Journal of General Microbiology (1982), **128**, 1785–1790. *Printed in Great Britain*

A New Class of Mutants of the *cysB* Regulatory Gene for Cysteine Biosynthesis in *Salmonella typhimurium*

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(*Received 23 July 1981; revised 2 October 1981*)

A new class of regulatory mutants in the *cysB* locus has been isolated by plating *cysM* strains, under anaerobic conditions, on medium containing 1,2,4-triazole. The isolated *cysB* mutants are cysteine prototrophs and triazole-resistant, although the levels of cysteine and *O*-acetyl-L-serine sulphydrylase are not changed. In contrast to the constitutive *cysB* mutants identified previously, the expression of the cysteine biosynthetic enzymes in the newly isolated mutants is regulated by the same factors as in wild-type strains. In the double mutant *cysE cysB2971*, the cysteine biosynthetic enzymes are absent with the exception of *O*-acetyl-L-serine sulphydrylase.

INTRODUCTION

The *cysB* region in *Salmonella typhimurium* and *Escherichia coli* is involved in the positive regulation of the cysteine regulon (Spencer *et al.*, 1967; Jones-Mortimer, 1968; Kredich, 1971). The detection of temperature-sensitive and amber mutations in *cysB* indicates that the gene product is a protein (Tully & Yudkin, 1975). The fine-structure genetic mapping of the *cysB* region suggests that it codes for a single polypeptide chain (Cheney & Kredich, 1975). Most known *cysB* mutations result in cysteine auxotrophy and these *cysB* strains cannot be derepressed for cysteine biosynthetic enzymes by any known nutritional means. A few prototrophic *cysB* constitutive mutants were found in the course of biochemical analysis of cysteine-requiring strains (Kredich, 1971). Recently, Śledziewska & Hulanicka (1978) described a method for selection of cysteine constitutive strains by plating *cysM* strains on medium containing 1,2,4-triazole. The *cysB*^{c} strains have high levels of cysteine biosynthetic enzymes even in the absence of *O*-acetyl-L-serine and these enzymes are not repressed by the addition of L-cysteine. The *cysB*^{c} strains are triazole- and selenate-resistant. They excrete sulphide which feeds cysteine auxotrophs and enables the growth of wild-type strains on triazole plates. An interesting, partially constitutive mutant, *cysB484*, has been described by Kredich (1971); this is a cysteine auxotroph with respect to the pathway of sulphate reduction but constitutive for the expression of *O*-acetylserine sulphydrylase. Sulphydrylation of *O*-acetyl-L-serine in *S. typhimurium* is catalysed by two sulphydrylases, A and B, coded by the genes *cysK* and *cysM*, respectively (Hulanicka *et al.*, 1979). Therefore, although cysteine auxotrophy occurs in strains lacking both enzymes, the *cysK* or *cysM* mutation alone does not lead to a requirement for cysteine. However, it has been observed that although *cysM* strains grow at a normal rate on minimal medium, they become cysteine bradytrophs under anaerobic conditions (Filutowicz & Hulanicka, 1978). We were therefore interested to see what kind of mutants might be obtained by plating *cysM* strains under anaerobic conditions on triazole plates. The present communication describes a regulatory *cysB* mutant of *S. typhimurium* with novel properties.

