TRANSPOSITION OF Tn*4560* IN *STREPTOMYCES AVERMITILIS*

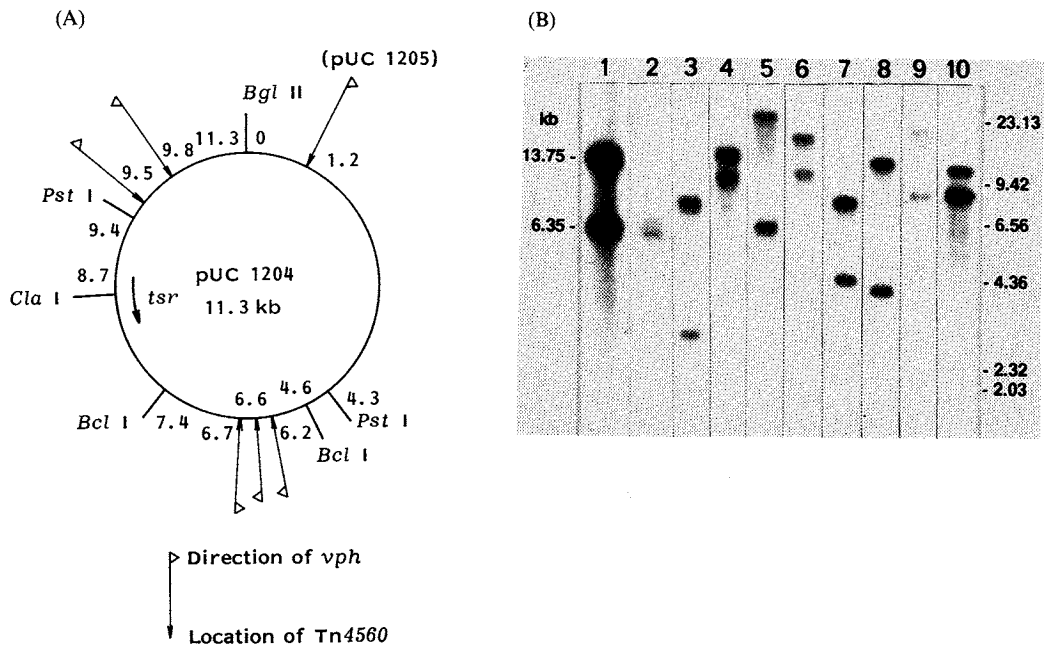
Sir:

A streptomycete transposon, Tn*4556*, was discovered in a neomycin-producing strain of *Streptomyces fradiae*.[1] The 6.6 kilobase pair (kb) transposon was modified to create Tn*4560* (8.6 kb), which now carries a viomycin-resistance gene (*vph*) as a selectable marker.[2] Employing pUC 1169, which contains Tn*4560*, CHUNG demonstrated that in *Streptomyces lividans* Tn*4560* was transposed from the plasmid to many different locations on the chromosome.[1]

In this communication, I would like to show that Tn*4560* is functional in *Streptomyces avermitilis*.[3] For studying transposition of Tn*4560* in this organism, Tn*4560* was first transposed from chromosome of *S. lividans* UC 8934 to a streptomycete plasmid pUC 1204 (11.3 kb; contains a thiostrepton-resistance marker, *tsr*[4]). Such transposition took place in different locations on pUC 1204 (Fig. 1A). One of the plasmids, designated pUC 1205, was then transformed into protoplasts of *S. avermitilis* UC 8346 (ATCC 31267) generating a viomycin/thiostrepton-resistant isolate UC 8936. Transposition of Tn*4560* was observed in this isolate after an extended period of time of cell growth; for 5 days at 30°C on Hickey-Tresner agar (HT; BBL) containing viomycin and thiostrepton at 15 μg/ml each, for additional 8 days at 28°C on HT, and finally 7 more days at 28°C on HT containing viomycin. Under this condition, 99.7% of the cells became sensitive to thiostrepton. In order to examine whether or not transposition of Tn*4560* from the plasmid to the chromosome took place in *S. avermitilis* UC 8936, chromosomal DNA was isolated by a SDS-NaCl method[5] from 12 randomly picked viomycin-resistant, thiostrepton-sensitive clones for Southern hybridization analysis.[4] The chromosome of the original strain UC 8346 does not contain homologous sequences to either pUC 1204 or Tn*4560* (data not shown). When *Bgl* II digested chromosomes from the above 12 isolates were probed with pUC 1205, at least one of the hybridized DNA fragments exhibited a molecular size greater than 5.7 kb while the other not less than 2.9 kb (Fig. 1B). This result reflects a fact that *Bgl* II digestion of Tn*4560* generates 2 fragments with a molecular size of 2.9 and 5.7 kb[1,2]. Tn*4560*

Fig. 1. Transposition of Tn*4560*.

(A) Locations of Tn*4560* on pUC 1204. (B) Southern hybridization of pUC 1205 to the chromosomes isolated from the transposed clones of *Streptomyces avermitilis*. Lane 1, pUC 1205; lanes 2~10, DNA from the clones. All samples were digested by *Bgl* II. Markers at right indicate length in kilobases.



transposition in *S. avermitilis* UC 8936 took place in various locations; 9 different places among 12 isolates examined (only different ones are shown in Fig. 1B).

CHUNG and CROSE reported isolation of 7 *S. lividans* auxotrophs out of 1,500 clones in which Tn*4560* was transposed on the chromosome.[2] Although approximately 7,000 viomycin-resistant, thiostrepton-sensitive clones of *S. avermitilis* UC 8936 were screened for Tn*4560* induced auxotrophs, no such mutants were identified. This suggests that Tn*4560* transposition on the chromosome of *S. avermitilis* is very likely not absolutely random. Another possible explanation could be that Tn*4560* was transposed from pUC 1205 to a cryptic plasmid in *S. avermitilis* which cannot be detected by ultra-centrifugation with cesium chloride employing a conventional SDS lysate and which has not yet been identified by other means.

Transposition of Tn*4560* from a plasmid to chromosome has so far been reported to occur only in *S. lividans* and *Streptomyces lincolnensis*.[1,2] The list is now expanded to include *S. avermitilis*; however, based on the observations presented in this communication, utility of Tn*4560* appears limited if one wishes to employ the transposon to generate mutations in the entire genome of this industrially important organism.

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YOSHIHIKO YAGI

Chemical & Biological Screening,
The Upjohn Company,
Kalamazoo, MI 49001, U.S.A.

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