The *Bacillus subtilis* *cysP* gene encodes a novel sulphate permease related to the inorganic phosphate transporter (Pit) family

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Sulphate permeases in the plasma membrane are responsible for uptake of environmental sulphate used in the sulphate assimilation pathway in bacteria and plants. Here it is reported that the ORF designated *cysP*, located on the *Bacillus subtilis* chromosome between *cysH* and five putative genes involved in sulphate assimilation, encodes a sulphate permease. *cysP* is able to complement *Escherichia coli* cysteine auxotrophs with mutations affecting either the membrane or periplasmic components of the sulphate-thiosulphate permease. Transport studies with cell suspensions of a *cysA97 E. coli* strain transformed with a plasmid expressing the *B. subtilis* *cysP* gene indicated that CysP catalyses sulphate uptake. Analysis of the primary sequence showed that CysP (354 amino acids, estimated molecular mass 24 kDa) is a highly hydrophobic protein which has 11 putative transmembrane helices. Sequence comparisons revealed that CysP, together with the phosphate permease of *Neurospora crassa*, Pho-4, and *E. coli* PitA, belongs to the family of related transporters, the inorganic phosphate transporter (Pit) family. Among the putative phosphate permeases, CysP shows a similar size and the same domain organization as the archaeal transporters. This is the first report of a sulphate permease in a Gram-positive organism.

Keywords: *Bacillus subtilis*, cysteine biosynthesis, sulphate, transport

INTRODUCTION

In many micro-organisms and higher plants sulphur metabolism is initiated by the uptake of sulphate from the environment. These organisms assimilate sulphate into cysteine, the first sulphur-containing amino acid, and various sulphur-containing secondary metabolites (Kredich, 1996). Thus, uptake of sulphate by cells is considered to be the key entry step of the sulphur cycle in nature. Because the sulphate transport system is involved in this initial step, it should play a central role in the regulation of the entire sulphur metabolism pathway by controlling the import of available sulphate. Among bacterial sulphate transporters, those from *Escherichia coli* and *Salmonella typhimurium* are the best studied. These micro-organisms possess an ATP-binding cassette (ABC)-type sulphate-thiosulphate transport system (Higgins, 1992), which is controlled in parallel with cysteine biosynthetic enzymes and which is a part of the cysteine regulon; gene expression of this

region requires sulphur limitation and a positive regulator, CysB, the product of the *cysB* gene (Kredich, 1996). Components of the sulphate-thiosulphate permease from *E. coli* and *S. typhimurium* are encoded by the contiguous genes *cysP*, *cysT*, *cysW* and *cysA*, and by the unlinked gene *sbp* (Kredich, 1996). The products of *cysT* and *cysW* span the membrane and form a channel for the passage of sulphate and related ions, *cysA* encodes a hydrophilic membrane-associated ATP-binding protein, and *sbp* and *cysP* encode the sulphate and thiosulphate periplasmic binding proteins, respectively (Hryniewicz *et al.*, 1990; Sirko *et al.*, 1990). Molecular analysis of sulphate transport in *Synechococcus* sp., a cyanobacterium, established a four-component membrane sulphate transport system, essentially similar to that of *E. coli* (Laudenbach & Grossman, 1991).

The *Neurospora crassa* *cys-14⁺* gene, the first eukaryotic sulphate transporter gene to be cloned, encodes a protein of approximately 90 kDa with 12 putative hydrophobic membrane-spanning domains (Ketter *et al.*, 1991). The CYS14 protein is localized within the plasma membrane fraction and its synthesis depends on sulphur depri-

Abbreviation: TMS, transmembrane segment.

Fig. 1. Genetic organization of the *B. subtilis* operon containing *ylnA*. The ORFs encoding enzymes similar to those of cysteine biosynthesis are shaded. The arrows indicate the direction of gene transcription. Relevant restriction sites are indicated.

vation. Additional genes that encode H^+ /sulphate co-transporters have been identified in *Homo sapiens* (Hastbacka *et al.*, 1994), the tropical legume *Stylosanthes hamata* (Smith *et al.*, 1995a), *Saccharomyces cerevisiae* (Smith *et al.*, 1995b), *Mus musculus* (Kobayashi *et al.*, 1997), *Arabidopsis thaliana* (Takahashi *et al.*, 1997) and *Yersinia enterocolitica* (Hoffmann *et al.*, 1998). All of them show significant homology to each other and, together with CYS14, represent a family of membrane transport proteins, the sulphate permease (SulP) family.

