JOURNAL OF BACTERIOLOGY, Jan. 1989, p. 16–23
0021-9193/89/010016-08$02.00/0
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Vol. 171, No. 1

Site-Specific Integration in *Streptomyces ambofaciens*: Localization of Integration Functions in *S. ambofaciens* Plasmid pSAM2

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Received 11 July 1988/Accepted 6 October 1988

In *Streptomyces ambofaciens* ATCC 15154, an 11.1-kilobase element, pSAM2, exists as a single integrated copy in the chromosome. In *S. ambofaciens* 3212 (a derivative of ATCC 15154), pSAM2 exists as a free, circular plasmid as well as an integrated element. *BclI* fragments from the free form of pSAM2 were cloned into an *Escherichia coli* plasmid vector. By using gene transplacement methods, the chromosomally integrated form of pSAM2 was marked with a gene coding for apramycin resistance. This enabled us to isolate both a segregant that had lost the integrated pSAM2 element and a cosmid clone containing integrated pSAM2 along with the flanking chromosomal sequences. One of the *BclI* fragments derived from free pSAM2 was shown to contain all the plasmid-specified information required to direct site-specific recombination in a derivative of *S. ambofaciens* lacking the resident pSAM2 element as well as in a number of other *Streptomyces* strains. The attachment sites used by the plasmid and the chromosome in site-specific recombination and the junctions created after integration were cloned and sequenced. Certain structural features in common with other integrating elements in actinomycetes were noted.

Streptomyces spp. contain a number of self-transmissable elements capable of site-specific excision, integration, and replication (3, 4, 7, 16, 26–30, 37); similar elements have also been reported in *Nocardia* (22, 25) and *Saccharopolyspora* (5) spp. These plasmid elements have served as sources of cloning vectors (SLP1, pIJ110, and pIJ408), as systems to study site-specific recombination in actinomycetes, as sources of promoter elements, and as sources of regulated gene systems (5, 15, 22, 27, 29). Some plasmid elements are found as free structures only when plasmid functions required for integration have been deleted (3, 26, 29), while others (e.g., pSAM2, pSG1, and pMEA100) can exist in both states with no obvious loss of their ability to integrate (7, 22, 25, 30, 37). Two different loci (*intA* and *intB*) required for integration yet separate from *attP* have been identified in SLP1 (29). When *intA* is present in *trans*, it allows an integration-defective derivative of SLP1 to integrate in *Streptomyces lividans* (29).

S. ambofaciens ATCC 15154 contains an 11.1-kilobase (kb) integrated element, pSAM2^{int} (30). In *S. ambofaciens* 3212 (30), pSAM2 exists as a free plasmid as well as a chromosomally integrated element. pSAM2 carries conjugal functions and can elicit the lethal zygosis (*ltz*) phenotype (30). It also specifies plasmid functions allowing it to integrate in a site-specific fashion in *Streptomyces* strains (4, 30, 37). We are interested in characterizing both the sites and the gene(s) required to direct pSAM2 integration.

In this paper, we describe the isolation of a strain of *S. ambofaciens* ATCC 15154 which has been cured of its resident pSAM2 element. We show that all the plasmid-specified information required for integration is carried on a 2.5-kb *BclI-BamHI* fragment cloned from free pSAM2. Plasmids carrying the above DNA fragment integrate by an apparently site-specific mechanism into a variety of different *Streptomyces* strains. The sequences of the plasmid and *S. ambofaciens att* sites have been determined, as well as the sequence of the left and right pSAM2-chromosome junctions. We have identified regions of limited similarity among pSAM2, SLP1, and pMEA100 *att* sites.

MATERIALS AND METHODS

Strains. *S. ambofaciens* ATCC 15154 (34), *S. griseofuscus* C581 (ATCC 23916) (20), *S. toyocaensis* MJ16 (unpublished), *S. lipmanii* PM87 (R. H. Baltz and P. Matsushima, manuscript in preparation), and the *Escherichia coli* K-12 strains DH1 (13) and JM109 (40) were from the Lilly Culture Collection. *S. ambofaciens* 3212 (30) and *S. lividans* TK64 (17) were obtained from D. A. Hopwood, John Innes Institute. Shuttle vectors pHJL302 (21) and pHJL401 (20) were obtained from C. Hershberger. Free pSAM2 was isolated from *S. ambofaciens* 3212. The *E. coli* apramycin resistance gene [*aac(3)IV*, Am^r gene] (18, 31) was obtained from plasmid pKC418 (unpublished).

