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Transposon Mutagenesis by Tn*4560* and Applications with Avermectin-Producing *Streptomyces avermitilis*

HARUO IKEDA, YOSHIO TAKADA, CHANG-HONG PANG, HARUO TANAKA, AND SATOSHI ŌMURA*

School of Pharmaceutical Sciences, Kitasato University, and Research Center for Biological Function, The Kitasato Institute, Tokyo 108, Japan

Received 1 October 1992/Accepted 27 January 1993

The Tn*3*-like *Streptomyces* transposon Tn*4560* was used to mutagenize *Streptomyces avermitilis*, the producer of anthelmintic avermectins and the cell growth inhibitor oligomycin. Tn*4560* transposed in this strain from a temperature-sensitive plasmid to the chromosome and from the chromosome to a plasmid with an apparent frequency of about 10^{-4} to 10^{-3} at both 30 and 39°C. Auxotrophic and antibiotic nonproducing mutations were, however, obtained only with cultures that were kept at 37 or 39°C. About 0.1% of the transposon inserts obtained at 39°C caused auxotrophy or abolished antibiotic production. The sites of insertion into the *S. avermitilis* chromosome were mapped. Chromosomal DNA fragments containing Tn*4560* insertions in antibiotic production genes were cloned onto a *Streptomyces* plasmid with temperature-sensitive replication and used to transport transposon mutations to other strains, using homologous recombination. This technique was used to construct an avermectin production strain that no longer makes the toxic oligomycin.

Transposable elements, including insertion sequences, transposons, and transposing bacteriophages, have been widely exploited in the genetic analysis of organisms. Transposons have been particularly valuable tools in the genetic analysis and molecular manipulation of gram-negative (1, 7, 19) and gram-positive (32) bacteria. For example, despite the large number of classical *spo* mutants of *Bacillus subtilis*, introduction of Tn*917* and Tn*917-lac* interrupted hitherto unknown sporulation genes (24, 32). The development of transposable elements for *Streptomyces* species should facilitate the genetic analysis and exploitation of these versatile secondary metabolite producers. The characteristics of some *Streptomyces* transposable elements, including insertion sequences and transposons, have been studied in detail (3).

Two transposable elements, Tn*4556* (4, 25) and IS*493* (27), have been sufficiently developed to be useful as genetic tools for transposon mutagenesis or for insertion of genetic information into the chromosome. The Tn*3*-like class II transposon Tn*4556*, found in a neomycin-producing strain of *Streptomyces fradiae* in single copy, was modified to create Tn*4560*, which carries a viomycin resistance gene (viomycin phosphotransferase; *vph*) as a selectable marker (6). IS*493* (a class I insertion sequence), found in *Streptomyces lividans* in three copies, was modified to create Tn*5096*, which carries an apramycin resistance gene (aminoglycoside acetyltransferase; *aac*(3)IV) (26). Both Tn*4560* and Tn*5096* transposed randomly from plasmid to chromosome and vice versa in *Streptomyces* spp. (5, 6, 26).

Streptomyces transposons have a number of potential applications for improving production of secondary metabolites. They might be used for gene disruption to identify and clone genes for biosynthesis of secondary metabolites and their global regulatory genes. They could also be used to block the production of unwanted secondary metabolites or unnecessary pathways to allow more efficient production of useful secondary metabolites.

* Corresponding author.

We describe here the conditions for efficient and random transposition of Tn*4560* in the avermectin-producing *Streptomyces avermitilis* (2) and applications for the efficient production of useful secondary metabolites. In addition to the anthelmintic and insecticidal avermectins, *S. avermitilis* produces oligomycin, a potent toxic inhibitor of oxidative phosphorylation in mammalian cells (23). We also describe the use of Tn*4560* to disrupt synthesis of oligomycin without affecting production of avermectins.

MATERIALS AND METHODS

Media. YMS agar and avermectin production medium were described previously (13, 14). Oligomycin production medium consisted of 20 g of glucose, 20 g of soybean meal, 10 g of corn steep liquor, 3 g of NaCl, 3 g of $CaCO_3$, and 20 g of agar per liter (pH 7.0). *S. avermitilis* and *S. lividans* protoplasts were regenerated by growing the strains on RM14 (16) and R2YE (12), respectively. Trypticase soy broth (BBL Microbiology Systems) was used for the preparation of plasmid. Luria agar was used for the selection of *Escherichia coli* transformants.

