Identification of the *Escherichia coli* *cysM* Gene Encoding *O*-Acetylserine Sulphydrylase B by Cloning with Mini-Mu-*lac* Containing a Plasmid Replicon

By AGNIESZKA E. SIRKO, MALGORZATA ZATYKA AND
M. DANUTA HULANICKA*

*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Rakowiecka 36,
02-532 Warsaw, Poland*

(Received 10 November 1986; revised 11 February 1987)

A region located at around 52' on the *Escherichia coli* chromosome was cloned by use of mini-Mu-*lac* containing a plasmid replicon and recloned into pBR322. Enzyme assays on transformants carrying the cloned fragments indicated the presence in the latter of the *cysA* and *cysM* genes coding for sulphate permease and *O*-acetylserine sulphydrylase B, respectively.

INTRODUCTION

In *Salmonella typhimurium* the presence of two genes, *cysK* and *cysM*, encoding *O*-acetylserine sulphydrylases A and B (OASS-A and OASS-B), respectively, is well documented (Hulanicka *et al.*, 1979). Either isoenzyme alone is sufficient for normal aerobic growth of bacteria on sulphate as sulphur source. The prototrophy of *cysK* mutants of *Escherichia coli* suggests the existence of two isoenzymes in this organism also (Fimmel & Loughlin, 1977; Wiater & Hulanicka, 1979). However, there is no published report of the existence of the second sulphydrylase-encoding gene. Boronat *et al.* (1984) located a gene specifying *O*-acetylserine (thiol)-lyase on the *E. coli* genome by analysing peptide synthesis directed by plasmids carrying DNA fragments from around 52' of the *E. coli* chromosome. Earlier, the gene order in this region had been established as: 52' *cysA* *gsr* *ptsI* *ptsH* *cysK* *cysZ* *lig* *dsd* 51' (Britton *et al.*, 1982, 1983). A similar gene order for this region in *S. typhimurium*, namely *cysK* *ptsHJ* *crr* *cysA*, was established by Nelson *et al.* (1984).

In this paper we report the results of attempts to locate the second gene, *cysM*, encoding OASS, in this region of the *E. coli* chromosome by cloning the region with the aid of mini-Mu-*lac* containing a plasmid replicon.

METHODS

Bacterial strains, plasmids and phages. These are listed in Table 1. Phage P1 lysates were prepared and transduction was performed according to Miller (1972). All procedures for handling Mu bacteriophage were as described by Bukhari & Ljungquist (1977).

Media and culture conditions. All Mu⁺ lysogenic cells were grown at 30 °C to avoid Mu repressor inactivation. Sulphur-free minimal medium (SF) was as described by Hulanicka *et al.* (1972) and 1 mM-djenkolic acid or 1 mM-sodium thiosulphate were used as sulphur sources. To check the triazole resistance of bacteria, 10 mM-1,2,4-triazole was used in the media. L-Broth (Luria & Burrows, 1957) was used routinely. Antibiotic supplements were ampicillin (50 µg ml⁻¹), tetracycline (25 µg ml⁻¹) or chloramphenicol (30 µg ml⁻¹). All solid media contained 1.5% (w/v) agar.

Abbreviations: OASS, *O*-acetylserine sulphydrylase; SP, sulphate permease; SF-medium, sulphur-free minimal medium.