0022-1287/82/0001-0071 $02.00

Table 1. *Designation and derivation of bacterial strains*

Strain	Genotype	Source or derivation*
Salmonella typhimurium LT2		
TK1000	Wild-type	N. D. Zinder
DW24	*cysE396*	N. M. Kredich
DW25	*cysB^{c}1352*	N. M. Kredich
DW221	*ara-9 his-340 thy trpA160 pyrF146 trpC109*	N. M. Kredich
TK1470	*cysA20*	K. E. Sanderson
TK1530	*trpA52 cysB12 pyrF146*	K. E. Sanderson
TK1513	*pyrE125*	K. E. Sanderson
TK1001	*pyrE125 trp-1*	NG-induced mutation in TK1513
SB 3751	Δ(*cysK-ptsHI182*) *trpB223*	J. C. Cordaro
TK2058	Δ(*cysK-ptsHI182*) *cysM2328 trpB223*	Spontaneous mutation to azaserine resistance in SB3751
TK2072	*cysM2328 trpB223*	Transductant from TK1000 lysate × TK2058
TK2078	*cysM2328*	Transductant from TK2072 lysate × TK1470
TK2087	*cysM2328 cysB2971*	Spontaneous mutation under anaerobic conditions in TK2078
TK2090	*cysM2328 cysB2972*	DES-induced mutation under anaerobic conditions in TK2078
TK2085	*trpB223 cysM2328 cysB2973*	DES-induced mutation under anaerobic conditions in TK2072
TK2098	*pyrF146 cysB2971*	Transductant from TK2087 lysate × TK1530
TK2135	*pyrE125 cysB2971*	Transductant from TK2087 lysate × TK1001
TK2140	*cysB2971 cysE396*	Transductant from DW24 lysate × TK2135
TK2176	*ara-9 hisC340 thy cysB2971*	Transductant from TK2087 lysate × DW221
TK2177	*ara-9 hisC340 thy cysB2972*	Transductant from TK2090 lysate × DW221
TK2029	*pyrF146 cysB2971/pyrF$^+$ cysB$^+$*	F-ductant TK2098 × CGSC 4256
Escherichia coli		
CGSC 4256	*thi-1 pyrD34 his-68 trp-45 recA1 mtl-2 xyl-7 malA1 galK35 strA118 λ^{R}λ$^-$*/KLF123 *pyrF$^+$ cysB$^+$ trp$^+$*	*E. coli* Genetic Stock Center

* NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; DES, diethyl sulphate.

METHODS

Bacterial strains and phages. Bacterial strains and their sources are listed in Table 1. The phage P22 mutant L4 was used for transduction (Smith & Levine, 1967). Culture media and culture conditions were as described previously (Hulanicka & Kłopotowski, 1972). The sulphate-free medium was supplemented with 0·1 mM-Na_2SO_4, 0·5 mM-L-cystine or 0·5 mM-L-djenkolic acid.

Enzyme assays. NADPH-sulphite reductase (EC 1.8.1.2) activity was assayed by the method of Vito & Dreyfuss (1964). *O*-Acetyl-L-serine sulphydrylase (EC 4.2.99.8) activity was determined according to Kredich (1971); one unit (U) of *O*-acetylserine sulphydrylase is defined as the amount of enzyme catalysing the formation of 1 μmol cysteine min^{-1}. Antibodies against *O*-acetylserine sulphydrylase-A were prepared and immunological determination of this enzyme was performed as described by Hulanicka *et al.* (1974).

Isolation of cysB mutants. The new mutants were isolated by spreading 0·5 ml of an overnight broth culture on 10 mM-1,2,4-triazole minimal agar plates. The Petri dishes were placed in a vacuum chamber which was evacuated and refilled with nitrogen, thus providing almost completely anaerobic conditions. After incubation for a few days at 37 °C colonies of mutants appeared; these were purified and their ability to grow on 10 mM-triazole was checked by replica plating.

Protein was determined by the biuret method (Gornall *et al.*, 1949).

Chemicals. O-Acetyl-L-serine was prepared by the method of Sakami & Toennies (1942). Other chemicals used were commercial products of reagent grade.

RESULTS

Isolation of mutants

The *cysM* mutants were plated on 10 mM-1,2,4-triazole solid agar media under anaerobic conditions. Since the *cysM* mutants have a long generation time under these conditions,

Table 2. *Mapping of newly isolated triazole-resistant mutants*

The recipient in the first and second crosses was TK1001 (*pyrE125 trp-1*); the recipient in the third cross was DW221 (*trpA160 pyrF146 trpC109*).

Donor strain	Relevant genotype	Selection	No. of recombinants: Total	No. of recombinants: Trz$^-$	Cotransduction (%)
TK2087	*cysM2328 cysB2971*	Trp$^+$	104	32	30
TK2090	*cysM2328 cysB2972*	Trp$^+$	104	27	26
TK2087	*cysM2328 cysB2971*	Trp$^+$ PyrF$^+$	54	54	100

Table 3. *Activity of O-acetylserine sulphydrylase and sulphite reductase in newly isolated cysB mutants and control strains grown on different sulphur sources*

Strain	Relevant genotype	Sulphur source ...	*O*-Acetylserine sulphydrylase [U (mg protein)$^{-1}$]: L-Djenkolate	Sulphate	L-Cystine	Sulphite reductase [nmol min^{-1} (mg protein^{-1})]: L-Djenkolate	Sulphate	L-Cystine
TK1001	*cysB*$^+$		14	5	0·7	90	90	5
TK2078	*cysM2328*		16	0·9	0·2	80	10	6
TK2176	*cysM*$^+$ *cysB2971*		16	8	1·4	108	72	12
TK2177	*cysM*$^+$ *cysB2972*		20	5	0·9	136	58	6
DW25	*cysB*^{c}*1352*		14	11	9·4	141	122	103

triazole-resistant colonies appeared after a few days. These colonies were purified and their phenotypes were checked by replica plating.

