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Sulfate Transport in *Penicillium chrysogenum*: Cloning and Characterization of the *sutA* and *sutB* Genes

MART VAN DE KAMP,† ENRICA PIZZININI, ARNOLD VOS, TED R. VAN DER LENDE, THEO A. SCHUURS, ROGER W. NEWBERT,‡ GEOFFREY TURNER, WIL N. KONINGS, AND ARNOLD J. M. DRIESSEN

Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9751 NN Haren, The Netherlands, and Department of Molecular Biology and Biotechnology, Krebs Institute for Biomolecular Research, University of Sheffield, Sheffield S10 2TN, United Kingdom.

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In industrial fermentations, *Penicillium chrysogenum* uses sulfate as the source of sulfur for the biosynthesis of penicillin. By a PCR-based approach, two genes, *sutA* and *sutB*, whose encoded products belong to the SulP superfamily of sulfate permeases were isolated. Transformation of a sulfate uptake-negative *sB3* mutant of *Aspergillus nidulans* with the *sutB* gene completely restored sulfate uptake activity. The *sutA* gene did not complement the *A. nidulans* sB3 mutation, even when expressed under control of the *sutB* promoter. Expression of both *sutA* and *sutB* in *P. chrysogenum* is induced by growth under sulfur starvation conditions. However, *sutA* is expressed to a much lower level than is *sutB*. Disruption of *sutB* resulted in a loss of sulfate uptake ability. Overall, the results show that *SutB* is the major sulfate permease involved in sulfate uptake by *P. chrysogenum*.

The filamentous fungus *Penicillium chrysogenum* is well known for its ability to produce penicillin (5, 39, 57). Penicillin biosynthesis starts with the condensation of the amino acids L-α-aminoacidic acid, L-Cys, and L-Val by the peptide synthetase δ(1-α-aminoacidyl)-L-cysteinyl-D-valine synthetase. The three precursor amino acids are synthesized in the cell as part of the primary metabolism of the fungus. To accommodate to the high demand for sulfur to be assimilated and incorporated into penicillin by high-producing strains (46, 54), inorganic sulfate is added to the medium as the source of sulfur for the formation of Cys (15, 39).

The uptake of sulfate, the first step in the pathway, has been studied by using mycelium and isolated plasma membrane vesicles from *P. chrysogenum* (4, 10, 17, 18, 46, 56, 60). These experiments indicated that sulfate is actively transported across the plasma membrane via a sulfate/proton symport mechanism.

Sulfate uptake is an important point of regulation of the sulfur metabolism in fungi. In *Neurospora crassa*, sulfate uptake is subject to a mechanism called sulfur (metabolite) repression or regulation, involving the action of positively and negatively acting regulatory proteins on the expression of sulfate permease-encoding genes (22, 27, 32). A similar situation holds for *Aspergillus nidulans* (30, 35, 36) and Saccharomyces cerevisiae (9, 55). In contrast, little is known about the mechanism and regulation of sulfate uptake in *P. chrysogenum* despite its possible significance in penicillin biosynthesis. Therefore, we set out to investigate sulfate permease-encoding genes from *P. chrysogenum*. The data show that *P. chrysogenum* has two genes, designated *sutA* and *sutB* (*sut* for “sulfate transporter”), that encode putative sulfate transporters. Whereas the function of *SutA* remains to be elucidated, *SutB* was shown to be a functional sulfate transporter responsible for sulfate uptake in *P. chrysogenum* mycelium.

**MATERIALS AND METHODS**

**Strains, plasmids, and libraries.** *Escherichia coli* LE392 [hdb574 (r~g~ m~g~ s~p~) supE44 supF58 lacY1 galK2 galT22 metB1 trp855] (34) and DH5α [802lacZAM15 recA1 endA1 gpt806 thi-1 hsdR17 (r~K~ m~K~) supE44 thi-1 deoR Δ(lacY1-argF1)169] (13) were used for plasmid handling and plasmid transformations, respectively. *P. chrysogenum* Wisconsin 54-1255 (Wis54-1255) has been described previously (8, 38). *P. chrysogenum* HP90 is a nicotinamide-requiring derivative of NRRL1951 (48). The construction of *P. chrysogenum* aux was described below. *Aspergillus nidulans* GI1 (sB3 prg98 pabA41), carrying the sB3 mutation (1, 52), was derived from a cross between strains G191 (2) and 0198 obtained from the Glasgow Stock Collection (J. Chutterly, University of Glasgow).