Bacterial strains and plasmids. *S. lividans* TK24 (12), *S. avermitilis* K139 (wild-type strain [13]), *S. avermitilis* K329 (13), and *S. avermitilis* K2038 (22) were used as the hosts for streptomycete plasmids. Host strains for *E. coli* plasmids were *E. coli* JM108 (K-12 F⁻ *recA1* Δ(*lac-proAB*) *endA1 gyrA96 thi-1 relA1 supE44 hsdR17*) (31) and *E. coli* GM2929 (K-12 F⁻ *galK2 galT22 ara-14 lacY1 xyl-5 thi-1 tonA31 rspL136 hisG4 tsx-78 mtl-1 glnV44 leuB6 rfbD1 dam-13*::Tn*9 dcm-6 hsdR2 recF143* McrA⁻ McrB⁻), obtained from M. G. Marinus. *Streptomyces* vector pIJ61 was described by Hopwood et al. (12). *E. coli-Streptomyces* shuttle vector pKU109 was derived from pIJ699 (18) and pUC19 (32). The temperature-sensitive replication plasmid pKU110 was constructed by in vitro hydroxylamine mutagenesis (10). Tn*4560* (Tn*4556-vph*) was delivered from plasmid pUC1169 (6).

Culture conditions and transformations. Propagation and transformation of *E. coli* and *Streptomyces* cultures have been described elsewhere (12, 20). *E. coli* transformants

were selected for resistance to 75 μg of ampicillin per ml. *S. avermitilis* transformants were selected for resistance to 15 μg of thiostrepton or viomycin per ml. For *S. avermitilis* transformants, tuberactinomycin N at 15 μg/ml could be used in place of viomycin.

Chemicals and enzymes. Thiostrepton, viomycin, and tuberactinomycin N were a gift from Asahi Chemical Company (Tokyo, Japan). Ampicillin was obtained from Nakalai tesque (Kyoto, Japan). Restriction enzymes and ligase were obtained from Takara Biochemicals (Kyoto, Japan) and New England Biolabs and used according to the specifications of the suppliers.

Transposition of Tn*4560*. Transposition of Tn*4560* from plasmid to chromosome was obtained by growing *S. avermitilis* carrying pUC1169 (5) or pKU112 for 5 to 7 days at various temperatures on YMS agar containing viomycin or tuberactinomycin N at 15 μg/ml. Spores were collected and screened for viomycin or tuberactinomycin N resistance and thiostrepton sensitivity as above. Transposition of Tn*4560* from chromosome to plasmid was obtained by growing *S. avermitilis* (chr::Tn*4560*) carrying pKU109 for 5 to 7 days at various temperature on YMS agar containing thiostrepton at 15 μg/ml. Spores were harvested and cultured for 2 days at 30°C in Trypticase soy broth plus thiostrepton at 5 μg/ml. Plasmid DNA isolated from mycelia was used to transform *S. lividans* TK24. Transformants carrying pKU109::Tn*4560* were obtained by selection for resistance to 15 μg of both thiostrepton and viomycin per ml.

Detection of secondary metabolites. Cultures containing transpositions of Tn*4560* were plated on YMS agar containing viomycin at 15 μg/ml to obtain 200 colonies per plate. Each colony was spread in about a 1-cm^2 patch on avermectin or oligomycin production agar medium, and the plate was incubated for 7 days at 28°C. Each patch colony was scraped and extracted with 0.5 ml of methanol for 15 min at room temperature. The methanol extract was concentrated under reduced pressure and applied to silica gel thin-layer chromatography plates, which were developed with *n*-hexane–isopropanol (85:15) or by reversed-phase high-performance liquid chromatography (HPLC) (22). Authentic samples of avermectins and oligomycin were used as internal standards.

Detection of auxotrophs. Cultures containing transpositions were plated on YMS agar containing viomycin at 15 μg/ml to obtain 500 colonies per plate. The plate was replicated onto minimum medium (12) containing viomycin at 15 μg/ml, and the plate was incubated for 2 days at 30°C. Auxotrophic mutants were identified by a lack of growth on minimum medium and further characterized for nutritional requirements.

Genetic mapping of transposon-induced mutations. Conventional genetic mapping methods for *Streptomyces* species (12) were used to map the transposon-induced auxotrophic mutations on the linkage map.

DNA isolation and recombinant DNA methods. Conventional DNA manipulations were performed as described by Maniatis et al. (20). *Streptomyces* plasmids were isolated by the method of Kieser (17). DNA fragments were purified from agarose gels for use in ligations or as probes by using GeneClean (Bio 101, Inc.). Southern hybridization was performed as described by Hopwood et al. (12). DNA probes were labeled with biotinylated-14-dATP by using a Random Primer Labeling Kit (Takara Biochemicals). Biotinylated DNAs on the filter were detected by the Southern-Light Chemiluminescent Detection System (Tropix) according to the manufacturer's recommendations.

Cloning of chromosomal DNA fragments containing inserted Tn*4560*. Chromosomal DNAs were purified from avermectin- or oligomycin-nonproducing mutants containing transpositions. Each chromosomal DNA was digested with *Bam*HI, and 5 μg of *Bam*HI-cut chromosomal DNA was ligated with 1 μg of *Bam*HI-cut pIJ61 in a total volume of 100 μl. *S. lividans* TK24 was transformed by the ligated mixture. Transformants containing the chromosomal DNA fragment with the inserted Tn*4560* were selected for resistance to 15 μg of both thiostrepton and viomycin per ml.