Genetic mapping of Trz$^-$ mutants

Mapping of the isolated mutants was performed by P22-mediated transduction. So far, the known triazole-resistant mutants have been linked by P22-mediated transduction to three chromosomal markers: the *cysK* mutants with the *pts* operon (Hulanicka *et al.*, 1974); the *cysB*^{c} constitutive mutants with the *trp* operon and *pyrF* gene (Śledziewska & Hulanicka, 1978); and the *cysE* promoter-up mutant with *pyrE* (Hulanicka & Kredich, 1976).

Phage lysates prepared on the isolated mutants were used as donors in crosses where the recipients carried the above markers, and the transductants were scored for their resistance phenotype. Linkage of the triazole resistance phenotype was found only with *trp* and *pyrF* genes, and the triazole resistance mutation was 26–30% cotransducible with the *trp* gene (Table 2). These results indicate that the mutations rendering colonies resistant to triazole are located in the *cysB* region, since a similar linkage has been found for the *cysB* locus (Cheney & Kredich, 1975). In some crosses *trp* and *pyrF* markers were transduced to prototrophy; this required transfer of the whole *cysB* gene and so the linkage cannot be calculated.

Biochemical analysis

To avoid any possible confusion due to the presence of secondary mutations, as well as the effect of the *cysM* mutation, the biochemical assays were also performed on non-lysogenic transductants such as PyrF$^+$Trp$^+$Trz$^-$.

The activities of *O*-acetylserine sulphydrylase and sulphite reductase in cells grown on different sulphur sources were assayed. All the enzymes of the reductive pathway are known to respond in parallel to each sulphur source (Kredich, 1971). Thus, sulphite reductase can be considered as representative of the entire reductive pathway. As shown in Table 3, activities of both enzymes (sulphite reductase and *O*-acetylserine sulphydrylase) in the newly isolated

Table 4. *Repression index of O-acetylserine sulphydrylase and sulphite reductase in the wild-type, cysB^{c}1352 and transductants of the newly isolated cysB mutants*

The repression index is defined as the ratio of the enzyme activity of bacteria grown on L-djenkolic acid to the enzyme activity of bacteria grown on L-cystine.

Strain	Relevant genotype	Repression index: *O*-Acetylserine sulphydrylase	Repression index: Sulphite reductase
TK1001	*cysB*$^+$	20	18
TK2078	*cysM2328*	80	13
TK2176	*cysM*$^+$ *cysB2971*	11	9
TK2177	*cysM*$^+$ *cysB2972*	22	23
DW25	*cysB*^{c}*1352*	1·5	1·4

Table 5. *Activity of O-acetylserine sulphydrylase and sulphite reductase in cysE396, cysB2971 and cysB2971 cysE396 mutants*

Strain	Relevant genotype	Sulphur source . . .	*O*-Acetylserine sulphydrylase: L-Djenkolate	*O*-Acetylserine sulphydrylase: L-Cystine	Sulphite reductase: L-Djenkolate	Sulphite reductase: L-Cystine
DW24	*cysE396*		1·9	0·1	2	2
TK2135	*cysB2971*		14·0	1·5	110	12
TK2140	*cysB2971 cysE396*		7·0	0·8	12	17

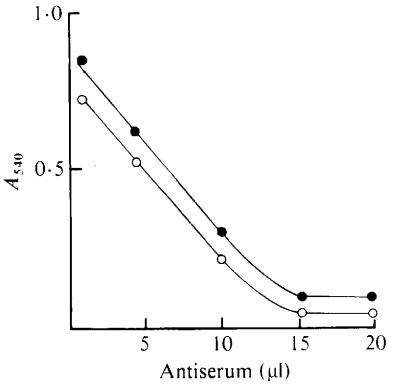

Fig. 1. Titration of *O*-acetylserine sulphydrylase-A of the *cysB2971* strain (●) and the double mutant *cysE396 cysB2971* (○) with antiserum to *O*-acetylserine sulphydrylase-A.

mutants were similar to those in the wild-type strain (TK1001). Growth on L-djenkolic acid caused derepression, whereas the presence of L-cystine repressed the enzyme activities. The normal regulation of cysteine biosynthetic enzymes in the Trz$^-$ mutants was clearly shown by comparison of the repression indexes of our mutants with that of the wild-type (Table 4). For both sulphite reductase and *O*-acetylserine sulphydrylase the repression indexes of our mutants were similar to that for the wild-type and varied from 9 to 23, whereas that for the *cysB*^{c} constitutive mutant was 1·4 (Śledziewska & Hulanicka, 1978).