Plasmid pBI2s was used for cloning and sequencing in *E. coli*. Plasmid pGEM-T-easy (Promega) was used to clone PCR products. Plasmid pDJ2B is an *A. nidulans* transformation vector carrying the *N. crassa* prr-4 gene as a selection marker (2). Plasmid pBSsutA contains a 4.5-kb PstI fragment (Fig. 1) cloned into the PstI site of pBI2s KS. Plasmid pBSsutB contains a 4.3-kb BamHI fragment cloned into the BamHI site of pBI2s KS. Plasmid pBSsutB contains a 2.1-kb Sall fragment cloned into the Sall site of pbScript II KS. Plasmid pBSsutA contains an internal 1.0-kb XhoI-SfuI fragment of sutB cloned into the MCS of pbScript II KS. Plasmid pBSsutA was constructed as follows. Plasmid pBSsutB was digested with EcoRV (in the multiple-cloning site) and HincII (28 bp upstream of the sutB ATG start codon), and a 737-bp EcoRV-HincII fragment containing part of the sutB promoter region was isolated (Fig. 1). This fragment was cloned into plasmid pBSsutB, from which the sutB promoter region was removed by digestion with BseII (49 bp upstream of the sutA ATG start codon), treatment with DNA polymerase (Klenow fragment), and digestion with EcoRV (in the multiple-cloning site). A genomic library of *P. chrysogenum* Q176 (38) DNA in phage λ-EMBL3a was a generous gift from H. Schwab, Technical University, Graz, Austria. DSM-Gist (Delft, The Netherlands) kindly provided the *P. chrysogenum* cDNA library.

**Media and growth conditions.** Manipulations with and growth of *E. coli* LE392 and DH5α were performed by standard methods (43). *P. chrysogenum* and *A. nidulans* growth media and conditions have been described previously (2, 16, 17).

Where appropriate, sulfur sources were replaced by chloride salts, and methionine was added as indicated.

**Gene cloning and sequencing.** Degenerate deoxyribonucleotide oligomers, designated *sR* -f orw (*5’-ACC TAC ACC GCA TGG[A][C][A][T] CTT[C][A][C][A][T][G][C] [AA][A][T][A][C][T][G][C] [AA][A][T][G][C][A][T][G][C][T][C][T]-3’*) and *sR*-rev (*5’-CC GAA [G][T][G][C][T][G][G][A][G][J][A][T][G][C][T][C][T][C]-3’*), were designed to correspond to two stretches of amino acid residues present in CYS-14p of *N. crassa* (TYK[VI][N][E][L][K] and EHAISKSFG) (23) and SUL1p and SUL2p of *S. cerevisiae* (TYK[VI][N][E][L][K] and EHAISKSFG) (9, 21, 49). PCR was performed on chromosomal *P. chrysogenum* DNA under standard conditions. PCR products of about 400 bp were isolated, treated with DNA polymerase (Klenow fragment), ligated into the NotI site of pbScript II KS, and sequenced. Of the 20 clones

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**Address correspondence to** W. N. Konings, Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, PO Box 415, 9700 AV Groningen, The Netherlands (konings@biochem.rug.nl).

**Corresponding author.** Mailing address: Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, P.O. Box 415, 9700 AV Groningen, The Netherlands. Phone: 31-50-3621250. Fax: 31-50-3621254. E-mail: A.J.M. Driesse@BIOL.RUG.NL.

