Method for Selection of Transposable DNA and Characterization of a New Insertion Sequence, IS493, from *Streptomyces lividans*

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A method to select for transposable elements from *Streptomyces* spp. by using insertional inactivation of a repressor gene that functions in *Escherichia coli* was developed. Plasmid pCZA126, which can replicate in *Streptomyces* spp. or *E. coli*, contains a gene coding for the lambda cI857 repressor and a gene, under repressor control, coding for apramycin resistance. *E. coli* cells containing the plasmid are apramycin sensitive but become apramycin resistant if the cI857 repressor gene is disrupted. Plasmids propagated in *Streptomyces* spp. can be screened for transposable elements that have disrupted the cI857 gene by transforming *E. coli* cells to apramycin resistance. This method was used to isolate a new 1.6-kilobase insertion sequence, IS493, from *Streptomyces lividans* CT2. IS493 duplicated host DNA at the target site, had inverted repeats at its ends, and contained two tandem open reading frames on each strand. IS493 was present in three copies in the same genomic locations in several *S. lividans* strains. Two of the copies appeared to be present in regions of similar DNA context that extended at least 11.5 kilobases. Several other *Streptomyces* spp. did not appear to contain copies of IS493.

Transposable elements, including insertion sequences, transposons, and transposing bacteriophages, have been widely exploited in the genetic and molecular analyses of *Escherichia coli*, *Salmonella typhimurium*, and other bacteria (4, 5, 21, 32). Transposable elements functional in *Streptomyces* spp. would be valuable in the genetic analysis and manipulation of these important industrial microorganisms.

Recently, a few transposable elements have been identified in *Streptomyces* spp., including the insertion sequences IS110 (8) and IS281 (36); a transposon, Tn4556 (9); and the 2.6-kilobase (kb) minicircle from *Streptomyces coelicolor* (24). To obtain a larger number of transposable elements from *Streptomyces* spp., a method for the selection of these elements was developed.

This report describes a plasmid, pCZA126, that can replicate in *Streptomyces* spp. or *E. coli* and that contains a gene coding for the lambda cI857 repressor and a gene, under repressor control, coding for apramycin resistance (Am^r). *E. coli* cells containing pCZA126 were Am^r. Am^r cells resulted when the cI857 gene was disrupted. Plasmids that were propagated in *Streptomyces* spp. were screened for transposable elements disrupting the cI857 gene by transforming *E. coli* to Am^r. Selection for the relatively rare event of transposition is feasible, since large numbers of *E. coli* cells can be transformed and selectively plated. By using this method, a new element, IS493, was isolated from *Streptomyces lividans* CT2.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1.

Culture conditions and transformations. *E. coli* K-12 JM109 cells were propagated in minimal A medium (27) or TY broth (30). Competent *E. coli* cells were prepared (39) or were purchased (Stratagene). For plasmid constructions, cells were transformed as described by Maniatis et al. (25) and were selected using TY agar (TY broth with 15 g of agar

per liter) supplemented with carbenicillin at 25 µg/ml. Transformed cells grew in the presence of carbenicillin since the plasmids contained the *bla* gene. For selection of Am^r transformants, the procedure was modified as follows: transformed cells were grown in TY broth for 45 min at 30°C; the transformation plates were supplemented with both apramycin and carbenicillin at 25 µg/ml each or with only carbenicillin at 25 µg/ml to determine transformation frequency; and plates were incubated at 30°C and examined for colonies after 2 days. Carbenicillin was included in plates containing apramycin to prevent the growth of spontaneous Am^r non-transformed cells. All isolated transformants were propagated at 37°C in TY broth containing carbenicillin at 50 µg/ml.

Methods for manipulation of *Streptomyces* strains have been described elsewhere (16). *S. lividans* CT2 was transformed as described previously (30), and transformed cells were plated on modified R2 bottom agar (1). Soft R2 top agar (2), overlaid at 16 to 24 h, contained thiostrepton at a final concentration on the plate of 25 µg/ml. *Streptomyces* transformants were propagated in Trypticase soy broth (BBL Microbiology Systems) supplemented with thiostrepton at 25 µg/ml.

Chemicals and Enzymes. Apramycin was a gift of K. Merkel of Eli Lilly & Co. Thiostrepton was a gift of S. J. Lucania of E. R. Squibb & Sons. Carbenicillin was obtained from Sigma Chemical Co. Restriction enzymes, ligase, and mung bean nuclease were used according to the specifications of the suppliers.

