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Short Communication

envB Mutations Confer UV-Sensitivity to *Salmonella typhimurium* and UV-Resistance to *Escherichia coli*

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Summary. An *envB* mutation isolated in *Salmonella typhimurium* LT2 was transferred by conjugation to *Escherichia coli* K-12. The mutation produced the same alterations in *E. coli* as in *S. typhimurium* concerning cell shape, sensitivity to drugs, autolysis, and fermentation of carbohydrates. However, although the mutation conferred sensitivity to UV irradiation in *Salmonella*, in *E. coli* it behaved as a genuine *envB* mutation producing resistance to UV inactivation. The fact that the mutation produced opposite effects in the survival of UV-irradiated *S. typhimurium* and *E. coli* discloses an intriguing difference between these closely related species.

A round cell mutation recently described in *Salmonella typhimurium* was assigned, on the basis of genetic location and general properties, to the gene known in *Escherichia coli* as *envB* (Antón 1972, 1978). However, the behavior of that *S. typhimurium envB* strain presented an important difference when compared with the reported properties of *E. coli envB* mutants. Thus, whereas the latter are characterized by increased resistance to UV inactivation (Adler et al. 1968; Westling-Häggström et al. 1975), the *Salmonella envB* mutant showed increased sensitivity to UV irradiation (Antón 1978).

Only one *envB* mutation has been identified up to now in *S. typhimurium*; therefore, to find out whether the discrepancy was due to the particular *envB* allele studied in *Salmonella* or to some intrinsic difference between the two species, the *Salmonella* mutation (*envB4*) was transferred to *E. coli* and its effects on the latter species were studied.

To transfer *envB4* to *E. coli* a cross was performed between strain DA223, an F^+ *Salmonella* donor carrying *envB4* (see Table 1 for details of the strains) and JC411, an *E. coli* recipient carrying an *argG* mutation. As gene *envB* is located halfway between markers *argG* and *rpsL* (Westling-Häggström et al. 1975; Antón 1978), $argG^+$ *rpsL* recombinants were selected. Only four recombinants were obtained, and two of them presented the typical round cell morphology conferred by *envB4* whereas the other two displayed a bacillar shape like the recipient. Except for the marker *argG6* employed for the selection and, as explained above, in two cases also the mutation *envB4*, the four recombinants retained all the other markers present in the recipient strain. The four strains displayed sensitivity to MS2 phage, thus demonstrating they had also received the F factor of the donor. Further studies were carried out with one strain of each kind: DA253, an *envB4* derivative, and DA252, which retained $envB^+$ character.

Many *E. coli-S. typhimurium* hybrids are unstable. Therefore, the two strains were tested for segregation of arginine-requiring cells. After prolonged growth in tryptone-yeast extract broth followed by penicillin enrichment, no arginine-requiring colony was found and only one spontaneous proline mutant was recovered from strain DA252. Treatment of strains DA252 and DA253 with acridine orange led to loss of the F factor, as demonstrated by recovery of resistance to phage MS2. Both cured strains retained their arg^+ character, and DA253 also maintained the *envB4* mutation, showing that those properties were not linked to the F factor.

Strain DA253 was able to transfer the *envB4* mutation back to *Salmonella*. In a cross employing DA253 as donor and DA475, an *aroE32* $rpsL^+$ derivative of *S. typhimurium* LT2 (Table 1), as recipient,

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Table 1. Bacterial strains used

Strain	Relevant markers	Source or derivation
S. typhimurium LT2		
DA223	*envB4 mal-591* F^+	Antón 1978
DA475	*aroE32 gal-1921*	Antón 1978
DA540	*aroE*$^+$ *envB4* F^+	DA253 × DA475
DA541	*aroE*$^+$ *envB*$^+$ F^+	DA253 × DA475
DA606	*envB4* F^-	DA540 treated with AO
DA607	*envB*$^+$ F^-	DA541 treated with AO
E. coli K-12		
JC411	*argG6 metB1 leu-6 his-1 rpsL104 malA1 mtl-2 xyl-4 gal-6*	B. Bachmann, Coli Genetic Stock Center
DA252	*envB*$^+$ *argG*$^+$*metB1 leu-6 his-1 rpsL104* F^+	DA223 × JC411
DA253	*envB4 argG*$^+$*metB1 leu-6 his-1 rpsL104* F^+	DA223 × JC411
DA537	*envB*$^+$ F^-	DA252 treated with AO
DA538	*envB4* F^-	DA253 treated with AO