Culture conditions and genetic manipulations. TY broth and TY agar (23) were used for growing *E. coli*. TS broth and TS agar (2) were used for growing *Streptomyces* strains. Modified R2 bottom agar and top agar (2, 24) were used for *Streptomyces* transformations. Growth and plasmid transformations of *E. coli* were done as described in Maniatis et al. (23). *Streptomyces* culture conditions followed procedures described in Hopwood et al. (15). *Streptomyces* strains were protoplasted and transformed as described by Larson and Hershberger (20) and Thompson et al. (38). In some cases protoplasts were frozen and used for transformation as described by Rao et al. (32). Selection for antibiotic resistance was done on TY, TS, or modified R2 agar supplemented with apramycin (100 μg/ml for *E. coli*, 50 μg/ml for *Streptomyces* strains), ampicillin (100 μg/ml for *E. coli*), or thiostrepton (50 μg/ml for *Streptomyces* strains).

Chemicals and enzymes. Ampicillin was obtained from Sigma Chemical Company; apramycin was a gift from K. Merkel of Eli Lilly and Company; thiostrepton was a gift from S. J. Lucania of E. R. Squibb and Sons. Restriction enzymes and T4 DNA ligase were obtained from New England Biolabs. Lysozyme and RNase A were obtained from Sigma Chemical Company.

Preparation of DNA. Plasmid DNA was prepared from *E.*

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coli and *Streptomyces* strains either by $CsCl_2$ centrifugation (23) or by a modification of other methods (19, 32). Total chromosomal DNA was prepared from *Streptomyces* strains as described by Rao et al. (32). For smaller amounts, total DNA was routinely prepared by the method of Hopwood et al. (15).

Plasmid constructions. A *Nar*I deletion of plasmid pHJL302 (21) (a shuttle vector containing the pUC18 *lac* region), removes 1,240 base pairs (bp) of SCP2* DNA. The resulting plasmid, pKC527, retains only 220 bp of SCP2* DNA and is unable to transform *Streptomyces* strains. pKC527 retains an ampicillin resistance (Ap^r) gene and a thiostrepton resistance (Th^r) gene.

DNA containing integrated pSAM2 which had been marked with the Am^r gene as well as flanking sequences from the *S. ambofaciens* chromosome was cloned in an *E. coli* cosmid vector, pKC608 (unpublished). One resulting cosmid, pKC623, was chosen for further analysis.

Southern hybridizations and plaque and colony hybridizations. Colony and plaque hybridizations were performed with the Colony/Plaque Screen (New England Nuclear) essentially as described by the manufacturer. Southern hybridizations were performed by the alkaline blot method described by Reed and Mann (33). Filters were washed four times at 60°C in 0.1× SSPE (23)–0.1% sodium dodecyl sulfate.

Probes were prepared from plasmids by nick translation with a kit supplied by Bethesda Research Laboratories (35) and from gel-purified fragments by the random primed method (11, 12) with a kit supplied by Boehringer Mannheim.

Sequence determination and analysis. DNA fragments to be sequenced were cloned into either M13mp18 or M13mp19 (40), and the DNA sequence was determined on both strands by the method of Sanger et al. (36). The nomenclature used for attachment sites is adapted from that used for lambda and SLP1 (28). The chromosomal DNA fragment containing the *attB* site was isolated from a cosmid library of strain BES2087.

Sequence analysis was performed with the programs in the University of Wisconsin Genetics Computer Group Sequence Analysis Software Package (10).

RESULTS

Subcloning of pSAM2. To localize the integration function(s) specified by pSAM2, *Bcl*I fragments from the free form of the plasmid were subcloned into *Bam*HI-cut pKC527, generating three plasmids: pKC541 [*Bcl*I(10)-*Bcl*I(3); Fig. 1], pKC542 [*Bcl*I(4)-*Bcl*I(10)], and pKC543 [*Bcl*I(3)-*Bcl*I(4)]. All three plasmids were able to transform *S. ambofaciens* ATCC 15154 to thiostrepton resistance at a low frequency (50 to 400/μg), whereas pKC527 gave no transformants in these experiments. Under similar experimental conditions, pHJL401 (a plasmid that replicates in *Streptomyces* strains [20]) gave about 1.5×10^4 transformantants per μg of DNA. Southern analysis revealed that all three plasmids had integrated into the resident pSAM2 in the *S. ambofaciens* chromosome. Transformation by pKC542 and pKC543 depended on DNA sequence homology; no transformants were obtained when a strain cured of $pSAM2^{int}$ was transformed with either pKC542 or pKC543. pKC541 transformed *S. ambofaciens* ATCC 15154 and cured strains at similar frequencies.

