Transposon mutagenesis with IS6100 in the avermectin-producer *Streptomyces avermitilis*

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The insertion sequence IS6100 was shown to undergo intermolecular transposition from a temperature-sensitive delivery plasmid to the genome of the avermectin-producer *Streptomyces avermitilis*, creating cointegrates. Evidence from both Southern hybridization and the range of auxotrophic mutations present in a transposon library was consistent with random transposition. It was not possible to increase transposase expression by readthrough transcription from a copy of the *tipA* promoter located adjacent to the insertion sequence. This was in part due to the absence of a homologue of the *Streptomyces lividans* transcriptional activator TipA_L in *S. avermitilis*. However, recombinant *S. avermitilis* strains carrying the *S. lividans* *tip* operon were also deficient for induction of the promoter. The frequency of reversion of different auxotrophic mutations by precise excision, involving recombination across 8 bp direct repeats, was shown to vary by at least five orders of magnitude. This dependence of recombination frequency on chromosomal location may contribute to the stability of repetitive modular type I polyketide biosynthetic genes.

Keywords: insertion sequence, transposon mutagenesis, reversion, *Streptomyces avermitilis*

INTRODUCTION

To date, the application of transposon mutagenesis in antibiotic-producing *Streptomyces* spp. has been quite limited. The number of different transposable elements which both function in the genus, and exhibit little target site specificity, is small. Moreover, the diversity of species within the genus has meant that transposon systems optimized for one species may be at best suboptimal, and sometimes non-functional, in another species. Transposons based on IS493, originally isolated from *Streptomyces lividans*, have been successfully utilized in several *Streptomyces* species (reviewed by Baltz *et al.*, 1997). The preferred target site for this element has been established as NgNCaNTgNNyN. Modified derivatives of Tn4556 isolated from *Streptomyces fradiae* have also been shown to transpose in several species (Chung & Crose, 1989; Ikeda *et al.*, 1993). Although nucleotide sequence data of the Tn4556 target site are not available, both Southern hybridization data and the variety of auxotrophic mutations obtained after transposon mutagenesis suggest that transposition of this element occurs reasonably randomly. Recently, an engineered version of the *Escherichia coli* transposon Tn5 has been demonstrated to transpose at high

frequency and at random in *S. lividans* (Vollf & Altenbuchner, 1997).

To complement these approaches, we have been assessing the potential of a mycobacterial insertion sequence IS6100 as a genetic tool in streptomycetes. This element is a member of the IS6 family and consequently forms cointegrates as an end-product of transposition. It was originally isolated as a component of the compound transposon Tn610, which confers sulphonamide resistance in *Mycobacterium fortuitum* (Martin *et al.*, 1990). It has also been found associated with nylon-catabolism determinants on a plasmid isolated from *Flavobacterium* (Kato *et al.*, 1994), suggesting that the element may have a broad host range. Our initial findings indicated that this element promotes random transposition in *S. lividans* (Smith & Dyson, 1995). The vector we employed for delivery of the insertion sequence, in common with all other means of transposon delivery so far utilized in *Streptomyces*, was a temperature-sensitive plasmid. It was subsequently important to assess the utility of both the element and the delivery system in other commercially important species.

Streptomyces avermitilis is an important producer of the potent broad-spectrum antiparasitic compounds termed

avermectins, which are type I polyketides. The species produces a complex of eight different variants, each containing a 16-membered macrocyclic lactone substituted with a disaccharide of L-oleandrose. The 95 kb biosynthetic gene cluster contains 12 modular repeats of the polyketide synthase necessary for the 12 acyl condensations required for formation of the avermectin polyketide backbone (MacNeil *et al.*, 1992; MacNeil *et al.*, 1993, 1994). Other aspects of the genetics of the species are less well characterized. A linkage map for several genetic markers has been constructed (Ikeda *et al.*, 1987; Ikeda & Ōmura, 1991), and physical characterization has revealed the presence of two giant linear plasmids (Evans *et al.*, 1994). Little is known about the regulatory networks which define morphological and physiological differentiation and which could be potentially manipulated to increase avermectin yields. The one transposon which has been successfully applied in the species is Tn4560 (Yagi, 1990; Ikeda *et al.*, 1993; Irnich & Cullum, 1993). Here we demonstrate random transposon mutagenesis using IS6100 in *S. avermitilis*, and investigate the reversion of different auxotrophic mutations.