†Present address: Synpac Pharmaceuticals, Cambois, Bedlington, Northumberland NE2 7DB, United Kingdom.
A cotransformation was carried out with 5 μg of pBC1003 (which carries a phleomycin resistance marker [a gift from E. Fricelien, Biochimie GmbH]) mixed with 5 μg of pBSsutB-XS. pBSsutB-XS contains an internal 1.0-kb XhoI/SstI fragment of pHBBac1 (Fig. 1). Transformants were selected on medium containing 50 μg of phleomycin per ml in 25 ml of bottom agar overlaid with 20 ml of drug-free top agar into which transformed protoplasts had been mixed. Phleomycin-resistant transformants were tested for the ability to grow on sulfate as the sole sulfur source 

Sulfate uptake and expression studies. A. nidulans strains were grown aerobically at 37°C for 16 h on glucose-containing minimal medium in which all sulfate salts were replaced by chloride salts. As required, 1-Met (0.25 or 5 mM) and MgSO₄ (0.1 or 2.0 mM) sulfate salts were added to the plate medium as appropriate, uridine, uracil, and p-aminobenzoate were added to the medium at 10 mM, 20 mM, and 1 μg/ml, respectively. P. chrysogenum Wis57-1255 was grown aerobically at 25°C on a sulfate-sufficient (≥20 mM sulfate) main culture medium with lactose as the C source. After 24 h, the medium was exchanged either for fresh original (S-rich) medium or for sulfateless medium in which all sulfate salts were replaced by chloride salts; this was followed by 16 h of growth. P. chrysogenum HP60 and HP45 were creatd on the same conditions at 37°C on sulfate-free medium supplemented with 10 mM 1-Met. The starter cultures were used to inoculate (at a 1:10 ratio) main culture medium with glucose as the C source, supplemented with 20 mM Met. After growth for 40 h, the medium was exchanged either for a medium containing 10 mM Met and 0.1% Met (S-rich medium) or for a medium lacking Met and with all sulfate salts replaced by chloride salts (S starvation medium), and growth was continued for 4 h. Mycelium for sulfate uptake studies was harvested by suction filtration, washed with 0.9% NaCl, and resuspended in 50 mM Tris-HCl buffer (pH 6.0) at approximately 10 mg/ml (wet weight). After the mycelium had been cooled for at least 30 min on ice, it was aerated for 15 min at that temperature prior to the uptake experiments. After preincubation of the mycelium at 25°C for 3 min, sulfate was added (0.05, 0.1, 0.15, or 0.20 mM), and at 20 min (150 mM; JCN Pharmaceuticals) was added to a final concentration of 10 mM. Samples were drawn and processed as described previously (17). To check the energy dependency of sulfate uptake, deenergization of the mycelium was performed with the protonophore carbonyl cyanide-m-chlorophenylhydrazone (10 mM final concentration), which was added at the start of the 3-min preincubation period. Dry weights of lyophilized samples were determined.

Mycelium for total-RNA isolation was harvested by suction filtration and immediately frozen in liquid nitrogen. The mycelium was ground in a mortar and pestle, and RNA was isolated from the powdered mycelium with Trizol (Gibco BRL) as specified by the manufacturer. Electrophoresis and Northern blotting were carried out essentially as described previously (45). To ensure that hybridization was specific for sutA or sutB, probes for sutA and sutB were made from gene regions which have the lowest sequence identity (less than 40%) (Fig. 1) and hybridization was performed under stringent conditions. A sutA-specific fragment was amplified by PCR with primers sutA-N-forw (5'-GCCTGCATGACCCTGACATGAG-3') and sutA-rev (5'-GTGGTGCCCAAAGCTGGATAGTTGGC-3') (Download full version) and a gutB-specific fragment was obtained with sutB-N-forw (5'-CATCTCCATAACTACATCGTC-3') and sutB-rev (5'-GAGTCAGAACCTGATGTCT-3') (for sutA, PCR was performed with the Expand High Fidelity PCR system (Boehringer Mannheim) as specified by the manufacturer. Specific PCR products were cloned into the pGEM-T-easy vector (Promega) and completely sequenced. For sutA, a fragment encompassing a tentative third intron was amplified with the primers sutA-in-forw (5'-CTGATGCCAGGAGGAGCCACAAAC-3') and the aforementioned sutA-rev. To determine the sequences of the 5'- and 3'-untranslated regions of the sutA and sutB genes, primers which annealed in the coding regions but were directed outward of the genes were designed. For sutA, the primers rev-sutA (5'-GGATGAAATGATGAAATGACGTGTC-3') and low-sutA (5'-CTGATGCCAGGAGGAGCCACAAAC-3') were used, and for sutB, the primers rev-sutB (5'-GGGCACAGACTCGTCAGTCTGTTGCAGAACCTGGAT-3') and low-sutB (5'-GGGCACAGACTCGTCAGTCTGTTGCAGAACCTGGAT-3') were used. By using PCR (Expand Long Template PCR; Boehringer Mannheim), the cDNA library, and the primers mentioned above, the sutA and sutB flanking sequences as well as the vector backbone were amplified, treated with DNA polymerase (Klenow fragment), self-ligated, and sequenced.

