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Short communication

Nitrofurantoin-resistant mutants of *Escherichia coli*: Isolation and mapping

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Summary. Mutants of *Escherichia coli* resistant to nitrofurantoin have been isolated. The mutations, designated *nfnA* and *nfnB* were introduced individually into a multiply auxotrophic *E. coli* F^- strain and mapped by conjugation and transduction. *nfnA* is located at 79.8 min and *nfnB* at 13.0 min on the *E. coli* chromosome.

Nitrofurans are a family of synthetic, broad spectrum antibiotics, widely used in medicine, food preservation etc., (Conklin 1978; Gleckman et al. 1979). Nitrofurantoin (NF; 1-[5-Nitro-2-furfurylidene) amino] hydantoin) is a prominent nitrofuran derivative used against human urinary tract infections (Kala and Ausborn 1971). These compounds have also been found to be mutagenic and carcinogenic (Cohen 1978). Although nitrofurans have been known for over 40 years their exact mode of action remains unclear (Gleckman et al. 1979; Delsarte et al. 1981; for reviews see Conklin 1978; Gleckman et al. 1979).

Asnis et al. (1952) and Asnis (1957) reported that nitrofurans are reduced to derivatives which are more toxic than the parent compounds in *E. coli*. McCalla et al. (1970) showed that reduction of nitrofurans is a crucial step in their mutagenic and carcinogenic activities. McCalla et al. (1970; 1978) and Bryant et al. (1981) showed that *E. coli* has at least two types of nitro-reductases: The oxygen-insensitive type I reductases and the oxygen sensitive type II reductase. McCalla et al. (1978) isolated nitrofuranzone-resistant mutants of *E. coli* deficient in type I nitro-reductases and mapped the mutations (*nfsA* and *nfsB*, presumably the genes coding for aerobic reductases) near the *gal* operon. However, their data do not give the precise map position of these mutations and they suggest that the probable gene order could be *lac-nfsB-gal-nfsA* (McCalla et al. 1978; Bachmann 1983). The isolation of nitrofuran-resistant mutants defective in the reduction of the drug, confirms that reduction is essential for the activity of these drugs. Moreover, it has been observed (Arai et al. 1975) that some R-factors could confer resistance to nitrofurans by suppressing cellular nitro-reductase activities.

In the present work we report the isolation and mapping of mutants of *E. coli* resistant to nitrofurantoin which still retain the ability to reduce the drug. These mutants are novel since they reduce the drug both in vivo and in vitro. The mutations are mapped at different chromosomal loci from those reported by McCalla and co-workers (1978).

Since we failed to isolate spontaneous mutants resistant to NF (20 μg/ml) on glucose-M9-medium, we resorted to nitrosoguanidine (100 μg/ml) mutagenesis (Adelberg et al. 1965) of *E. coli* KL16 (*Hfr*). Each of the mutants SSJ-1 through SSJ-5 is an independent isolate. SSJ-2A and SSJ-2B (both derivatives of CSH57 F^-) were constructed by P1 transduction using SSJ-2 as donor. All these strains except SSJ-2A are resistant to 25 μg NF per ml in glucose minimal medium. SSJ-2A is resistant to 12.5 μg NF per ml while the parental strains KL16 and CSH57 are sensitive to more than 3–5 μg NF per ml in minimal medium. Before undertaking further characterization of these mutants it was necessary to know their capacity to reduce NF since the nitrofurazone-resistant mutants isolated previously (McCalla et al. 1978) were reported to be deficient in this property. It was also necessary to rule out that resistance was due to defects in the entry of the drug into the cell. Table 1 shows the rate of reduction of NF by intact cells and cell-free extracts of the parent and the mutants. It is important to note that all the mutants reduced the drug in vivo and in vitro. Mutant SSJ-2, in particular, reduced the drug at

Table 1. Rates of NF-reduction by intact cells and cell-free extracts[a]

Strain	Rate of reduction	
	Intact cells[b]	Cell-free extract[c]
KL16	11.72	235
SSJ-1	3.60	48
SSJ-2	12.50	226
SSJ-3	3.90	65
SSJ-4	3.22	59
SSJ-5	3.30	78
SSJ-2A	3.65	102
SSJ-2B	3.22	82