Introduction of the *aac(3)IV* gene into $pSAM2^{int}$. To help in the isolation of a pSAM2-free segregant of *S. ambofaciens*

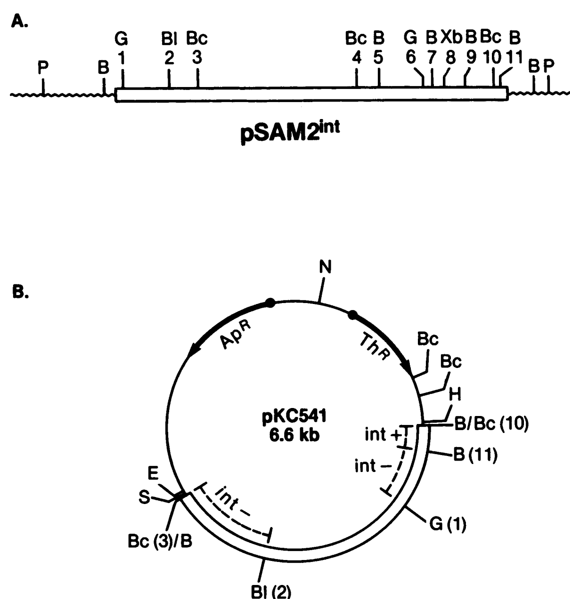

FIG. 1. (A) Restriction site map of pSAM2 integrated in *S. ambofaciens* ATCC 15154. The locations of some sites as described by Pernodet et al. (30) are shown. Abbreviations: B, *Bam*HI; Bl, *Bal*I, Bc, *Bcl*I; G, *Bgl*II; P, *Pst*I, Xb, *Xba*I. Open box, pSAM2 sequences; wavy line, chromosomal sequences. (B) Restriction site map of plasmid pKC541. Not all sites present in the *lac* α multiple cloning site have been shown (sites for *Sph*I, *Pst*I, *Sal*I, and *Xba*I lie between H and B/Bc [10], and sites for *Sac*I and *Kpn*I lie between E and S). Abbreviations are as above, plus: E, *Eco*RI; H, *Hin*dIII; S, *Sma*I. Solid line, pKC527; solid arrows, structural gene with its promoter; broken line, deleted region (int^-, plasmid cannot integrate when the indicated region is deleted; int^+, plasmid can integrate when the indicated region is deleted).

ATCC 15154 and to aid in the cloning of the integrated structure, a selectable marker was introduced internal to pSAM2 as follows. A *Bam*HI fragment carrying the *aac(3)IV* gene (Am^r) (18, 31) was cloned into the unique *Bgl*II site of pKC542 (Fig. 1A), and this plasmid (pKC555) was transformed into *S. ambofaciens* ATCC 15154, selecting for Am^r. Transformants were obtained at a frequency of about 10^2/μg. The transformants were then screened for their resistance to thiostrepton (the unselected marker). If the plasmid integrates by a single crossover, the transformants will be Am^r Th^r. However, if the plasmid integrates by a double crossover, the transformants will be Am^r Th^s. About 7% of the original Am^r transformants were Th^s. Southern analysis of transformants scored as having resulted from single and double crossovers confirmed the structures in the chromosome (Fig. 2).

Isolation of *S. ambofaciens* lacking $pSAM2^{int}$. Insertion of the Am^r gene into $pSAM2^{int}$ provided us with an easy method to screen for the loss of $pSAM2^{int}$. Strain BES1958 (Fig. 2; a double-crossover mutant) was allowed to sporulate, and the colonies generated from the spores were replicated onto TS plates containing apramycin. Am^s colonies were detected at a frequency of about 0.1% of the total number replicated. One such colony, BES2087, was characterized further. Southern analysis confirmed that pSAM2 was no longer present in the chromosome (Fig. 3). When pKC623 (a cosmid carrying $pSAM2^{int}$ and flanking chromosomal sequences) was used as the probe, bands hybridizing to pSAM2 junction fragments and to the Am^r gene disappeared and a new band of the expected size appeared.

