genome digested with *AseI* and *DraI*. Lane 1, lambda concatemers; lane 2, *DraI* single digest; lane 3, *AseI*-*DraI* double digest; lane 4, *AseI* single digest. Running conditions were 5 to 30 s, 24 h, 200 V. The smallest band in the lambda concatemer ladder is a 48.5-kb monomer.

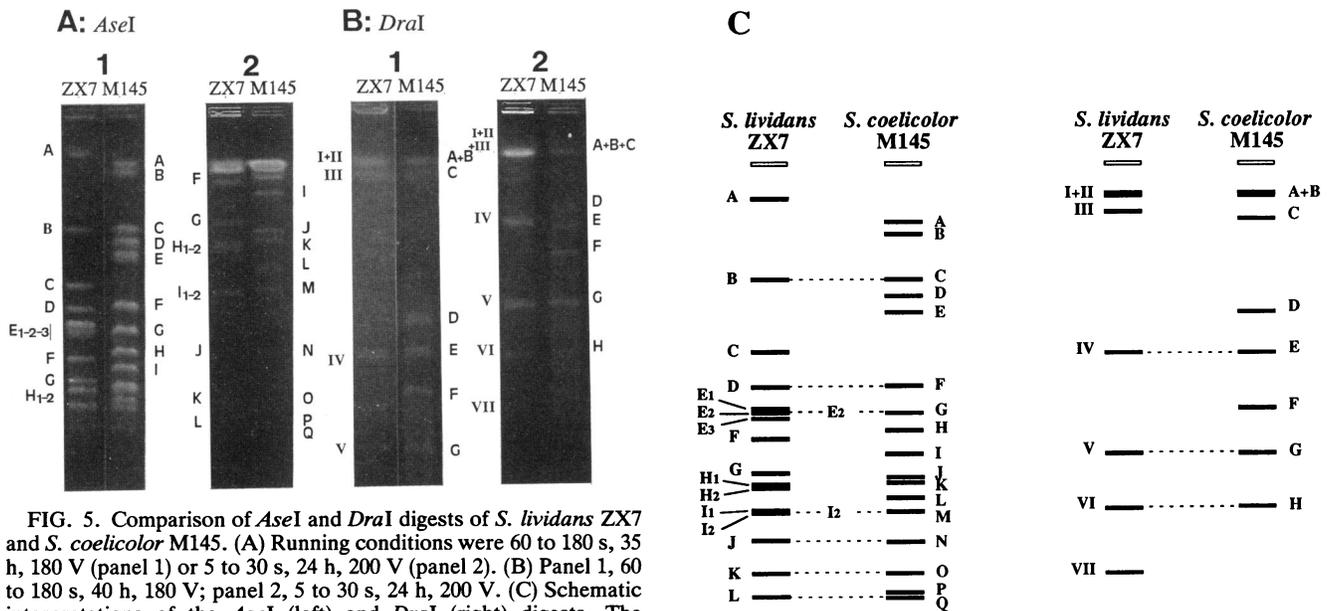


FIG. 5. Comparison of *AseI* and *DraI* digests of *S. lividans* ZX7 and *S. coelicolor* M145. (A) Running conditions were 60 to 180 s, 35 h, 180 V (panel 1) or 5 to 30 s, 24 h, 200 V (panel 2). (B) Panel 1, 60 to 180 s, 40 h, 180 V; panel 2, 5 to 30 s, 24 h, 200 V. (C) Schematic interpretations of the *AseI* (left) and *DraI* (right) digests. The comigrating fragments are indicated (dashes).

hybridization of C-4 to an *AseI*-*DraI* digest revealed *AseI* flanking fragment A and a new 30-kb fragment, allowing the localization of the *DraI* site 30 kb away from the *AseI* site. As confirmation, C-14, overlapping the *AseI* J and H₂ fragments, revealed the H₂ flanking fragment and a new 50-kb fragment (not shown). In the same way, *AseI* fragment J disappeared in the double *AseI*-*DraI* digest, demonstrating the presence of a *DraI* site in this fragment (Fig. 5A). This was expected from the *DraI* restriction analysis of cosmid C-4, overlapping *AseI* fragments J and A.