[a] The rates were calculated from the linear portions of the reaction curves
[b] Aerobic reduction, expressed as μ moles of NF reduced/h/$A_{600}=1.5$
[c] Aerobic reduction, expressed as n moles of NF reduced/min/mg protein

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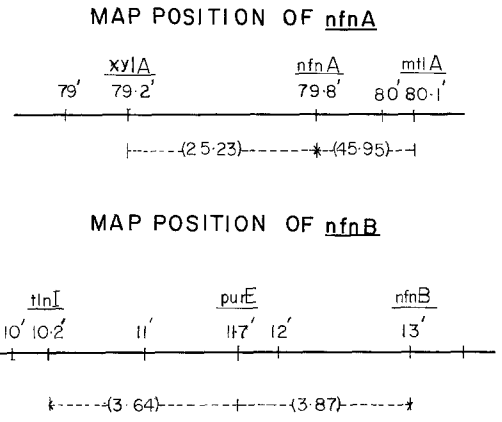

Fig. 1. Map position of *nfnA* and *nfnB*. The figure shows the relevant portions of the linkage map. The arrows indicate unselected markers. Numbers in parantheses are cotransduction frequencies. For transductional mapping of *nfnA*; the donor was *E. coli* KL16 (*Hfr: thi relA*) and the recipient was SSJ-2A (NF-resistant derivative of CSH57:*F⁻ ara leu lacY purE gal trp his argG malA rpsL xylA mtlA ilvD metA thi*). NF-sensitive transductants were scored on minimal medium among the Mtl^+ and Xyl^+ transductants. The linkage was determined from cotransduction frequencies (mean of four independent experiments; Wu 1966). Similarly for transductional mapping of *nfnB*, both KL16 and NSJ74 (Sivasubramanian and Jayaraman 1980) were used as donors and SSJ-2B (NF-resistant derivative of CSH57) as the recipient. The percentage of NF-sensitive and thiolutin-resistant transductants were calculated (mean of two independent experiments)

the same rate as the parent (KL16). Since the pattern of NF reduction both in vivo and in vitro is qualitatively similar there is no impairment to the entry of the drug into the cell. This finding was also corroborated by other studies such as the uptake of radioactively labelled compounds (unpublished).

Next, we mapped these mutants as follows: First, the approximate location of the mutation in SSJ-2A (*nfnA*) was determined by crossing it with different *Hfr's* (Miller 1972) and scoring for NF-sensitive-recombinants using various markers. This suggested that *nfnA* was near the *ilv-xyl* segment (79′–80′). Transductional mapping (Miller 1972) was done using P1 *vir* propagated on *E. coli* KL16 and scoring for NF-sensitives among Mtl^+ and Xyl^+ transductants of SSJ-2A. From the transductional frequencies (Wu 1966), it was calculated that *nfnA-mtlA* (45.95%) and *nfnA-xylA* (25.23%) distances were 0.3 min 0.6 min respectively. The gene order was therefore either *xylA-mtlA-nfnA* or *xylA-nfnA-mtlA*. However, the correct gene order is *xylA-nfnA-mtlA* because *nfnA* is also cotransducible at an appreciable frequency with *xylA* (Fig. 1). Similarly, the mutation in SSJ-2B (*nfnB*) was mapped at 13.0 min (Fig. 1).

To our knowledge, this is the first time, that novel mutants of *E. coli*, resistant to nitrofurantoin, have been isolated and mapped. The two mutations governing resistance to NF map at 79.8 min (*nfnA*) and 13.0 (*nfnB*), the positions of the mutations being different from those reported by McCalla et al. (1978). Moreover, these mutants reduce NF both in vivo and in vitro. The fact that SSJ-2 reduced NF as efficiently as the parent (*E. coli* KL16) made us study its properties in greater detail. Further studies have shown that SSJ-1, 3, 4 and 5 are of the SSJ-2B type (probably carrying the same mutation, Sastry and Jayaraman 1984).

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