Table 1. *E. coli* strains and plasmids

Strain	Genotype/phenotype	Reference
XPh43	F ⁻ Δ (argF lacIPOZYA) U169 trp Δ (brnQ phoA proC phoB phoR) Mucls (pBC4042)	M. C. Casadaban, Univ. of Chicago, USA
JA199	leu-6 trpE5 thi r ⁻ m _K ⁺	Stock collection
NK3	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK	Hulanicka <i>et al.</i> (1986)
EC1184	leu-6 trpE5 thi r ⁻ m _K ⁺ cysA15 Mucls	Stock collection
EC1185	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK Mucls	Mucls lysogen of NK3
EC2242	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK Mucls (pAMH2)	Transformant of EC1185
EC2243	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK Mucls (pAMH1)	Transformant of EC1185
EC2245	leu-6 trpE5 thi r ⁻ m _K ⁺ cysA15 Mucls (pAMH1)	Transformant of EC1184
EC2246	leu-6 trpE5 thi r ⁻ m _K ⁺ cysA15 Mucls (pAMH2)	Transformant of EC1184
EC2351	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK (pASH8)	Transformant of NK3
EC2352	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK (pASH11)	Transformant of NK3
EC2353	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK (pASH13)	Transformant of NK3
EC2356	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK ⁺	Transformant of NK3*
EC2357	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM ⁺ cysK ⁺	Transformant of NK3*
EC2358	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM ⁺ cysK	Transformant of NK3*
EC2359	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK (pAMH1)	Transformant of NK3
EC2360	leu-6 trpE5 thi r ⁻ m _K ⁺ cysM cysK (pAMH2)	Transformant of NK3
Plasmid		
pBC4042	pBCO::MudII4042	Groisman <i>et al.</i> (1984).
pAMH1	cysM	This work
pAMH2	cysM cysA	This work
pASH8	cysM cysA	This work
pASH11	cysM cysA	This work
pASH13	cysM	This work

* Transductant from JA199 lysate $\times$ NK3.

Enzyme assays. OASS activity was assayed as described by Kredich (1971). Sulphate permease (SP) activity was assayed according to Dreyfuss & Pardee (1966). Protein was determined by the biuret method using bovine serum albumin as a standard.

In vivo cloning. This was done by use of mini-Mu containing a plasmid replicon, as described by Groisman *et al.* (1984). The essential feature of this method is thermal derepression of the mini-Mu-lac MudII4042 carried on the pUC9 derivative pBCO. MudII4042 contains, as well as Mucls and lac markers, a chloramphenicol resistance gene and the plasmid pACYC184 replicon. Strain EC1185, which is a Mucls lysogen of the double mutant NK3 (cysK cysM), was used as recipient.

In vitro recombinant DNA techniques. Plasmid DNA isolated by the boiling method (Holmes & Quigley, 1981) was purified in a CsCl gradient. Restriction endonuclease digestions and ligation reactions were carried out in buffers recommended by Maniatis *et al.* (1982). Transformation was performed according to Cohen *et al.* (1972). Transformants were selected either by antibiotic resistance on LB plates or by cysteine prototrophy on minimal plates.

Agarose gel electrophoresis. DNA fragments were separated in 1% (w/v) agarose gels in Tris/acetate buffer (40 mM-Tris, 20 mM-sodium acetate, 1 mM-EDTA, pH 8.2) and their lengths were estimated in comparison with standard DNA fragments.

RESULTS

Presence of two genes coding for OASS activity on the E. coli chromosome

In order to demonstrate the presence of two genes coding for OASS we transduced the cysteine auxotroph NK3 (lacking OASS activity) to cysteine prototrophy using P1 phage grown on the wild-type strain. All transductants selected on minimal medium containing SO₄²⁻ as sulphur source were further characterized on the basis of their resistance to 10 mM-1,2,4-triazole and their ability to grow on 1 mM-S₂O₃²⁻ as sulphur source. Those discriminate between the genotypes as follows. OASS-A encoded by the cysK gene catalyses the reaction between 1,2,4-triazole and O-acetylserine producing 1,2,4-triazole-1-alanine, a compound not toxic for cells. However, the presence of this activity leads in parallel to exhaustion of the substrate for cysteine

Table 2. OASS activities

Bacteria were grown on SF medium with 1 mM-L-djenkolic acid or 0.1 mM-L-cysteine. Results are means of three to five experiments; the deviations from these means were less than 10%.