METHODS

Bacterial strains and plasmids. *E. coli* strain F⁻ Z⁻ ΔM15 (Rüther *et al.*, 1981) was routinely employed as a host for recombinant plasmids used and constructed in this study. *S. avermitilis* 12804 (National Collections of Industrial and Marine Bacteria, Aberdeen, UK) was transformed with non-methylated DNA obtained by passaging plasmids through the *dam dcm* *E. coli* strain GM2929 (kindly provided by M. Marinus, University of Massachusetts). The transposon vector pUCS30 and plasmid pUCS19, used as a source of the IS6100 hybridization probe, were previously constructed by ourselves (Smith & Dyson, 1995). Both pAK114, used to assay thiostrepton induction, and pAK109, which carries a cloned copy of the *tipA* operon, have been previously described (Murakami *et al.*, 1989) and were kindly supplied by C. J. Thompson (Biozentrum, Basel).

Growth conditions and transformation. *E. coli* strains were transformed by a standard calcium-chloride-mediated procedure (Sambrook *et al.*, 1989). Transformants were cultured in L-broth or plated on L-agar (Sambrook *et al.*, 1989), supplemented with appropriate antibiotics (kanamycin 25 µg ml⁻¹, ampicillin 50 µg ml⁻¹, spectinomycin 25 µg ml⁻¹, streptomycin 25 µg ml⁻¹). *S. avermitilis* was cultured in tryptic soy broth or on GHM agar, a complete medium containing glucose, yeast extract and malt extract (Dyson & Schrempf, 1987). Plasmid DNA was introduced by polyethylene-glycol-mediated transformation of protoplasts which were subsequently regenerated on RM14 medium, as described by MacNeil & Klapko (1987). To select transformants, plates were overlaid with selective concentrations of appropriate antibiotics (final concentrations: kanamycin 20 µg ml⁻¹, spectinomycin 20 µg ml⁻¹, streptomycin 8 µg ml⁻¹, thiostrepton 5 µg ml⁻¹). Transposition assays were carried out using GHM agar containing kanamycin, streptomycin, spectinomycin and, when indicated, thiostrepton, all at the concentrations specified above. Thiostrepton-induction of kanamycin resistance conferred by plasmids pAK114 and pJW100 was tested using the plate-disc assay described by Murakami *et al.* (1989). Auxotrophic mutants were identified by transferring mycelia

to both GHM and minimal media (MM; Hopwood *et al.*, 1985). Mutants characterized by their inability to grow on MM were subsequently tested for growth on MM supplemented with appropriate amino acids and growth factors, as described in Hopwood *et al.* (1985).

DNA manipulations and construction of pTIP4. *S. avermitilis* chromosomal DNA was isolated as previously described (Dyson & Schrempf, 1987). Plasmid DNA was isolated by the alkaline-lysis technique (Birnboim, 1983). Restriction enzyme digests were performed as recommended by the supplier (Life Technologies or New England Biolabs). Restricted chromosomal DNA was electrophoresed in 0.8% agarose gels run in HEPES buffer (Ray *et al.*, 1992) to avoid Tris-dependent DNA cleavage. The DNA was transferred to Hybond-N nylon membrane (Amersham) using a vacuum-blotting system (Stratagene). The IS6100-specific hybridization probe was prepared by gel purification of a 0.9 kb *Sma*I fragment of pUCS19, and then labelled with digoxigenin-11-dUTP by random priming using a kit from Boehringer Mannheim. Hybridization conditions and subsequent detection by colour reaction with 4-nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate were according to the manufacturer's instructions (Boehringer Mannheim). Inserts for pulsed-field gel electrophoresis (PFGE) were prepared from 4-d-old cultures following the method of Leblond *et al.* (1993). Undigested or *A*flII-digested DNA was separated by PFGE in 1% agarose gels in a contour-clamped homogeneous electric field system (Chu *et al.*, 1986) supplied by Bio-Rad. Pulse times were optimized with respect to the length of DNA to be separated. In all experiments, the running buffer, 0.5 × TBE (Sambrook *et al.*, 1989), was supplemented with 50 µM thiourea to prevent Tris-dependent DNA cleavage (Evans & Dyson, 1986). pTIP4 was constructed by fusing pAK109 and pAK114 at their unique *Xba*I sites. *Xba*I-digested pAK109 was treated with calf intestinal alkaline phosphatase prior to ligating with linearized pAK114.