DNA isolation and recombinant DNA methods. Conventional DNA manipulations were performed as described by Maniatis et al. (25), unless otherwise noted. Large-scale *E. coli* plasmid isolations were performed by Triton X-100 lysis of the cells and CsCl gradient purification of the plasmid (30). *Streptomyces* plasmids were isolated by the methods of Kieser (20). Total genomic DNA was isolated from *E. coli* and *Streptomyces* spp. as described elsewhere (12).

DNA fragments were purified from gels for use in ligations by using GENECLAN (Bio 101, Inc.). DNA fragments

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TABLE 1. Strains used

Strain	Source	Outstanding characteristics	Reference
<i>E. coli</i> K-12 JM109	Stratagene		40
<i>S. ambofaciens</i> (ATCC 15154)	Lilly Culture Collection		26
<i>S. clavuligerus</i>	Lilly Culture Collection		19
<i>S. coelicolor</i> A3(2)	John Innes Institute	Closely related to <i>S. lividans</i>	17
<i>S. lividans</i> 1326	John Innes Institute	SLP2 SLP3	17
<i>S. lividans</i> CT2	John Innes Institute	Sm ^r mutant of <i>S. lividans</i> 1326	Charles Thompson, unpublished data
<i>S. lividans</i> TK21	John Innes Institute	SLP2 ⁻ SLP3 ⁻	17
<i>S. lividans</i> TK24	John Innes Institute	SLP2 ⁻ SLP3 ⁻	17
<i>S. fradiae</i> T59235	Lilly Culture Collection		3
<i>S. griseofuscus</i> (ATCC 23916)	Lilly Culture Collection		10
<i>S. thermotolerans</i> A57744.2	Lilly Culture Collection		
<i>S. lipmanii</i> LE32	P. Matsushima		14

were purified from gels for use as probes by using ELUTRAP (Schleicher & Schuell, Inc.). Southern hybridization was performed as described by Reed and Mann (31). Filters were washed four times at 60°C in 0.1× SSPE (1× SSPE is 0.18 M NaCl, 10 mM sodium phosphate, and 1 mM EDTA [pH 7.7])–0.1% sodium dodecyl sulfate.

DNA probes were labeled with [α -³²P]dCTP (3,000 Ci/mmol; New England Nuclear Corp.) by using a Random Primed DNA Labeling Kit (Boehringer Mannheim Biochemicals).

Oligonucleotides were synthesized with a DNA synthesizer (model 380A; Applied Biosystems) according to the recommended procedure of the manufacturer. The oligonucleotides were purified by polyacrylamide gel electrophoresis, desalted on a DE-52 ion-exchange column, lyophilized, and then dissolved in 10 mM Tris hydrochloride (pH 7.5). After the oligonucleotides were mixed in an equal molar ratio, they were heated to 95°C and then cooled slowly to permit annealing.

DNA sequencing was performed on both strands by the method of Sanger et al. (33).

DNA and protein sequence analysis was performed with the University of Wisconsin Genetics Computer Group (UWCGC) Sequence Analysis Software Package (11) and a program (John Shepherd, personal communication) that predicts protein-coding regions based on the distribution of G+C bases in the three positions of the codons, as described by Bibb et al. (6).

Construction of vectors. An Am^r gene, *aac(3)IV* (29), was isolated from plasmid pKC462a (37) on a 1.5-kb *Pst*I-*Eco*RI fragment and ligated into pUC19 (40) digested with *Pst*I and *Eco*RI, resulting in pCZA122. Plasmid pCZA122 was partially digested with *Sac*I and then completely digested with *Sph*I. The resulting mixture of fragments was separated on a gel of 0.8% agarose. The DNA at 3.9 kb, which lacks the native promoter of *aac(3)VI* and a 5' portion of this gene, was isolated.

A 0.44-kb *Sph*I-*Xba*I DNA fragment containing the lambda *p*_L promoter sequence was isolated from plasmid pL110, constructed by Jaskunas and Daugherty (R. Jaskunas, personal communication). An *Xba*I-*Sac*I DNA fragment that coded for a Shine-Dalgarno sequence (35) and a possible 5' sequence of the *aac(3)VI* gene was synthesized (Fig. 1A) on the basis of sequence data for a similar Am^r gene (7).

