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# PROPERTIES OF *cysK* MUTANTS OF *ESCHERICHIA COLI K12**

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Triazole and azaserine resistant mutants of *E. coli K12* affecting *cysK* gene coding for *O*-acetylserine sulphydrylase were isolated. The *cysK* gene in *E. coli* is located in the same region of chromosome as the *cysK* gene in *Salmonella typhimurium*. All azaserine and some triazole resistant mutants require cysteine for growth at a normal rate. The *cysK* mutants have reduced sulphate uptake. Stability and transfer by conjugation of triazole resistant phenotype were checked. Differences in sulphate metabolism between closely related organisms *E. coli* and *S. typhimurium* are discussed.

The final step in cysteine biosynthesis is sulphydrylation of *O*-acetyl-L-serine. In *Salmonella typhimurium*, this reaction is catalysed by two sulphydrylases, A and B, coded for by genes *cysK* and *cysM*, respectively (Becker & Tomkins, 1969; Śledziewska & Hulanicka, 1978). Mutations in one of these sulphydrylases do not lead to cysteine auxotrophy, as one enzyme is sufficient to fulfil cysteine requirement. Mutations in *cysK* gene render cells resistant to 1,2,4-triazole (triazole), (Hulanicka *et al.*, 1974).

In our studies on *cysK* mutants in *E. coli K12* we have observed that some of them require cysteine for normal growth rate. However, isolation of tight auxotrophs on the basis of their resistance to triazole is not possible as L-cysteine reverses inhibition of bacterial growth by triazole (Bogusławski *et al.*, 1967). Therefore, a new method of isolation of *cysK* mutants of *E. coli K12* was worked out with the use of azaserine. Decomposition of this compound by *O*-acetylserine sulphydrylase to a toxic compound, diazoacetic acid, results in inhibition of growth (N.M. Kredich, personal communication). Strains with decreased *O*-acetylserine sulphydrylase activity are resistant to azaserine. Cysteine does not relieve the azaserine inhibition and therefore does not interfere with selection of azaserine-resistant mutants.

The present communication describes the isolation of *cysK* mutants. All these mutants show a pleiotropic effect, namely a decrease of sulphate permease activity, and some among them require cysteine or methionine for growth at a normal rate. Preliminary results of this work were reported previously (Wiater *et al.*, 1976).

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## MATERIALS AND METHODS

*Bacterial strains and phages.* All strains used were derivatives of *E. coli K12*. Their characteristics and origin are described in Table 1. Phage *P1CM clr* 100 was used for transduction (Rosner, 1972).

Table 1

*Escherichia coli K12 strains*

| Strain designation | Genotype | Source or derivation |
|---|---|---|
| EC437 | *thiA79 thyA54* HfrH5 | W.Kunicki-Goldfinger |
| EC438 | *thr leu met purC lam tsx strA phx* | Phabagen Collection |
| EC440 | *bgl thi ptsI* HfrKL16 | W. Epstein |
| EC630 | *thiA79 thyA54 cysK67* HfrH5 | Spontaneous 1,2,4-tria-zole resistant mutants of EC437 |
| EC632 | *thiA79 thyA54 cysK68* HfrH5 | |
| EC638 | *thiA79 thyA54 cysK69* HfrH5 | |
| EC640 | *thiA79 thyA54 cysK70* HfrH5 | |
| EC651 | *thiA79 thyA54 cysK71* HfrH5 | |
| EC446 | *thr leu thi phx rel* HfrH | Phabagen Collection |
| EC664 | *thr leu thi cysK72* HfrH | Spontaneous triazole-resistant mutants of EC446 |
| EC665 | *thr leu thi cysK*73 HfrH | |
| EC668 | *thr leu thi cysK74* HfrH | |
| EC452 | *thi purC lacY gal xyl mal lam strA phx* | Phabagen Collection |
| EC669 | *thi purC lacY gal xyl mal lam strA phx cysK75* | Spontaneous triazole-resistant mutants of EC452 |
| EC671 | *thi purC lacY gal xyl mal lam strA phx cysK76* | |
| EC672 | *thi purC lacY gal xyl mal lam strA phx cysK77* | |
| EC677 | *thiA79 thyA54 cysK78* HfrH5 | Spontaneous azaserine-resistant mutants of EC437 |
| EC678 | *thiA79 thyA54 cysK79* HfrH5 | |
| EC679 | *thiA79 thyA54 cysK80* HfrH5 | |
| EC680 | *thiA79 thyA54 cysK81* HfrH5 | |
| EC502 | *thiA79 thyA54 cysA61* HfrH5 | resistant to $CrO_4$ in EC437 |

Culture media, culture conditions and preparation of cell-free extracts were as described previously (Hulanicka *et al.*, 1972). The sulphate-free medium was denoted as SF.