RESULTS

Transposition of Tn*4560* from chromosome to plasmid. To study transposition in *S. avermitilis*, we first transposed Tn*4560* from the chromosome of *S. avermitilis* K139-TP to a *Streptomyces-E. coli* bifunctional vector, pKU109. *S. avermitilis* K139-TP containing the transposon (K139Ω0 [chr::Tn*4560*]) was obtained by introducing pUC1169 and then allowing transposition and curing of the plasmid to occur. K139-TP was then transformed with pKU109. The transformants were spread on YMS plates containing 15 μg of viomycin per ml, and the plates were incubated at 30 or 39°C until sporulation occurred. Plasmid DNAs from each condition were isolated and used to transform *S. lividans*. In both cases, transposition events were observed, but the apparent transposition frequency was about twofold higher after growth at 39°C than at 30°C. Transposition occurred in various locations on pKU109, including both the *Streptomyces* and *E. coli* DNA regions.

Transposition of Tn*4560* from plasmid to chromosome. pUC1169 consists of the temperature-sensitive replication plasmid pMT660 and transposon Tn*4560*. However, because it was difficult to cure the plasmid by high-temperature incubation in *S. avermitilis*, it was not easy to obtain cultures containing transpositions. We therefore constructed a new derivative of the temperature-sensitive plasmid pKU110 by in vitro mutagenesis. For this construction, a DNA fragment containing an *E. coli* plasmid carrying Tn*4560* was isolated by digestion of pKU109::Tn*4560* with *Hin*dIII, and this fragment was ligated with the large *Hin*dIII fragment of pKU110 (Fig. 1). One of the resulting plasmids obtained, designated pKU112, was used for transposition of Tn*4560* from plasmid to chromosome.

pKU112 was introduced by transformation into the wild-type strain of *S. avermitilis* K139. Transformants were spread on YMS agar containing 15 μg of viomycin per ml and incubated at 37 or 39°C until sporulation took place, then replicated to YMS agar containing 15 μg of both thiostrepton and viomycin per ml. In parallel, YMS agar was used for growing transformants at 30°C with two rounds of sporulation in the absence of selection, and the plates were replicated to YMS agar containing viomycin or viomycin and thiostrepton. About 90% of the colonies obtained by growing transformants at 30°C and 99.9% of the colonies obtained by growing transformants above 37°C were viomycin resistant and thiostrepton sensitive. Some of the viomycin-resistant and thiostrepton-resistant colonies obtained by growing transformants above 37°C segregated viomycin-resistant and thiostrepton-sensitive colonies, likely reflecting resolution of pKU112 from the Tn*4560*-mediated cointegrates of the plasmid and the chromosome and subsequent loss from the cells during purification of clones. Transpositions to the chromosome were detected by the appearance of Tn*4560*-induced auxotrophs. No auxotrophs were found among 4,689 independent viomycin-resistant and thiostrepton-sensitive clones obtained by growing transformants at 30°C. In con-