The 3.9-kb *Sac*I-*Sph*I vector fragment, the *Sph*I-*Xba*I fragment containing the *p*_L promoter, and the synthetic *Xba*I-*Sac*I fragment were ligated to produce plasmid pCZA123. A 0.97-kb *Hind*III-*Eco*RI fragment containing the *p*_L and *aac(3)VI* sequences was isolated from pCZA123.

After the ends were blunted with mung bean nuclease, the fragment was ligated into *Sma*I-digested pUC19, resulting in pCZA124. Plasmid pCZA124 was digested with *Pst*I and ligated with a 1.2-kb *Pst*I fragment from pOU57 (22) which contained the *cI857* repressor allele and the *p*_R promoter from phage lambda, resulting in pCZA125. A 2.8-kb fragment containing the *cI857* and *aac(3)VI* genes was isolated from pCZA125 by complete digestion with *Eco*RI and partial digestion with *Hind*III. This fragment was ligated with *Hind*III- and *Eco*RI-digested pHJL400 (23), resulting in plasmid pCZA126 (Fig. 1B). PCZA126 derivatives, which have the *cI857* gene disrupted, were made by ligating *Nsi*I-digested pCZA126 with *Pst*I-digested lambda DNA.

RESULTS

Plasmid-conferred Am^r in *E. coli* cells. *E. coli* JM109 transformants containing plasmid pCZA126 did not grow at 30°C on TY agar supplemented with apramycin at 50 µg/ml. However, Am^r was conferred when the *cI857* protein encoded by this plasmid was nonfunctional. The temperature-sensitive *cI857* protein was made nonfunctional by either incubating the cells at 40°C or making plasmid derivatives that contained a disrupted *cI857* gene sequence.

Isolation of transposable elements from *E. coli*. *E. coli* JM109 cells containing plasmid pCZA126 were streaked onto

FIG. 1. Plasmids pCZA126 and pCZA140. (A) *Xba*I-to-*Sac*I synthetic DNA sequence. (B) ■, Synthetic DNA; □, IS493.