In contrast to the knowledge of the sulphate transport system in Gram-negative bacteria and several eukaryotes, no information is available about the genes or the transport proteins involved in sulphate uptake in *Bacillus subtilis* or other Gram-positive organisms. We have recently reported the isolation of the *B. subtilis* *cysH* gene, whose product is 3'-phosphoadenosine 5'-phosphosulphate (PAPS) sulphotransferase and whose expression is repressed by cysteine and sulphide and induced by sulphur limitation (Mansilla & de Mendoza, 1997). A partial DNA sequence downstream of *cysH* revealed a second ORF, called ORF2, that gave a deduced amino acid sequence similar to that of a putative phosphate permease. As a result of the *B. subtilis* genome sequencing project, the complete nucleotide sequence of ORF2 was determined (Kunst *et al.*, 1997). This ORF was named *ylnA*; its gene product has 354 amino acids and it has a predicted molecular mass of 42.3 kDa. Both the *cysH* and *ylnA* genes form part of a 6074 bp putative operon containing seven ORFs (Fig. 1). It has been demonstrated that three of these ORFs encode enzymes involved in cysteine biosynthesis: *cysH* encodes the PAPS sulphotransferase (Mansilla & de Mendoza, 1997), whilst *ylnD* and *ylnF* encode proteins involved in synthesis of sirohaem, a cofactor of sulphite reductase (Johansson & Hederstedt, 1999). *ylnB* and *ylnC* appear to be homologous to ATP-sulphurylase and adenosine 5'-phosphosulphate (APS) kinase, respectively, whilst *ylnE* does not show significant homology with any known protein contained in the databases.

In this report we show that *ylnA* encodes a membrane-associated protein able to restore the capacity of *E. coli*

mutants unable to transport sulphate to grow in minimal media with sulphate as a sulphur source. In addition, we demonstrate that the *B. subtilis* *ylnA* gene encodes a permease required for sulphate uptake, rather than a phosphate permease.

METHODS

Bacterial strains, plasmids and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* and *B. subtilis* strains were routinely grown in Luria-Bertani (LB) broth (Sambrook *et al.*, 1989). The minimal medium used for *E. coli* was M9 (Miller, 1972) supplemented with 0.4% glucose, 2 mM $MgSO_4$, 0.001 mM $CaCl_2$ and 0.01% thiamin. For nutritional studies, $MgCl_2$ replaced $MgSO_4$, and glutathione (1 mM) or cystine (1 mM) were used as sulphur sources. Antibiotics were added to media as follows: 100 μg ampicillin ml^{-1} , 5 μg chloramphenicol ml^{-1} . Spizizen salts (Spizizen, 1958), supplemented with 0.5% glucose and the required L-amino acids, was used as the minimal medium for *B. subtilis*. In nutritional studies, where different sulphur sources were tested, $MgSO_4$ and $(NH_4)_2SO_4$ were substituted by an equimolar amount of $MgCl_2$ and NH_4Cl , respectively.

Chromate resistance was tested on LB agar plates to which sterile 0.25 mM K_2CrO_4 was added (Pardee *et al.*, 1966).

Plasmid constructions. Plasmid preparations, restriction enzyme digestions and agarose gel electrophoresis were carried out according to methods described by Sambrook *et al.* (1989). Competent *E. coli* cells were transformed with supercoiled plasmid DNA by using the calcium chloride procedure or by electroporation (Sambrook *et al.*, 1989).

Expression analysis. Recombinant plasmids containing the appropriate DNA fragments under T7 promoter control were transformed into *E. coli* BL21(λ DE3). Induction of gene expression was done as described by Studier *et al.* (1990). Briefly, mid-exponential-phase cultures (1 ml) of BL21(λ DE3) carrying plasmids pBluescript II SK(+) or pBS181 were induced by adding 1 mM IPTG. After 10 min the cultures received rifampicin (100 μg ml^{-1}) to inhibit mRNA synthesis from the *E. coli* chromosome. After further incubation for 1 h, they were labelled with 1 μCi [^{35}S]methionine [specific activity 1000 Ci $mmol^{-1}$ (37 TBq $mmol^{-1}$)]. After 10 min, the cells were harvested by centrifugation, washed twice with 10 mM Tris/HCl buffer (pH 7.4), and stored frozen until analysis. Cells were resuspended in 30 mM Tris/HCl buffer (pH 7.4) containing 10 mM $MgCl_2$ and 1 mM PMSF, and disrupted by sonication (four or five 10 s bursts) using a VibraCell Ultrasonic Processor (Sonics & Materials). The extracts were centrifuged at 190000 g for 1 h, and labelled proteins present in either soluble or membrane fractions were separated by SDS-PAGE and detected by autoradiography.

