JOURNAL OF BACTERIOLOGY, June 1969, p. 1274-1277
Vol. 98, No. 3

Mutation in *Escherichia coli* K-12 Mediating Spherelike Envelopes and Changed Tolerance to Ultraviolet Irradiation and Some Antibiotics

STAFFAN NORMARK
Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden

Received for publication 20 March 1969

In a mutation experiment with a rough, ampicillin-resistant strain of *Escherichia coli* K-12, two smooth ampicillin-sensitive mutants were isolated. One of the mutants (with the *envA* gene) was recently described. The second mutant (strain D23) with the *envB* gene which has been mapped to a position close to streptomycin resistance (*strA*) at 64 min is described. The *envB* gene gives rise to spherelike cells. Electron microscopy revealed an abnormal septum formation and a circular distribution of the nuclear material. Strain D23 (with *envB*) showed a changed resistance to several antibiotics as well as an increased tolerance to ultraviolet irradiation. The *envB* gene decreased the ampicillin resistance mediated by the *ampA* gene (at 82 min), but no effect was found on episomally mediated penicillin resistance.

In a previous genetic study of a highly ampicillin-resistant mutant of *Escherichia coli* K-12, we observed some classes of recombinants which always produced smooth colonies (2). To investigate a possible relationship between smooth-colony formation and ampicillin resistance, a number of smooth mutants were isolated from a rough parent strain (D21) containing the *ampA* gene for ampicillin resistance. Two mutants were found which both showed a decreased ampicillin resistance. One of them, the chain-forming mutant D22 with the *envA* gene, was recently described (5).

Here I describe another envelope mutant (strain D23) which was obtained in the same mutation experiment as the chain-forming mutant (D22). The gene mutated in D23, designated *envB*, maps near streptomycin resistance (*strA*) at 64 min [time scale and gene designation of Taylor and Trotter (6)]. The presence of *envB* gives rise to spherelike cells resembling those recently described by Adler et al. (1).

MATERIALS AND METHODS

The mutation experiment and all methods and media were described recently (5). The donor strains used were described by Low (4); AB673 is believed to be the same strain as J4 (6).

RESULTS AND DISCUSSION

The *envA* gene was earlier found to counteract both episomally and chromosomally mediated resistance (5). Table 1 shows that the main effects of *envB* were the suppressing of the *ampA* phenotype and the "natural" resistance to actinomycin D. The latter effect is known to be due to a permeability barrier in the ethylenediaminetetraacetate-sensitive surface layer of the bacteria

TABLE 1. *Effect of the envB gene on chromosomally and episomally mediated resistance to antibiotics*

Antibiotic tested	Resistance[a] on plates (μg/ml)			
	D21[b]	D23[c]	D21-R1a[b]	D23-R1a[c]
D-Ampicillin	18	6	100	100
Penicillin G	150	30	200	200
D-Cycloserine	20	30		
Kanamycin	1	0.3		
Chloramphenicol	2	2	400	400
Nalidixic acid	3	6		
Streptomycin	1,000	1,000		
Spectinomycin	4	2		
Actinomycin D	>10	1		

[a] Resistance was determined as the maximum concentration permitting single-cell colony formation. The resistance factor R1a mediates resistance to ampicillin, chloramphenicol, and sulfonamide. All strains carry the genes *ampA* and *strA* which mediate resistance to ampicillin and streptomycin, respectively.
[b] Genotype: +.
[c] Genotype: *envB*.