...	AGCGTTTTTCAACCTCAGGCTGACGGGTGAGGGTCAGAGGCGGGTCTGGATCAGCGCGAGCGGTCGTGAGGTAGTACCGGTTGCGCTCGAAGCTCAC	100
101	TGCCGTCTAGGACAGAAGCTGACCCCTAGCTCACGCCTACCGTCGCTGCTGGCAAGCAACATCAGCGAAAGGACCGCGATGCCCCAGCAGACCAGCCCCGA	200
	ORF A → M P Q Q T S P E	
201	GCCGGAGGGCTACACCACGTTGCGCCCTGGGTCGTACCGACGACACGGGGGCTTCTCGACTTCGTACCCAGGCATTGCGGGGCGAGGAACTCGGG	300
	P E G Y T T V A P W V V T D D T G A F L D F V T Q A F G G E E L G	
301	CGGGTGTGACCGAGGAGGGCTTGATCGGTGACGCTGAGATCCGAGTCGGCGACACCGTCGTGTTGGCCTTCGACCGACACACGGACTGGCCCGCCATGC	400
	R V S T E E G L I G H A E I R V G D T V V L A F D R H T D W P A M P	
401	CGAGCCTGCTGCGCGTATTTGTCGCCGATGCGGACGAGGCGTTTTCCAGGCCGTCGACGCTGGCGGCTATGTCGTCACTTCCCTGGCCGACGACGCTT	500
	S L L R V F V A D A D E A F S Q A V A A G G Y V V T S L A D D A F	
501	CGGACAGCGCGGAGGGCGCATCAAGGACCCCTTCGGCAATATCTGGTGGGTGGTCAGCTGCGTTGAGGACGTCTCTGAGGGCGAGATGTGAAGCGGCTG	600
	G Q R G G R I K D P F G N I W W V V S C V E D V S E G E M W K R L	
601	CAGGACCCCGTATACGCCGAGGCCATGCGCGTGGCTCAGGAGACACTCGACGCCGAGCTCAGCGGTCGACGCCACGGGCGCAGCAGCGCACCCGTCAGGA	700
	Q D P V Y A E A M R V A Q E T L D A E L S G R R H G R S S A P V R T	
701	CAACCAGCTAGACCCACGACGTCGGGGTTCGCATGCGCCAGACCGGTAGCCAAGGAGCGTCTTCATTCTGCTTCGGCAGCTTGGGGTTGGCTGACACA	800
	T S *	
801	GGGGTGAAGCCCCTGGTAGATGGGTTTTGACCAAGAAATCCGCTCTACTAGAGGCTTCAGTGCCTTGTTACCCCTCCGGGCTGGACGTGCCAGTTC	900
	ORF B → V L V Y P S G L D V S S S	
901	TGCCCTCCGCTTCTCTCCGCCGTCTGCGGGAACACCGCGTGGTCTCGGCACCCGGTGGCGGCGCTGAGCGCAGGTGCGCAGGCTCTGCTCGCCCTC	1000
	A L R F L S A R L R E H R R G L G T R W R R L S A G R Q A L L A L	
1001	GCCCATCTCCGCAACGGTCATCCGTATGTGCAGCTCGCGGCGGGTTCGGCGTGGGCACCACCACTGCCTACCGCTACGTACCCGAGGCAGCTGAGGTTTC	1100
	A H L R N G H P Y V Q L A A G F G V G T T T A Y R Y V T E A A E V L	
1101	TGGCCGCCCTTGCCCCGACGCTCGCCGAGGCGGTCCGGGCGGCGTCGATGAAGGCGTTCGTCCTGCTGGACGGGACGCTGCTGCCGATCGACCGAATCGC	1200
	A A L A P T L A E A V R A A S M K A F V L L D G T L L P I D R I A	
1201	CGCCGACCGGCTTTCTACTCGGGCAAGCACAAGAAACACGGGTGAACGTACAGGTGATCGCAGATCCTTCGGGCCGCTGCTGTGGGCTCGCCGGCC	1300
	A D R P F Y S G K H K K H G M N V Q V I A D P S G R L L W A S P A	
1301	CTGCCCCGAGCCGTCACGACGTCCTGCGGCCCGGAGCAGCGCATCATCGACAGCTTGCCACAGCCGACGTCAACTGCTGGGCAGACAAGGGCTACC	1400
	L P G A V H D V R A A R E H G I I D T L A T A D V N C W A D K G Y Q	
1401	AGGGCGCCGAGGCACGGTCCGTGTTCCCTACCGCGCCGTTGGGAGACGCTCTGTCAGGGCAGCAGGCGTCAATCGATCCCACGCGAAGATCCGGGC	1500
	G A G G T V R V P Y R G R W E T L S A G Q Q A V N R S H A K I R A	
1501	GCTCGTCGAGCAAGCGTCGCGACCCCTCAAGTCCTGGCGACTCCTTCGAGGCTCCGTTGCTCGACACCCGCGTCACGGTCTCGTCCAGGCCGTCCTC	1600
	L V E Q A V A T L K S W R L L R R L R C S T T R V T V L V Q A V L	
1601	TGCCTGCATCAGGCCAGCTCAGCTTGAGGATGGAAAACGCT...	
	C L H Q A S S A *	

FIG. 2. Sequence of IS₄₉₃. A sequence of 1,641 bp is shown, but it may include an additional G or TG on the 5' end and an additional C or CA on the 3' end. ORF A and ORF B are translated. Possible initiation codons in ORF A and ORF B that would result in proteins of 50 amino acids or more are underlined. Possible ribosome-binding sites (15) upstream of ORFs A and B are overlined.

TY agar plates containing a 0- to 100-μg/ml gradient of apramycin (38). After a 2-day incubation at 30°C, Am^r colonies were picked and their plasmids were analyzed for insertions in the *cI857* gene. Judged on size, restriction

patterns, and, in some cases, sequence data, the observed elements included several occurrences of IS₁ and IS₅, gamma-delta, and an IS₁₀ element, which had features of both IS₁₀-right and IS₁₀-left. (IS₁₀ is not native to *E. coli*

FIG. 5. (A) ORFs in IS493. Arrowheads indicate possible initiation codons (ATG or GTG) and directions of translation. Vertical bars represent stop codons. Only ORFs with coding capacities of 50 amino acids or more are shown. The four ORFs larger than 500 bp are labeled (A, B, C, and D). (B) Restriction map of IS493. (C) G+C content of the three positions in the codons of the first reading frame, determined by using a sliding window of 60 codons. Analysis of known *Streptomyces* genes shows a strong bias for G+C content in the codons to approximate 70, 50, and 90% in positions 1, 2, and 3 (6). Two regions of IS493 containing codons with this characteristic content are indicated by arrows. The first region, in frame 1, and the second region, in frame 2, must be translated from left to right to result in the appropriate bias. Since the calculation at each position used an average of the next 60 codons, a lag of about 180 bp is expected before changes in G+C content are fully observed. 1, 2, and 3. Positions in codons of frame 1.