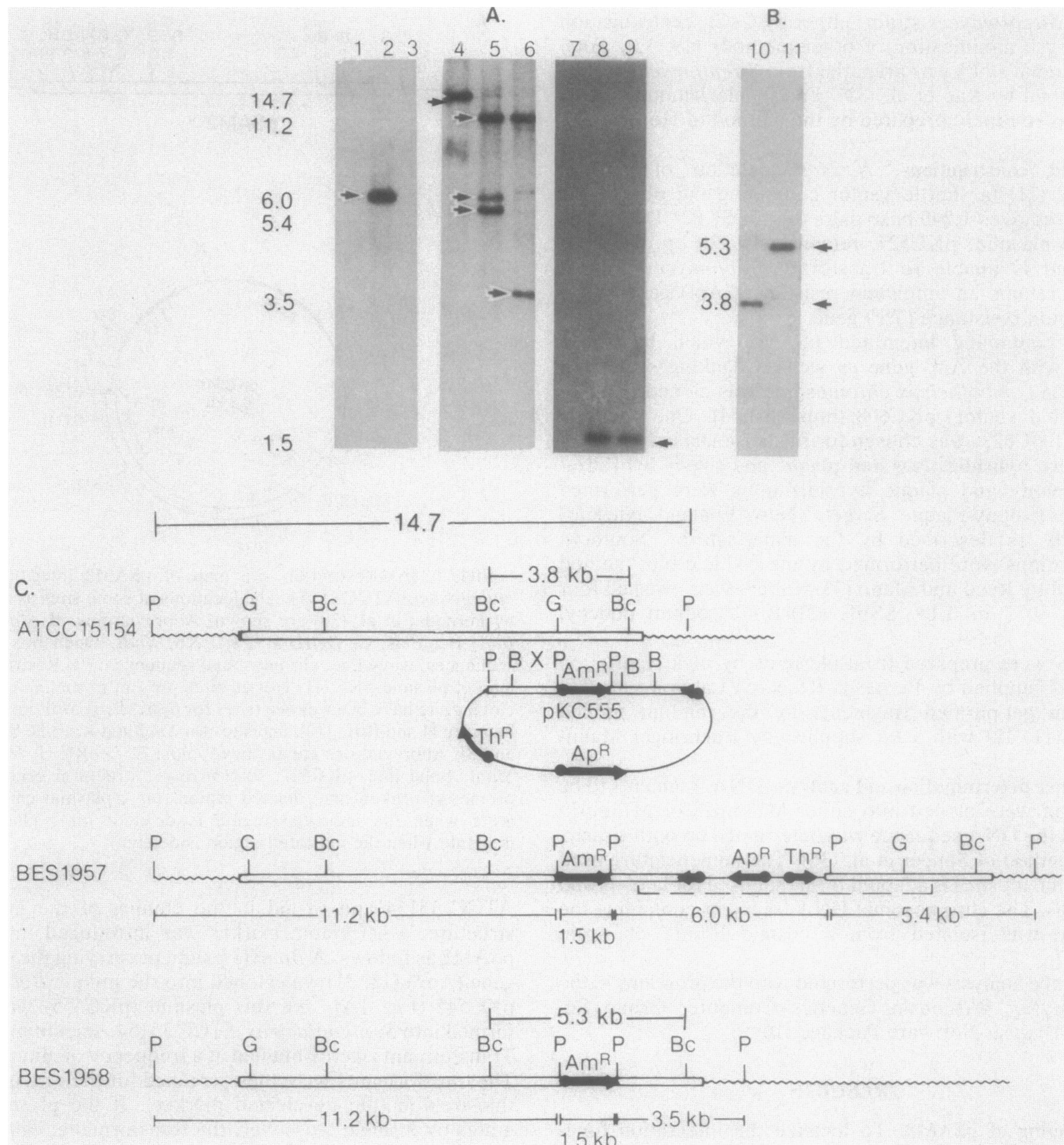

FIG. 2. (A) *Pst*I-cut *S. ambofaciens* ATCC 15154 (lanes 1, 4, 7), BES1957 (a single crossover; lanes 2, 5, 8), and BES1958 (a double crossover; lanes 3, 6, 9). Probed with pKC527 (lanes 1, 2, 3), pSAM2 (lanes 4, 5, 6), and purified Am^r fragment (lanes 7, 8, 9). Size markers are in kilobases. Arrows indicate *Pst*I fragments corresponding with those shown in panel C. (B) *Bcl*I-cut *S. ambofaciens* ATCC 15154 (lane 10) and BES1958 (double crossover, lane 11) probed with pKC542. Arrows indicate *Bcl*I fragments corresponding to those shown in panel C. (C) Restriction site map of the integrated structures which resulted from single (BES1957) and double (BES1958) crossover events with pKC555. Abbreviations and symbols are as in Fig. 1; Am^r, apramycin resistance. X indicates the region where a single crossover can generate the structure shown for BES1957; the dash-line bracket indicates a second crossover. Sizes of fragments generated by *Pst*I or *Bcl*I cutting are indicated.

Further work allowed us to demonstrate that BES2087 retained a functional locus into which pKC541 could integrate.