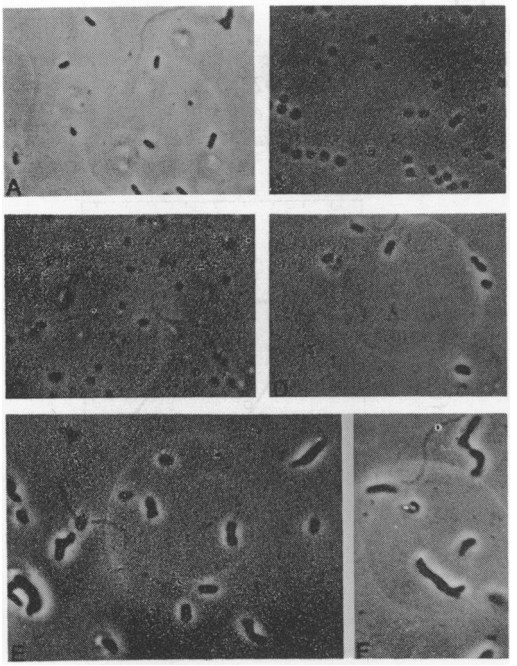

FIG. 1. *Phase-contrast photomicrographs of (A) the parent strain D21 grown on rich medium and the envB mutant D23 grown (B) on minimal medium, (C) on rich medium, (D) on plates containing nalidixic acid (3 μg/ml), (E) on plates containing nalidixic acid (5 μg/ml), and (F) in liquid culture for 3 hr with ampicillin (10 μg/ml). Magnification approximately × 920.*

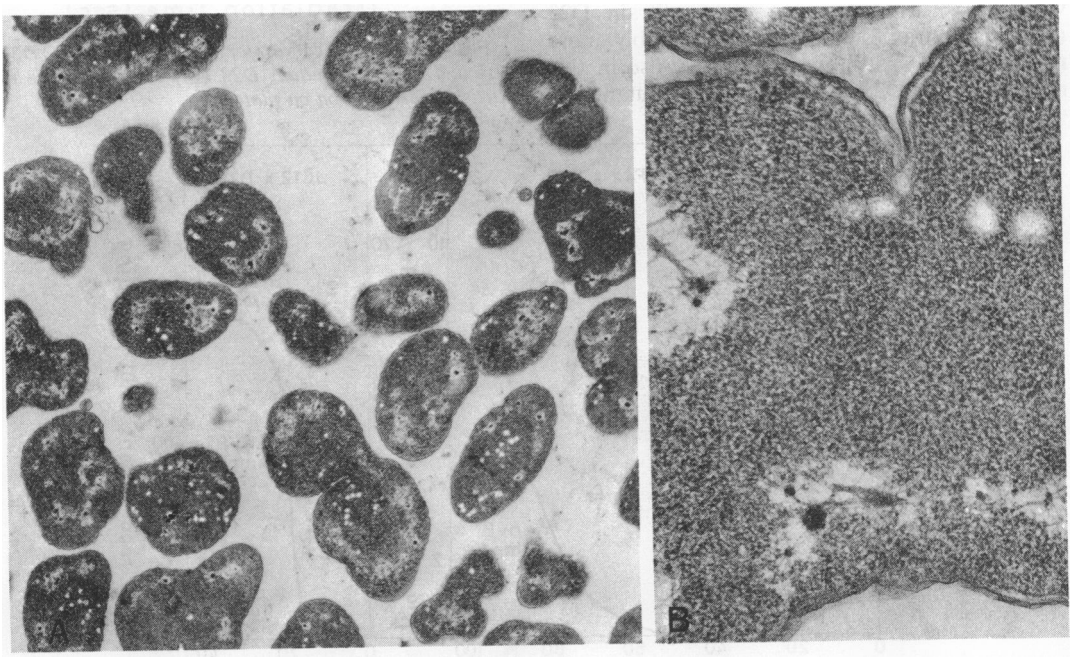

FIG. 2. *Electron micrographs of sections of the envB mutant D23. An overnight culture of D23 was diluted with a rich medium to an optical density of 10^8 cells/ml. The cells were then grown at 37 C for 90 min, harvested by centrifugation, and fixed directly by suspension in ice cold 4% glutaraldehyde in 0.1 M phosphate buffer. The cells were postfixed in 1% osmium tetroxide and processed according to standard procedures for electron microscopy. Embedding was performed in Epon 812 and sectioning was carried out on an LKB-ultrotome III electron microscope. Sections were stained with uranyl acetate and lead citrate and examined in a Philips EM300 electron microscope. (A) Cells of strain D23. ×10,000. (B) Assymetrical constriction in strain D23. ×45,400.*

(3). The actinomycin D sensitivity of strain D23 therefore indicates that *envB* in some way affects the envelope. The tolerance to nalidixic acid was increased, whereas decreased resistance was observed towards kanamycin and spectinomycin. The gene-mediating resistance toward spectinomycin is linked to *strA* (6).

The spherelike shape of strain D23 cells is shown in Fig. 1B and C. Growth in minimal or rich medium at 30 or 37 C did not produce any major differences in morphology. However, antibiotics known to induce filament formation in *E. coli* K-12 caused D23 cells to assume an elongated form (Fig. 1D-F). *E. coli* strain C normally has almost spherical cells, but after mitomycin C treatment it forms cells similar in appearance to those of strain D23 after growth with nalidixic acid [cf. Fig. 1D–E, and Suit et al. (7)].