Strain	Relevant genotype	[U(mg protein) ⁻¹]	
		Sulphur source: cysteine	djenkolic acid
EC2356	<i>cysM cysK</i> ⁺	0.52	7.7
EC2357	<i>cysM</i> ⁺ <i>cysK</i> ⁺	0.92	8.0
EC2358	<i>cysM</i> ⁺ <i>cysK</i>	0.18	0.93
EC2243	<i>cysM cysK</i> (pAMH1)	—	0.28
EC2242	<i>cysM cysK</i> (pAMH2)	—	0.46
EC2359	<i>cysM cysK</i> (pAMH1)	0.15	0.33
EC2360	<i>cysM cysK</i> (pAMH2)	0.16	0.40
EC2351	<i>cysM cysK</i> (pASH8)	—	0.31
EC2352	<i>cysM cysK</i> (pASH11)	—	0.36
EC2353	<i>cysM cysK</i> (pASH13)	—	0.49
NK3	<i>cysM cysK</i>	0.11	0.17
EC1185	<i>cysM cysK</i> Mucls	0.12	0.10
JA199	Wild-type	0.68	10.6

synthesis and of an internal inducer of this regulon. Therefore, mutants impaired in the *cysK* gene are resistant to triazole (Trz^r) (Hulanicka *et al.*, 1974; Kredich *et al.*, 1975). Nakamura *et al.* (1983) reported that in *S. typhimurium* thiosulphate is assimilated exclusively by OASS-B encoded by the *cysM* gene; *cysM* mutants are unable to grow on thiosulphate as sulphur source.

Three classes of Cys⁺ transductants of the NK3 (*cysM cysK*) double mutant were found: (1) *cysM*⁺ *cysK*, (2) *cysM cysK*⁺ and (3) *cysM*⁺ *cysK*⁺. OASS activity in representative transductants picked out of these three classes are summarized in Table 2. Transductants of class 2 and 3 are triazole-sensitive; they cannot be distinguished on the basis of the sulphhydrylase activity assay. Mutations in the *cysM* gene do not lead to a measurable decrease of sulphhydrylase activity in *cysK*⁺ strains. However, transductants of class 3 differ from transductants of class 2 by their ability to grow on thiosulphate as sulphur source. The observed OASS activity in *cysM*⁺ *cysK* transductants was very low. However, their activity depended on the sulphur source in the medium (Table 2).

Cloning with mini-Mu-lac containing a plasmid replicon

Strain XPh43 lysogenic for Mucls and harbouring MudII4042 on plasmid pBCO was grown at 30 °C and transferred to 42 °C to induce transposition-replication and lysis. The lysate obtained (10⁹ p.f.u. ml⁻¹) was used in transduction to cysteine prototrophy with the *cysM cysK* Mucls strain EC1184 as recipient. Chloramphenicol-resistant (Cm^r) transductants were obtained at a frequency of 10⁻⁵ p.f.u. Cysteine-independent (Cys⁺) transductants were found among Cm^r colonies at a frequency of 1.5 × 10⁻².

Plasmid DNA was isolated from a few Cys⁺ Cm^r transductants and used for transformation of strain EC1185 to cysteine prototrophy and Cm^r. In this case all Cm^r colonies were Cys⁺ and all Cys⁺ colonies were Cm^r. These results indicated that both markers are carried on the same plasmid. Two transformants, EC2242 and EC2243, were chosen for further studies. Plasmid DNA from these strains was isolated and analysed on agarose gels. Strain EC2243 carries plasmid pAMH1 containing a 3.0 kb fragment of the *E. coli* chromosome, and strain EC2242 carries plasmid pAMH2 containing an 8.0 kb fragment of *E. coli* chromosome. Restriction mapping of the chromosomal fragments of these plasmids was made difficult due to the length of MudII4042 (Groisman *et al.*, 1984). Therefore, in order to obtain more precise maps of these fragments they were recloned in pBR322. Plasmids complementing cysteine auxotrophy of the *cysM cysK* mutant NK3 were selected. The construction of these plasmids is presented in Fig. 1. In pASH13 derived from pAMH1 the chromosomal insert is flanked by 1.0 kb (on the *Hind*III side) and 0.1 kb (on the *Bam*HI side) of mini-Mu-lac DNA. The chromosomal fragment of

Fig. 1. Scheme of plasmid construction. Double lines represent fragments of *E. coli* chromosome, filled bars the vector MudII4042 and single lines the vector pBR322; *bla*, ampicillin resistance gene; *tet*, tetracycline resistance gene. Restriction enzymes: H, *Hind*III, E, *Eco*RI, B, *Bam*HI.

pAMH2 was recloned either at the *Bam*HI site (pASH8) or the *Eco*RI site (pASH11) of pBR322. Plasmids pASH8 and pASH11 contain 0.1 kb and 3.1 kb DNA of mini-Mu-*lac* vector, respectively. Digestion of the above plasmids with available restriction enzymes enabled us to establish the restriction maps of the cloned fragments (Fig. 2).