*Genetic procedure.* Preparation of phage lysates and transduction were performed as described by Mojica-A (1975).

*Enzymes assays.* Sulphate permease activity was determined as reported previously (Karbonowska *et al.*, 1977). Sulphite reductase was assayed by the method of Siegel & Kamin (1971), and *O*-acetylserine sulphydrylase according to Kredich (1971). Protein was determined by the biuret method (Gornall *et al.*, 1949).

*Isolation of cysK mutants.* The *cysK* mutants were isolated by spreading 0.2 ml of overnight broth culture on the 10 mM-triazole - minimal agar plates or on the plates containing 4.5 μg/ml of azaserine and 0.1 mM-L-cysteine. Mutants arised spontaneously; all colonies which appeared after a few days on plates containing inhibitors were resistant to the inhibitor used. In some cases, triazole plates were supplemented with L-methionine to a final concentration of 0.1 mM.

*Chemicals. O*-Acetylserine was prepared by the method of Sakami & Toennies (1942). Other chemicals used were commercial products of reagent grade. Carrier-free sodium [$^{35}$S]sulphate was purchased from the Instytut Badań Jądrowych (Świerk, Poland).

## RESULTS

*Isolation of cysK mutants and their properties.* Some of the isolated triazole-resistant mutants showed poor growth on minimal media. Addition of L-cysteine or L-methionine normalized the growth behaviour. It has been observed that L-methionine in *E. coli K12*, unlike in *S. typhimurium*, did not relieve triazole inhibition of bacterial growth. The presence of this amino acid during isolation of mutants increased the number of cysteine bradytrophic *cysK* mutants. The use of azaserine selection (see Methods) enabled the isolation of mutants practically unable to grow on minimal media. The division times of the *cysK* mutants obtained are presented in Table 2. As can be seen from the data included, *cyskK67* and *cysK68* grew in sulphate minimal medium at a normal or slightly decreased rate. The growth rate of the third mutant of this class, *cysK71*, was reduced by a half. The division time of *cysK80* and *cysK81* isolated as azaserine-resistant mutants was about three times longer than that of wild type. Substitution of L-cysteine or L-methionine for sulphate, shortened generation time of *cysK* mutants to that observed for *cysK*$^{+}$ strains.

Table 2

*Division time of E. coli K12 cysK mutants*

The bacterial growth at 37°C was followed by measuring light transmittance at 650 nm of the cultures made in photometer tubes.

| Collection designation | Relevant genotype | Sulphur source | | |
|---|---|---|---|---|
| | | sulphate | L-cysteine | L-methionine |
| EC437 | wild type | 75 | 75 | 75 |
| EC632 | *cysK68* | 75 | 75 | 75 |
| EC630 | *cysK67* | 90 | 90 | 90 |
| EC651 | *cysK71* | 150 | 90 | 90 |
| EC679 | *cysK80* | 215 | 90 | 90 |
| EC680 | *cysK81* | 250 | 75 | 85 |

Mutants *cysK* isolated as triazole-resistant clones were sensitive to azaserine. The cross-resistance test of azaserine-resistant mutants was not feasible because these strains require cysteine for growth at a normal rate.

*Mapping*. Taking advantage of the homology between the chromosomes of *E. coli* and *S. typhimurium*, we started mapping triazole or azaserine resistance by determining its linkage with *cysA* and *ptsI* markers. In the case of azaserine-resistant strains, only linkage with *ptsI* locus could be checked as these strains are cysteine bradytrophs. The P1 transductants were selected on L-methionine plates for selection of *cysA*$^+$ or on mannitol plates containing either L-methionine or L-cysteine. As it was mentioned above, the presence of L-methionine did not interfere with scoring of triazole resistance phenotype by replica plating. The data presented in Table 3 show that azaserine or triazole resistance marker was cotransduced with *cysA* and *ptsI* alleles in 70% and 90%, respectively.

Table 3

*Linkage of cysK mutations by P1 transduction*

Transductions were performed to *cysA*$^+$ (the recipient strain EC502) on plates containing L-methionine, and to *pts*$^+$ (the recipient strain EC440) on mannitol plates containing either L-methionine or L-cysteine. Transductants were scored for either triazole or azaserine resistant phenotype by replica plating.