RESULTS

Transposition of IS6100 in *S. avermitilis*

To test transposition, the temperature-sensitive IS6100 delivery vector pUCS30 (Smith & Dyson, 1995) was introduced by transformation into *S. avermitilis*. We found that the most reliable method for isolating independently derived insertions of IS6100 within the genome was to obtain outgrowing sectors of colonies grown at the non-permissive temperature for replication of the delivery vector, as originally developed by Solenberg & Baltz (1991) to test transposition of IS493 in *S. griseofuscus*, and subsequently employed by Smith & Dyson (1995) to observe transposition of IS6100 in *S. lividans*. The optimized assay involved selective plating at a density of less than 100 viable spores per plate and growing at 28 °C for 3–4 d until individual colonies were about 2 mm in diameter. The plates were then transferred to 40 °C and incubation continued for a further 9 d. At this point outgrowing sectors were clearly visible at a frequency of 0.4 sectors per colony. The frequency of sector formation was found to depend on three factors: (i) the age of the original spore stock, (ii) the number of colonies per plate, and (iii) the maturity of colonies when shifted to the non-permissive temperature. The number of sectors gradually dwindled to none

after storage of spores over a period of 3–4 months, and so spore stocks from fresh transformants were routinely used. We could not detect evidence for observable plasmid rearrangements over prolonged storage, and the basis for the loss of transposition competence is not understood.

The experiments below describe analysis of transposants derived from a single transformant containing pUCS30, and are representative of results obtained with other fresh transformants. Spores raised from the transformant were plated at low density. Following 2–3 d incubation at 40 °C, the master colonies from which sectors were derived changed appearance, becoming translucent. After 4 d incubation at 40 °C, mycelia from 100 translucent colonies were transferred to fresh non-selective media and incubated at 28 °C; only one sample grew which, after DNA extraction and Southern hybridization, was shown to be due to transposition. Presumably, a very small sector, not visible to the naked eye, had been established on this colony. In contrast, mycelia transferred from all outgrowing sectors continued to grow when transferred to fresh selective media and incubated at 28 °C. Genomic DNA was isolated from a total of 68 individual cultures for Southern hybridization analysis. Duplication of the insertion sequence during cointegrate formation is a good criterion that can be applied as evidence for transposition, and the hybridization pattern obtained using an IS6100 probe was consistent with this in 91% of the samples analysed, as shown in Fig. 1 for 13 independent isolates. Four samples (6%) contained single hybridizing fragments of different molecular masses and also not the same size as linearized pUCS30: these could have arisen either due to integration of the vector in a transposition-independent manner (although no significant homology between vector and *S. avermitilis* DNA could be detected by hybridization), or due to cointegrate formation and subsequent recombination between duplicated copies of the insertion sequence (see below). The remaining two samples (3%) did not exhibit evidence for transposition, but instead possessed a single hybridizing fragment of similar molecular mass to linearized pUCS30, consistent with retention of the free plasmid.

Lack of induction of the *tipA* promoter in *S. avermitilis*

pUCS30 contains a copy of the *tipA* promoter (Murakami *et al.*, 1989) adjacent to IS6100, which is itself oriented in such a manner that the transposase gene is potentially susceptible to readthrough transcription emanating from this inducible promoter. Previous studies in *S. lividans* have indicated that IS6100 is unusual in not possessing a mechanism to protect its transposase gene from external activation from this promoter, and consequently the transposition frequency can be increased 100-fold as a result of induction of the promoter in this species (Smith & Dyson, 1995). However, using outgrowth frequency as a measure of transposition in *S. avermitilis*, we could observe no

increase in the numbers of sectors when they were generated on media containing the inducer thiostrepton. To test activity of the promoter, we introduced plasmid pAK114 (Murakami *et al.*, 1989) into *S. avermitilis*. This plasmid carries an *aphII* gene under control of the promoter, so that promoter induction can be measured using a disc assay: the strain is plated on medium containing kanamycin and induction of the promoter is observed by a zone of growth around a disc impregnated with thiostrepton. However, no growth was observed. To test if lack of induction could be due to a mutation affecting the plasmid, it was reisolated and introduced into *S. lividans* where normal induction, as measured using the disc assay, was observed. Together, these results indicated that the promoter cannot be induced in *S. avermitilis*.