Only relevant markers are shown. For explanation of the genetic symbols, which are the same for both species, see Sanderson and Hartman (1978). In the crosses the donor appears first. Gene *rpsL* was formerly known as *strA*. AO is acridine orange. Hybrid strains like DA252 and DA253 are depicted as *E. coli* because most of their chromosome belongs to this species; although the presence of some *E. coli* markers in strains like DA540 and DA541 has not been excluded, they are assumed to be *S. typhimurium*.

aroE$^+$ *leu*$^+$ *his*$^+$ *met*$^+$ recombinants were selected. Of 24 colonies obtained, 4 displayed *envB4* phenotype; in spite of the close proximity of the *aroE* and *rpsL* loci, none acquired the *rpsL* allele of the donor, which proceeded from the original *E. coli* JC411 strain and was probably selected against by the *Salmonella* recipient. It is concluded that strains DA252 and DA253 are stable *E. coli-S. typhimurium* hybrids.

From the last cross described, one *envB4* (DA540) and one *envB*$^+$ derivative (DA541) were saved to compare their properties with those of *E. coli* strains. All four strains were previously treated with acridine orange to eliminate the F factor, since it somehow appears to affect some of the characteristics studied.

It was found that most of the properties of the *envB4 E. coli* strains (DA538) corresponded to those displayed by *envB4 Salmonella* strains (i.e. DA606). As shown in Table 2, strain DA538 showed increased sensitivity to deoxycholate, penicillin, and cycloserine. Furthermore, it showed enhanced autolytic activity in TRIS-HCl, 0.05 M, pH8.5, and anomalous fermentation of mannose on EMB plates, as was also the case with *Salmonella envB4* strains (Table 2).

On the other hand, submission of the four strains to the lethal action of UV light demonstrated, as reported before (Antón 1978), that the *envB4* allele increases the sensitivity of *S. typhimurium* strains (Fig. 1). However, like genuine *E. coli envB* mutations, *envB4* increased UV resistance when present in *E. coli* strains (Fig. 1).

These results demonstrate that the opposite phenotype reported for *envB* mutants of *E. coli* and *S. typhimurium* in regard to UV inactivation is due to intrinsic differences between those species. Such differences manifest themselves only in this aspect, since other alterations produced by *envB* mutations are similarly expressed by the two species.

The enzymatic repair of UV-damaged DNA has not been studied in *S. typhimurium* as extensively as in *E. coli*; however, the same main mechanisms appear to operate since, like in *E. coli*, *Salmonella* mutants affected in excision repair and mutants with alterations in post-replication repair have been isolat-

Table 2. Properties of *envB4* and *envB*$^+$ derivatives of *E. coli* and *S. typhimurium*

Species	Allele	Cell shape	Response to			Autolysis (%)	Fermentation of mannose
			DOC	Pen	Cyc		
E. coli	*envB4*	Round	S	S	S	39.5	Anomalous
	envB$^+$	Rod	R	R	R	12.1	Normal
S. typhimurium	*envB4*	Round	S	S	S	52.6	Anomalous
	envB$^+$	Rod	R	R	R	23.7	Normal