FIG. 6. Southern hybridization of *S. lividans* CT2 genomic DNA digests with an IS493 probe. *, Enzymes which do not cleave the IS493 sequence.

(ORFs), a restriction map, and the G+C content of the codons are shown in Fig. 5.

When WORDSEARCH was used, a comparison of polypeptides encoded by ORFs A and B did not show significant similarity to any transposon-encoded proteins in the National Biomedical Research Foundation Protein Identification Resource, release 17.0 (13), or to the possible protein encoded by the major ORF of IS110.

Analysis of genomic DNAs by Southern hybridization. A 1.6-kb *Ksp632I-SauI* fragment isolated from pCZA142 lacked only 14 bp of IS493 and contained only 5 bp of adjacent *cI857* sequence. This fragment was hybridized to *S. lividans* CT2 genomic DNA digests (Fig. 6). Three bands of similar intensity were seen in the lane containing the *BglII* digest. A shorter exposure of the film also showed three bands in the lane containing the *NcoI* digest (results not shown). In lanes containing digests with *BamHI*, *EcoRI*, *KpnI*, *SauI*, *SmaI*, and *XhoI*, only two bands of hybridization were present, and in each case one of the bands appeared to be more intense. In the case of *BsmI*, which had one site within the IS493 sequence, four bands were seen. A separate experiment (results not shown) showed three identical bands of hybridization to the *BglII* genomic digests of *S. lividans* CT2, *S. lividans* 1326, *S. lividans* TK21, and *S. lividans* TK23. There was only faint hybridization to the genomic digests of *S. griseofuscus* C581, *S. fradiae* C373.1, *S. lipmanii* LE32, and *S. thermotolerans* and no apparent hybridization to the genomic digest of *S. ambifaciens*, *S. clavuligerus*, or *S. coelicolor* A3(2), an organism closely related to *S. lividans* 1326.

DISCUSSION

Plasmid pCZA126 can be used to select for transposable elements from *Streptomyces* spp., as shown by the isolation of a new element, IS493, from *S. lividans* CT2. The plasmid contains an *Am^r* gene controlled by the lambda *cI857* repressor. This feature permitted plasmids propagated in *Streptomyces* spp. to be screened for transposable elements by transforming *E. coli* to *Am^r*.

It appears that much of the *cI857* gene is a viable target for insertional inactivation. In the case of IS493, insertion into the 3' end of the *cI857* gene resulted in a stop codon that would shorten the 237-amino-acid repressor protein by only 6 amino acids, apparently causing inactivation. In addition, the *E. coli* transposable elements isolated were inserted at several different locations throughout the *cI857* gene.

In general, transposable elements with the most utility have highly random insertion site specificity. The *cI857* gene should serve well as a target for transposable elements that insert randomly or for those that prefer A+T-rich DNA. The *cI857* gene is only 45% G+C. However, some elements with G+C-rich or specific target sites may not insert into the *cI857* gene. Theoretically, a more G+C-rich target and some specific target sites could be made by synthetically constructing a *cI857* repressor gene using alternative codons.

Plasmid pCZA126 could also be modified by incorporating origins of replication for other species and other selectable markers, if needed. The ability to select in *E. coli* for transposable elements from other organisms obviates the need to develop specific selection methods for each organism to be screened.

Selective plating for the isolation of *S. lividans* transposable elements resulted in two *Am^r* transformants that did not appear to contain an insert in the *cI857* gene. These transformants may contain point mutations, deletions, or frame shifts in the *cI857* sequence, resulting in the lack of a functional repressor, or the transformants may be spontaneous chromosomal mutations that result in *Am^r*. No plasmids of *Am^r* transformants that had gross rearrangements or deletions that resulted in loss of repressor function were found. Thus, pCZA126 may be relatively immune from the problems that occur when some bifunctional plasmids replicate in *Streptomyces* spp.