Induction of the *tipA* promoter requires a functional TipA_L protein, the N-terminus of which contains a DNA-binding domain responsible for binding to sequences overlapping the promoter, whereas the C-terminus contains two cysteine residues either of which may form a covalent linkage with a dehydroalanine residue of thiostrepton (Chiu *et al.*, 1996). However, using Southern hybridization with a probe of plasmid pAK109 containing the *S. lividans tip* operon, we could not detect homologous sequences in the *S. avermitilis* genome (results not shown). Thus, lack of *P_{tipA}* induction in this species could be attributable to the absence of a functional TipA_L protein. We therefore expected that by introducing a copy of the *S. lividans tip* operon into *S. avermitilis*, we might then observe induction of the promoter. To test this, we introduced plasmid pTIP4 (Fig. 2), consisting of a fusion of pAK109 and pAK114. However, thiostrepton induction as measured using the disc assay also proved negative with the strain harbouring this plasmid. Moreover, the *S. lividans tip* operon has been stably integrated into the *S. avermitilis* chromosome, but *P_{tipA}* could also not be induced in this recombinant strain (J. Weaden, unpublished). These results indicate that other factors expressed in *S. lividans* but not *S. avermitilis* are required for *P_{tipA}* induction.

The random nature of transposition

The patterns of hybridizing fragments evident from analysis of the 68 independent putative transposants gave no indication of any preferred target site for insertion. For example, the hybridization profile of each isolate in Fig. 1 is unique. We also assessed the distribution of insertions throughout the genome using PFGE. Wild-type *S. avermitilis* possesses two large linear plasmids, pSA1 and pSA2 (Evans *et al.*, 1994). To examine if transposition occurred into either plasmid or the linear chromosome, unrestricted DNA was separated by PFGE and hybridized against an IS6100 probe. This revealed that all transposants had in fact lost pSA2, the larger of the two linear plasmids, which was subsequently shown to be typically absent in *S. avermitilis* pUCS30 transformants used to generate the initial

Fig. 1. Southern hybridization analysis of transposant DNA. (a) Schematic representation of the cointegrate structure formed by intermolecular transposition of IS6100 into the *S. avermitilis* genome. (b) Southern blot of DNA from independently isolated transposants hybridized with an IS6100 probe. Lane 1, λ HindIII marker; lane 2, pUCS30 linearized with BamHI; lane 3, *S. avermitilis* 12804 (Sa); lane 4, Sa::pUCS30 (free plasmid); lanes 5-17, independent Sa::pUCS30 transposants (samples in lanes 3-17 are total DNA extractions restricted with BamHI). Due to underloading of DNA in lane 16, the hybridizing bands of approximately 12 and 4.2 kb are poorly visible.

Fig. 2. Map of the *tip* operon-promoter plasmid, pTIP4. The arc in bold corresponds to the 4.25 kb *KpnI* fragment of *S. lividans* DNA containing the *tip* operon.

spore stocks. It appears that this plasmid is very easily lost during protoplasting and regeneration. pSA1 was also absent in 40% of transposants tested. Loss of this

plasmid is believed to occur during formation of outgrowing sectors at elevated temperature as original transformants had generally maintained it. However, in two isolates from 15 which contained the linear plasmid we observed transposition into pSA1, evident both from its larger size in these mutants and from positive hybridization with the probe. The small sample size did not allow us to derive any conclusion about the relative frequencies of transposition into either the chromosome or the plasmid. In the other strains, the probe hybridized uniquely to the large linear chromosomal band, and the distribution of insertions in these isolates was further analysed by hybridization to *Afl*II-digested fragments separated by PFGE. This enzyme does not have a recognition sequence within pUCS30, so we expected a single hybridizing fragment in each isolate. The hybridization data indicated that insertion had taken place into different regions of the chromosome in different isolates; for example, Fig. 3 illustrates insertion into different *Afl*II fragments in at least seven out of nine samples.

To examine target specificity further, a transposon library derived from 4500 independently isolated outgrowing sectors was examined for auxotrophic mutations. Twenty-four auxotrophic mutants were iso-

Fig. 3. Pulsed-field gel (a) and corresponding Southern blot (b) of nine independent *Sa::pUCS30* transposants demonstrating insertion into various genomic sites. The DNA was restricted with *Afl*II and hybridized with an *IS6100* probe. Running conditions: 180 V, 16 °C, pulse times 10–60 s for 22 h. YM, yeast marker.

