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A Third Kasugamycin Resistance Locus, *ksgC*, Affecting Ribosomal Protein S2 in *Escherichia coli* K-12

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A third kasugamycin-resistant mutant affecting ribosomal protein S2 has been isolated from *Escherichia coli* K-12. Mating and transduction revealed that this newly recognized kasugamycin resistance locus, designated as *ksgC*, is located at 0.1 to 0.2 min from *purE*.

This note describes the mapping of a third locus, *ksgC*, for kasugamycin resistance affecting ribosomal protein S2 (9).

Kasugamycin, an antibiotic formed by *Streptomyces kasugaensis*, is used to inhibit *Pericularia oryzae*, which causes rice blast disease (14). It is classified as an aminoglycoside antibiotic and is known to inhibit protein synthesis in vitro at high concentrations with an *Escherichia coli* subcellular protein synthesizing system.

Several kasugamycin-resistant mutants of *E. coli* K-12, strain YC80 (a fusidic acid-resistant derivative of AB312:Hfr, PO12, *lac*, *thr*, *leu*, *thi*, *strA*) (12) were isolated by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1) followed by selection on a gradient plate (2) containing kasugamycin at 0 to 4,000 μg/ml. Several resistant mutants thus obtained were shown to have mutated from Lac$^-$ to Lac$^+$. This simultaneous mutation in the *lac* gene may be related to the map position of this additional locus for kasugamycin resistance in consideration of the nature of the mutagen used. One of the kasugamycin resistance mutations was found to be associated with the alteration of ribosomal protein S2 (9). The mutant (YC81) had lost the ability to serve as an Hfr but still exerted entry exclusion when used as a recipient for mating. The kasugamycin resistance of this strain was transduced by P1 *vir* (6) to another Hfr strain, W1895 (a stable clone selected from Cavalli Hfr:PO2A, *met*). Thus, a kasugamycin-resistant transductant designated as YC82 was obtained. The minimal inhibitory concentration values to kasugamycin of strains YC80, YC81, W1895, and YC82 are 320, 2,560, 80 and 160 μg/ml, respectively. Thus, the level of resistance was lower for this transductant than that for the original mutant. However, the strain YC82 was adopted for the present study because the molecular alteration of ribosomal protein S2 was observed also in this strain (9, unpublished data), and the possibility of multisite mutations as the consequence of mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine should have been decreased by transduction of the resistance marker into the strain W1895.

For mating, one volume of the Hfr culture at exponential growth phase was added to 9 volumes of the F$^-$ culture and incubated at 37 C for 75 min. Transduction was performed by the method of Lennox (8) using P1 *vir* (6). The method of selecting kasugamycin-resistant transductants was the same as that used for streptomycin-resistant transductant in our previous report (12). L broth, L agar, and L soft agar (10) were used in transduction and for growth of cells to prepare P1 *vir* lysates. Penassay broth (Difco) was used as an ordinary growth medium and for mating. Media for selection and scoring of the recombinants or transductants were either EMB-sugar agar or EM-sugar agar (5, 7) supplemented with appropriate nutritional requirements at 10 μg/ml. For phage sensitivity tests, EMB agar without added sugar was used. For scoring kasugamycin resistance, the drug was added at 50 μg/ml.

The mutagen, *N*-methyl-*N'*-nitro-*N*-nitroguanidine has been known to provoke multisite mutations at adjacent loci. As the original kasugamycin-resistant mutant from strain YC80 had been reverted to Lac$^+$, it seemed likely that the kasugamycin locus might be located near the *lac* gene. Therefore, a mating of the kasugamycin-resistant transductant, strain YC82, with the F$^-$ strain JE346, which harbors several mutations near *lac*, was carried out. The markers *lac*$^+$, *ara*$^+$, *purE*$^+$, and *mtl*$^+$ were selected separately, with counterselection for *met*$^+$. Recombinants were purified by successive single colony isolations and scored for unselected markers. The results obtained revealed a very close linkage of this kasugamycin resistance locus to *purE* (12 min on the Taylor and Trotter map [13]). This was further confirmed by transduction. In the cross between

strains YC82 and JE346 (F^-, *purE*, *tsx*, *lac*, *ara*, *mtl*, *strA*, *trp*, *gal*), 93.3% of Pur^+ transductants obtained were found to be resistant to kasugamycin. On the other hand, more than 90% of kasugamycin-resistant transductants obtained in the same cross were purine nonrequiring. These figures indicate that the responsible locus, *ksgC*, is located at 0.1 to 0.2 min from *purE*.

Thus, a newly recognized kasugamycin resistance locus, *ksgC*, was found near *purE*. Two other kasugamycin resistance loci were earlier reported. One of them, *ksgA*, is located at about 0.5 min (10) and is known to affect 16*S* RNA of 30*S* ribosome subunit (3, 4). In KsgA mutants, two adjacent adenosine nucleotides in 16*S* RNA molecules which are known to be methylated in the wild type were found not to be methylated. The lack of a specific RNA methylase is the molecular basis of this mutation. Another kasugamycin resistance locus called *ksgB* was shown not to affect ribosomes. This mutation is at 25 to 39 min and is thought to be associated with an alteration in permeability to kasugamycin (11).

It is interesting that this additional locus, *ksgC*, affects the ribosomal protein S2, but the location of this gene is distinct from the *strA* gene, where ribosomal mutations lie in a cluster.

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