OASS assay of transformants

In order to check if the cysteine prototrophy of all transformants results from restoration of OASS activity, crude extracts of several transformants were assayed for OASS. The representative results shown in Table 2 demonstrate a slight increase in OASS activity by comparison with that observed in the *cysM cysK* mutant NK3. Nevertheless, the generation time of transformants was only slightly longer than that of the wild-type (100 and 90 min respectively, on minimal medium – data not shown). Since OASS-A activity constitutes the main fraction of sulphhydrylase activity in the cell, a low level of OASS in transformants strongly suggests the presence of the *cysM* gene on the plasmids (Hulanicka *et al.*, 1979).

Presence of the *cysA* gene on pAMH2 and its derivatives

In *S. typhimurium* the *cysA* gene coding for SP is closely linked to the *cysM* gene (Hulanicka *et al.*, 1979). It was of interest to check if the constructed plasmids also carry the *cysA* gene. The *cysA* mutant EC1184 was transformed to chloramphenicol or ampicillin resistance with the

Fig. 2. Location of *cysA* and *cysM* genes and restriction map of the studied fragments of the *E. coli* chromosome. Hc, *HincII*; B, *BamHI*; E, *EcoRI*; P, *PstI*. *HincII* and *PstI* were only mapped in the region between 2.2 kb and 8.0 kb. No restriction sites for the following enzymes were found: *BglII*, *XhoI*, *HindIII* and *SalI*. The restriction site for *EcoRI* (left-hand site) was arbitrarily designated zero.

Table 3. *SP activities*

Bacteria were grown on SF medium with 1 mM-L-djenkolic acid.

Strain	Relevant genotype	[nmol SO ₄ ²⁻ min ⁻¹ ml ⁻¹ (OD ₆₅₀ unit) ⁻¹]
EC2245	<i>cysA</i> (pAMH1)	0.7
EC2246	<i>cysA</i> (pAMH2)	5.5
EC1184	<i>cysA</i>	0.0
JA199	Wild-type	2.7

constructed plasmids. Only pAMH2 and its derivatives pASH8 and pASH11 complemented the cysteine auxotrophy of the *cysA* mutant. Results of the SP assay are presented in Table 3. The level of SP activity in the transformant carrying pAMH2 is similar to that observed in the wild-type strain JA199, the parental strain of the *cysA* mutant. In the transformant carrying pAMH1 the SP activity was low and similar to that found in the *cysA* mutant.

Chromate inhibits the growth of *Enterobacteriaceae*. Strains impaired in *cysA* are resistant to chromate because there is no uptake of the inhibitor. Therefore, we checked chromate resistance by measuring the growth inhibition zones caused by various chromate concentrations (data not shown). As expected, the *cysA* mutant and the *cysA*/pAMH1 transformant were resistant to chromate. The strain transformed with pAMH2 showed about 10 times higher sensitivity to the inhibitor than the wild-type strain JA199.

DISCUSSION

The final step of L-cysteine biosynthesis in *S. typhimurium* and *E. coli* consists of the sulphhydrylation of *O*-acetylserine by free sulphide (Kredich, 1971). Two enzymes, encoded by the *cysK* and *cysM* genes, catalysing this reaction have been described in *S. typhimurium* (Hulanicka *et al.*, 1979). These genes are cotransducible but separated by genes unrelated to sulphur metabolism (Hulanicka *et al.*, 1986). Separation of the *cysK* gene from other cysteine genes of the cysteine regulon in this region was previously reported (Nelson *et al.*, 1984).

In this paper we have studied whether in *E. coli* sulphhydrylation of *O*-acetylserine is also catalysed by two separate enzymes, OASS-A and OASS-B, encoded by the genes *cysK* and *cysM*, respectively.