Table 1. Nutritional requirements and reversion frequencies of auxotrophic mutants

Nutritional requirements	Mutant strain no.	Reversion frequency
Adenine	1278	ND
Arginine	2166	ND
	4292	9.2×10^{-8}
Cysteine or methionine	794	1.4×10^{-3}
	912	$< 4.4 \times 10^{-8}$
Histidine	3070	ND
Leucine	416	ND
Methionine	3100	ND
Methionine and threonine	1110	4.7×10^{-5}
Niacinamide	1483, 2739	ND
Pyridoxine	3911	ND
Thiamin	2	$< 1.3 \times 10^{-7}$
	138	$< 9.6 \times 10^{-8}$
	302	$< 3.9 \times 10^{-8}$
	31, 241, 1530	ND
Undetermined	2819, 2884, 3765,	ND
	3808, 3782, 4413	

ND, Not determined.

lated, indicating a frequency of insertion into genes involved in essential anabolic pathways of 5.3×10^{-8} . The nutritional requirements of each mutant were then established (Table 1). In some cases it was found that a given nutritional requirement was represented by more than one mutant, which could have resulted from insertion into a preferred target site in each mutant of the same class. This possibility was further examined by analysis of the *IS6100* hybridization profiles of *Bam*HI-restricted DNA isolated from each mutant. For example, different profiles were observed in three independently isolated thiamin auxotrophs, and two independently isolated methionine- or cysteine-requiring mutants.

These data are consistent with *IS6100* exhibiting very little target site specificity.

Precise excision and cointegrate stability

At least two types of recombination event can affect the stability of *IS6100* cointegrates. Recombination between two copies of *IS6100* can result in loss of the integrated plasmid, leaving one copy of the insertion sequence as a 'footprint' at the original site of cointegration. Alternatively, reversion of a mutation caused by insertional mutagenesis can occur by recombination across the direct repeats of target DNA caused by the transposition

mechanism, resulting in precise excision of the cointegrate. IS6100, like other members of the IS6 family, generates 8 bp target site duplications (P. Herron & P. Dyson, unpublished). A sensitive assay to measure precise excision was to examine the reversion frequency of different auxotrophic mutants. As these mutants sporulated poorly or not at all, mycelia from liquid cultures were fragmented by intense vortexing and thoroughly washed prior to plating on minimal media with and without the relevant nutritional requirement. As Table 1 indicates, of the seven auxotrophic mutants studied, three generated prototrophic revertants at a measurable frequency. Mutant 794, a cysteine auxotroph, was particularly unstable, reverting at a frequency of 1.4×10^{-3} . Checks on antibiotic resistance revealed that all revertants had lost the plasmid-encoded resistance markers. DNA was isolated from a sample of revertants derived from each mutant and analysed by Southern hybridization with the IS6100 probe. This failed to reveal any hybridizing signal in each of the revertants tested (results not shown).

To carry out a qualitative investigation into recombination involving the 880 bp direct repeats of IS6100 flanking the cointegrates, seven randomly selected transposants were subjected to prolonged growth without antibiotic selection for retention of the integrated plasmid. The individual mutants were subcultured ten times over a period of 40 d, prior to testing their antibiotic resistance phenotypes. In each case they retained the antibiotic resistance of the original transposant. Moreover, DNA was recovered from the cultures which had not been exposed to antibiotic selection during this period and the hybridization patterns obtained with the IS6100 probe compared to those of the original isolates. In each case similar hybridization profiles had been maintained in the absence of selection (results not shown), indicating that, at least in the small sample analysed, recombination affecting cointegrate stability was infrequent. Moreover, the cointegrate strains presumably exhibited no growth disadvantage compared to segregants in which rearrangements may have occurred.

DISCUSSION

We have demonstrated transposition of IS6100 in *S. avermitilis*. The method of delivery and transposant selection is reliable and ensures that a negligible number of sibling isolates are generated, facilitating construction of transposant libraries. Southern hybridization analysis of transposant DNA separated by both conventional and pulsed-field gel electrophoresis gave no evidence for any preferred target site utilized by the insertion sequence. The variety of auxotrophic mutations isolated in a library of 4500 transposants was also consistent with random transposition, and was in line with the range of auxotrophic mutations isolated in *Mycobacterium smegmatis* as a result of transposition of the compound transposon Tn611, which contains two copies of IS6100 flanking a kanamycin-resistance gene (Guilhot *et al.*, 1994). Sequence analysis of target sites

