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*Short communication*

# *cysB* and *cysE* mutants of *Escherichia coli* K12 show increased resistance to novobiocin

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**Summary.** Mutations in the *cysB* and *cysE* genes of *Escherichia coli* K12 cause an increase in resistance to the gyrase inhibitor novobiocin but not to coumermycin, acriflavine and rifampicin. This unusual relationship was also observed among spontaneous novobiocin resistant (Nov$^r$) mutants: 10% of Nov$^r$ mutants isolated on rich (LA) plates with novobiocin could not grow on minimal plates, and among those approximately half were *cysB* or *cysE* mutants. Further analyses demonstrated that *cysB* and *cysE* negative alleles neither interfere with transport of novobiocin nor affect DNA supercoiling.

**Key words:** *Escherichia coli* – *cysB* and *cysE* genes – Resistance to novobiocin

Biosynthesis of cysteine in *Escherichia coli* is controlled by a set of 16 genes found in five different chromosomal regions (Bachmann and Low 1990; Sirko et al. 1990). The biosynthetic pathway is convergent, sulphate uptake and its reduction to sulfide comprising one branch and O-acetylserine synthesis the other. Regulation of the *cys* gene regulon is achieved through feedback inhibition of O-acetylserine synthesis by cysteine and by a system of positive genetic control. A complex of CysB protein and O-acetylserine activates transcription at various *cys* promoters (with the exception of the *cysE* promoter) in a manner analogous to that described for other systems of positive control. CysB is coded for by the *cysB* gene located at minute 28 on the *E. coli* map. Serine transacetylase, encoded by the gene *cysE* (80 min), catalyses the synthesis of O-acetylserine from serine and O-acetyl coenzyme A (Denk and Böck 1987). Both genes are not linked to other known *cys* genes (reviewed by Kredich 1987).

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In our recent mapping by P1 transduction of a new gene which affects DNA supercoiling, and is located in the *osmZ-opp-trp* region, we used a Trp$^-$ derivative of strain C600 in which a *cysB* auxotrophic mutation had been isolated by selection for azaserine resistance (SY376). (For details of the genotypes and isolation of these and other strains described below, see Table 1.) This *cysB* mutation increased resistance of the parental strain to the antibiotic novobiocin (Nov). To investigate this phenomenon further, we isolated 30 spontaneous C600 Cys$^-$ auxotrophic mutants by the same method (only *cysE* mutants cannot be isolated by selection for azaserine resistance; Hulanicka et al. 1979). Cysteine mutants were isolated from the inhibition zone around a 1 cm filter paper disk containing 1 μmol of azaserine. The disk was placed on a minimal agar plate containing 0.2 mM tryptophane, 0.5 mM glutathione, 0.3 mM threonine, 0.3 mM leucine and 0.05 mM thiamine, on which approximately $10^8$ cells of a fresh overnight culture had been previously spread. Approximately 40 azaserine resistant colonies appeared per inhibition zone after 48 h incubation at 37° C. Thirty resistant colonies (taken from three independent experiments; 10 from each plate) were purified on plates lacking azaserine. Among those, 4 were *cysB* mutants and 26 were other *cys* mutants. *cysB* mutants were identified by complementation with F'123 *trp*$^+$ *cysB*$^+$ *pyrF*$^+$ (KL701). All clones were further tested by streaking undiluted overnight cultures grown in LB supplemented with cysteine (0.5 mM) on LA plates with cysteine (0.05 mM) and different amounts of Nov (50–400 μg/ml). Three independently isolated *cysB* mutants (isolated from three independent cultures), each showed an increase in resistance to Nov from 80 μg/ml to 400 μg/ml. In contrast, other *cys* mutants (26 mutants tested) retained the level of resistance to Nov characteristic of the parental strain C600 (80 μg/ml). In addition, spontaneous fast-growing *cysB*$^+$ revertants regained their original resistance level to Nov. They all appeared to be true revertants; it was not possible to cotransduce *cysB* mutations in crosses in which phage