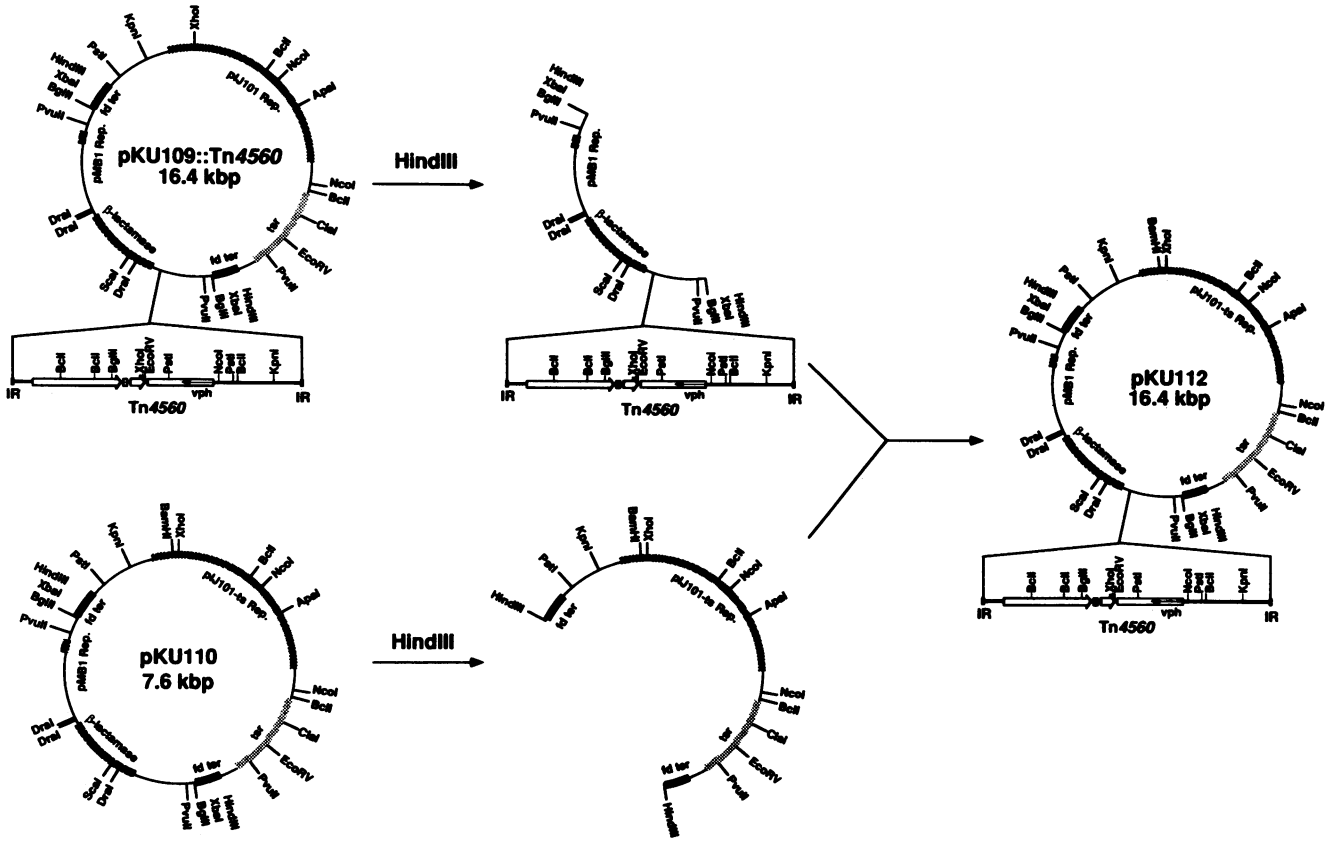

FIG. 1. Construction of pKU112. Large *Bgl*II fragment of pKU109 is derived from 4.9-kbp *Bgl*II fragment of pIJ699 containing pIJ101 replication origin and thiostrepton resistance determinant, and small *Bgl*II fragment of pKU109 is derived from 2.7-kbp *Bgl*II-linearized pUC19 via a *Bgl*II linker. pKU110 was constructed by joining the large *Pst*I-*Cla*I fragment of pKU109 and the small *Pst*I-*Cla*I fragment of a temperature-sensitive derivative of pIJ703 which had been isolated by in vitro hydroxylamine mutagenesis. Abbreviations: *tsr*, thiostrepton-resistance gene; fd ter, filamentous phage fd transcriptional terminator; *vph*, viomycin phosphotransferase gene; IR, inverted repeat sequence of Tn*4556*.

trast, several auxotrophs were isolated among clones containing transpositions obtained by growing transformants above 37°C (frequency, 6.7×10^{-4} to 1.2×10^{-3}). The highest auxotroph frequency was among clones growing at 39°C. Of eight independent auxotrophs, seven auxotrophs, A1 (Ade^-), A2 (Arg^-), A3 (Cys^-), A4 (Ilv^-), A6 (Pdx^-), A7 (Ser^-), and A8 (Cys^-), were stable, giving no prototrophic revertants ($<5 \times 10^{-8}$), but A5 (Met^-) reverted at a frequency of 3×10^{-8}.

The chromosomal insertion site of Tn*4560* in each auxotroph was determined by genetic mapping (Fig. 2). Strain A8 (Cys^-) required cysteine or methionine for growth on minimum medium and was resistant to 1 mM sodium selenate, a new phenotype in this organism. The cosegregation of viomycin resistance with each auxotrophy in all crosses was observed.

Transposition of Tn*4560* to genes involving secondary metabolite production. **(i) Avermectin.** Over 4,000 independent clones containing transpositions of Tn*4560* into the chromosome were screened for the production of avermectins. Ten avermectin-nonproducing mutants were isolated, and their morphology was identical to that of the wild-type strain K139. All 10 mutants produced oligomycin. Southern hybridization analysis of genomic DNA of the 10 mutants indicated that in 5 of the mutants, transpositions occurred

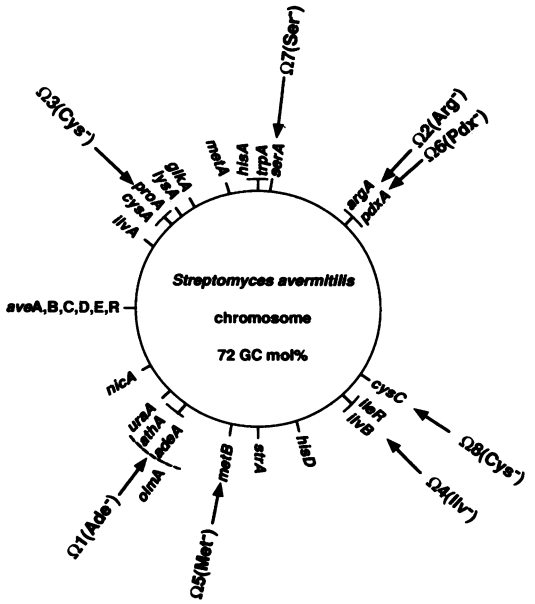

FIG. 2. Distribution of Tn*4560* insertions on the *S. avermitilis* chromosome. Arrows indicate the map positions of independent Tn*4560* insertions. The linkage map and gene symbols were described previously (13, 15).

