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Genetic and Physiological Analysis of an *envB* Spherelike Mutant of *Escherichia coli* K-12 and Characterization of Its Transductants

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The *envB1* mutation mediating a distorted cell morphology of *Escherichia coli* K-12 was cotransducible with *strA*, *aroE*, *aspB*, and *argG*. The mapping data is consistent with a gene location for *envB* around 62.5 min. In partial diploids *envB1* was recessive to its wild-type allele. The original *envB* mutant contained a second mutation in a locus denoted *sloB* close to *strA*. The following gene order is suggested: *sloB-strA-aroE-envB-aspB-argG*. The *sloB1* mutation caused a marked reduction in the growth rate of both *envB* and *envB*$^+$ strains. Moreover, this mutation in the presence of *envB1* appears to increase the ratio between deoxyribonucleic acid and protein in cells growing in rich medium. The phenotypic properties of *envB1*, *sloB*$^+$ and *envB*$^+$ transductants were characterized. Cells with *envB1*, *sloB*$^+$ genotype were hypersensitive to several penicillins including the β-lactam compound, amidino penicillin. Penicillin hypersensitivity could not be explained by increased outer membrane penetrability. The original *envB* mutant (*envB1,sloB1*), as well as *envB1*, *sloB1* or *envB*$^+$, *sloB1* transductants were resistant to amidino penicillin. Resistance was explained by the slow growth rate mediated by the *sloB1* mutation. The similarity between *envB* cells and wild-type cells treated with sublethal concentrations of amidino penicillin was emphasized.

Bacterial cellular morphology is, as pointed out by Henning and Schwarz, a multivalent term which in itself conceals several aspects (10). Morphology can be looked upon from a static point of view in the hope of finding the shape-determining principle of the cell (8, 10). To us it has seemed natural to regard cellular morphology in a dynamic way as one aspect of growth and division. It is evident that in *Escherichia coli*, rod shape can only be maintained if the orientation of the length axes is unchanged during growth and if consecutive septations are parallel and perpendicular to the length axis of the cells. We have previously described an *envB* mutant of *E. coli* where asymmetrical constrictions are associated with a changed orientation of length axes. Therefore, consecutive division planes are not parallel, leading to a heterogeneous population of irregular and spherelike cells (2).

Morphology mutants are generally isolated after treatment of wild-type cells with mutagens, which may affect several loci simultaneously. This may hamper biochemical studies (9, 12, 13, 20, 23). It therefore appears necessary that a careful genetic analysis should be carried out before physiological and biochemical investigations. The original spherelike mutant D23 was obtained after ethyl methane sulphonate treatment. This mutant exhibited a changed tolerance to certain antibiotics, a multihit response upon ultraviolet (UV) irradiation, and a considerably lower growth rate than its parental strain (23). In this report we have shown that this changed phenotype was associated with mutations in two loci, *envB* and *sloB*, respectively. The phenotypic properties of transductants carrying both or either of these two mutations have been analyzed.

MATERIALS AND METHODS

Bacterial strains. All strains used were derivatives of *E. coli* K-12 (Table 1).

Media and growth conditions. The complete medium used was LB of Bertani (1) supplemented with medium E of Vogel and Bonner (30) and 0.2% glucose. LA plates contained LB, medium E, 0.2% glucose, and 2.5×10^{-3} M $CaCl_2$, solidified with 1.5% agar. The minimal medium was medium E supplemented with 0.2% glucose or glycerol, 1 μg of thiamine per ml, and 50 μg of the L-epimer of the required amino acid per ml. The Casamino Acids medium contained the basal minimal medium, 0.2% Casamino

TABLE 1. *Strains of E. coli K-12*

Strain	Source	Relevant markers
D21	H. G. Boman	*proA, trp, his, strA, ampA1*
D23	Normark (23)	*proA, trp, his, strA, ampA1, envB1, sloB1*
Jc411	Low (15)	*his, leu, met, argG, strA*
RE113	E. C. R. Reeve	*aroE, malA*
G11	H. G. Boman	*ilv, metB, ampA*$^+$
AA1	Reiner (25)	*aspB, argG, strA*, Pur$^-$
KLF41/Jc1553	Low (15)	*argG*$^+$*/his, leu, met, argG, recA*
Jc411-57	D23 × Jc411	*his, leu, met, strA*
Jc411-39	D23 × Jc411	*his, leu, met, strA, envB1*
RE113-60	D23 × RE113	*malA, strA, envB1, sloB1*
BWO1	RE113-60 × RE113	*malA*
BWO2	RE113-60 × RE113	*malA, envB1*
BWO3	RE113-60 × RE113	*malA, strA, sloB1*
BWO31	*ampA* transductant of BWO3	*malA, strA, sloB1, ampA1*
KLF41/RE113-60	KLF41/Jc1553 × RE113-60	*malA*$^+$, *strA*$^+$, *envB*$^+$, *sloB*$^+$*/ malA, strA, envB1, sloB1*