Assay of sulphate transport. Cultures of strain JM2314 carrying relevant plasmids were grown in M9 minimal medium supplemented with glutathione as sulphur source to exponential phase. Cells were collected, washed and then resuspended in Davis salts (Miller, 1972) and incubated for 5 min at 30 °C. The measurement of sulphate transport was performed by incubating at 30 °C a cell suspension containing 10^8 cells ml^{-1} , 30 μg chloramphenicol ml^{-1} , 0.01 mM sodium sulphate and approximately 10^6 c.p.m. $^{35}SO_4^{2-}$ ml^{-1} (1050 Ci $mmol^{-1}$). The incubation period was terminated by filtering the cell suspension through a 0.45 μm Millipore filter. The filters were washed with 5 ml Davis salts. Filters were

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Source or reference
<i>Bacillus subtilis</i>		
JH642	<i>trpC2 pheA1</i>	Laboratory stock
<i>Escherichia coli</i>		
DH5 α	<i>supE44 thi-1 ΔlacU169(ϕ80dlacZΔM15) <i>endA1 recA1</i> <i>hsdR17 gyrA96 relA1</i></i>	Laboratory stock
BL21(λ DE3)	F ⁻ <i>ompT</i> r _B ⁻ m _B ⁻ pLysS	Laboratory stock
JM2314	<i>cysA97</i>	CGSC*
EC2402	<i>sbp cysP</i>	Sirko <i>et al.</i> (1995)
EC2256	F ⁺ <i>araD319 ΔlacU169 rpsL thi fla trp6 cysT329::lac</i> <i>imm</i> ^{λp1(209)}	Hryniewicz <i>et al.</i> (1990)
Plasmids		
pBluescript II SK(+)	<i>E. coli</i> cloning vector, Amp ^R	Stratagene
pBS170	3.4 kb <i>EcoRI</i> – <i>EcoRI</i> fragment of chromosomal DNA of JH642 cloned into pBluescript II SK(+)	Mansilla & de Mendoza (1997)
pBS181	1.98 kb <i>BglII</i> – <i>HindIII</i> fragment of pBS170 cloned into pBluescript II KS(+) digested with <i>Bam</i> HI/ <i>Hind</i> III	This study
pBS184	2.2 kb <i>PstI</i> – <i>PstI</i> fragment of pBS170 (extending from the <i>PstI</i> site in <i>ylnA</i> to the <i>PstI</i> site in the pBS170 polylinker) cloned into pBluescript II KS(+) digested with <i>PstI</i>	This study
pBS188	1.32 kb <i>BglII</i> – <i>ClaI</i> fragment of pBS170 cloned into pBluescript II SK(+) digested with <i>Bam</i> HI/ <i>ClaI</i>	This study
pBS190	pBS181 digested with <i>PstI</i> , the protruding ends digested with Klenow DNA polymerase and the fragment religated	This study

* Strain obtained from the *Escherichia coli* Genetic Stock Center.

transferred to polyethylene vials containing 2 ml Optiphase 'HiSafe 3' scintillation fluid (Wallac) and the radioactivity counted in an LKB Primo liquid scintillation counter. Uptake rates are expressed in nmol sulphate min⁻¹ (g cellular protein)⁻¹.

RESULTS AND DISCUSSION

The *ylnA* gene encodes a sulphate transporter

The *ylnA* ORF from *B. subtilis* shows homology with phosphate permeases (Mansilla & de Mendoza, 1997) but no significant homology with proteins involved in sulphate uptake (see below). However, we have found that *ylnA* is transcriptionally induced by sulphate starvation, together with *cysH* and other genes involved in cysteine biosynthesis (unpublished results), suggesting that its gene product is a sulphate permease. To test the possibility that the *ylnA* gene product is involved in sulphate transport, we tried complementation of different classes of *E. coli* mutants unable to transport sulphate and thus to use sulphate as a sole sulphur source. Mutants affected in *cysT* as well as the uncharacterized *cysA97* mutant are cysteine auxotrophs and they are unable to utilize sulphate as a sole sulphur source (Kredich, 1996). Strains carrying a single mutation in *cysP* or *sbp* are able to utilize both sulphate and

thiosulphate as a sole sulphur source, whilst the inactivation of both genes leads to cysteine auxotrophy resulting from the block in the transport of both ions (Sirko *et al.*, 1995).