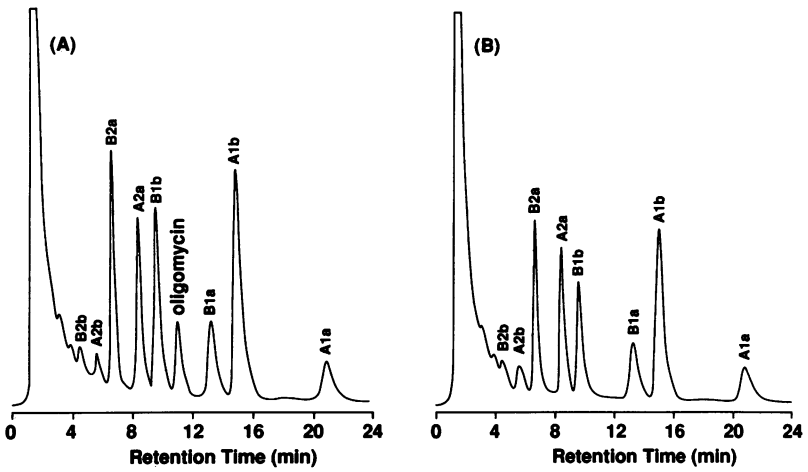

FIG. 3. Chromatograms of analytical HPLC of the mycelial extracts from the wild-type strain (A) and oligomycin-nonproducing mutant 6-13 containing Tn*4560* (B). Each strain was cultured in the liquid production medium (13) at 28°C for 7 days. Products were extracted with an equal volume of methanol for 30 min. After the removal of mycelia by centrifugation, mycelial extracts were directly applied to the HPLC column. The column was developed with acetonitrile-methanol-water (60:16:24), and the flow rate was 0.6 ml/min. Avermectins and oligomycin were detected by UV absorption at 244 nm. A1a, A1b, A2a, A2b, B1a, B1b, B2a, and B2b are eight components of avermectins.

within the same *Bam*HI fragment of the gene cluster for avermectin biosynthesis, suggesting that Tn*4560* transposition to genes involving avermectin production is not absolutely random.

(ii) Oligomycin. Among 2,400 independent clones containing transpositions, 5 oligomycin-nonproducing mutants were detected. All still produced avermectins (see, for example, mutant 6-13 in Fig. 3). Each *Bam*HI-digested chromosomal DNA was analyzed by Southern hybridization analysis, using Tn*4560* as a probe. Each mutant had a single Tn*4560* insertion, but they were mapped to four different *Bam*HI fragments (data not shown). Mutants 6-13 and 10-16 appeared to be identical.

Recombination between a cloned chromosomal DNA fragment carrying inserted Tn*4560* on a plasmid and the corresponding homologous region on the chromosome. If a cloned chromosomal DNA region containing Tn*4560* on a plasmid recombines with the corresponding homologous region on the chromosome by two rounds of reciprocal recombination or a double-crossover event, the cloned chromosomal fragment carrying Tn*4560* will replace the corresponding homologous region on the chromosome. Thus, the phenotype caused by a Tn*4560* insertion mutation in wild-type *S. avermitilis* can be transferred to another wild-type strain or other mutants of *S. avermitilis*.

Chromosomal DNA fragments of four independent oligomycin-nonproducing mutants (5-10, 6-13, 9-15, and 10-17) carrying Tn*4560* were cloned. The cloned DNA fragments were ligated with *Bgl*II-cut temperature-sensitive plasmid pKU110, and the wild-type strain *S. avermitilis* K139 was transformed by the ligated DNA. After the recombinant plasmids in the transformants were cured by high-temperature incubation, viomycin-resistant and thiostrepton-sensitive clones were obtained at a frequency of about 10^{-3}. Six independent clones derived from each cloned DNA fragment were examined for their oligomycin productivity. Only clones derived from the cloned DNA fragment of strain 6-13 failed to produce oligomycin, suggesting that it was only in mutant 6-13 that the defect in oligomycin production was due to the transposition events. The transposition sites in six clones from each mutant were identical to those in the original clones (data not shown). These results indicate that random transposition of Tn*4560* to the chromosome occurs at a frequency significantly lower than that of homologous recombination between the cloned chromosomal DNA fragment containing Tn*4560* on the plasmid and the homologous chromosomal region.