Acids, and 0.2% glucose. The bacteria were grown at 37 C. Growth was recorded by optical density readings using a Klett-Summerson colorimeter with a W66 filter, or by a Zeiss spectrophotometer at a wavelength of 450 nm.

Materials. D-ampicillin (α-aminobenzylpenicillin) and penicillin G (benzylpenicillin) were kindly provided by AB Astra, Södertälje, Sweden. Amidino penicillin (FL1060) {[6-β-(hexahydro-1 H-azepin-1-ly)-methyleneamino]-penicillanic acid (17) was a gift from Leo Pharmaceutical Products, Ballerup, Denmark. Rifampicin was kindly obtained from Lepetit, Milano, Italy. Nalidixic acid was obtained from Winthrop Ltd., Surbiton on Thames, England, [^{14}C]thymidine (62 mCi/mmol) and methyl-[^{3}H]-thymidine (5 Ci/mmol) were from the Radiochemical Centre, Amersham, England.

Mating and transduction procedure. The transduction procedure with phage P1bt was according to Eriksson-Grennberg (6).

Transfer of the episome F'KLF41 to F$^-$ recipients was performed as follows. About 0.1 ml from an overnight culture of the F$^-$ recipient was inoculated into 10 ml of LB in an 250-ml flask and incubated with rapid shaking at 37 C to an optical density of 50 Klett units (about 2 × 10^8 cells/ml). The donor strain KLF41/Jc1553 was grown overnight in minimal medium to avoid loss of the episome. About 0.2 ml of the overnight culture was inoculated into 10 ml of LB and grown at 37 C to 50 Klett units. The donor and recipient were mixed in a ratio 1:1 and the mating mixture was incubated for 60 min with gentle shaking.

Determination of deoxyribonucleic acid (DNA) and protein content. Cultures were grown to a cell density of 50 Klett units (about 2 × 10^8 cells/ml) or to the stationary phase. The cells were immediately chilled, centrifuged, and washed twice in 0.15 M NaCl. The pellet was treated twice with ice cold 5% trichloroacetic acid. DNA was extracted with 0.5 M perchloric acid at 90 C for 20 min. After two extractions, DNA content was determined in the pooled supernatants by the diphenylamine method (27). Calf thymus DNA was used as a standard. The protein fraction was solubilized in 1 M NaOH and protein content was measured by the method of Lowry (16) with bovine serum albumin as a standard.

To assay for acid-insoluble radioactivity in DNA, cells were grown in Casamino Acids medium supplemented with adenosine (250 μg/ml) and [^{14}C]thymidine (0.05 μCi/ml, 1.2 μCi/μmol). Samples (0.1 ml) were taken at intervals into 5 ml of ice-cold 5% trichloroacetic acid and allowed to precipitate at 0 C for at least 30 min. Each sample was then filtered on a Whatman glass filter (23 mm), washed 10 times with ice-cold 0.1 M HCl, and dried by one washing with acetone. The samples were then counted in a liquid scintillation counter (Nuclear-Chicago Mark I).

Plasmid DNA analysis. Cells were grown to an optical density of 100 Klett units (4 × 10^8 cells/ml) in Casamino Acids medium, supplemented with adenosine (250 μg/ml) and [^{3}H]thymidine (5 μCi/ml, 5 Ci/mmol). Further processing was according to Silver and Falkow (28).

UV irradiation. Cells were grown in 10 ml of LB medium to a density of 50 Klett units (2 × 10^8 cells/ml), centrifuged, and suspended in 10 ml 0.15 M NaCl. The suspension was kept in a petri dish and irradiated by a UV lamp mounted at a distance of 18 cm in a box lined with aluminium foil. The bulb was of type TUV 6W from Philips, Netherlands. Samples were taken at intervals and appropriate dilutions were spread on LA plates. Photoreactivation was prevented by keeping the plates wrapped in aluminium foil during incubation.