A 2.5-kb *Bcl*I-*Bam*HI fragment has all the plasmid-specified functions required for integration into the chromosome. Plasmid pKC541 transformed *S. ambofaciens* ATCC 15154 (pSAM2^{int} present) and BES2087 (no pSAM2^{int}) at similar frequencies. Two independent integrants generated in strain BES2087 were analyzed by Southern analysis of their total DNA (Fig. 4). The probe used was the 421-bp *Bam*HI(11)-*Bgl*II(1) fragment, which includes *attP*. In the first case (lane 4), both a 1.1-kb *Bam*HI fragment and a 1.6-kb *Bam*HI fragment disappeared when BES2087 was transformed with pKC541, and four new bands appeared (1.7, 0.3, 0.95, and 0.54 kb). This is consistent with the interpretation that pKC541 integrated into two different fragments: the 1.1-kb fragment that appeared when pSAM2^{int} was lost from *S. ambofaciens* (compare lanes 1, 2, 3, and 4) and also a 1.6-kb

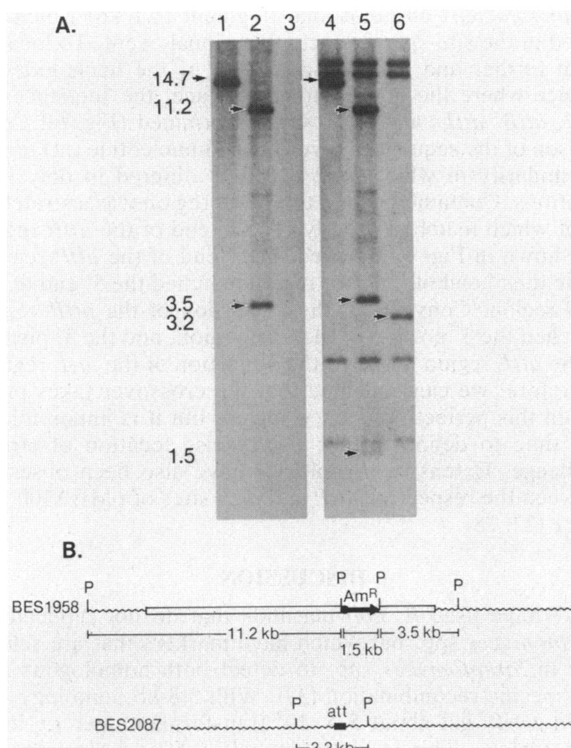

FIG. 3. (A) *Pst*I-cut *S. ambofaciens* ATCC 1515 (Lanes 1, 4), BES1958 (a double crossover with pKC555, lanes 2, 5), and BES2087 (a derivative of ATCC 15154 cured of integrated pSAM2, lanes 3, 6). Probed with pSAM2 (lanes 1, 2, 3) and pKC623 (lanes 4, 5, 6; pKC623 is a cosmid clone isolated from BES1958 that contains pSAM2^{int} marked with Am^r plus chromosomal sequences). Size markers are in kilobases. Arrows indicate *Pst*I fragments corresponding to those shown in panel B. (B) Restriction site map of BES1958 and BES2087 showing *Pst*I fragments when pSAM2 is integrated (BES1958) and excised (BES2087). Sizes of fragments generated by *Pst*I cutting are indicated. Abbreviations and symbols are as in Fig. 1.