Application of transposon mutagenesis for designing a selective antibiotic producer. *S. avermitilis* mutants K329 (13) and K2038 (22) produce the avermectin aglycone and the two most effective components, avermectins B1a and B2a, respectively; both mutants also produce oligomycin. A recombinant plasmid, constructed by joining the temperature-sensitive plasmid pKU110 and the cloned chromosomal DNA fragment carrying Tn*4560* of mutant 6-13, was introduced into both mutants, K329 and K2038. Transformants were grown on YMS plate containing 15 μg of viomycin per ml for 4 days at 39°C to cure the recombinant plasmid. At this temperature, cells containing Tn*4560* in the *S. avermitilis* genome continued to grow. A few colonies appeared on the plate, and then the plate was replicated to a YMS plate containing thiostrepton and viomycin at 15 μg/ml. Thiostrepton-sensitive and viomycin-resistant clones were isolated, and the products of each were analyzed by HPLC. All clones derived from K329 and K2038 failed to produce oligomycin (Fig. 4), and clones derived from K2038 produced avermectins B1a and B2a alone.

DISCUSSION

Insertional specificity of a transposon must be low for efficient transposon-induced mutagenesis. The insertional specificity of Tn*4560* in *S. avermitilis* on a macro level was demonstrated by determining the distribution of Tn*4560*-induced mutations to auxotrophy. At 37°C, insertions of Tn*4560* caused multiple types of auxotrophy at loci widely distributed on the *S. avermitilis* genome. Recently, Yagi (30) reported the transposition of Tn*4560* in *S. avermitilis*, but no auxotrophic mutants were detected among about 7,000 independent Tn*4560* insertion mutants. Yagi (30) isolated Tn*4560* insertion mutants by growing cells at 30°C, and he concluded that Tn*4560* transposition on the *S. avermitilis* genome was

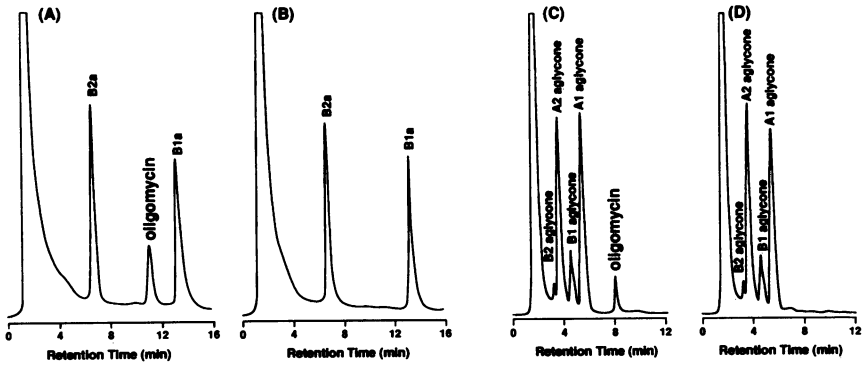

FIG. 4. Chromatograms of analytical HPLC of the mycelial extracts from K2038 (A) K2086(K2038Ω1; *olmA*::Tn*4560*) (B), K329 (C), and K2085(K329Ω1; *olmA*::Tn*4560*) (D). Analytical procedures are described in the legend to Fig. 3, except that the column was developed with acetonitrile-methanol-water (62:18:20) in panels C and D.

not absolutely randomly distributed. We observed similar results when Tn*4560* transposition to the genome was performed at 30°C. Our results suggest that transpositions of Tn*4560* to the *S. avermitilis* genome are stimulated and randomly distributed by growth at temperatures of 37°C or higher. This phenomenon might be exploited to isolate Tn*4560*-induced mutants in other *Streptomyces* species.

Eight Tn*4560*-induced auxotrophic mutants obtained in this study were stable, while reversion to prototrophy, presumably by precise excision of Tn*4560*, occurred at a very low frequency in another mutant, A5 (*metB*::Tn*4560*). Interestingly, the prototrophic revertants were still resistant to viomycin. This mutation may be caused by Tn*4560* insertion outside the essential coding sequence of the structure gene, and DNA rearrangement in or around this insertion might allow the relief of polar effects of Tn*4560* insertion or make functional but truncated proteins. Similar mechanisms of reversion have been reported in a Tn*3* insertion mutation in the plasmid ColE1 (8), in a Tn*1* insertion mutation in the bacteriophage P22 (29), in the plasmid R702 (11), in the *E. coli* chromosome (9), in a Tn*5* insertion mutation in the *Rhizobium meliloti* chromosome (21), and in a Tn*501* insertion mutation in *Pseudomonas aeruginosa* (28). Alternatively, Tn*4560* might excise precisely while transposing to a different location in the chromosome.

Chung and Crose (6) have reported that the frequency of occurrence of auxotrophs by Tn*4560*-induced mutations in *S. lividans* was 4.7×10^{-3}. Recently, Solenberg and Baltz (26) have reported that auxotrophs by Tn*5096*-induced mutations in *S. griseofuscus* occurred at a frequency of 2×10^{-3}. Auxotrophy occurred at a frequency of 1.2×10^{-3} among clones containing Tn*4560* transpositions in *S. avermitilis*.