Complementation analysis was performed with plasmid pBS170 (which contains *cysH*, *ylnA* and the 5' portion of *ylnB*, Table 1) and its derivatives: pBS181 (which includes a complete copy of *ylnA*), pBS184 (containing *ylnA* lacking the first 60 bp of the ORF), pBS188 (which includes *ylnA* without the last 140 bp of the ORF) and pBS190, a derivative of pBS181 possessing a frameshift mutation in *ylnA* (Table 1). As shown in Fig. 2, only plasmids pBS170 and pBS181, containing a complete functional copy of *ylnA*, restored the capacity of the *cysA97*, *cysT* and *cysP sbp* *E. coli* mutants to grow in minimal medium with sulphate as a sulphur source. These data strongly suggested that *ylnA* encodes a protein involved in sulphate transport. Moreover, the protein encoded by this gene should have a substrate recognition site since *ylnA* is able to bypass the requirement of *E. coli* for the *sbp* and *cysP* genes, which encode the sulphate and thiosulphate periplasmic binding proteins (Sirko *et al.*, 1995).

To directly prove that *ylnA* encodes a sulphate transporter we tested whether plasmid pBS181 could restore

Plasmid	SO_4^{2-}			CrO_4^{2-}		
	<i>cysA97</i>	<i>cysT</i>	<i>cysP sbp</i>	<i>cysA97</i>	<i>cysT</i>	<i>cysP sbp</i>
pBS170	+	+	+	-	-	-
pBS181	+	+	+	-	-	-
pBS184	-	-	-	+	+	+
pBS188	-	-	-	+	+	+
pBS190	-	-	-	+	+	+
<i>ylnA</i>						

Fig. 2. Growth of *E. coli* mutants deficient in sulphate transport in the presence of different *ylnA* region plasmids. Strains JM2314 (*cysA97*), EC2256 (*cysT*) and EC2402 (*cysP sbp*) transformed with the appropriate plasmid were tested for growth in minimal medium supplemented with 1 mM MgSO_4 as a sulphur source or in LB medium with 0.25 mM K_2CrO_4 . -, No growth; +, good growth.

Fig. 3. Uptake of $^{35}\text{SO}_4^{2-}$ by plasmid-carrying strains. Sulphate transport was measured as described in Methods. Each datum point is the mean of three separate experiments with a mean error in transport values of less than 5%. ●, JM2314 [pBluescript II SK(+)] ; ▼, JM2314 (pBS181).

the capacity to transport sulphate to the *E. coli cysA97* strain JM2314. To this end, cultures of strain JM2314 carrying plasmids pBluescript II SK(+) or pBS181 were assayed. In whole-cell transport assays, strain JM2314 bearing pBS181 showed $^{35}\text{SO}_4^{2-}$ transport activity, whereas no measurable accumulation of radioactive sulphate was observed in strain JM2314 transformed with pBluescript II SK(+) (Fig. 3). This experiment clearly demonstrates that the *B. subtilis ylnA* gene product possesses sulphate permease activity. Thus, we have renamed this gene *cysP*, the letter P denoting a permease.

Chromate sensitivity in *E. coli* and *B. subtilis*

In *Pseudomonas aeruginosa* and *Alcaligenes eutrophus*, chromate is accumulated by sulphate uptake systems, and expression of ChrA (chromate resistance protein A)

leads to reduced accumulation of chromium (Cervantes *et al.*, 1990; Nies & Silver, 1989). Since the genome of *E. coli* does not encode ChrA homologues, resistance to chromate in this micro-organism has been associated with a deficiency in sulphate uptake. We tested whether plasmids containing *B. subtilis cysP* could confer chromate sensitivity to *E. coli* strains unable to transport sulphate. The result of this experiment showed that plasmids pBS170 and pBS181 conferred sensitivity to chromate to the *cysT*, *cysP sbp* and *cysA97* mutants (Fig. 2), indicating that the *cysP* gene product can mediate the transport of chromate, as does the *E. coli* sulphate transporter system.