**Table 1.** Bacterial strains and plasmids used

| Strain or plasmid | Genotype or isolation | Source or reference |
|---|---|---|
| *Escherichia coli* K12 | | |
| C600 | *thi-1 thr-1 leuB6 lacY1 tonA 21 supE44 rfbD1* | Appleyard (1954) |
| AB1157 | *thi-1 thr-1 leuB6 Δ(gpt-proA)62 argE3 hisG4 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 rpsL31 supE44 rfbD1 mgl-51 kdgK51 rac⁻* | DeWitt and Adelberg (1962) |
| KL701 | F'123 (*trp⁺ cysB⁺ pyrF⁺*)/ *pyrD34 trp-45 his-68 recA1 thi-1 galK35 malA1 xyl-7 mtl-2 rpsL118* | Low (1972) |
| CBK130 | *proC*::Tn*5* | Shaw and Berg (1979) |
| SD108 | *trpE63 pyrF287 acrA13* | DiNardo et al. (1982) |
| JC10240 | HfrP045 *recA56 srlC300*::Tn*10 relA1 thr-300 rpsE2300 spoT1 ilv-318 thi-1* | Csonka and Clark (1980) |
| SY375 | C600 Trp⁻ spontaneous by penicillin selection | Laboratory collection |
| SY376 | SY375 *cysB* spontaneous by selection to azaserine; mutation mapped by complementation with F'123 | This work |
| SY380 | C600 *cysB* isolated and mapped as SY376 | This work |
| SY381 | AB1157 *cysB*(Ts) isolated and mapped as SY376 | This work |
| SY400 | C600 *cysE* isolated as novobiocin resistant mutant; mapped by differential growth on sulphate and O-acetylserine | This work |
| SY401 | C600 *proC*::Tn*5* by P1 transduction from CBK130 | This work |
| SY402 | SY401 *acrA13 proC⁺* by P1 transduction from SD108 | This work |
| SY403 | SY380 *proC*::Tn*5* by P1 transduction from CBK130 | This work |
| SY404 | SY403 *acrA13 proC⁺* by P1 transduction from SD108 | This work |
| SY405 | SY400 *proC*::Tn*5* by P1 transduction from CBK130 | This work |
| SY406 | SY405 *acrA13 proC⁺* by P1 transduction from SD108 | This work |
| SY407 | SY380 *recA56 srlC300*::Tn*10* by P1 transduction from JC10240 | This work |
| SY408 | SY381 *recA56 srl300*::Tn*10* by P1 transduction from JC10240 | This work |
| DG37 | HfrP045 Δ(*pstI-cysA*)*127 relA1 spoT1 thi-1* | Gottesman (1976) |
| JM246 | *cysI53* | Jones-Mortimer (1973) |
| AT2455 | HfrP01 *cysG44 relA1 spoT1 mal-18 thi-1* | Taylor and Trotter (1967) |
| AT2427 | HfrP01 *cysJ43 relA1 spoT1 mcrA thi-1 phoM510* | Taylor and Trotter (1967) |
| AB1369 | *cysB38* Δ(*gpt-proA*) *62 lacY1 tsx-29 galK2 rac⁻ hisG4 rfbD1 kdgK51 xyl-5 mtl-1 argE3 thi-1* | Taylor strain; via B. Bachmann |
| JM15 | *cysE50 trf-8* | Jones-Mortimer (1968) |
| JA199 | *trpE5 leu-6 thi hsdR hsdM⁺* | Hryniewicz et al. (1988) |
| NK1 | JA199 *cysB* | Hryniewicz et al. (1988) |
| EC1119 | JA199 *cysA* | Hryniewicz et al. (1988) |
| NK3 | JA199 (Δ*cysMK*) | D. Hulanicka (personal communication) |
| EC1801 | JA199 *cysE* | D. Hulanicka (personal communication) |
| EC1250 | *araD139 ΔlacU169 rpsL thi fla trp-1* | Hryniewicz et al. (1988) |
| EC2256 | EC1250 *cysT329*::*lac imm* | Hryniewicz et al. (1988, 1990) |
| EC2297 | EC1250 *cysP* | Hryniewicz et al. (1990) |
| Plasmid | | |
| pHV2810 | pBR322::*cysB⁺* 3.2 kb *Eco*RI-*Sal*I fragment from AB1157 chromosome cloned into pBR322 | This work (same as pJOH; Ostrowski et al. 1987) |