Transformation of A. nidulans. A. nidulans IG1 was transformed as described by Baille and Turner (2), using 2 μg of pDJ2 mixed with 2 μg of pBSsutA, pBSsutB, or pBSPut/sutB. Transformsants were selected on minimal medium lacking uridine and uracil but supplemented with 1-Met and p-aminobenzoic acid. After 5 days at 37°C, the transformants were tested for growth in the absence of methionine.

Cloning of the sutA and sutB genes. Two putative sulfate transporter-encoding genes, sutA and sutB, were cloned from P. chrysogenum genomic DNA by a PCR-based approach as described in Materials and Methods. The nucleotide sequences of a 5.5-kb region encompassing the sutA gene and of a 5.8-kb region encompassing the sutB gene were determined. The sutA and sutB genes encode single polypeptides of 746 and 842 amino acid residues, respectively, with predicted molecular masses of 81.5 kDa (SutA) and 91.9 kDa (SutB). cDNA analysis showed that the coding regions are interrupted by two introns (63 and 60 nucleotides (nt) [nt 260 to 322 and 469 to 528]) for sutA and by one intron (59 nt [nt 457 to 515]) for sutB. These regions fit the intron consensus sequence 5'-GTN NGT......CT[G/A]CA...YAG-3' (numbering is relative to the ATG start codon). The intron in sutB is at exactly the same position as the second intron of sutA with respect to the amino acid sequence.
FIG. 2. Sulfate uptake by four different strains under high-sulfate (5 mM Met and 2 mM MgSO₄) or low-sulfate (0.25 mM Met) conditions. Shown are the wild-type strain A. nidulans R21 (high, ○; low, ●), carrying the mutant sB3 allele of the sulfate permease gene sB, and strains M27 (high, △; low, ▽) and M63 (high, □; low, ▪), both of which are A. nidulans IG1 strains complemented with the P. chrysogenum sutB gene. The lower panel is a partial magnification of the upper panel to indicate that some residual sulfate uptake can be detected under high-sulfate conditions. dw, dry weight.

acid sequence. The sutA gene was suspected to contain an additional intron in the 3′ region (nt 2279 to 2332 [5′-GTC AGAN₉₂CTGAAN₉₂TAG-3′]). Splicing out of this putative intron would extend the amino acid sequence identity between SutA and SutB (see below). Therefore, three independently isolated cDNAs were analyzed, with special attention paid to this region. From none of these cDNAs was the suspected intron spliced out. Furthermore, a fragment encompassing the putative intron was amplified by PCR with the cDNA library. One major band was detected, with the suspected intron not spliced out. A very faint band was detected (<5% abundance) from which the intron was putatively spliced out. cDNA analysis showed that the 5′ untranslated regions are ≥60 nt (sutA) and ≥171 nt (sutB). Sequences directly upstream of the transcribed but untranslated regions of sutA and sutB are particularly CT rich and contain TATA- and CCAAT-like sequences that may be involved in transcription. cDNA analysis showed that the 3′ untranslated regions are 342 nt (sutA) and 423 nt (sutB), not including the poly(A) tails.