The four *DraI* sites (linking fragments II and V, V and IV, IV and VI, and VI and III) could be localized and mapped in *AseI* fragments B and E₃ by using the same approach. B was restricted in three fragments, 200, 500, and 300 kb, with the last fragment being *DraI* fragment V. In the same way, E₃ generated three fragments, 120, 200, and 150 kb, with the last one being *DraI* fragment VI. Thus, *DraI* fragment IV (700 kb) was shown to overlap B and E₃.

Although C-68 contained only one *DraI* site, it hybridized to the three *DraI* fragments III, VII, and I (data not shown). Furthermore, despite lacking an *AseI* site, it hybridized to both *AseI* fragment H₁ and *AseI* fragment A, which lie at the ends of the map (Fig. 3). The location of C-68 was established by chromosome walking, and it was possible to isolate an overlapping clone that hybridized solely to *DraI* fragment I and *AseI* fragment A. The extra hybridization signals are, thus, due to repeated sequences in clone C-68, which are also present in *DraI* fragment III and *AseI* fragment H₁. We had initially expected that the map would be circular, so that it would be possible to isolate linking clones carrying an *AseI* site (linking fragments A and H₁) and a *DraI* site (linking fragments VII and III). These sites must be very close together, as no fragment corresponding to the region between these sites was seen in the *DraI*-*AseI* double digest. As the sites had not been isolated in the 3,000 cosmids screened for *AseI* or *DraI* sites (see above), the 30-kb *DraI* fragment was used as a hybridization probe against a further 4,000 clones from the cosmid gene bank. None of the 44 positive clones carried an *AseI* site. Ten of the cosmids

carried a *DraI* site, but this site corresponded to that already cloned in C-68 and not to a site linking fragments III and VII.

Comparison of the genome structures of closely related strains *S. lividans* 66 and *S. coelicolor* A3(2). Recently, a combined genetic and physical map of the well-characterized strain *S. coelicolor* A3(2) was reported (29). *S. lividans* 66 and *S. coelicolor* A3(2) are closely related and belong to the same taxonomic group (cluster 21 [45, 46]). In order to compare the basic genomic structures and genetic organizations of these two organisms, we used several approaches. First, we analyzed the comigrating fragments of *AseI* and *DraI* digests in PFGE (Fig. 5A and B). The comigrating fragments are shown in the schematic interpretation in Fig. 5C. In agreement with Kieser et al. (29), only seven *AseI* fragments (B, D, E₂, I₂, J, K, and L) seemed to be conserved. With *DraI*, three of the seven fragments (IV, V, and VI) appeared to be conserved. The comigration of the largest *DraI* fragments does not prove conservation of these fragments because of the poor resolution of fragments in this size range.

There are significant differences between the size estimations of the low-molecular-weight fragments (10 to 200 kb) and those reported by Kieser et al. (29). However, restriction analysis of an *AseI* digest of *S. lividans* DNA in classical electrophoresis confirmed the sizes of the smallest two fragments (K and L) (not shown). It seems that the size estimation can depend on the PFGE system used.

We hybridized the *AseI* linking clones isolated from *S. lividans* 66 to *AseI* digests of *S. coelicolor* M145 resolved with PFGE. All of these recombinant cosmids revealed DNA fragments in *S. coelicolor* (Table 2). Most of them revealed two fragments, as in *S. lividans*, and could correspond to linking clones in *S. coelicolor*. C-107 and C-6 hybridized to only one fragment in *S. coelicolor* instead of two and three, respectively, in *S. lividans*. Moreover, C-44 hybridized to the three fragments H, Q, and D in *S. coelicolor* while only two, I₁ and E₁, were revealed in *S. lividans*. In contrast, C-5, which links fragments B and C in *S. lividans*, hybridized only to the two fragments C and E in *S. coelicolor* but not the intervening Q fragment. The map

TABLE 2. Hybridization of *AseI* linking clones to the *S. lividans* ZX7 and *S. coelicolor* M145 DNAs