Cysteine prototrophy of *cysK* mutants of *E. coli* suggested the existence of a second OASS enzyme in *E. coli* (Wiater & Hulanicka, 1979). The results presented in this paper, i.e. the

presence of three classes of Cys⁺ transductants of the NK3 strain (which completely lacks OASS activity) and the complementation of the cysteine auxotrophy of the NK3 strain by the constructed plasmids clearly indicate that indeed a *cysM* gene, coding for the second OASS enzyme, exists in *E. coli*.

In *S. typhimurium* the product of the *cysM* gene, OASS-B, enables utilization of thiosulphate as a sulphur source. The finding that the intact *cysM* gene is required for growth on thiosulphate as a sulphur source indicates that OASS-B of *E. coli* also displays the second activity. It catalyses the reaction between *O*-acetylserine and thiosulphate to give *S*-sulphocysteine.

In *S. typhimurium* the *cysM* gene is situated close to the *cysA* gene and the *pts* operon (Nelson *et al.*, 1984; Hulanicka *et al.*, 1986). The *cysA20* deletion covers *cysM* (Hulanicka *et al.*, 1979). The close linkage of the *cysA* gene with the *pts* operon in *E. coli* was previously established by P1 transduction (Karbonska *et al.*, 1977). Therefore, it was of interest to check if constructed plasmids also carry the *cysA* gene. Complementation by pAMH2 of *cysA* mutant EC1184, sulphate permease assay (Table 3) and chromate resistance tests indicate that the chromosomal fragment of pAMH2 carries the *cysA* gene.

The restriction maps of cloned fragments and their comparison with the map of this region reported by Boronat *et al.* (1983) allowed location of the *cysM* gene between *cysA* and the *pts* operon (Fig. 2).

Assay of OASS activities in crude extracts of *cysM*⁺ *cysK* transductants of NK3 strain and its transformants carrying plasmids with the *cysM* gene showed a low increase of enzyme activity in comparison with the level found in the NK3 mutant. However, even such a low increase of enzyme activity was sufficient to restore cysteine prototrophy.

The level of cysteine enzymes in the wild-type cells depends on the sulphur source in the medium (Kredich, 1971). L-Cysteine causes repression whereas growth on a poor sulphur source such as L-djenkolate leads to a derepressed level of cysteine enzymes. Results presented in Table 2 show that growth of the transductant *cysM*⁺ *cysK* on L-djenkolate gives an OASS-B level higher than that found in L-cysteine-grown cells. The dependence of OASS-B activity on the sulphur source indicates that the expression of the *cysM* gene is regulated by the same factors that control other enzyme activities of the cysteine regulon.

A similar regulation of OASS activities was found in transformants carrying plasmids with a cloned *cysM* gene. This finding indicates that the expression of the *cysM* gene proceeds from its own promoter. Interestingly, the presence of the *cysM* gene on a high copy number plasmid did not lead to an increase of OASS activity. Similar observations were made with plasmids carrying other cysteine genes (unpublished results). Plasmid pGBK5 carrying the *cysJIH* operon of *S. typhimurium* on pBR322 did not cause a higher level of sulphate reductase in crude extracts of transformants, probably because of limitation of the positive regulatory protein, the *cysB* gene product.

This work was supported by the Polish Academy of Sciences within the project 3.13.

REFERENCES

- BORONAT, A., BRITTON, P., JONES-MORTIMER, M. C., KORNBERG, H. L., LEC, L. G., MURFITT, D. & PARRA, F. (1984). Location on the *Escherichia coli* genome of a gene specifying *O*-acetylserine (thiol)-lyase. *Journal of General Microbiology* **130**, 673–685.
- BRITTON, P., MURFITT, D., PARRA, F., JONES-MORTIMER, M. C. & KORNBERG, H. L. (1982). Phosphotransferase-mediated regulation of carbohydrate utilization in *Escherichia coli* K12: identification of the products of genes on the specialized transducing phages *iex* (*crr*) and *gsr* (*tgs*). *EMBO Journal* **1**, 907–911.
- BRITTON, P., BORONAT, A., HARTLEY, D. A., JONES-MORTIMER, M. C., KORNBERG, H. L. & PARRA, F. (1983). Phosphotransferase-mediated regulation of carbohydrate utilization in *E. coli* K12: location of the *gsr* (*tgs*) and *iex* (*crr*) genes by specialized transduction. *Journal of General Microbiology* **129**, 349–356.
- BUKHARI, A. J. & LJUNGQUIST, E. (1977). In *DNA Insertion Elements, Plasmids and Episomes*. Edited by A. J. Bukhari, J. A. Shapiro & S. L. Adhya. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- COHEN, S. N., CHANG, A. C. Y. & HSU, L. (1972). Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. *Proceedings of the National Academy of Sciences of the United States of America* **69**, 2110–2114.