Determination of resistance. The bacteria to be tested by single-cell test were grown in LB at 37 C in a rotary shaker and harvested in the logarithmic growth phase. About 100 to 400 cells were spread on LA plates containing different concentrations of the antibacterial agent. Resistance was determined as the highest

concentration permitting 100% colony formation. The viability on LA plates without antibiotics was 100%.

Determination of β-lactamase activity of intact cells and spheroplast lysates. Cells growing exponentially in Casamino Acids medium were harvested and suspended in 0.05 M phosphate buffer. β-Lactamase activity of intact cells or cells lysed by lysozyme (100 μg/ml) and ethylenediaminetetraacetate (5 mM) was assayed by the automated micro-iodometric method (14). Different concentrations of penicillin G (benzylpenicillin) were used as substrate.

RESULTS

Transduction analysis of the envB and sloB loci. Previous studies (23) have shown that the distorted shape in strain D23 was due to one or several mutations in a gene designated *envB*, which by conjugation was located to the *strA* region of the chromosome. In the transduction analysis presented in Table 2 we have selected for markers closely linked to *strA* and examined all transductants for their cell morphology under the phase-contrast microscope. Transductants showing a spherelike or irregular cell shape analogous to strain D23 (2) were scored as *envB1* transductants. Using strains Jc411, RE113, and AA1 as recipients, the *envB* locus was found to be cotransducible with *strA*, *argG*, *aspB*, and *aroE* (Table 2). The genetical analysis reveals the following gene order: *strA-aroE-envB-aspB-argG*. As *aroE* and *argG* are well mapped at 63.6 and 61.0 min, respectively (29), *envB* must be located close to 62.5 min.

Several lines of evidence indicated that strain D23 contained a second mutation close to *strA*, which affected the phenotype of the mutant strain. In cross 4 (Table 2), wild-type transductants were selected from strain D23. Among these, 42% had also received the *strA*$^+$ allele of the donor strain. This high cotransduction frequency was in contrast to the low (3%) linkage found between *strA* and *envB* (cross 3, Table 2). A further evidence for a second mutation in strain D23 was the finding that *argG*$^+$, *envB1* transductants grew considerably faster than strain D23 and some *aroE*$^+$, *envB1* transductants. The mutation in strain D23 mediating slow growth rate was denoted *sloB1* (*sloA1* is localized close to *rifA*, Glenn Björk, personal communication). To localize the *sloB1* mutation, a transduction experiment was performed using strain RE113 as recipient. Table 3 shows the number of the eight possible genotypes among selected *aroE*$^+$ transductants. The result infers that *sloB* is located very close to *strA* and most likely to the left of this marker.

To obtain transductants as isogenic as possible, a slow growing *aroE*$^+$, *envB1*, *sloB1* transductant RE113-60 was used as donor in a cross with RE113 as recipient. From this cross three *aroE*$^+$ transductants, BWO1 (*envB*$^+$, *sloB*$^+$), BWO2 (*envB1*, *sloB*$^+$), and BWO3 (*envB*$^+$, *sloB1*), were further studied.

Dominance studies of the envB mutation. The episome F'KLF41 covering the region 60 to

TABLE 2. *Transduction experiments with the envB gene using phage P1bt*[a]

Cross	Donor	Recipient	Selected phenotype	No. of transductants tested	Cotransduction between genes (%)
1	D23	Jc411	Arg$^+$	163	*argG*$^+$-*envB* (4)
2	D23	AA1	Arg$^+$	100	*argG*$^+$-*asp*$^+$ (95) *argG*$^+$-*envB* (3) *argG*$^+$-*asp*$^+$-*envB* (3)
			Asp$^+$	199	*asp*$^+$-*argG*$^+$ (30) *asp*$^+$-*envB* (2) *asp*$^+$-*argG*$^+$-*envB* (0.5)
3	D23	RE113	Str^r	172	*strA-aroE*$^+$ (54) *strA-envB* (3) *strA-aroE*$^+$-*envB* (3)
			Aro$^+$	246	*aroE*$^+$-*strA* (52) *aroE*$^+$-*envB* (9) *strA-aroE*$^+$-*envB* (2)
4	G11	D23	Amp-r[b]	32	Amp-r-*strA*$^+$ (42)

[a] Strain D23 is not resistant to phage P1bt as earlier reported (23). When incubating phage P1bt-infected D23 cells for 24 h instead of 12 h as previously tried, very small plaques are discerned. Reasonably good phage stocks could be obtained. Transduction were performed as described in text.

