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Correspondence

A unique aminoglycoside-O-phosphorylating activity mediating resistance to aminoglycosides in *Escherichia coli*

Sir,

The limitation of disc diffusion tests was recently illustrated by a report on the failure of the test to detect moderate levels of resistance to tobramycin in kanamycin-resistant strains of *Escherichia coli* (Santanam, 1984). The resistance in two representative strains was found to be mediated by an aminoglycoside-O-phosphorylating enzymatic activity having a novel spectrum of substrates. This report presents the results of additional studies on the phosphorylating activity and its genetic basis in the two strains.

The two strains PS 5 and PS 10, having a moderate level of resistance to gentamicin and tobramycin (MICs 5 mg/l), were susceptible to amikacin (MIC 13 mg/l) and netilmicin (MIC 6 mg/l) (National Committee for Clinical Laboratory Standards, 1982). Both the strains transferred the kanamycin resistance along with ampicillin resistance to the recipient strain HK 254 (F^-, R^-, Lac^-) by conjugation (Table I), at a frequency of 10^{-2} and 10^{-3} respectively. However, strain PS 5 alone transferred resistance to tetracycline as well. The transfer of resistance markers in *E. coli* PS 5 was mediated by a plasmid that was >54·2 kb and that could be eliminated by treatment with acriflavin. The resulting derivative was phenotypically susceptible to kanamycin, streptomycin, ampicillin and tetracycline by disc diffusion tests (Table I). Strain PS 10, in addition to a large plasmid similar to the one found in PS 5, had two other detectable plasmids with a size of 6·1 kb and 5·4 kb. However, treatment of PS 10 with acriflavin selectively eliminated resistance only to kanamycin. The resulting derivative was phenotypically susceptible only to kanamycin, but not to streptomycin, ampicillin or tetracycline. It retained all the plasmids found in the wild strain, without any apparent change due

Table I. Resistance pattern of *E. coli* strains used in mating experiments[c]

E. coli strain		[a]Disc diffusion test result					
		[b]S	K	AM	CF	C	T
HK 254 (F^-, R^-) Lac^-	Recipient	R	S	S	S	S	S
PS 5 Lac^+	Donor	R	R	R	S	S	R
PS 5 – HK 254	Trans-conj	R	R	R	S	S	R
PS 5 (E)	Eliminant[d]	S	S	S	S	S	S
PS 10 Lac^+	Donor	R	R	R	R	R	R
PS 10 – HK 254	Trans-conj	R	R	R	S	S	S
PS 10 (E)	Eliminant	R	S	R	R	S	S

[a]R, Resistant; S, susceptible

[b]S, Streptomycin; K, kanamycin; AM, ampicillin; CF, cephalothin; C, chloramphenicol; T, tetracycline

[c]Mating was performed first by growing for 4 h at 37°C cultures of the donor and the recipient grown overnight and diluted in brain-heart infusion (BHI) to 1:10 and 1:100 respectively. One millilitre of the donor culture was mixed with 4·5 ml of the recipient, the volume made up to 10 ml in BHI and incubated at 37°C. Transconjugants were selected from 100 µl samples taken after 2 h and 24 h of incubation, on Mackonkey agar incorporating 4000 mg/l of streptomycin and 500 mg/l of kanamycin.

[d]Eliminants were selected from BHI cultures incorporating with 16 mg/l of acriflavin stored at 37°C for 4 weeks.

Table II. Phosphorylation of aminoglycosides by the cell extract of *E. coli* PS 10

Drug	^{32}P incorporated [a](cpm/3 nmol)	Efficiency (%)	[b]MIC (mg/l)	[c]Total inactivation
Ribostamycin	46,154	100	>100	3 nmoles
Paromomycin	38,827	84·12	>100	3 nmoles
Neomycin	35,811	77·59	>100	3 nmoles
Butirosin	22,041	47·76	>100	3 nmoles
Kanamycin	21,423	46·42	>100	3 nmoles
Lividomycin	16,428	35·59	>100	3 nmoles
Streptomycin	11,797	25·56	>100	3 nmoles
Netilmicin	8740	18·94	6	80 pmoles
Amikacin	8106	17·56	13	80 pmoles
Gentamicin	7722	16·73	5	80 pmoles
Sisomicin	7519	16·29	—	80 pmoles
Tobramycin	2940	6·37	5	80 pmoles
Dibekacin	2941	6·37	—	80 pmoles
Spectinomycin	0	0	—	not tested

[a] A value of 978 cpm from the control without drug has been deducted from the experimental values.
[b] MIC was tested by spreading a suspension of 1×10^5 to 4×10^5 cells in 100 µl of Mueller-Hinton broth on Mueller-Hinton agar containing an aminoglycoside as described elsewhere (Santanam, 1984).
[c] Total inactivation was determined by the microbiological assay (Santanam & Kayser, 1976*a*).

to loss of the kanamycin resistance as discerned by agarose gel electrophoresis of plasmid DNA (Kieser, 1984).

The cell extract obtained by the osmotic shock procedure (Santanam & Kayser, 1976*b*), phosphorylated ribostamycin with maximum efficiency (Table II). The efficiency of phosphorylation of netilmicin and amikacin was 16% that of ribostamycin and that of tobramycin and dibekacin was only 6%. Phosphorylation resulted in the inactivation of the drugs tested in the range of 3 nmoles to 80 pmoles (Santanam & Kayser, 1976*a*).

The phosphorylating activity described here has a unique spectrum of substrates that encompasses the spectrum of substrates of both aminoglycoside-O-phosphotransferase (3′) III and aminoglycoside-O-phosphotransferase (2″) (Foster, 1983) both of which are usually associated with Gram-positive organisms. Since 3′, 4′-dideoxykanamycin B (dibekacin) and gentamicin, in addition to tobramycin, are substrates for the enzymatic activity, phosphorylation occurs presumably in the deoxystreptamine moiety (ring II) or aminohexose III of the aminoglycoside molecule. Whether phosphorylation of the different hydroxyl groups is mediated by a single novel enzyme or by a combination of enzymes remains to be determined, as does the exact molecular weight of the large plasmid.

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