Electron micrographs of strain D23 (Fig. 2A and B) exhibited a great variety of sizes and shapes. A characteristic feature was the peripheral distribution of the chromosomal material (Fig. 2A). There were also numerous examples of invaginations without a symmetrical counterpart as well as many round, electron-lucid areas (Fig. 2B).

Figure 3 shows a survival curve after ultraviolet (UV) irradiation on plates. Strain D23 (with *envB*) was found to be considerably more resistant than was the parent strain. Also in this respect, strain D23 is similar to the mutant described by Adler et al. (1). With D23 the shape of the UV killing curve resembles that obtained with the *envA* mutant D22 (5).

The mapping of the *envB* gene in strain D23 (F^-, *proB*, *trp*, *his*, *ampA*, *envB*, *strA*) was

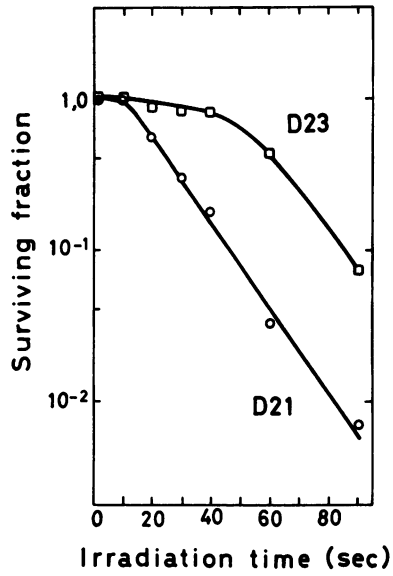

FIG. 3. *Survival curves for the parent strain D21 (○) and the envB mutant D23 (□) after exposure to ultraviolet irradiation on plates.*

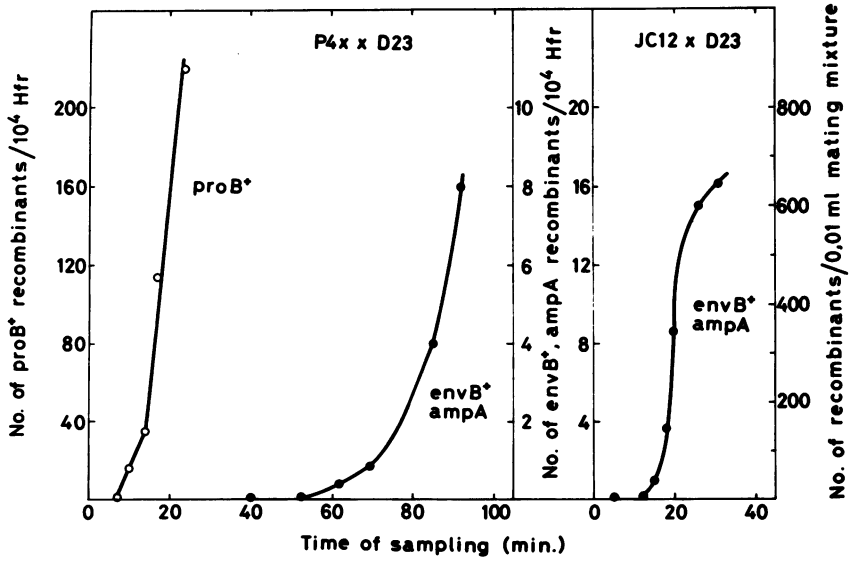

FIG. 4. *Two interrupted mating experiments in which the recipient D23 (envB, ampA) was crossed to two wild-type donor strains with the reversed order of transfering of the chromosome (see Fig. 5). Since envB phenotypically suppressed the resistance mediated by ampA, it was possible to select for envB+/ampA recombinants by using plates containing a D-ampicillin concentration of 10 μg/ml. The use of streptomycin for counter selection gave no envB+ recombinants.*

TABLE 2. *Analysis of crosses between the ampA containing envB mutant D23 and wild-type donor strains*[a]

Cross (donor × F⁻)	Selection on D-ampicillin (μg/ml)	Entrance of *envB*⁺ (min)	No. of recombinants tested	Unselected characters			
				Response to str		Cell shape	
				r	s	Sphere	Rod
P4X × D23	10	56	100	0	100	0	100
AB673 × D23	10	40	100	1	99	0	100
Jc12 × D23	10	12	100	0	100	0	100
KL41/Jc1553 × D23	15		100	0	100	0	100

[a] The properties of the donor strain are illustrated in Fig. 5. Entrance times for *envB*⁺ were determined by interrupted mating experiments as shown in Fig. 4. The recombinants from the first cross all maintained the *his*⁻ gene of the recipient; the other recombinants all grew on the minimal medium required by the recipient.