IS493 had features typical of insertion sequences. It was 1.6 kb in size, had inverted repeats at its ends, and duplicated up to 6 bp of host DNA at the target site. If the duplication was 6 bp, *EndI* of IS493 would contain a terminal A-T base pair that would not be part of the inverted repeat. Duplications of 5, 3, or 1 bp, that is, CAGTG, AGT, or G, would be the result if the element contained inverted repeats that were terminal. Since the exact extent of the duplication cannot be determined by observing one transposition, the ends of IS493 may include one or more base pairs not shown in the given sequence (Fig. 2 legend). Additional IS493 target sequences need to be examined to determine the ends of the element, the extent of host DNA duplication, and the target site specificity.

IS493 had four ORFs, A, B, C, and D, that were larger than 500 bp. The possible ORFs were identified by using both ATG and GTG as potential start codons, and the ORFs described contained internal ATG and GTG sequences that may code for the actual start of proteins produced. The sizes, locations, reading frames, and directions of translation of ORFs A and B, as determined by FRAMES of the UWGCG software package, agreed with the two probable coding regions, as determined by analysis of G+C content of the positions in the codons. Most insertion sequences contain a large ORF that encodes a putative transposase of about 300 to 400 amino acids (34). IS493, like IS1, was an exception that contained tandem ORFs in different frames. ORFs A and B had the capacity to code for proteins of 177 and 253 amino acids. The 92- and 125-amino-acid proteins of IS1 are both essential for IS1-mediated cointegrate formation (18). The functions of the putative IS493 proteins are not

known. Deduced amino acid sequences of ORFs A and B did not show significant similarity to known transposases.

On the opposite strand, IS493 contained ORFs C and D, which overlapped and were in the same frame as ORFs A and B. This organization is observed in other elements (28) and may permit effective use of a limited coding capacity. Alternatively, ORFs C and D may be the results of the scarcity of A+T-rich stop codons in regions of high G+C content.

IS493 appeared to be present in three copies in the *S. lividans* CT2 genome, as indicated by the presence of three bands of hybridization in the *Bgl*II and *Nco*I genomic digests. In several digests, only two bands of hybridization were present, and in each case one of the bands appeared to be more intense than the other. Coincidental similar migration of two unique restriction fragments that contain IS493 was a possibility. However, other possibilities included close linkage of two elements so that they comigrated on the same fragment for some digests or that two elements existed in regions of the genome with similar contexts, so that certain digests produced two similar fragments per genome, each with one copy of IS493.

The similar-context explanation was supported by the fact that a *Bam*HI genomic digest had the same two-band hybridization pattern as a *Bam*HI-*Bgl*II genomic digest (results not shown). Digestion with *Bgl*II was shown to separate the elements onto three distinct fragments. Thus, two elements could not be linked on 5-kb fragments in the more intense band observed in the *Bam*HI digest, since this band was not cut into two smaller bands upon double digestion with *Bam*HI and *Bgl*II. It appeared that this region of similar context extended at least 11.5 kb, since that was the size of the largest observed band of greater intensity. In addition, the formation of four bands of hybridization in the *Bsm*I genomic digest suggested that *Bsm*I, which had one recognition site in IS493, generated four kinds of junction fragments. This would occur if two of the copies of IS493 were in regions of similar context. A *Pst*I genomic digest showed only two apparent bands of hybridization in addition to the 0.85-kb band which represented the internal *Pst*I fragment of IS493. A total of five bands would be expected if there were four kinds of junction fragments. It is possible that the *Pst*I bands with short regions of homology to the probe were not detected.

The three copies of IS493 observed in *S. lividans* CT2 appeared to be present in the same genomic locations in other closely related *S. lividans* strains, as indicated by similar bands of hybridization to these strains. *S. lividans* TK21 and *S. lividans* TK24 lack the native plasmids SLP2 and SLP3, which indicated that these plasmids did not affect IS493 copy number or location. IS493 does not appear to exist in the other *Streptomyces* species examined, although faint hybridization in some strains may indicate the presence of other insertion elements with some similarity to IS493.

We are currently attempting to develop IS493 into a transposable element containing a selectable antibiotic resistance marker.

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