P1 prepared on revertants was used to transduce the *trp⁺* allele into Trp⁻ recipients (SY375 and EC1250). One of the *cysB* mutants (SY380) was chosen for further study.

The behaviour of *cysB* mutants with respect to Nov resistance was confirmed in another widely used *E. coli* K12 strain AB1157. The AB1157 *cysB* mutant (SY381) carries a *cysB*(Ts) mutation. At the permissive temperature (30° C), the mutant was Cys⁺ and exhibited the normal wild-type level of resistance to Nov of AB1157 (100 μg/ml). A temperature shift to 42° C induced complete cysteine auxotrophy; at the same time, resistance to Nov increased to 400 μg/ml. This finding, which suggested that the CysB protein may play some role in cellular tolerance to Nov, was further strengthened by complementation. Introduction of F'123 or pHV2810 (pBR322; *cysB⁺*) into both strains (SY380 and SY381) produced a Cys⁺ phenotype and also lowered resistance to Nov to its normal level (80 μg/ml and 100 μg/ml, respectively). The same pattern of complementation was obtained when RecA⁻ variants of SY380 and SY381 (SY407 and SY408) were used as recipients.

Our observation was verified by testing other well-characterised cysteine mutants. Revertants and transductants to Cys⁺ of a set of mutants (*cysA*, *cysI*, *cysG* and *cysJ*) showed the same level of resistance to Nov as their auxotrophic progenitors (DG37, JM246, AT2455 and AT2427). Surprisingly, Cys⁺ transductants of a *cysE* mutant (JM15) showed (similarly to Cys⁺ revertants and transductants of a *cysB* mutant AB1369) a

**Table 2.** Resistance level of known *cys*$^-$ mutants and their P1 transductants and revertants to Cys$^+$

| Strain | | Native resistance to novobiocin[a] | Revertant to Cys$^+$[a] | Transductant to Cys$^+$[a] |
|---|---|---|---|---|
| DG37 | Δ(*cysA*) | 50 | NR | 50 |
| JM246 | (*cysI*) | 50 | 50 | 50 |
| AT2455 | (*cysG*) | 50 | 50 | 50 |
| AT2427 | (*cysJ*) | 40 | 40 | 40 |
| AB1369 | (*cysB*) | 200 | 50 | 50 |
| JM15 | Δ(*cysE*) | 300 | NR | 50 |
| JA199 | (*cys*$^+$) | 50 | | |
| NK3 | Δ(*cysMK*) | 50 | | 50 |
| EC1119 | (*cysA*) | 50 | | 50 |
| NK1 | (*cysB*) | 300 | | 50 |
| EC1801 | (*cysE*) | 300 | | 50 |
| EC1250 | (*cys*$^+$) | 100 | | |
| EC2297 | (*cysP*) | 100 | | |
| EC2256 | (*cysT*) | 100 | | 100 |

All transductions were performed by phage P1 propagated on C600 (Miller 1972)
Cys$^+$ revertant of the strain AB1369 is a true *cysB*$^+$ revertant; it was not possible to recover *cysB*$^-$ mutation in the cross in which phage P1 prepared on the revertant was used to transduce the *trp*$^+$ allele into Trp$^-$ recipients (SY375 and EC1250)
EC2297 is a prototroph (Hryniewicz et al. 1990)
NR, not revertable