| Strain | Pertinent genotype | Recipient strains: EC502 *thiA79 thyA54 cysA61* — Number of transductants *cysA*$^+$ | *trz*$^-$ | Cotransduction (%) | Recipient strains: EC440 *bgl thi ptsI* — Number of transductants *pts*$^+$ | *trz*$^-$ | Cotransduction (%) |
|---|---|---|---|---|---|---|---|
| EC630 | *cysK67* | 206 | 139 | 68 | 87 | 78 | 90 |
| EC632 | *cysK68* | 194 | 137 | 70 | 87 | 82 | 94 |
| EC638 | *cysK69* | 173 | 125 | 72 | 73 | 64 | 88 |
| EC640 | *cysK70* | 170 | 125 | 73 | 69 | 62 | 90 |
| | | | | | *pts*$^+$ | *azas*$^r$ | Cotransduction (%) |
| EC677 | *cysK78* | — | — | — | 95 | 90 | 94 |
| EC678 | *cysK79* | — | — | — | 82 | 75 | 91 |
| EC679 | *cysK80* | — | — | — | 76 | 65 | 85 |
| EC680 | *cysK81* | — | — | — | 67 | 58 | 86 |

The above values of cotransduction and lack of *O*-acetylserine sulphydrylase activity (see below) suggest that triazole or azaserine resistance of the isolated mutants result from mutation in *cysK* gene coding foı *O*-acetylserine sulphydrylase. Therefore, the isolated mutants were denoted as *cysK*.

*Stability of cysK mutants.* In *S. typhimurium* there are two types of triazole-resistant mutants, *trzA* and *trzB*. We have recently demonstrated that *trzA* strains bear mutation in the structural gene for *O*-acetylserine sulphydrylase *A*, and this locus is denoted as *cysK* (Hulanicka *et al.*, 1974). The *trzB* mutants show the same linkage by transduction with *cysA* and *ptsI* markers as *cysK* mutants but, unlike *trzA* mutants, they are weakly linked by conjugation (Hulanicka & Kłopotowski, 1971). Furthermore, *trzB* mutants are unstable, readily segregate forming *trz*$^+$ colonies. In order to establish whether in *E. coli K12* two similar types of triazole-resistant mutants occur, properties of isolated mutants were studied in detail. Stability of triazole resistance phenotype was checked by replica plating of single colonies on a plate with or without the inhibitor. The segregation was never higher than 0.1%. In further experiments, transfer of triazole resistance marker by conjugation was examined. In all crosses the linkage of triazole resistance marker with the *purC* strain as a recipient was similar to the linkage found in the *trzA* mutants of *S. typhimurium*. These results indicate that in *E. coli*, unlike in *S. typhimurium*, only one type of triazole-resistant mutants could be isolated.

Table 4

*Specific activities of cysteine biosynthetic enzymes of E.coli K12 cysK mutants and their parental strains*

Bacteria were grown on L-djenkolate as sulphur source.

| Strain | Pertinent genotype | *O*-Acetylserine sulphydrylase (u/mg protein/min) | Sulphate permease (pmole/mg dry wt/min), substrate $^{35}SO_4$ | Sulphite reductase (nmole/mg protein/min) |
|---|---|---|---|---|
| EC437 | wild type | 14.8 | 20.0 | 4.0 |
| EC630 | *cysK67* | 0.7 | 2.0 | 2.7 |
| EC632 | *cysK68* | 0.1 | 2.1 | 4.0 |
| EC651 | *cysK71* | 0.18 | 4.5 | 2.0 |
| EC446 | wild type | 16.8 | 24.0 | 2.3 |
| EC664 | *cysK72* | 0.5 | 12.0 | 3.4 |
| EC665 | *cysK73* | 0.5 | 9.0 | 2.9 |
| EC668 | *cysK74* | 0.6 | 10.0 | 2.5 |
| EC452 | wild type | 6.2 | 21.0 | 2.6 |
| EC669 | *cysK75* | 0.2 | 10.1 | 2.2 |
| EC671 | *cysK76* | 0.1 | 2.0 | 2.1 |
| EC672 | *cysK77* | 0.01 | 1.6 | 2.7 |
| EC677 | *cysK78** | 0.05 | 0.6 | 4.5 |
| EC678 | *cysK79** | 0.06 | 1.4 | 3.5 |
| EC679 | *cysK80** | 0.01 | 0.8 | 4.1 |
| EC680 | *cysK81** | 0.03 | 1.0 | 4.6 |

* The mutants isolated as azaserine resistant.

*Cysteine enzymes in cysK mutants.* Cell-free extracts of the mutants isolated were assayed for cysteine biosynthetic enzymes (Table 4). As it could be expected from the results of mapping, the activities of *O*-acetylserine sulphydrylase of the mutants were low or nearly unmeasurable. A negative correlation exists between enzymatic activity of *O*-acetylserine sulphydrylase and generation time. *cysK80* and *cysK81*, characterized by the longest generation times, had the lowest *O*-acetylserine sulphydrylase activity (0.01 - 0.03 u/mg protein). The *cysK* mutant in which generation time was the same (*cysK68*), or slightly longer (*cysK67*) than that of the wild type, showed higher *O*-acetylserine sulphydrylase activity than cysteine bradytrophic mutants. The assay of sulphate permease showed that mutations in *cysK* gene affected the activity of this enzyme; in all *cysK* mutants studied, the uptake of sulphate was decreased by a factor from 0.5 to 10.