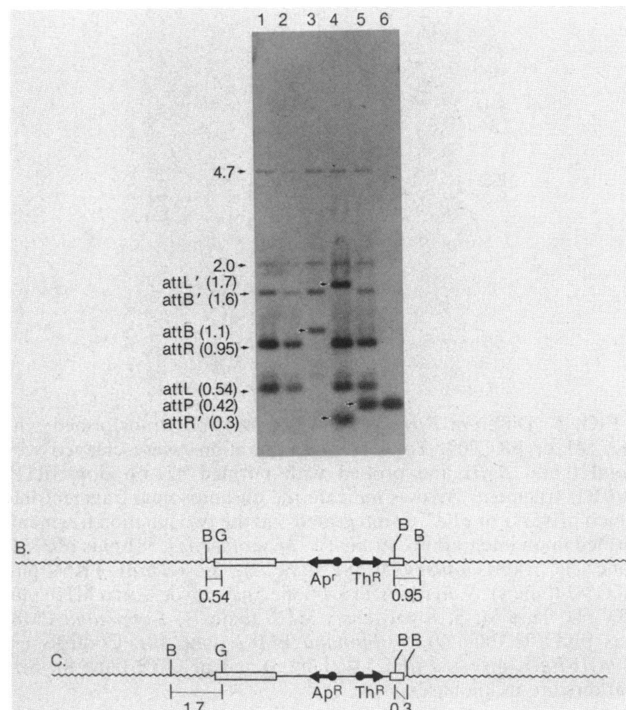

FIG. 4. (A) BES2087 (cured of pSAM2) was transformed with pKC541, and two Th^r transformants were chosen for further analysis. Total DNA preparations were cleaved with *Bam*HI and *Bgl*II and probed with purified 421-bp *Bam*HI(11)-*Bgl*II(1) fragment. Lanes: 1, BES1958 (a double crossover, see Fig. 2); 2, *S. ambofaciens* ATCC 15154; 3, BES2087 (a derivative of BES1958 cured of pSAM2^{int}); 4, BES2087 plus pKC541 (isolate 1); 5, BES2087 plus pKC541 (isolate 2); 6, pKC541. In isolate 1 (lane 4), pKC541 has integrated in two fragments—a 1.1-kb *Bam*HI fragment (*attB*, the site where pSAM2 is integrated in ATCC 15154), generating junction fragments *attL* (0.54 kb) and *attR* (0.95 kb), and a 1.6-kb *Bam*HI fragment (*attB'*), generating junction fragments *attL'* (1.7 kb) and *attR'* (0.3 kb). In isolate 2 (lane 5), both integrated and free pKC541 are present. Size markers are in kilobases. (B) Structure generated upon pKC541 integration into *attB*. (C) Structure generated upon pKC541 integration into *attB'*.

*Bam*HI fragment. In the second case (lane 5), the 1.1-kb *Bam*HI fragment disappeared and the 0.95-kb and 0.54-kb bands appeared. In this case, a 0.42-kb fragment corresponding to the *attP* fragment was also apparent. Southern analysis of uncut total DNA preparations showed free plasmid in the second case but not the first (data not shown). Free and integrated forms of pKC541 have also been seen in *S. ambofaciens* ATCC 15154 transformants. These experiments demonstrate that pSAM2 excision regenerated a functional *attB* site. Analysis of more integrants may reveal the utilization of the other two hybridizing bands as integration sites for pKC541.

The plasmid-specified functions involved in chromosomal integration of pKC541 were localized by subcloning experiments. Deletion of the *Bam*HI(11)-*Bgl*II(1) region, which contains the actual site of integration, eliminated the ability to transform BES2087. When the *Bam*HI(11)-*Bgl*II(1) fragment was subcloned in another *E. coli* plasmid, the recombinant plasmid was unable to transform BES2087 (transformation was reduced by a factor of at least 10^3). Deletion of the region between *Bcl*I(3) and *Bal*I(2) (Fig. 1B) also destroyed the ability to transform. However, deletion of the *Bam*HI(11)-*Bcl*I(10) region did not affect the ability of the plasmid to transform and integrate (Fig. 1B). Together, these data indicate that loci contained on pKC541, in addition to the region homologous to the chromosomal bands, are involved in the integration of the plasmid. Thus, a 2.5-kb *Bam*HI-*Bcl*I fragment contains all the plasmid-specified functions needed to direct site-specific recombination in *S. ambofaciens*.

pSAM2-directed integration occurs in a number of streptomycetes. To test the generality of this site-specific integration system, plasmids pKC541 and pKC702 (a plasmid similar to pKC541 but carrying the Am^r marker instead of the Th^r marker) were used to transform *S. lividans* TK64 (17), *S. griseofuscus* C581 (ATCC 23916 [20]), *S. toyocaensis* MJ16 (unpublished), and *S. lipmanii* PM87 (R. H. Baltz and P. Matsushima, manuscript in preparation). All these strains could be transformed with various efficiencies (ranging from only a few to ca. $10^2/\mu g$). Total DNA of representative transformants was prepared and analyzed by Southern hybridization (Fig. 5). As with *S. ambofaciens* strain BES2087, all strains tested showed multiple fragments which hybridized to the 421-bp *Bam*HI(11)-*Bgl*II(1) probe, although integration occurred in only one chromosomal fragment in each of these other strains. In all cases, integration utilized the 421-bp *Bam*HI(11)-*Bgl*II(1) fragment from pSAM2, the same fragment used in *S. ambofaciens* integration. Because of the