[a] Concentration of novobiocin (μg/ml) in LA plates

significant decrease of resistance to Nov (Table 2). Similar results were obtained in a parallel study with two other sets of defined cysteine mutants with isogenic Cys$^+$ parental strains: *cysB, cysA, cysMK* and *cysE* (parental strain JA199), and *cysT* and *cysP* (parental strain EC1250). *cysMK, cysA, cysP* and *cysT* mutants showed the same level of resistance to Nov as JA199 and EC1250, while *cysB* and *cysE* mutants were substantially more resistant to Nov than JA199 (Table 2). In addition, Cys$^+$ transductants of *cysB* and *cysE* mutants acquired the Nov resistance level of JA199. In a parallel control experiment we also managed to demonstrate the relationship between *cysB* and *cysE* mutations and resistance to Nov. Among 500 tested Nov$^r$ clones, picked from 650 Nov$^r$ clones obtained on two LA plates containing 0.5 mM cysteine and 400 μg/ml novobiocin, on which approximately $10^8$ cells of a fresh overnight culture had been spread, 52 clones appeared to be auxotrophs. Among those, 11 were *cysB* mutants, 14 were *cysE* mutants, while the nature of the other 27 could not be identified by the method of auxanography (Davis et al. 1980). Finally, we did not manage to separate CysB$^-$ or CysE$^-$ characters from resistance to novobiocin, which also suggested that both characters were due to the single mutation. An analysis of 300 *cysB*$^+$ transductants, taken from the cross C600 (P1) × SY380, showed that all were Nov$^s$. Similarly, none of 100 tested *cysE*$^+$ revertants of the strain SY400 appeared to be Nov$^r$.

The lethal effect of Nov is exerted through the inhibition of DNA gyrase (Gellert et al. 1976; Drlica and Franco 1988). Possible relationships of the *cysB* and *cysE* genes with DNA supercoiling were studied by measuring the expression of the supercoiling-sensitive *lac* promoter (Borowiec and Gralla 1985; Borowiec et al. 1987) in strains C600, SY380 (C600 *cysB*) and SY400 (C600 *cysE*). No substantial difference of β-galactosidase activity in the tested strains was found. This finding was confirmed in a parallel experiment in which assay of the levels of supercoiling of pBR322 isolated from the cultures of the strains C600, SY380 and SY400 (Pruss and Drlica 1986) did not demonstrate any difference in average linking numbers (results not presented).

Another possibility that we considered was that the outer membrane permeability for large hydrophobic molecules such as Nov (Nikaido and Vaara 1987) could be affected in *cysB* and *cysE* mutants. We examined this possibility by monitoring the efficiency of plating