Genetic complementation of the A. nidulans sB3 mutant. To investigate the function of the proteins encoded by the sutA and sutB genes, their ability to complement the sB3 (sulfate permease) mutation of A. nidulans was tested by cotransformation with pDBJ2 and plasmids pBSsutA or pBSsutB, using the pyr-4 gene of pDBJ2 as a selectable marker. Plasmids pBSsutA and pBSsutB contain about 2.5 and 0.8 kb of the respective promoter regions (Fig. 1). Of 50 Pyr+ transformants, 14 showed complementation of the sB3 mutation by the sutB gene from their ability to grow on a medium with sulfate as the sole sulfur source. Of these 14 clones, 2, named M27 and M63, were used for sulfate uptake studies. Strains M27 and M63, as well as the parental sB3 mutant strain IG1 and strain R21 (wild type for sB), were grown for 16 h on an S-poor medium containing 0.25 mM Met as the sole S source. After being harvested, mycelium was resuspended in phosphate buffer and sulfate uptake was studied. Strains M27 and M63 showed uptake levels comparable to that of R21, whereas sulfate uptake by IG1 was undetectably low (Fig. 2). When the strains were grown on an S-rich medium containing 5 mM Met and 2 mM MgSO₄, sulfate uptake by R21 was repressed more than 500-fold, while sulfate uptake of the sutBΔ strains M27 and M63 was repressed approximately 50-fold (Fig. 2).

In contrast to the results for sutB, of 50 tested Pyr+ transformants cotransformed with pBSsutA and pDBJ2, none showed complementation of the sB3 phenotype. To circumvent the possibility that sutA did not complement the A. nidulans IG1 sB3 mutation because of low expression of the sutA gene (see below), the 2.5-kb promoter region of sutA present in pBSsutA was replaced by the 0.8-kb promoter region upstream of sutB on pBSsutB, yielding plasmid pBSputB. When A. nidulans IG1 was cotransformed with pDBJ2 and pBSputB sutA, none of the tested Pyr+ clones showed complementation of the sB3 mutation as judged by their ability to grow on a medium with sulfate as the sole sulfur source. Northern analysis showed that in some of these transformants the sutA gene was expressed under the control of the sutB promoter during growth on S-poor medium. One of these clones (designated BA2) was used for sulfate uptake studies. After growth for 16 h on an S-poor medium containing 0.25 mM Met as the sole S source, sulfate uptake was measured, but not detected (not shown). These data demonstrate that SutB is a sulfate transporter. The function of SutA remains to be elucidated.

Expression of sutA and sutB, and disruption of sutB in P. chrysogenum. Northern analysis with P. chrysogenum Ws54-1255 showed that transcription of sutA and sutB was almost completely repressed when the strain was grown (for 40 h) under S-sufficient conditions (i.e., normal levels of sulfate) on main culture medium with lactose as the C source. When the mycelium was starved for sulfur for 16 h, after 24 h of growth on main culture medium, expression of both sutA and sutB was induced. However, expression of sutB was an order of magnitude stronger than that of sutA (Fig. 3A). The level of sutA and sutB expression corresponded to sulfate uptake by mycelium grown under S-rich and S starvation conditions (Fig. 3B).

To study the function of sutB and sutA in P. chrysogenum, the sutB gene of P. chrysogenum HP60 was disrupted by homologous integration of an internal fragment of sutB following

FIG. 3. Northern blots showing the expression of sutA and sutB in P. chrysogenum Ws54-1255 (equal amounts of RNA were loaded [not shown]) (A) and corresponding sulfate uptake levels (B). Ws54-1255 was grown for 24 h in a sulfur-sufficient medium with lactose as the C source, after which the medium was exchanged either for fresh medium of the same composition (+S) or for fresh medium in which all sulfate salts were replaced by chloride salts (−S), and growth was continued for 16 h. dw, dry weight.
cotransformation with a phleomycin resistance vector. Of 100 phleomycin-resistant transformants tested, only one, nr45, failed to grow normally on sulfate as the sole sulfur source. This strain, which grew normally on medium supplemented with methionine, also showed resistance to selenite, indicative of a lesion in an early step in sulfate assimilation (1). DNA extracted from this strain and probed with pBSsutB-XS confirmed the disruption of sutB by homologous integration of pBSsutB-XS (data not shown). Sulfate uptake by strain HP60 grown for 44 h on an S-rich main culture medium (containing normal amounts of sulfate salts and 10 to 20 mM Met) was completely repressed compared to that by strain HP90 grown for 40 h under S-sufficient conditions and then starved for 4 h on S-less main culture medium (no sulfate salts, no l-Met) (Fig. 4). No sulfate uptake was detected for the sutB mutant strain nr45, grown either under S-rich or under S starvation conditions (Fig. 4). These data demonstrate that SutB is the major sulfate permease involved in sulfate uptake by P. chrysogenum.