Cosmid	<i>AseI</i> fragment(s)	
	<i>S. lividans</i> ZX7	<i>S. coelicolor</i> M145
C-33	H ₁ , D	J, F
C-17	D, I ₂	F, M
C-2	I ₂ , E ₂	M, G
C-24	E ₂ , E ₃	G, L
C-21	E ₃ , B	I, C
C-5	B, C	C, E
C-13	C, G	E, H
C-107	G, I ₁	H
C-44	I ₁ , E ₁	H, Q, D
C-6	E ₁ , L, F	D
C-27	F, K, H ₂	D, O, K
C-14	H ₂ , J	K, N
C-4	J, A	N, P

position seems to be correct, as Kieser et al. (29) reported the cloning of the *AseI* linking fragment between C and Q (clone ML10) and there must be a sequence rearrangement between the two organisms in this region. C-4 hybridized to fragments A and J in *S. lividans* and N and P in *S. coelicolor*, suggesting the loss of an *AseI* site in this area in *S. lividans*. The two additional sites between I and L and between A and B in *S. coelicolor* can be explained by the insertion of two IS117 elements carrying an *AseI* site (23, 29). IS117 is not present in *S. lividans*, and the fusion of fragments I (290 kb) and L (140 kb) of *S. coelicolor* A3(2) could correspond to E₃ (485 kb) in *S. lividans*. The same explanation is likely for fragments A (1,500 kb) and B (1,300 kb), giving rise to fragment A (2,600 kb) in *S. lividans*. Taking into account some additional rearrangements detected in this region with the *DraI* analysis (absence of the 30-kb fragment in *S. coelicolor*, for example), these data supported the size estimation of the largest *AseI* fragment in *S. lividans* as about 2,600 kb (2,600 ≈ 1,500 + 1,300). The alignment of the two genomic maps with *AseI* is summarized in Fig. 6A.

A similar analysis was performed with the *DraI* linking clones of *S. lividans* on *DraI* digests of *S. coelicolor*. The sites between fragments II and V, IV and VI, VI and III, and I and II in *S. lividans* were recovered in *S. coelicolor* (not shown). C-68 (linking fragments VII and I in *S. lividans*) hybridized only with *DraI* fragment A of *S. coelicolor*. The comparison of the two *DraI* maps is presented in Fig. 6B.

The genetic organizations of the two related strains were further compared by hybridizing cloned marker genes to Southern blots of *AseI* patterns of *S. lividans* and *S. coelicolor* (Table 3). Each of the markers except the rDNA probe, which gave four *AseI* fragments in *S. lividans* (C, E₁, E₂, and E₃) and five (B, D, E, G, and I) in *S. coelicolor*, hybridized to one DNA fragment in each strain. All probes except *dnaA* (19) could be localized unambiguously to an *S. lividans* fragment. However, the *dnaA* gene hybridized to the double band I_{1,2}. We had previously shown that this double band did not contain any *BfrI* site. We therefore hybridized cosmids C-107 and C-17, flanking the two *AseI* fragments I₁ and I₂ (Fig. 3A, outer circle), to identify *BfrI* fragments including these *AseI* fragments. C-17 and C-107 revealed two different *BfrI* fragments, of 650 and 470 kb, respectively. The *dnaA* DNA hybridized the 470-kb *BfrI* fragment, demonstrating unambiguously that the *dnaA* gene was located on *AseI* fragment I₁. This marker was localized in fragment H of *S. coelicolor* M145 (29).