As expected, Tn*4560* could transpose into genes involved in secondary metabolite production. *S. avermitilis* produces two kinds of secondary metabolites, oligomycin and a family of avermectins. The insertion sites of Tn*4560* in two nonproducing mutants were in the *aveA* region involved in polyketide synthesis, as shown by Southern hybridization analysis with cloned genes for avermectin biosynthesis (data not shown), causing loss of synthesis of the avermectin aglycone (13). In five nonproducing mutants, Tn*4560* transposed to the *aveR* region involved in the regulation of avermectin biosynthesis. Nonproducing mutants containing transpositions in other *ave* regions were not observed. Thus, these results indicate that the insertional specificity of Tn*4560* in the gene cluster for avermectin biosynthesis is not low.

Five oligomycin-nonproducing mutants were found among over 2,400 independent Tn*4560* insertion mutants tested, but in only two of them, 6-13 and 10-16, was the Tn*4560* insertion responsible for the loss of oligomycin synthesis. The mutants 6-13 and 10-16 were independently isolated but appeared to be the result of transpositions into the same site and with the same orientation. Mutants which were constructed by transreplacement of cloned chromosomal fragments containing Tn*4560* at its site in mutants 6-13 and 10-16 did not produce oligomycin.

Although transpositions were randomly distributed in transformants carrying the plasmid containing Tn*4560* (pKU112), recombination events between a cloned chromosomal fragment carrying Tn*4560* on a plasmid and the corresponding homologous region on the chromosome occurred in the absence of further random transpositions, even though the frequency of homologous recombination (about 10^{-3}) was similar to that of Tn*4560* transposition on the chromosome.

The wild-type strain of *S. avermitilis* K139 produces not only the anthelmintic and insecticidal antibiotic avermectin, which consists of eight closely related components, but also the toxic compound oligomycin. We have previously designed an important recombinant strain, K2038 (22), which produces two components of avermectins, the most effective compounds B1a and B2a, but the strain still produced oligomycin. Using the strategy designed above, it was possible to produce *S. avermitilis* K2086(K2038Ω1[*olmA*::Tn*4560*]), an extremely useful strain for the industrial production of avermectins because the strain produces the most effective avermectin components, B1a and B2a, and does not produce oligomycin. This obviates the need for chemical separation of avermectins and oligomycin.

In summary, our data suggest that Tn*4560* transpositions are induced by growth at high temperature, that transpositions occur at many locations on the chromosome with relatively low insertional specificity, and that Tn*4560*-based mutagenesis can be applied to clone genes involved in secondary metabolite production (biosynthetic and regulatory genes) without isolating biosynthetically blocked mutants in *S. avermitilis*. Tn*4560*-based mutagenesis has been demonstrated in *S. lividans* (6), *S. lincolnensis* (6), and *S. avermitilis*. We have also recently shown that Tn*4560* transposes in *S. griseofuscus* and *S. ambofaciens*, suggesting that it may have broad utility in *Streptomyces* species.