**DISCUSSION**

We have cloned two *P. chrysogenum* genes, sutA and sutB, one of which, sutB, encodes a functional sulfate permease. sutB complements the sB3 mutation of *A. nidulans*, and disruption of sutB in *P. chrysogenum* abolishes sulfate uptake. SutB appears to be the major sulfate permease present during mycelial growth and should therefore be located at the plasma membrane. SutB probably represents the high-affinity high-capacity $2\text{H}^+/\text{SO}_4^{2-}$ symport system, which has been kinetically characterized by Hillenga et al. (17). In line with this, both the $K_m$ for SutB (20 to 30 mM for $\text{SO}_4^{2-}$) and the inhibition profile for SutB ($\text{SO}_4^{2-} > \text{S}_2\text{O}_3^{2-} > \text{SO}_3^{2-} > \text{SO}_4^{2-}$) after expression of the sutB gene in the *A. nidulans* sB3 strain (57a), resemble the characteristics of the *P. chrysogenum* system in mycelium (4, 17, 60). The physiological function of SutA remains unclear. Expression of SutA in mycelium is low and is not enhanced in the early growth stages (57b), unlike the situation indicated for *N. crassa* cys-13 (31). It is unlikely that SutA represents a low-affinity system, since *P. chrysogenum* nr45 (sutB disruptant) does not grow on a medium with high sulfate concentrations without methionine. sutA may encode a thiolsulfate, tetrathionate, or sulfate transporter (56) or may function as a sulfate transporter in the vacuolar membrane (18).

SutA seems to be truncated at its C terminus in comparison to SutB. Although the sutA genomic sequence suggests that an intron (nt 2279 to 2332 with respect to the ATG start codon) runs over the stop codon, cDNA analysis showed that it is not removed from the mRNA. If this intron were spliced out, SutA would be extended by 54 amino acid residues, which is very similar to the C terminus of SutB (5) and to the C termini of SUL1p and SUL2p from *S. cerevisiae* (9, 49). It will be interesting to see whether (physiological) conditions exist that facilitate the removal of the putative intron from the primary transcript, resulting in SutA proteins with greater similarity to SutB and other sulfate transporters.

Expression of both sutA and sutB is induced when *P. chrysogenum* is grown under S starvation conditions (Fig. 3 and 4). Also, in *A. nidulans*, SutB-mediated sulfate uptake is subject to sulfur regulation (Fig. 2). S regulation is a well-documented phenomenon in *N. crassa*, *A. nidulans*, and *S. cerevisiae* (32, 55). For SutB, the 800 nt upstream of the start codon that are present on pBSsutB are almost completely sufficient for S regulation in *A. nidulans* (Fig. 2 and 4). In *N. crassa*, S regulation is positively mediated by the DNA-binding protein CYS-3p (22, 23, 26, 27, 32). Recently, a positively acting CYS-3p homologue has been found in *A. nidulans* (37). No CYS-3p homologue has been reported for *P. chrysogenum*. Sequences that weakly resemble the CYS-3p binding-site consensus ATGR YRYCAT (26, 27) are present upstream of sutA and sutB at positions $-2481$ (ATTGTAACAAT), $-1871$ (ATTACGTGTT), $-1513$ (GTGCAGGTGAC), $-813$ (GTGACGTACC), and $-312$ (GTGACGTTCG) (sutA) and $-1516$ (ATGACGTGTA), $-983$ (ATTATGTAAT), $-394$ (ACAACGTGGA), and $-231$ (ATTGGCAGCAT) (sutB) with respect to the ATG start codon. Other sequences in the sutA and sutB promoter regions resemble the consensus binding site TCAGTGT, which is recognized in *S. cerevisiae* by the Cbf1p-Met4p-Met28p complex (24, 25, 55) (sutA, positions $-2209$, $-2105$, $-1870$, $-1512$, $-904$, and $-812$; sutB, positions $-1909$, and $-1515$ [note that some of these sites are part of putative CYS3p homologue-binding sites]), or the consensus binding site AAAAGTGT of the positive regulators Met31p and Met32p (3) (sutA, positions $-2129$, $-1600$, and $-1000$; sutB, positions $-1483$, $-1475$, and $-876$). A possible function for these cis-acting elements and their proposed trans-acting binding factors remains to be investigated.