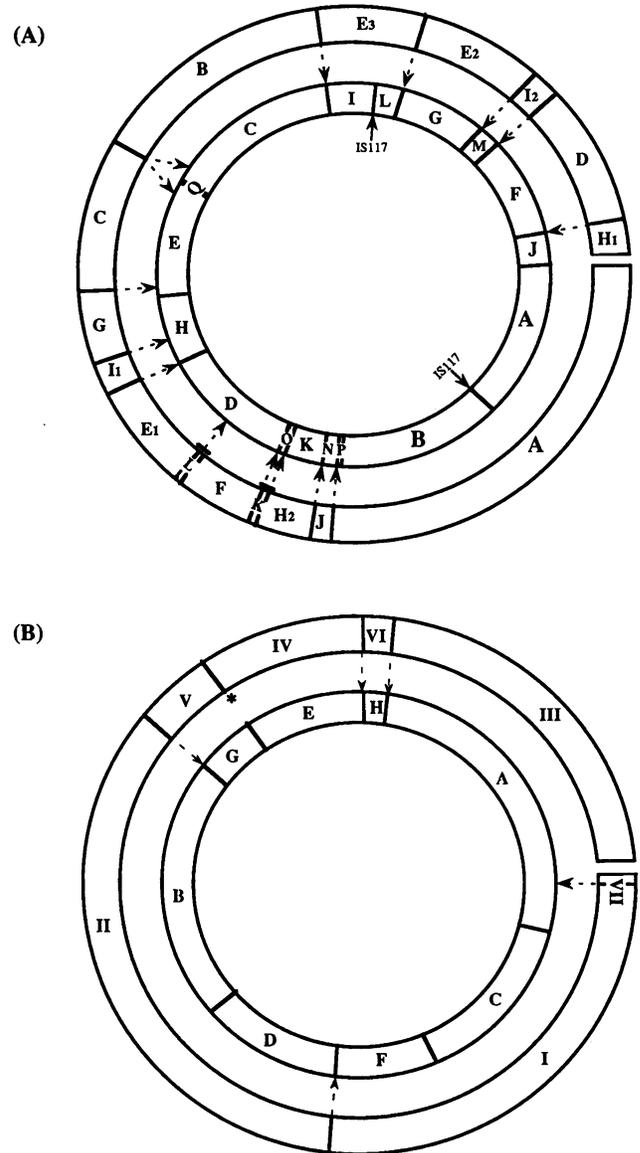


FIG. 6. Comparison of the *AseI* (A) and *DraI* (B) physical maps of *S. lividans* ZX7 (outer circles) and *S. coelicolor* M145 (inner circles). Fragments in *S. coelicolor* revealed by the linking clones of *S. lividans* (see Table 2 and text) are indicated (arrows). The *DraI* site marked with an asterisk was not cloned. The positions of the IS117 sequences on the *S. coelicolor* M145 chromosome are also shown. The *S. coelicolor* maps are according to reference 29.

DISCUSSION

***S. lividans* 66 genome mapping.** A complete restriction map for the rare cutting restriction enzymes *AseI* and *DraI* was constructed for *S. lividans* 66 ZX7 by ordering the large restriction fragments resolved in PFGE experiments; 16 *AseI* and 7 *DraI* fragments could be ordered on the genome. The data were consistent with a linear genome of about 8,000 kb. The chosen mapping method had three main advantages: it allowed accurate mapping of the restriction sites, it avoided problems in the detection and localization of small restriction fragments, and it avoided the problems caused by multiple bands. The *S. lividans* 66 genetic map containing

TABLE 3. Genetic markers and DNA fragments localized on the *S. lividans* ZX7 and *S. coelicolor* M145 *AseI* physical maps

Genetic marker	Origin	<i>AseI</i> fragment(s)		Reference
		<i>S. lividans</i> ZX7	<i>S. coelicolor</i> M145	
<i>actIII</i>	<i>S. coelicolor</i>	J	N ^a	20
<i>argG</i>	<i>S. lividans</i> , <i>S. coelicolor</i>	A	A ^a	27
AUD type I	<i>S. lividans</i>	A	A	1
AUD type II	<i>S. lividans</i>	D	F	38
<i>bldA</i>	<i>S. coelicolor</i>	C	E ^a	36
<i>bldB</i>	<i>S. coelicolor</i>	A	B ^a	21
<i>cysCD</i>	<i>S. coelicolor</i>	A	B ^a	34
<i>dnaA</i>	<i>S. lividans</i>	I ₁	H ^a	19
<i>galK</i>	<i>S. coelicolor</i>	C	E	28
<i>gyl</i>	<i>S. coelicolor</i>	E ₃	I ^a	41
<i>leuA</i>	<i>Streptomyces rochei</i>	B	C	24
rDNA	<i>Streptomyces rimosus</i>	C, E ₁ , E ₂ , E ₃	B, D, E, G, I	37
<i>redBFE</i>	<i>S. coelicolor</i>	A	B ^a	16
<i>whiB</i>	<i>S. coelicolor</i>	C	E ^a	13
<i>whiE</i>	<i>S. coelicolor</i>	A	B ^a	12
<i>whiG</i>	<i>S. coelicolor</i>	A	B ^a	35

^a From reference 29.

only 10 markers (26) could not be compared with the physical map because of the lack of cloned markers.