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REFERENCES

1. **Berg, C. M., D. E. Berg, and E. A. Groisman.** 1989. Transposable elements and the genetic engineering of bacteria, p. 879–925. *In* D. E. Berd and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
2. **Burg, R. W., B. M. Miller, E. E. Baker, J. Birnbaum, S. A. Currie, R. Hartman, Y.-L. Kong, R. L. Monaghan, G. Olson, I. Putter, J. B. Tunac, H. Wallick, E. O. Stapley, R. Ōiwa, and S. Ōmura.** 1979. Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. Antimicrob. Agents Chemother. **15**:361–367.
3. **Chater, K. F., D. J. Henderson, M. J. Bibb, and D. A. Hopwood.** 1988. Genome flux in *Streptomyces coelicolor* and other streptomycetes and its possible relevance to the evolution of mobile antibiotic resistance determinants, p. 7–42. *In* A. J. Kingsman, K. F. Chater, and S. M. Kingsman (ed.), Transposition. Cambridge University Press, Cambridge.
4. **Chung, S.-T.** 1987. Tn*4556*, a 6.8-kilobase-pair transposable element of *Streptomyces fradiae*. J. Bacteriol. **169**:4436–4441.
5. **Chung, S.-T.** 1988. Transposition of Tn*4556* in *Streptomyces*. J. Ind. Microbiol. **29**:81–88.
6. **Chung, S.-T., and L. L. Crose.** 1989. *Streptomyces* transposon Tn*4556* and its applications, p. 168–175. *In* C. L. Hershberger, S. W. Queener, and G. Hegeman (ed.), Genetics and molecular biology of industrial microorganisms, 4th ed. American Society for Microbiology, Washington, D.C.
7. **de Bruijin, F. J., and J. R. Lupski.** 1984. The use of transposon Tn*5* mutagenesis in the rapid generation of correlated physical and genetic maps of DNA segments cloned into multicopy plasmids. Gene **27**:131–149.
8. **Emerick, A. W.** 1982. Read-through transcription from a derepressed Tn*3* promoter affects ColE1 functions on a ColE1::Tn*3* composite plasmid. Mol. Gen. Genet. **185**:408–417.
9. **Harayama, S., M. Tsuda, and T. Iino.** 1981. Tn*1* insertion mutagenesis in *Escherichia coli* K-12 using a temperature-sensitive mutant of RP4. Mol. Gen. Genet. **184**:52–55.
10. **Hashimoto-Gotoh, T., and M. Sekiguchi.** 1976. Isolation of temperature-sensitive mutants of R plasmid by in vitro mutagenesis with hydroxylamine. J. Bacteriol. **127**:1561–1563.
11. **Hernalsteens, J. P., R. Villarroel-Madiola, M. van Montage, and J. Shell.** 1977. Transposition of Tn*1* to a broad-host-range drug resistance plasmid. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, J. M. Ward, and H. Schrempf.** 1985. Genetic manipulation of *Streptomyces*: a laboratory manual. The John Innes Foundation, Norwich, England.
13. **Ikeda, H., H. Kotaki, and S. Ōmura.** 1987. Genetic studies of avermectin biosynthesis in *Streptomyces avermitilis*. J. Bacteriol. **169**:5615–5621.
14. **Ikeda, H., H. Kotaki, H. Tanaka, and S. Ōmura.** 1988. Involvement of glucose catabolism in avermectin production in *Streptomyces avermitilis*. Antimicrob. Agents Chemother. **32**:282–284.
15. **Ikeda, H., and S. Ōmura.** 1991. Strategic strain improvement of antibiotic producer. Actinomycetologica **5**:86–99.
16. **Joyce, K., and G. Darland.** 1988. Protoplasts fusion in *Streptomyces avermitilis*. J. Ind. Microbiol. **3**:311–320.
17. **Kieser, T.** 1984. Factors affecting the isolation of ccc DNA from *Streptomyces lividans* and *Escherichia coli*. Plasmid **12**:19–36.
18. **Kieser, T., and R. E. Melton.** 1988. Plasmid pIJ699, a multi-copy positive-selection vector for *Streptomyces*. Gene **65**:83–91.
19. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. J. Mol. Biol. **116**:125–159.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Meade, H. M., S. R. Long, G. B. Ruvkin, S. E. Brown, and F. M. Ausbel.** 1982. Physical and genetic characterization of symbiotic and auxotrophic mutants of *Rhizobium meliloti* induced by transposon Tn*5* mutagenesis. J. Bacteriol. **149**:114–122.
22. **Ōmura, S., H. Ikeda, and H. Tanaka.** 1991. Selective production of specific components of avermectins in *Streptomyces avermitilis*. J. Antibiot. **44**:560–563.
23. **Pinna, L. A., M. Lorini, V. Moret, and N. Siliprand.** 1967. Effect of oligomycin and succinate on mitochondrial metabolism of adenine nucleotides. Biochim. Biophys. Acta **143**:18–25.
24. **Sandman, K., R. Losick, and P. Youngman.** 1987. Genetic analysis of *Bacillus subtilis spo* mutations generated by Tn*917*-mediated insertional mutagenesis. Genetics **117**:603–617.
25. **Siemieniak, D. R., J. L. Slightom, and S.-T. Chung.** 1990. Nucleotide sequence of *Streptomyces fradiae* transposable element Tn*4556*: a class-II transposon related to Tn*3*. Gene **86**:1–9.
26. **Solenberg, P. J., and R. H. Baltz.** 1991. Transposition of Tn*5096* and other IS*493* derivatives in *Streptomyces griseofuscus*. J. Bacteriol. **173**:1096–1104.
27. **Solenberg, P. J., and S. G. Burgett.** 1989. Method for selection of transposable DNA and characterization of a new insertion sequence, IS*493*, from *Streptomyces lividans*. J. Bacteriol. **171**:4807–4813.
28. **Tsuda, M., S. Harayama, and T. Iino.** 1984. Tn*501* insertion mutagenesis in *Pseudomonas aeruginosa* PAO. Mol. Gen. Genet. **196**:494–500.
29. **Weinstock, G. M., M. M. Susskind, and D. Botstein.** 1979. Regional specificity of illegitimate recombination by transposable ampicillin-resistance element Tn*1* in the genome of phage P22. Genetics **92**:685–710.
30. **Yagi, Y.** 1990. Transposition of Tn*4560* in *Streptomyces avermitilis*. J. Antibiot. **43**:1204–1205.
31. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–199.
32. **Youngman, P. J., J. B. Perkins, and R. Losick.** 1983. Genetic transposition and insertional mutagenesis in *Bacillus subtilis* with *Streptococcus faecalis* transposon Tn*917*. Proc. Natl. Acad. Sci. USA **80**:2305–2309.