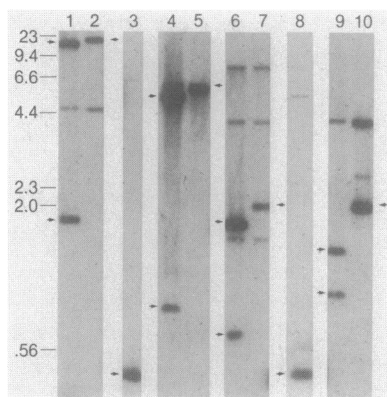

FIG. 5. Different *Streptomyces* strains were transformed with pKC541 or pKC702. Total DNA preparations were cleaved with *Bam*HI and *Bgl*II and probed with purified 421-bp *Bam*HI(11)-*Bgl*II(1) fragment. Arrows indicate the chromosomal fragment into which pKC541 or pKC702 integrated and the two junction fragments formed upon integration. Strains: *S. griseofuscus* C581 plus pKC541 (lane 1), *S. griseofuscus* C581 (lane 2), *S. lividans* TK64 plus pKC541 (lane 4), *S. lividans* TK64 (lane 5), *S. toyocaensis* MJ16 plus pKC541 (lane 6), *S. toyocaensis* MJ16 (lane 7), *S. lipmanii* PM87 plus pKC702 (lane 9), *S. lipmanii* PM87 (lane 10). Controls are *Bam*HI-*Bgl*II-digested pKC541 (lane 3) and pKC702 (lane 8). Size markers are in kilobases.

small sample size, we cannot exclude the possibility that the other chromosomal fragments detected by hybridization could also serve as integration sites.

Sequences of *attP*, *attB*, *attL*, and *attR*. In a previous section, we localized the plasmid fragment (421 bp) and the *S. ambofaciens* chromosomal fragment (1.1 kb) which engaged in the site-specific recombinational event. To localize them further and to gain some idea of the nucleotide sequence where the crossover takes place, the sequences of *attP*, *attB*, *attL*, and *attR* were determined (Fig. 6). Comparison of the sequences revealed a 66-nucleotide (nt) region of similarity in which *attP* and *attB* differed in only four locations. Contained within this 66-nt region was a stretch of 45 nt which matched exactly. The 5′ end of the *attR* region (as shown in Fig. 6) matched the 5′ end of the *attP* region, while the 5′ end of the *attL* region matched the 5′ end of the *attB* region. Conversely, the 3′ portion of the *attR* region matched the 3′ portion of the *attB* region, and the 3′ portion of the *attL* region matched the 3′ portion of the *attL* region. Therefore, we can conclude that the crossover takes place within this perfectly matched region, but it is impossible at this time to define further the precise location of strand exchange. Extensive homologies have also been observed between the respective *attP* and *attB* sites of pMEA100 and SLP1 (22, 28).

DISCUSSION

We have used *E. coli* plasmids that do not replicate in *Streptomyces* spp. but which have markers that are selectable in *Streptomyces* spp. to detect both homologous and site-specific recombination (39). With 3.8-kb homology, we could easily get about 5×10^2 transformants per μg in *S. ambofaciens*. This is approximately 0.5% of the transformants observed with replicating plasmids. About 7% of the transformants resulted from double crossover events. Attempts to induce a second recombinational event in a single-crossover transformant were unsuccessful (less than 1%

FIG. 6. DNA sequences of the four pSAM2 attachment regions. Position 1 is the first nucleotide in the region which is common to all four. The common region is underlined. Dots indicate mismatched bases in the homologous region.

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pMEA100  CACAACCCAAGACCTGAAAAGCGGAAGGTCGCCGGTTCGACCCCGGCCCCTGGCCACCGTGGTTACCTGCGAAAACAGGCCCCCCACCCATCGGGTAGGGGGCCTGATTC
pSAM2    CCACCCAAGTTGCTTTCGGGACGAAGAGGTCGTGGGTTCAAATCCCCGCCCACCCCGGACAGAGAAACACCAGGTGAGGCAGACCGTAACGTTACGGTCTGCCTCACCTGTTTTCT
SLP1     GCAGACTGTAAATCTTGCCGGCTCAGCCTTTCCCAGGTTCGAATCCTGGCCGCCCGGCCACACGACCGAAAGGGTCTGTGACCAGCAGCAATGCTGATCGCAGACCCTTTCGTCGTT
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FIG. 7. Comparison of pMEA100, pSAM2, and SLP1 sequences around their respective plasmid *att* sites. Boxed nucleotides indicate regions of exact match. Shaded areas indicate regions in which recombination with *attB* occurred. Arrows indicate inverted repeats; dots indicate mismatches. Sequences are aligned with respect to the common nucleotides.

segregation of the marker). This suggests that the second crossover event is more likely to occur during the initial transformation than during subsequent growth. The stability of the duplication in the single-crossover transformants is similar to that observed with ΦC31 insertions (6). All the transformants studied in this paper were obtained by using circular plasmid DNAs. Similar experiments with linearized plasmid DNAs were unsuccessful. This may be explained in part by the decreased frequency of transformation by linear DNA (about 10^{-3}) and by the possibility that linear DNA is more unstable than circular DNA in *Streptomyces* spp. (8). The success of our method relied on the nonrestricting nature of *S. ambofaciens* (9) and the relative ease of getting efficient transformation (24).