We have found that sulphate is able to relieve the toxicity of chromate in *cysP*⁺ *B. subtilis*. In fact, strain JH642 is resistant to chromate in LB medium or in minimal media supplemented with 1 mM sulphate (data not shown). However, in minimal media supplemented with 1 mM glutathione as a sole sulphur source, the growth of strain JH642 is inhibited by chromate (data not shown). The toxicity of chromate in this case is relieved by the addition of 1 mM sulphate, thus indicating that sulphate by itself leads to reduced accumulation of chromate (data not shown). These results agree with the suggestion that the *B. subtilis* ChrA homologues YwrA and YwrB could catalyse a chromate/sulphate antiport, exchanging sulphate for the toxic accumulated chromate (Nies *et al.*, 1998).

Expression of *cysP* in *E. coli*

To analyse the protein encoded by *cysP*, we used plasmid pBS181 containing *cysP* under the control of the T7 promoter (Table 1). This plasmid was transformed into *E. coli* BL21(λ DE3). Induction of gene expression was done according to the method of Studier *et al.* (1990). Expression of the DNA insert containing *cysP* in pBS181 resulted in the detection of a protein of 24 kDa (Fig. 4, lane 2). As described in the Introduction, the molecular mass calculated from the deduced primary sequence of CysP (YlnA) is 42.3 kDa, larger than the molecular mass of 24 kDa estimated by the mobility of the *cysP* gene product. Such an aberrant migration on SDS-PAGE is

Fig. 4. Expression of *B. subtilis* *cysP* in *E. coli* BL21(ΔDE3). Cultures harbouring plasmid pBluescript II SK(+) (lane 1) and pBS181 (lane 2) were labelled with [³⁵S]methionine as described in the text. Samples were analysed by SDS-PAGE. The positions of molecular mass markers (kDa) are indicated at the left.

Fig. 5. Hydropathy profile of *B. subtilis* CysP. Hydropathy indices were calculated by the algorithm of Kyte & Doolittle (1982) with a window size of 11 residues. The regions indicated by the numbered bars are the most likely to form TMSs. Amino acid residue numbers are shown on the horizontal scale.

well documented for a variety of hydrophobic proteins (Buchel *et al.*, 1980; David *et al.*, 1990; Maiden *et al.*, 1987).

To analyse if indeed CysP is localized in the membrane fraction, strain BL21(ΔDE3) carrying plasmid pBS181 was labelled with [³⁵S]methionine. Cells were disrupted by sonication, the extracts centrifuged and labelled proteins present either in the soluble or membrane fractions subjected to SDS-PAGE. Autoradiography of the gel showed that the bulk of the 24 kDa protein encoded by *cysP* is localized in the membrane fraction (data not shown).

Predicted structure of CysP

The hydropathy profile of the product of *cysP*, determined as described by Kyte & Doolittle (1982), shows a hydrophobic peptide with 10–12 transmembrane segments (TMSs) (Fig. 5), resembling those of other membrane-associated proteins. Moreover, a putative cleavable N-terminal signal peptide, characteristic of

membrane-bound proteins, was determined in CysP using the program PSORT (Nakai, 1996).

Charge bias analysis of membrane protein topology, performed using the program TOP PRED (von Heijne, 1992) was used to predict the most likely putative TMSs of CysP. This analysis revealed that the polypeptide would contain 11 TMSs, where the C terminus is cytoplasmic and the N terminus is on the outside surface of the membrane. According to this model of CysP, several basic amino acids residues, such as lysine and arginine, would be asymmetrically distributed on both sides of the membrane. We suggest that CysP contains 20 basic amino acids located in the cytoplasmic loops of the protein, whilst the loops located on the outside face of the membrane contain two positively charged amino acids. This charge distribution is in agreement with the positive-inside rule (von Heijne, 1992). Nevertheless, the actual number of TMSs should be determined experimentally.

Analysis of the amino acid sequence of the *cysP* gene product using the BLAST algorithm (Altschul *et al.*, 1997) revealed that this protein is composed of two homologous domains, called D1 and D2, possessing 69% similarity. Domain D1 extends from residue 1 to 150 and contains five TMSs (TMS 1–TMS 5). Domain D2 extends from residue 163 to 313 and possesses TMS 6–TMS 10. This topological organization of the transporter suggests that CysP might have arisen by a tandem internal gene duplication event.