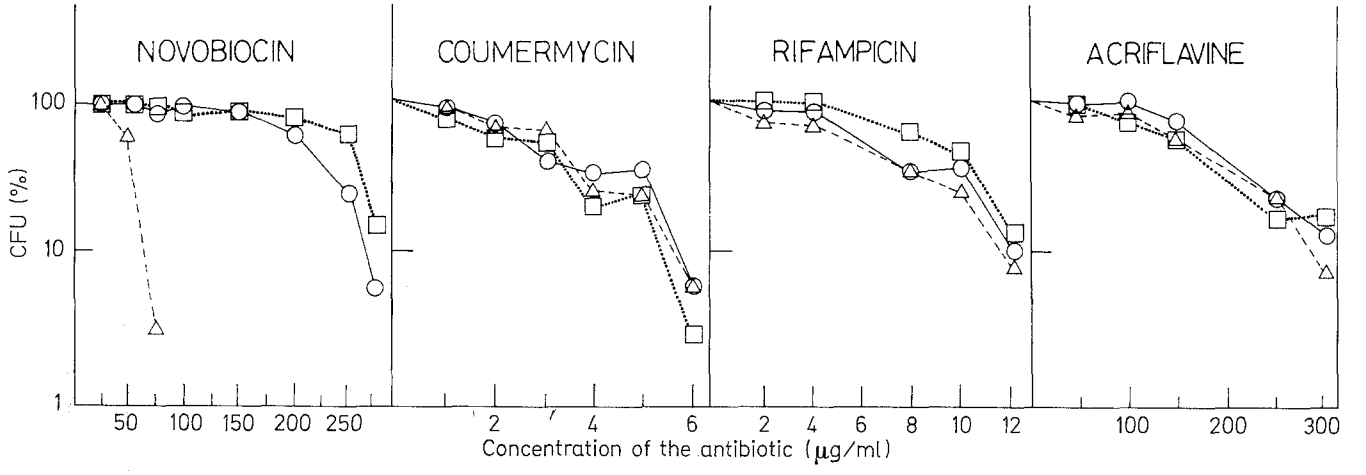


**Fig. 1.** Efficiency of plating, expressed as colony forming units (CFU) of C600 (*cys*$^+$), SY380 (*cysB*) and SY400 (*cysE*) in the presence of novobiocin, coumermycin, rifampicin and acriflavine. Overnight cultures grown in LB with 0.5 mM cysteine were diluted and plated on LA plates supplemented with 0.5 mM cysteine and indicated amounts of the antibiotics. Colonies were counted after 24 h of incubation at 37° C. No additional colonies appeared after 4 days of incubation. Presented percentages were computed from efficiencies of plating on LA plates without antibiotics. C600 (△); SY380 (○); SY400 (□)

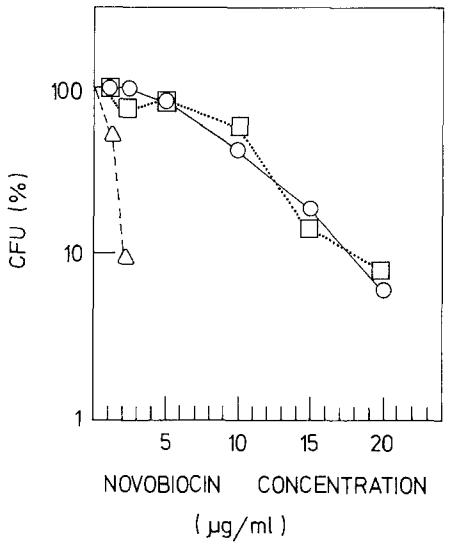


**Fig. 2.** Efficiency of plating of SY402 (*cys*$^+$ *acrA13*), SY404 (*cysB acrA13*), and SY406 (*cysE acrA13*) on novobiocin. Values of CFU were calculated as described in Fig. 1. SY402 (△); SY404 (○); SY406 (□)

of strains C600, SY380 and SY400 in the presence of rifampicin and acriflavine, drugs which share similar modes of transport with Nov (Nikaido and Vaara 1987). Coumermycin, which is practically a dimer of Nov and which presumably enters *E. coli* more readily (Gellert et al. 1976), as well as Nov, was also employed in this analysis. As shown, increased resistance to Nov of the strains SY380 (*cysB*) and SY400 (*cysE*) was reproducible (Fig. 1). However, no effect of these mutations on *E. coli* resistance to rifampicin, acriflavine, and surprisingly also to coumermycin, was observed. That *cysB* and *cysE* mutations do not interfere with the transport of Nov was demonstrated in a comparable experiment with *cysB* and *cysE* mutants also harboring an *acrA* mutation. Mutations in the *acrA* gene result in supersensitivity to Nov and other hydrophobic drugs, as a result of increased permeability of the outer membrane (Coleman and Leive 1979). Double *cysB acrA* and *cysE acrA* mutants were constructed by transducing the *acrA13* mutation into *proC*::Tn*5* derivatives of the strains C600, SY380 (*cysB*) and SY400 (*cysE*). As shown (Fig. 2), the increase in sensitivity to Nov of *acrA* mutants was not accompanied by a change of curve profile relative to those obtained with AcrA$^+$ strains (Fig. 1). Finally, increased resistance to Nov of *cysB* and *cysE* mutants was also demonstrated in an alternative experiment in which growth rates of the strains C600, SY380 and SY400 in the presence of different amounts of Nov were monitored (Fig. 3). This time, higher resistance of the *cysE* mutant relative to the *cysB* mutant (indicated also in Fig. 1), appeared in more pronounced form. The nature of this difference remains for the moment unclear.