The *cysB*^{c} constitutive mutants are characterized by a derepressed, unregulated level of cysteine biosynthetic enzymes and the expression of the cysteine structural genes even in the absence of the internal inducer, *O*-acetylserine (Kredich, 1971). The introduction into the

cysB^c mutant of a mutation in the *cysE* gene results in a requirement for cysteine. However, the cysteine biosynthetic enzymes in this double mutant, *cysE cysB*^{c}, show the same activities as the parental strain, the *cysB*^{c} mutant (Kredich, 1971).

It was of interest to determine whether the presence of the inducer is dispensable for the expression of cysteine biosynthetic enzymes in the newly isolated mutants. The *cysE396 cysB2971* strain, which is a non-reverting cysteine auxotroph totally lacking serine transacetylase (EC 2.3.1.30) activity, was grown on media with different sulphur sources and the activities of *O*-acetylserine sulphydrylase and sulphite reductase in crude extracts were assayed. The results (Table 5) show that the *cysE396* mutation slightly affects the activity of *O*-acetylserine sulphydrylase, whereas it leads to a lack of sulphite reductase activity.

In the wild-type, total sulphydrylase activity consists of two sulphydrylases: *O*-acetylserine sulphydrylase-A and *O*-acetylserine sulphydrylase-B, coded by *cysK* and *cysM*, respectively (Hulanicka *et al.*, 1979). To check whether the *cysK* gene is expressed in the *cysE cysB2971* strain, the crude extract of this strain was titrated with antibodies against the wild-type *O*-acetylserine sulphydrylase-A (Fig. 1). The decrease in enzyme activity after addition of antibodies indicates that *O*-acetylserine sulphydrylase was present in the assayed extracts. Thus the *cysK* gene is expressed in the *cysE396 cysB2971* mutant.

DISCUSSION

This paper describes the isolation and characterization of a new class of *cysB* mutants of *S. typhimurium*. The newly isolated triazole-resistant mutants differ from the previously described *cysB*^{c} strains. The cysteine biosynthetic enzymes of these mutants are regulated normally and their activities are the same as in wild-type strains (Table 3). They are not resistant to selenate and they can neither support growth of wild-type strains on triazole plates, nor feed cysteine auxotrophs (data not shown). Another difference was observed in the phenotype of merodiploids (data not shown). Analysis of the merodiploids *cysB*^{c}/*cysB*$^+$ showed that *cysB*^{c} is dominant to *cysB*$^+$ (Jagura & Hulanicka, 1978), whereas *cysB2971* is recessive to *cysB*$^+$. Introduction of the *cysB*$^+$ allele on the plasmid rendered cells sensitive to triazole. After segregation of the plasmid carrying the *cysB*$^+$ allele, the cells regained their resistance to triazole.

The newly isolated mutants are similar to that peculiar *cysB484* mutant in one way. The *cysB484* allele is unusual in that it results in a Cys$^-$ phenotype with a *cysB* effect on all the enzymes of the reductive pathway of cysteine biosynthesis, while causing the constitutive expression of *O*-acetylserine sulphydrylase (Kredich, 1971). We found that in the *cysB2971* mutant carrying the *cysE* mutation (TK2140) only the *cysK* gene is expressed (Table 5). This means that the impaired *cysB2971* product does not require the internal inducer *O*-acetyl-L-serine to permit binding of the RNA polymerase to the promoter of the *cysK* gene, whereas the presence of *O*-acetyl-L-serine is obligatory for the binding to other cysteine regulatory regions. However, the level of *O*-acetylserine sulphydrylase in TK2140 is regulated normally, whereas in the *cysB484* mutant it is expressed constitutively.

It is difficult to explain the triazole resistance of the *cysB2971* and *cysB2972* strains since they are characterized by a normal level of *O*-acetylserine sulphydrylase and lack of derepressed cysteine biosynthetic enzymes. As previously reported, a lack of *O*-acetylserine sulphydrylase or a high level of cysteine enzymes causes resistance to 1,2,4-triazole (Kredich *et al.*, 1975). One possible explanation is that the mutated *cysB* protein reacts with *O*-acetylserine sulphydrylase-A and alters the site for triazolylase, which confers cell resistance to triazole.

This work was supported by the Polish Academy of Sciences within Project 09.7.

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