Phylogenetic relationship

A comparison of the primary structure of CysP with protein sequences in the databases revealed a high level of similarity to those of several phosphate permeases of both prokaryotic and eukaryotic origin. However, no significant homology was found between CysP and proteins belonging to the SulP family.

Among the putative phosphate permeases, CysP shows a similar size and the same domain organization as the archaeal transporters (Table 2). The CysP gene product of *B. subtilis* showed a high level of similarity (67–72%) to the putative phosphate transporters of *Pyrococcus horikoshii*, *Archaeoglobus fulgidus* and *Methanobacterium thermoautotrophicum*. We also found that *Neurospora crassa* Pho-4 and *E. coli* PitA proteins show significant homology with D1 in the N-terminal region and with D2 in the C-terminal region (Table 2). Both proteins have a central region which is not homologous to CysP, which accounts for the difference in size, since both Pho-4 and PitA are larger than CysP (Mann *et al.*, 1989; Sofia *et al.*, 1994).

All the proteins that show significant homology with *B. subtilis* CysP belong to the inorganic phosphate transporter (Pit) family (Saier *et al.*, 1999). Functionally characterized members of this family appear to catalyse inorganic phosphate uptake, either by proton or sodium symport (Versaw & Metzenberg, 1995; Martinez & Persson, 1998).

Table 2. Amino acid identity and similarity of the CysP domains of *B. subtilis* to corresponding proteins of other organisms

Organism	Name or description	GenBank accession no.	Size (no. of amino acids)	D1*		D2*	
				I (%)	S (%)	I (%)	S (%)
<i>Archaeoglobus fulgidus</i>	Putative phosphate permease	AAB89449	333	35	71	31	67
<i>Pyrococcus horikoshii</i>	Putative sodium-dependent phosphate permease	BAA30231	318	33	69	30	72
<i>Methanobacterium thermoautotrophicum</i>	Sodium-dependent phosphate permease	AAB86351	326	33	69	28	68
<i>Haemophilus influenzae</i>	Putative phosphate permease HI1604	P45268	420	31	69	31	69
<i>Neurospora crassa</i>	Pho4, phosphate-repressible phosphate permease	P15710	590	25	67	21	68
<i>Escherichia coli</i>	PitA, low-affinity inorganic phosphate permease	P37308	499	23	66	23	59
<i>Bacillus subtilis</i>	YkaB, putative phosphate permease	CAA05564	328	25	66	23	60

* Domain D1 of CysP extends from residue 1 to 150 and domain D2 extends from residue 163 to 313. Alignments were created with the CLUSTAL method (Thompson *et al.*, 1994). I, identity; S, similarity.

Concluding remarks

The results presented here show that the *cysP* gene product is a membrane-bound protein with a gel electrophoresis migration corresponding to an apparent molecular mass of 24 kDa. The fact that *cysP* is able to restore the capacity to transport sulphate to the *cysA97* strain JM2314 and to relieve cysteine auxotrophy of mutants affected in both the periplasmic and membrane components of the sulphate-thiosulphate permease of *E. coli* demonstrates that its gene product is a sulphate permease. It is interesting to note that in *E. coli* and *S. typhimurium*, sulphate and thiosulphate share the same periplasmic transport system (Kredich, 1996). However, in *B. subtilis* thiosulphate might be transported by a different permease, since strain MC2620, which possesses a Tn917 insertion downstream of *cysH*, exerting a polar effect on expression of *cysP*, is able to grow with thiosulphate as sole sulphur source (Mansilla & de Mendoza, 1997).

The *B. subtilis* *cysP* gene encodes a novel membrane-associated sulphate transporter, without significant homology to sulphate transporters from both prokaryotic and eukaryotic organisms. This sulphate transporter belongs to a different class of carriers, the Pit family [Transport Commission (TC) no. 2.20; Paulsen *et al.*, 1998]. Taking into account the homology of CysP to inorganic phosphate permeases, it remains to be determined if in addition to functioning as a sulphate permease, CysP is able to transport inorganic phosphate.

It is believed that proteins of the Pit family, as well as the members of other ancient families of secondary active transporters, such as the CaCA family (TC no. 2.19) and the Amt family (TC no. 2.49), are very restricted with respect to their substrate specificities (Paulsen *et al.*, 1998). In this work we demonstrated that CysP is able to

transport sulphate; thus, the assumed function of the uncharacterized members of the Pit family as putative phosphate transporters should be reconsidered.

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