[b] Strain D23 was forming smaller colonies and was more sensitive to ampicillin than the parental strain (23). The wild-type phenotype of strain G11 could therefore be transduced into strain D23 by selection on LA-plates containing ampicillin (20 μg/ml) scoring for rapid growing colonies with wild type rod morphology. Only few transductants were obtained in this cross.

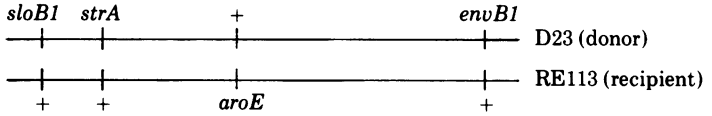
66 min (15) was introduced into the *sloB1 envB1* transductant RE113-60 by selecting for Mal$^+$ clones. Out of 44 clones 39 were forming rods. To prove that the *envB1* mutation was still present in these rod-forming clones, we made use of the fact that *strA* is recessive to *strA*$^+$ in diploids (11). As all rod-forming clones were streptomycin sensitive, haploid segregants with *envB* morphology could be obtained by selection for streptomycin resistance. This suggests that the *envB1* mutation is recessive to its wild-type allele. The dominance relationship of the *sloB1* mutation was not investigated.

DNA and protein content of strains D21 (wild type) and D23 (envB1, sloB1). The *envB1, sloB1* mutant D23 was previously found to be more tolerant to nalidixic acid and to show a multihit response upon UV irradiation (23). As an increased DNA content in the mutant could be the explanation for these phenotypic alterations, the DNA and protein content of mutant and parental strain was determined. During steady state growth in Casamino Acids medium strain D23 exhibited a DNA to protein ratio considerably higher than that of the parental strain (Table 4). However, when stationary-phase cultures were compared, no differences were observed. An independent evidence for an increased DNA content in the mutant D23 was obtained by continuous labeling with [^{14}C]thymidine where strain D23 contained per mass unit, 1.7 times more trichloroacetic acid precipitable-labeled material than the parental strain D21. During exponential growth in Casamino Acids medium the cellular DNA content of strain D23 was 3.1×10^{-11} mg, whereas that of strain D21 was 1.2×10^{-11} mg. This marked difference in DNA content per cell was due to higher DNA to protein ratio in the mutant, and to the fact that the average cell mass of strain

TABLE 3. *Genotypes from a transduction cross between strains D23 and RE113*[a]

Donor allele selected	Genotype of transductant				No.	No. of crossovers required for the gene order	
	sloB1	*strA*	*aroE*	*envB1*		*sloB-strA-aroE-envB*	*strA-sloB-aroE-envB*
aroE$^+$	0	0	1	0	64	2	2
	1	0	1	0	0	4	2
	0	1	1	0	12	2	4
	1	1	1	0	160	2	2
	0	0	1	1	6	2	2
	1	0	1	1	1	4	2
	0	1	1	1	1	2	4
	1	1	1	1	6	2	2
					250		

[a] The genotypes of the parents are as follows, with the loci placed in the order inferred from the results of Table 2, this Table, and from Taylor and Trotter (29)



In the body of the Table, the donor allele was always labeled "1", and the recipient allele "0". The presence of *sloB1* was determined by the colony size on LA plates after 18 h of incubation (<0.5 mm). The presence of *envB1* was determined by phase-contrast microscopy.

TABLE 4. *DNA to protein ratio in strains D21 and D23*[a]

Strains	Genotype	Dcublings per h	DNA per protein (μg/μg)	
			Log phase	Stationary phase
D21	*envB*$^+$, *sloB*$^+$	1.9	0.06 ± 0.01	0.06 ± 0.01
D23	*envB1, sloB1*	0.9	0.09 ± 0.01	0.06 ± 0.01

[a] Cells were growing in Casamino Acids medium. Determination of DNA and protein content was performed as described in text.

D23 was 1.5 times that of the parental strain.

The DNA distribution of both strains were also analyzed in an alkaline sucrose gradient. In none of the strains did we find any evidence for plasmid DNA.

In the *envB1*, *sloB1* mutant D23, the DNA to protein ratio increased considerably with growth rate. This was in contrast to the parent strain D21 where this function was constant (Fig. 1).