A similar decrease of sulphate permease activity was observed in the experiments in which $^{51}CrO_4$ was used as a substrate (results not shown). The decrease of sulphate permease activity was not related to the degree of cysteine bradytrophy of the mutants, *e.g.* the *cysK67* mutant had the generation time only slightly longer, but the activity of sulphate permease was lower by one order of magnitude. These observations suggest that cysteine bradytrophy in some *cysK* mutants depends on the remaining activity of *O*-acetylserine sulphydrylase. The activity of the third enzyme assayed, sulphate reductase, did not show any difference in comparison with the activity in the wild type.

## DISCUSSION

Isolation of *cysK* mutants in *E. coli K12* and studies of their properties demonstrated some differences in sulphate metabolism of so closely related organisms as *E. coli* and *S. typhimurium*. In contrast to the *S. typhimurium* mutants impaired in *cysK* gene, some *cysK* mutants of *E. coli* showed cysteine requirement for normal growth. The use of azaserine for isolation of the mutants with impaired *O*-acetylserine sulphydrylase activity enabled us to obtain mutants practically unable to grow on sulphate as a sole sulphur source (Table 2). Biochemical analysis of these mutants showed that mutations in *cysK* gene affect the activity of sulphate permease. Cysteine bradytrophy of the mutants seems to be related to the remaining *O*-acetylserine activity but not to the decreased level of sulphate permease. In the *cysK67* mutants growing at a normal rate on minimal medium, sulphate permease activity was decreased by a factor of 10. The activity of *O*-acetylserine sulphydrylase in this mutant (0.7 u/mg protein) was higher in comparison with that observed in cysteine bradytrophic *cysK* mutants, *e.g. cysK80* and *cysK81*.

Correlation between cysteine requirement and the decrease of *O*-acetylserine sulphydrylase activity in the mutants suggests the presence of only one sulphydrylase in *E. coli K12*. Methionine relieves the inhibition of growth in *S. typhimurium* by triazole equally effectively as L-cysteine, whereas in *E. coli* L-methionine had no effect. Since one molecule of L-cysteine is used for the synthesis of one molecule of L-methionine, the release of triazole toxicity by this amino acid is due to sparing of cysteine molecules. The lack of methionine effect in triazole inhibition could suggest higher sensitivity of *E. coli K12* cells to triazole. However, the growth experiments showed the same degree of sensitivity to triazole in both organisms (data not shown).

The lack of segregation of the triazole resistance marker and the transfer of triazole phenotype in *E. coli* by conjugation suggested the lack of *trzB* mutation, which is at variance with *trz* mutation in *S. typhimurium*. However, it is possible that in *E. coli K12*, *trzB* type of mutants should be sought among cysteine auxotrophs.

When our study was in progress, Fimmel & Loughlin (1977) reported on isolation of *cysK* mutants in *E. coli*; their mutants were isolated as the strains resistant to selenite or as colonies giving a black colour reaction on the bismuth indicator medium. The mutants showed an elevated level of sulphate reductase. Derepression of this enzymatic activity was caused by higher concentration of *O*-acetyl-L-serine, which was due to the decreased activity of sulphydrylase. In contrast, none of our 12 mutants isolated as directly resistant to triazole, and none of the azaserine-resistant mutants, showed the derepressed level of sulphite reductase. The results obtained by Fimmel & Loughlin (1977) are consistent with our data. However, these authors did not mention either cysteine bradytrophy of *cysK* mutants, or the pleiotropic effect of *cysK* mutations on the activity of sulphate permease.

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## WŁASNOŚCI MUTANTÓW *cysK ESCHERICHIA COLI K12*

### Streszczenie

Wyizolowano mutanty *cysK E. coli K12* jako kolonie oporne na triazol lub azaserynę. Gen *cysK* mapuje się w tym samym miejscu chromosomu co gen *cysK* u *Salmonella typhimurium*. Wszystkie mutanty wyizolowane jako oporne na azaserynę i niektóre otrzymane jako oporne na triazol wykazują zapotrzebowanie na cysteinę dla normalnego wzrostu. Mutanty *cysK* wykazują obniżone pobieranie siarczanu. Sprawdzono stabilność i zdolność do przenoszenia cechy oporności na triazol metodą koniugacji. Przedyskutowano zaobserwowane różnice w metabolizmie siarczanowym między *E. coli* a *S. typhimurium*.

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