utilized by a mini-IS6100-based transposon, Tn1792, in *S. lividans* has failed to reveal any preferred sequence motif or choice for regions with an atypical G+C content (P. Herron & P. Dyson, unpublished). Southern hybridization data supported the interpretation that auxotrophic mutants sharing the same nutritional requirements arose from independent insertions in each case. Alternatively, the auxotrophic mutations could have been unlinked to the sites of cointegration and could have arisen due to instability of certain primary metabolic genes, by analogy with the unstable nature of the *argG* gene in certain *Streptomyces* spp. (Altenbuchner & Cullum, 1984; Meade, 1985). However, for the two Cys⁻ mutants, not only did the differing reversion frequencies suggest independent mutagenic events in each strain, but the fact that reversion was associated with loss of the cointegrate in mutant 794 was also consistent with the initial mutation being caused by insertional inactivation. In the case of the Thi⁻ mutants, it is unclear whether these could have arisen due to genetic instability of a biosynthetic gene unlinked to the insertions. Indeed, one of the Thi⁻ mutants, mutant 2, contained a 7.6 kb amplified DNA sequence (results not shown). Amplified DNA sequences typically arise in *Streptomyces* spp. coincidentally with gross rearrangements and deletions affecting the integrity of the chromosome ends (reviewed by Chen, 1996). Conceivably, a thiamin biosynthetic gene could be located close to a chromosome end in *S. avermitilis* and be subject to high-frequency deletion, as is the case for *argG* in *S. lividans*. However, we are not aware of any other reports concerning instability of this phenotype in *S. avermitilis* and have never observed it ourselves in cultures not employed for transposon mutagenesis. It should be noted that growth at elevated temperatures, as applied to cure non-integrated forms of the temperature-sensitive delivery plasmid, is known to induce instability affecting chromosome ends (Leblond & Decaris, 1994). We detected amplified DNA in 2% of *S. avermitilis* transposants and these strains were typically non-sporulating (data not shown). If the objective of transposon mutagenesis is to isolate mutations conferring phenotypes similar to those associated with known unstable traits, affecting morphological development and secondary metabolism for example, use of a temperature-sensitive delivery plasmid may give an unacceptably high level of mutation unlinked to the insertion.

Other auxotrophic reversions were also accompanied by loss of the cointegrate, this being consistent with the mutations arising by insertional inactivation in each case. Previous reversion analysis of a Met⁻ mutant generated after transposition of Tn4560 in *S. avermitilis* was not so clear cut, as the revertants retained the antibiotic resistance conferred by the transposon (Ikeda *et al.*, 1993). Auxotrophic reversion provides a sensitive selectable 'forward' assay to measure recombination, even though precise excision involves recombination across only 8 bp direct repeats. The mechanism of precise excision of transposons has been studied in

enteric bacteria, and shown to rely on a RecA-independent process involving 'copy-choice' replication slippage. A variety of evidence supports a model of intrastrand pairing between the terminal inverted repeats of the transposon, creating a secondary structure which blocks progress of DNA polymerase III (Foster *et al.*, 1981). *In vivo* studies demonstrate a heterogeneity of excision frequency, scored by measuring auxotrophic reversion, dependent on the chromosomal context, varying, for Tn10, between 10^{-6} and less than 10^{-10} (Kleckner *et al.*, 1979). The high reversion frequency we observed for some mutants may be attributable to the G+C content of both the surrounding DNA and inverted repeats favouring intrastrand pairing. Alternatively, excision in *S. avermitilis* may be RecA dependent. Several enhanced transposon excision, *tex*, mutants of *E. coli* contain either special alleles of the *recB* and *recC* genes, or null mutations in genes involved in mismatch repair (Lundblad & Kleckner, 1985; Lundblad *et al.*, 1984). Typically, these mutations render transposon excision RecA dependent. Variation in excision frequency dependent on location could be a consequence of differences in overall recombination frequency in certain chromosomal regions. The evolution of repeated polyketide synthase modules which share up to 80% homology, as for example in the avermectin biosynthetic gene cluster (MacNeil *et al.*, 1994), may require that a mechanism to suppress recombination operates in these chromosomal regions. A system of recombinational suppression could explain the large variance in the frequencies of precise excision that we observed.

As described, IS6100 and transposons derived from this element are useful tools for transposon mutagenesis in *S. avermitilis*. The fact that the element can generate stable cointegrates means that it can also be employed to integrate adventitious genes into the chromosome. By this means it may be possible to introduce genes to overcome rate-limiting steps in product formation, for example in the biosynthesis of avermectins, or genes whose products can modify the structure of a metabolite. We assayed a small number of transposants for avermectin production and while no non-producing mutants were identified, the analysis revealed stable integration sites which have a neutral effect on antibiotic biosynthesis (data not shown). This demonstrates the potential for this particular application.

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