DNA to protein ratio of sloB1 and envB1 transductants. The *sloB1*, *envB1* transductant RE113-60 showed the same high DNA to protein ratio as the parental strain D23 (Table 5). On the other hand, *envB1* transductants lacking the *sloB1* mutation (Jc411-39 and BWO2), as well as the rod-forming *sloB1*, *envB*$^+$ transductant BWO3, exhibited a normal DNA to protein ratio. Thus, it appears that both the *sloB1* and *envB1* mutations are required to obtain a high DNA to protein ratio. From these analyses we may conclude with certainty that in strain D23 the changed macromolecular composition as such did not cause the distorted cell shape.

UV irradiation of transductants BWO1 (wild type), BWO2 (envB1), and BWO3 (sloB1). The *envB1* transductant BWO2 was exhibiting a multihit response upon UV irradiation in contrast to transductants BWO1 and BWO3 (Fig. 2). With strain BWO2, the shape of the UV-killing curve resembled that obtained with the original *envB* mutant D23 (23).

Antibiotic tolerance of strain D23 and its transductants. Compared to the parental strain D21, the *envB1*, *sloB1* mutant D23 was considerably more sensitive to ampicillin and penicillin G (Table 6). It was therefore surprising that the mutant was totally resistant to amidino penicillin (FL1060). This compound, in contrast to other penicillins, converts *E. coli* to osmotically stable round or irregular cells (19, 21, 24). As strain D23 contains at least three mutations that might affect penicillin resistance (*ampA1*, *envB1*, and *sloB1*), transductants with different genotypes were compared. Resistance to amidino penicillin was not due to the *envB1* mutation as strain BWO2 (*ampA*$^+$,

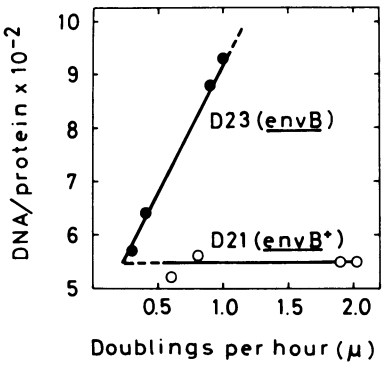


FIG. 1. *DNA to protein ratio of D21 (wild type) and D23 (envB1, sloB1) cells during steady state growth in different media. The following media were used: LB, Casamino Acids, minimal medium with 0.2% glucose, and minimal medium with 0.2% glycerol.*

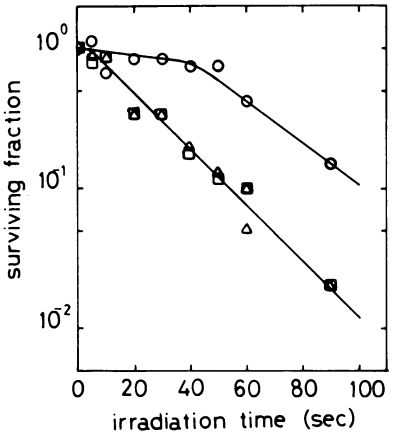


FIG. 2. *Surviving curves after exposure to UV irradiation. Symbols: BWO1 (wild type), □; BWO2 (envB1), ○; BWO3 (sloB1), △.*

TABLE 5. *Phenotypic characteristics of transductants*[a]

Transductants	Derivation	Genotype	Shape	Doublings per h	DNA per protein (μg/μg)
RE113-60	D23 × RE113	*envB1*, *sloB1*	spherelike	0.8	0.09 ± 0.01
Jc411-57	D23 × Jc411	*envB*$^+$, *sloB*$^+$	rod	1.9	0.06 ± 0.01
Jc411-39	D23 × Jc411	*envB1*, *sloB*$^+$	spherelike	1.4	0.06 ± 0.01
BWO1	RE113-60 × RE113	*envB*$^+$, *sloB*$^+$	rod	1.5	0.06 ± 0.01
BWO2	RE113-60 × RE113	*envB1*, *sloB*$^+$	spherelike	1.3	0.05 ± 0.01
BWO3	RE113-60 × RE113	*envB*$^+$, *sloB1*	rod	0.8	0.06 ± 0.01

[a] Cells were growing to steady state in Casamino Acids medium. The DNA and protein content, as well as the growth rate, was determined as described in text.

