TRANSPOSITION OF Tn4560 IN
STREPTOMYCES AVERMITILIS

Sir:

A streptomycete transposon, Tn4556, was dis-
covered in a neomycin-producing strain of Strepto-
myces fradiae. The 6.6 kilobase pair (kb) transposon was modified to create Tn4560 (8.6 kb),
which now carries a viomycin-resistance gene (vph)
as a selectable marker. Employing pUC 1169,
which contains Tn4560, Chung demonstrated that in Streptomyces lividans Tn4560 was transposed
from the plasmid to many different locations on the
chromosome.

In this communication, I would like to show that Tn4560 is functional in Streptomyces avermitilis. For studying transposition of Tn4560 in this
organism, Tn4560 was first transposed from chromosome of S. lividans UC 8934 to a strepto-
mycete plasmid pUC 1204 (11.3 kb; contains a
thiostrepton-resistance marker, tsr). Such trans-
position 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 Tn4560 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 exam-
ine whether or not transposition of Tn4560 from
the plasmid to the chromosome took place in S. avermitilis UC 8936, chromosomal DNA was
isolated by a SDS-NaCl method from 12 randomly
picked viomycin-resistant, thiostrepton-sensitive
clones for Southern hybridization analysis. The
chromosome of the original strain UC 8346 does
not contain homologous sequences to either pUC
1204 or Tn4560 (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 Tn4560 generates 2 fragments with
a molecular size of 2.9 and 5.7 kb. Tn4560

Fig. 1. Transposition of Tn4560.

(A) Locations of Tn4560 on pUC 1204. (B) Southern hybridization of pUC 1205 to the chromo-
somes 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 kilo-
bases.
transposition in \textit{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 \textit{S. lividans} auxotrophs out of 1,500 clones in which Tn4560 was transposed on the chromosome.\textsuperscript{2} Although approximately 7,000 viomycin-resistant, thiostrepton-sensitive clones of \textit{S. avermitilis} UC 8936 were screened for Tn4560 induced auxotrophs, no such mutants were identified. This suggests that Tn4560 transposition on the chromosome of \textit{S. avermitilis} is very likely not absolutely random. Another possible explanation could be that Tn4560 was transposed from pUC 1205 to a cryptic plasmid in \textit{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 Tn4560 from a plasmid to chromosome has so far been reported to occur only in \textit{S. lividans} and \textit{Streptomyces lincolnensis}.\textsuperscript{1,2} The list is now expanded to include \textit{S. avermitilis}; however, based on the observations presented in this communication, utility of Tn4560 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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References