Although the data were also consistent with a circular map, we were unable to clone the *AseI* site linking fragments H₁ and A or the *DraI* site linking fragments III and VII despite screening more than 7,000 cosmid clones. These two postulated sites would be very close together, as no extra fragment was observed in *DraI*-*AseI* double digests. Clone C-68 hybridized to the *AseI* and *DraI* fragments on both sides of this gap. Further analyses of cosmids from this region (38) show that the gap is flanked by large inverted repeats. The suggestion that the chromosome is linear, as has been shown for *Borrelia burgdorferi* (18), is strongly supported by work from the laboratory of C. Chen (9) that suggests that the linear plasmid SLP2 has integrated in this region to generate a linear chromosome.

Comparison of *S. lividans* 66 and *S. coelicolor* M145. Recently, the genome of *S. coelicolor* A3(2) was reported as a circular chromosome of about 8,000 kb (29). Our estimation and recent estimations of the genome size of *Streptomyces ambofaciens* isolates using PFGE (33) are consistent with this value.

S. lividans 66 and *S. coelicolor* A3(2) are closely related strains (45, 46), and *S. lividans* 66 ZX7 (29, 47) and *S. coelicolor* M145 (25), which are derived from these parental strains, were expected to share common structural and genetic organization traits.

Thus, we undertook a structural comparison of the two genomes by hybridizing the *AseI* and *DraI* linking clones of *S. lividans* to *S. coelicolor*. These experiments revealed very similar organizations, despite considerable differences in the restriction patterns with *AseI* (as already noted in reference 29) and *DraI*. The majority of the *AseI* (10 of 15) and *DraI* (5 of 6) sites were present in *S. coelicolor*. The remaining clones from *S. lividans* hybridized to fragments in *S. coelicolor* A3(2) expected to be hybridized from alignment of the two maps (Fig. 6). The only exception was the hybridization of clone C-44 (linking *AseI* fragments I₁ and E₁) to *AseI* fragment Q of *S. coelicolor*. Two of the additional *AseI* sites can be accounted for by the presence of IS117 in *S. coelicolor* (23, 29). The unstable region (38) consisting of a 1,000-kb region in *S. lividans* (*AseI* fragments A, H1, and D or *DraI* fragments I, VII and III) was recovered for the most

part in *S. coelicolor*. Nevertheless, the missing *DraI* fragment VII in *S. coelicolor* revealed a rearrangement in this region. These data corroborate the close relationship of the two strains deduced from hybridizations of randomly chosen cosmid clones from *S. coelicolor* A3(2) to *S. lividans* 1326 (the original *S. lividans* isolate). Indeed, more than 70% of the cosmids used (statistically spanning about half of the genome) cross-hybridized with *S. lividans* DNA (17).

We also compared *S. lividans* and *S. coelicolor* by localizing genetic markers on the two genomes (Table 3). We used markers already localized on the genetic map of *S. coelicolor* and investigated the presence of the unstable region characterized in *S. lividans* on the *S. coelicolor* genome. The unstable region corresponded to the 3 o'clock silent arc reported for *S. coelicolor* (29). The rDNA probe hybridized to four *AseI* fragments (Table 3) in *S. lividans*, demonstrating a relative dispersion of the clusters of genes coding for rRNA in the genome since six operons were identified in *S. lividans* (42). Five hybridizing fragments were seen in *S. coelicolor* (Table 3), which is reported to also have six copies of the ribosomal operon (2); there is hybridization to the *S. coelicolor* *AseI* fragment B but not to the corresponding *S. lividans* *AseI* fragment A. All of the other genetic markers used gave indistinguishable map positions in the two strains and, thus, confirmed the similarities deduced from the structural analysis.

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