- DREYFUSS, J. & PARDEE, A. B. (1966). Regulation of sulphate transport in *S. typhimurium*. *Journal of Bacteriology* **91**, 2275–2280.
- FIMMEL, A. L. & LOUGHIN, R. E. (1977). Isolation and characterization of *cysK* mutants of *Escherichia coli* K12. *Journal of General Microbiology* **103**, 37–43.
- GROISMAN, E. A., CASTILHO, B. A. & CASADABAN, M. J. (1984). In vivo DNA cloning and adjacent gene fusing with a mini-Mu-lac bacteriophage containing a plasmid replicon. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 1480–1483.
- HOLMES, D. S. & QUIGLEY, M. (1981). Rapid boiling method for the preparation of bacterial plasmids. *Analytical Biochemistry* **114**, 193–197.
- HULANICKA, M. D., KLOPOTOWSKI, T. & SMITH, D. A. (1972). The effect of triazole on cysteine biosynthesis in *Salmonella typhimurium*. *Journal of General Microbiology* **72**, 291–301.
- HULANICKA, M. D., KREDICH, N. M. & TREIMAN, D. M. (1974). The structural gene for *O*-acetylserine sulphydrylase A in *Salmonella typhimurium*. *Journal of Biological Chemistry* **249**, 867–872.
- HULANICKA, M. D., HALLOQUIST, S. G., KREDICH, N. M. & MOJICA, A. T. (1979). Regulation of *O*-acetylserine sulphydrylase B by L-cysteine in *Salmonella typhimurium*. *Journal of Bacteriology* **140**, 141–146.
- HULANICKA, M. D., GARRET, CH., JAGURA-BURDZY, G. & KREDICH, N. M. (1986). Cloning and characterization of the *cysAMK* region of *Salmonella typhimurium*. *Journal of Bacteriology* **168**, 322–327.
- KARBONOWSKA, H., WIATER, A. & HULANICKA, M. D. (1977). Sulphate permease of *Escherichia coli* K12. *Acta biochimica polonica* **24**, 329–334.
- KREDICH, N. M. (1971). Regulation of L-cysteine biosynthesis in *Salmonella typhimurium*. *Journal of Biological Chemistry* **246**, 3474–3484.
- KREDICH, N. M., FOOTE, L. J. & HULANICKA, M. D. (1975). Studies of the mechanism of inhibition of *Salmonella typhimurium* by 1,2,4-triazole. *Journal of Biological Chemistry* **250**, 7324–7331.
- LURIA, S. E. & BURROWS, J. W. (1957). Hybridization between *Escherichia coli* and *Shigella*. *Journal of Bacteriology* **74**, 461–476.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning. A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- MILLER, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- NAKAMURA, T., KON, Y., IWASHI, H. & EQUIHI, Y. (1983). Evidence that thiosulphate assimilation by *Salmonella typhimurium* is catalysed by cysteine synthase B. *Journal of Bacteriology* **156**, 656–662.
- NELSON, S. O., SCHUITEMA, A. R. J., BENNE, R., VAN DER PLOEG, L. H. T., PLIJTER, J. S., AAN, F. & POSTMA, P. W. (1984). Molecular cloning, sequencing and expression of the *crr* gene: the structural gene for III^{Glc} of the bacterial PEP:glucose phosphotransferase system. *EMBO Journal* **3**, 1587–1593.
- WIATER, A. & HULANICKA, D. (1979). Properties of *cysK* mutants of *Escherichia coli* K12. *Acta biochimica polonica* **26**, 21–28.