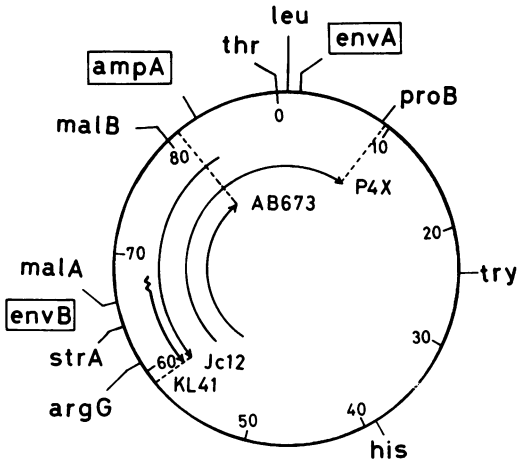

FIG. 5. *The circular chromosome of E. coli K-12 with envB, envA, and some other relevant markers. The arrowhead arcs represent the order of gene transfer of the Hfr strains and the episome KL41 (4). Time scale and reference markers according to Taylor and Trotter (6).*

achieved by crosses with three Hfr strains and one F′ strain, all four sensitive to ampicillin. Since in D23 the *envB* gene decreases the ampicillin resistance mediated by the *ampA* gene, *envB*⁺/*ampA* recombinants could be selected on ampicillin plates. Two kinetic experiments showing the time of entrance of the *envB* gene are presented in Fig. 4. The results of the crosses are summarized in Table 2 and Fig. 5. Both the time of entrance of the *envB*⁺ gene and the linkage data from the first three crosses suggest that *envB* is closely linked to streptomycin resistance (*strA*) at 64 min. Strain KL41/J1553 is rec⁻ and can only transfer an episome which covers the region 59 to 66 min (4). The fact that this strain produced ampicillin-resistant recombinants is additional evidence that *envB* is located in this region of the chromosome. Since strain D23 is resistant to phage P1, no transduction analysis can be performed. Further physiological and genetic characterization of the strain is in progress.

ACKNOWLEDGMENTS

I thank B. Low for placing strains at my disposal and for helpful discussions, and G. Bloom for preparing the electron micrographs. This work was supported by grants to H. G. Boman from The Swedish Cancer Society and The Swedish Natural Science Research Council.

LITERATURE CITED

1. Adler, H. I., C. E. Terry, and A. A. Hardigree. 1968. Giant cells of *Escherichia coli*. J. Bacteriol. 95:139–142.
2. Boman, H. G., K. G. Eriksson-Grennberg, S. Normark, and E. Matsson. 1968. Resistance of *Escherichia coli* to penicillins. IV. Genetic study of mutants resistant to D,L-ampicillin concentrations of 100 μg/ml. Genet. Res. 12:169–185.
3. Leive, L. 1968. Studies on the permeability change produced in coliform bacteria by ethylenediaminetetraacetate. J. Biol. Chem. 243:2373–2380.
4. Low, B. 1968. Formation of merodiploids in matings with a class of *rec*⁻ recipient strains of *Escherichia coli* K12. Proc. Nat. Acad. Sci. U.S.A. 60:160–167.
5. Normark, S., H. G. Boman, and E. Matsson. 1969. Mutant of *Escherichia coli* K-12 with anomalous cell division and ability to decrease episomally and chromosomally mediated resistance to ampicillin and several other antibiotics. J. Bacteriol. 97:1334–1342.
6. Taylor, A. L., and C. D. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. 31:332–353.
7. Suit, J. C., T. Barbee, and S. Jetton. 1967. Morphological changes in *Escherichia coli* strain C produced by treatments affecting deoxyribonucleic acid synthesis. J. Gen. Microbiol. 49:165–173.