Finally, two lines of evidence argue against the possibility that the effect of *cysB* negative alleles is exerted via the cysteine transport system: (1) in all experiments the functioning of the CysB-dependent cysteine "general" transport system was inhibited by the presence of 0.5 mM cysteine (Kredich 1987); and (2) differential inhibition by Nov of the CysB$^+$ and CysB$^-$ strains was observed even when cells were grown anaerobically in an atmosphere of $H_2$ and $CO_2$ (data not shown), a condition which bypasses the need of the regulon for the CysB positive regulator (Kredich 1987).

The results presented demonstrate an effect of *cysB* and *cysE* mutations on *E. coli* tolerance to Nov, the mechanism of the effect remaining for the moment obscure. The data available indicate that neither DNA supercoiling, nor transport of Nov, the two most logical metabolic aspects related to cellular resistance to Nov, are affected by these mutations. The CysB protein belongs to the LysR family, a group of similarly folded DNA-binding proteins of common ancestry known to activate other genes (Henikoff et al. 1988). This suggests that CysB, besides acting as a positive regulator of the cysteine operon, could be involved in some other regulatory processes. This, however, cannot be said for the

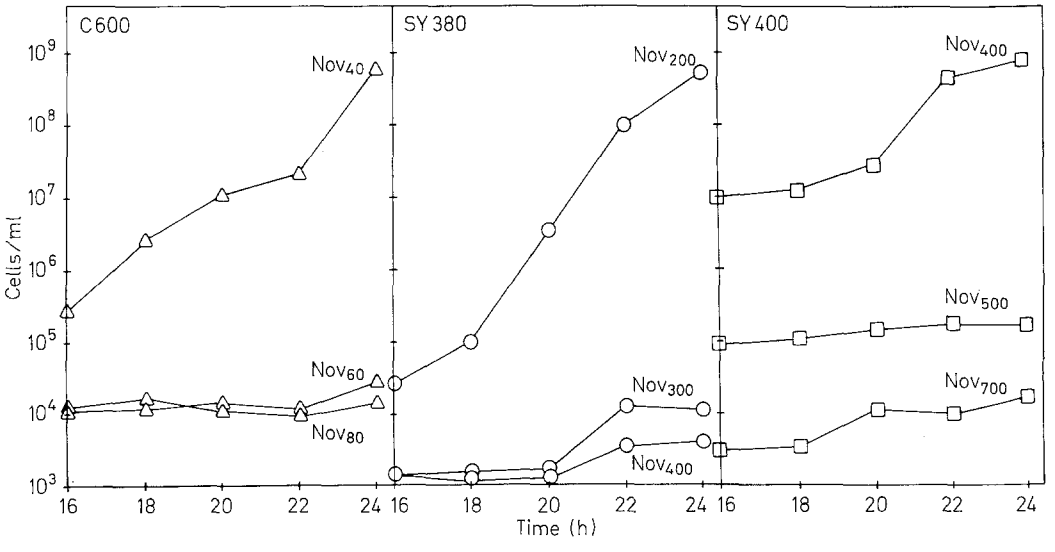


**Fig. 3.** Growth curves of C600 (*cys*$^+$), SY380 (*cysB*) and SY400 (*cysE*) in LB with different ammounts of novobiocin. Overnight cultures in LB with 0.5 mM cysteine were diluted to approximately $10^3$ cells/ml in LB with 0.5 mM cysteine and indicated amounts of novobiocin. Cultures were grown with aeration at 37° C and after 16 h of incubation titers were determined on LA plates. C600 (△); SY380 (○); SY400 (□)

CysE protein (serine transacetylase) which seems to be implicated solely in the synthesis of O-acetylserine (Denk and Böck 1987; Kredich 1987). Obviously, identification of new gene(s) affected by *cysB* and *cysE* mutations is necessary to open new directions of thinking. Such experiments, employing genetic fusions, are underway.

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