TABLE 6. *Effect of the envB1 and sloB1 mutations on the resistance to antibiotics*[a]

Strains	Genotype	Resistance on plates (μg/ml)						Resistance (mg/ml)
		Amp	PenG	Ceph	FL1060	Rif	Nal	Cholate
D21	*envB*$^+$, *sloB*$^+$, *ampA1*	17	150	2	0.2	1	2	NT
D23	*envB1*, *sloB1*, *ampA1*	7	50	1	>200	1	7	NT
RE113	*envB*$^+$, *sloB*$^+$, *ampA*$^+$	1	10	NT	0.1	1	2	NT
RE113-60	*envB1*, *sloB1*, *ampA*$^+$	0.5	2	NT	10	1	7	NT
BWO1	*envB*$^+$ *sloB*$^+$, *ampA*$^+$	1	10	1	0.1	1	2	50
BWO2	*envB1*, *sloB*$^+$, *ampA*$^+$	0.3	1	0.2	0.01	1	2	3
BWO3	*envB*$^+$, *sloB1*, *ampA*$^+$	1	10	1	10	1	7	50
BWO31	*envB*$^+$, *sloB1*, *ampA1*	10	150	2	>200	1	7	50

[a] Amp, D-Ampicillin (α-aminobenzylpenicillin); PenG, penicillin G (benzylpenicillin); Ceph, cephaloridine; FL1060, amidino penicillin (6β-[hexahydro-1 H-azepin-1-yl]methyleneamino)-penicillanic acid; Rif, rifampicin; Nal: nalidixic acid; Cholate, sodium deoxycholate; NT, not tested. Resistance was determined as described in text.

sloB$^+$, *envB1*) was hypersensitive to all penicillins tested including amidino penicillin (Table 6). The *envB*$^+$, *sloB1* transductant, BWO3 as well as the *envB1*, *sloB1* strain RE113-60, were not as resistant to amidino penicillin as strain D23. However, an *ampA1* transductant of strain BWO3 (strain BWO31) was totally resistant to this agent (>200 μg/ml). It therefore appears likely that the amidino penicillin resistance of strain D23 is due to both the *ampA1* and the *sloB1* mutations.

When strain BWO1 (wild type) was treated with sublethal concentrations of amidino penicillin, hypersensitivity to other penicillins was induced, whereas rifampicin tolerance was unaffected (Table 7). It should be noted that amidino penicillin-treated wild-type cells were morphologically indistinguishable from *envB* cells.

One explanation for the penicillin hypersensitivity of strain BWO2 (*envB1*) could be an increased penetration through the outer membrane (3, 7). The penetrability barrier to penicillins can only be determined indirectly by measuring the ability of penicillins to reach β-lactamases in the periplasmic space (3, 26). Figure 3 illustrates how the activity of a R factor-mediated β-lactamase varies with the substrate concentration in the presence (intact cells) or the absence (lysed cells) of the penetration barrier. For both BWO1 (wild type) and BWO2 (*envB1*), the β-lactamase activity was considerably higher in lysed than in intact cells. This result would imply that the *envB1* mutation of strain BWO2 does not affect the penetration barrier to penicillin G.

TABLE 7. *Effect of amidino penicillin (FL1060) on the tolerance to other antibiotics*[a]

Strain	FL1060 in plates (μg/ml)	Resistance on plates (μg/ml)			
		Amp	PenG	Ceph	Rif
BWO1[b]	0	1	10	1	1
BWO1[b]	0.05	0.3	5	0.1	1
BWO1[b]	0.1	0.05	0.2	0.05	1
BWO2 (*envB1*)	0	0.3	1	0.2	1

[a] A LB culture of strain BWO1 (wild type) was divided into two aliquots, one was kept as control and to the other amidino penicillin (1 μg/ml) was added.

[b] The cultures were grown for three generations and thereafter spread on plates containing different concentrations of the respective antibiotic. In addition, amidino penicillin (0, 05 or 0, 1 μg/ml) was included in the plates for the pretreated culture. Abbreviations were as in Table 6.