All the plasmid-specified information required to direct site-specific integration in a number of different *Streptomyces* strains was contained on a 2.5-kb *Bcl*I-*Bam*HI fragment of pSAM2. All the strains probed with the *attP* fragment showed more than one hybridizing band. We and Boccard et al. (4) have observed pSAM2-directed integration into more than one locus in strains of *S. ambofaciens* lacking pSAM2^{int}. Sequencing different *attB* regions may allow the determination of the minimal similarity required by pSAM2 to recognize a sequence as an *attB* site.

In *S. ambofaciens* and *S. toyocaensis*, but not in *S. griseofuscus*, *S. lividans*, or *S. lipmanii*, pKC541 has been seen in free as well as integrated form, although it has never been seen to exist only as the free form. Our interpretation is that there is an equilibrium between the integrated and free states, with little or no replication in the free state. This interpretation is based on the following observations. (i) Deletion of the 421-bp *Bam*HI(11)-*Bgl*II(1) fragment (Fig. 1) abolished the ability of pKC541 and its derivatives to transform *S. ambofaciens* lacking pSAM2. (ii) The large *Bgl*II fragment (1 to 6) was sufficient to direct replication in *Streptomyces* spp. (unpublished observations). (iii) It is unlikely that the 220 bp of SCP2* sequences remaining in pKC541 are functioning as a replicon, because the parental vector, pKC527, has been seen to transform *Streptomyces* spp. only in rare cases, and in these transformants the vector-hybridizing sequences were altered. (iv) The *Bam*HI(11)-*Bcl*I(3) fragment on an *E. coli* vector with no other *Streptomyces* DNA transformed and integrated into *Streptomyces* spp.

One of the best-studied *Streptomyces* integrating elements, SLP1, is seen as a free form only when regions involved with plasmid integration are lost (3, 26–29). Transformation of *S. lividans* with intact SLP1 gives rise only to the integrated state (28, 29). When intact pSAM2 is used to transform *Streptomyces* spp., both integrated and free pSAM2 are detected (data not shown) (4, 30, 37). Strains with integrated SLP1 are difficult to transform with the plasmid (3, 16, 27). It has been suggested (3, 16, 27) that this difficulty may be due to a control mechanism expressed in *trans* from the integrated form which represses replication. However, pSAM2 transformed *S. ambofaciens* ATCC 15154 and BES2087 at a similar frequency and with roughly the same efficiency as plasmids carrying the SCP2* replicon, even though a copy of pSAM2 was integrated in *S. ambofaciens* ATCC 15154. The significance of these differences is not clear.

Comparison of SLP1 and pMEA100 with pSAM2 attachment regions showed a conserved sequence which lay within the regions that showed homology to their respective chromosomal *att* sites (Fig. 7). However, SLP1 did not integrate at the pSAM2 locus in *S. ambofaciens* (data not shown).

Alignment of these attachment regions with respect to the conserved regions gave alignment of inverted repeats which were present in all three cases. The inverted repeats identified in pSAM2 and pMEA100 *att* regions lay outside the region of shared plasmid-chromosome sequences (Fig. 7). Therefore, these systems are unlike the recombinational systems of *loxP* or the 2μm plasmid (1, 14), which cross over at the loop in their inverted repeats. It seems reasonable to suppose that SLP1 may also cross over outside its inverted repeat. At this time, the function of the inverted repeats is not known.

Site-specific recombination directed by pSAM2 makes it possible to stably insert heterologous DNA in various *Streptomyces* strains. This would be desirable in a number of cases: genetic complementation studies; studies of gene regulation under unit copy conditions; and stable maintenance of heterologous genes in the absence of selective pressure.

ACKNOWLEDGMENTS

We thank K. Chater for helpful discussions, C. Hershberger and D. A. Hopwood for DNAs and strains, M. Ballou for pKC702, M. Jones and P. Matsushima for strains and help with *S. toyocaensis* and *S. lipmanii* transformations, S. Burgett for DNA sequencing, and J. P. Burnett and R. H. Baltz for their support.

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