E. coli strains used: DA538 (*envB4*) and DA537 (*envB*$^+$); *S. typhimurium* strains used: DA606 (*envB4*) and DA607 (*envB*$^+$). All the tests were performed as described by Antón (1979). Media contained Deoxycholate (DOC) 3 mg/ml; penicillin (Pen) 7 IU/ml; cycloserine (Cyc) 20 µg/ml with *Salmonella* strains and 15 µg/ml with *E. coli* strains. S: sensitive; R: resistant. Autolysis is expressed as the percentage decrease in OD (650 nm) of cells maintained for 2 h in 0.05 M TRIS-HCl, pH 8.5, at 37° C. Mutation *envB4* alters the fermentation of several carbohydrates in *S. typhimurium* (Antón 1978, 1979); however, as most of them are not fermented by the original *E. coli* strain employed, JC411 (Table 1), only mannose was tested. Mannose 0.1% was used in EMB agar. Normal fermentation leads to dark colonies with green metallic sheen; anomalous fermentation leads to opaque reddish colonies

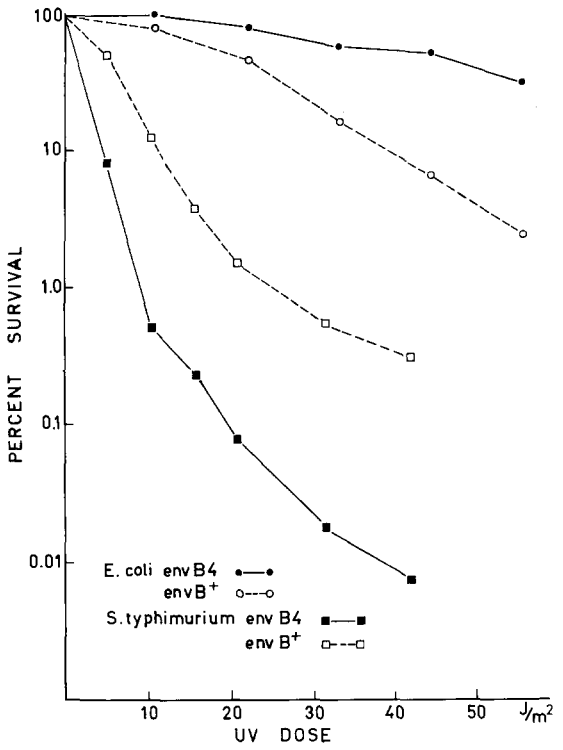

Fig. 1. Exponential cells grown in tryptone-yeast extract (TY) broth were centrifuged and resuspended at OD (650 nm) 0.100 (about $5–10 \times 10^7$ cells/ml) in 0.5% saline. Three milliliters of each suspension were placed in 60-mm glass petri dishes and irradiated with a General Electric germicidal lamp G15 T8, (General Electric, Schenectady, USA). The cells were constantly stirred during irradiation and, at intervals, samples were withdrawn, suitably diluted, and plated on TY agar for viable cell count. The whole procedure was carried out under dim light and the plates were incubated in the dark to avoid photoreactivation. UV intensity was measured with an Ultraviolet Products UV-meter (Ultraviolet Products, San Gabriel, USA). *E. coli* strains used: DA538 (*envB4*), DA537 (*envB*$^+$); *S. typhimurium* strains used: DA606 (*envB4*), DA607 (*envB*$^+$)

ed (Wing et al. 1968; Stouthamer 1969; Eisenstark et al. 1969; Andreeva et al. 1972; Skavronskaya et al. 1974). Notwithstanding, wild-type *S. typhimurium* is markedly more sensitive to UV irradiation than wild-type *E. coli* (Kondratiev et al. 1977), as can also be seen in Fig. 1. Other recent data appear to have bearing on this point inasmuch as it has been observed that the sealing of single-strand breaks produced in UV-irradiated DNA is defective in wild-type *S. typhimurium* LT2 (Kondratiev et al.1977). Moreover, a low level of activity of inducible error-prone repair has been observed in *S. typhimurium* (Walker 1978), in agreement with reports on poor mutagenicity by UV in that species (Skavronskaya 1978).

Further investigation is needed to understand the connection between cell envelope and UV lethality, and to find out whether *envB* contradictory behavior in such related species as *E. coli* and *S. typhimurium* is caused by differences in their cell envelope, the mechanisms they employ for repairing UV-damaged DNA, or both.

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