DISCUSSION

Phenotypes associated with the envB1 and sloB1 mutations. One approach to the under-

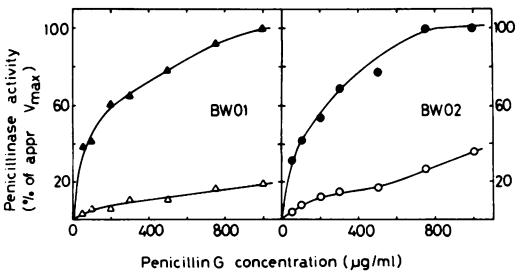


FIG. 3. *Hydrolysis of penicillin G by strains BWO1-R1drd-19 (wild type) and BWO2-R1drd-19 (envB1). The R factor that codes for a β-lactamase and a chloramphenicol acetylase (22) was introduced by conjugation selecting for chloramphenicol resistance. Single-cell penicillin G resistance for BWO1-R1drd-19 was 60 μg/ml, and for BWO2-R1drd-19 2 μg/ml. Symbols: Intact cells, Δ and O; spheroplast lysate ▲ and ●.*

standing of bacterial morphology is the isolation of mutants exhibiting a changed cell shape. However, without genetic analyses such mutants are of limited value as they often have been obtained after heavy mutagenesis (9, 12, 20, 23). Here, we have clearly shown that strain D23 differs from the parental strain D21 by mutations in at least two loci, *sloB* and *envB*, respectively, both of which contribute to the physiology of the mutant. The loss of rod shape is due to a mutation in the *envB* locus located at 62.5 min. This mutation also mediates hypersensitivity to penicillins including amidino penicillin and to deoxycholate. The *sloB1* mutation close to *strA* causes a considerable decrease of growth rate. This mutation confers increased tolerance to amidino penicillin and nalidixic acid. Together the *sloB1* and *envB1* mutations appear to affect the macromolecular composition of cells growing in rich medium resulting in an increased DNA to protein ratio. Cells growing in minimal medium showed a normal DNA to protein ratio. This is in contrast to wild-type *Salmonella* and *E. coli*, where this ratio is not affected by growth rate (5, 18). The nature of this phenomenon in strain D23 (*envB1*, *sloB1*) is not known, but could be due to a decreased ribosome efficiency in rich media. The high DNA content of strain D23 was suspected because of a higher nalidixic acid tolerance and a multihit response upon UV irradiation (23). However, an increased tolerance to UV was also found in *sloB*$^+$, *envB1* transductants (Fig. 2). Such cells do have a normal DNA to protein ratio, but are larger. The increased UV tolerance is therefore probably due to a higher cellular content of DNA.

Penicillin hypersensitivity mediated by the envB1 mutation. The *envB1* mutation alone mediates an increased sensitivity to both penicillins and cephalosporins, whereas resistance to other antibiotics appear to be virtually unaffected. The *envB1* mutant BWO2 shows the same penicillin sensitivity as *envA* strains. The latter mutation causes an increased penicillin sensitivity by affecting the outer membrane penetrability (3). In an *envA* strain the β-lactamase activity is virtually the same in intact and lyzed cells (3), indicating that penicillin G has almost free access to the periplasmic space. However, in strain BWO2-R1*drd-19* (*envB1*) the barrier to penicillin G was as efficient as in the wild-type strain BWO1-R1*drd-19*.

Richmond and co-workers have shown that in *E. coli* no penetration barrier exists against cephaloridine (26). This is supported by the fact that an *envA* mutant is as tolerant as wild-type strains to this agent (unpublished observation). Following this concept, the hypersensitivity to cephaloridine observed in strain BWO2 (*envB1*) should be caused by other mechanisms than increased penetrability.

The original mutant D23 (*ampA1*, *envB1*, *sloB1*) was totally resistant to amidino penicillin. We suggest that this resistance is due to a synergistic effect between the *ampA1* mutation, mediating β-lactamase production (3), and the slow growth rate caused by the *sloB1* mutation.

Recently Matsuhashi et al. (19) reported on mutants resistant to amidino penicillin that showed a rounded cell morphology even in the absence of the antibiotic. Phenotypically, these mutants resemble strain D23. In their work nitrosoguanidine was used, which is known to give multiple closely linked mutations (4). Therefore, some of these mutants may have a more complex genotype analogous to that of strain D23.

The target for amidino penicillin is not known. It is noticeable that this penicillin does neither inhibit endopeptidase, carboxypeptidase, nor transpeptidase activities (19, 24). It has been suggested that an enzyme affecting the outer membrane may be the target for this penicillin (19). The shape and physiology of amidino penicillin-treated wild-type cells and *envB1* cells were very similar. It is therefore possible that the *envB1* mutation does affect an amidino penicillin-sensitive target. A biochemical comparison between *envB1*, wild type, and amidino penicillin-treated wild-type cells has therefore been initiated.

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