Hydropathy analysis (28, 29, 47, 58) of SutA and SutB shows a pattern typical for a polytopic membrane protein, with 14 putative hydrophobic transmembrane (TM) helices in the N-terminal part of the protein followed by a long C-terminal extension (Fig. 5A). The overall sequence identity of the SutA and SutB proteins is $66\%$ (Fig. 5A). Both proteins show significant homology to eukaryotic sulfate permeases from fungi, plants, and animals (data not shown). These proteins are clustered, together with a number of prokaryotic proteins, in the so-called SulP superfamily of sulfate permeases, which belongs to the class of secondary transporters (40, 42). These proteins all contain a motif which has become known as the sulfate permease signature. Originally this motif was defined as P-x-Y-[GS]-L-Y-[STAG][2-4]-[LV][M][F][2-4]-Y-x(3)-[GSTA][2-4]-S-[KC][R] (44, 50), and it runs over the TM helix 3 (depicted in Fig. 5B). This motif is present in both SutA and SutB. However, a database search with this motif fails to recognize many
putative and experimentally proven sulfate permeases, including SutB (Fig. 5B). Therefore, a new motif is proposed with the sequence D-[LIVFM](2)-[GAS]-G-[ILV]-x(7)-[PL]-x(15,16)-[GS]-L-[YWFIL], which starts at TM helix 2 and runs into TM helix 3 (depicted in Fig. 5B). This motif is both complete and specific in the recognition of (putative) sulfate permeases in sequence databases.

According to the topology model of the whole SulP family, based on hydropathy profile analysis (data not shown), the N termini of both SutA and SutB are located in the cytosol.
FIG. 5. (A) Alignment of SutA and SutB amino acid sequences. Indicated are identical residues (•); charges (+, −) predicted protein kinase C phosphorylation sites ([ST]VIRK) (59), and predicted N-glycosylation sites (N [ST]X) (12, 33) which are present in both SutA and SutB, predicted transmembrane helices (black background), and predicted extracellular loops (bold arrow shown is the alignment of an extended version of SutA, named SutsA (see Fig. 1 and Discussion). Alignments were made with ClustalX (20), and hydropathy profile analysis was done with MemGen version 4.08 (28, 29). (B) Alignment of SutA and SutB amino acid sequences with sequences of some examples of the Sul family of sulfate permeases whose function has been proven experimentally (except SutA). Shown are the regions around the old sulfate permease motif (44) and the region around the proposed new sulfate permease motif. Alignments were made with the ClustalX program (20). SUTA, P. chrysogenum (access no. AF163975) (this work); SUTB, P. chrysogenum (AF163974) (this work); CSS14, N. crassa (YLR026w) (9); HVST1, Hordeum vulgare (YJ4587) (51); AST56, Arabidopsis thaliana (AB012050) (63); DTDST, Homo sapiens (P05045) (14, 41). The old motif fails to recognize SutB, Sul2, and HVST1.

REFERENCES


C-terminal domain of both systems is predicted to be located in the cytosol as well, in line with topology data for the human DRA-encoded sulfate transporter (7). Previously published models were based on alignments of a small number of eukaryotic sulfate permeases (see e.g., references 9, 11, 50, and 51) and predicted 12 or fewer TM helices. However, a hydropathy profile based on the 50 presently available sequences (not shown) predicts 14 TM helices for most eukaryotic sulfate permeases and 13 TM helices for the prokaryotic sulfate permeases. The predicted TM helix 1 appears to be present in a subset of eukaryotic sulfate permeases, including SutA and SutB (P. chrysogenum), Sul1p, Sul2p, and SulXp (S. cerevisiae), Cys14p (N. crassa), and some plant, nematode, and mammalian sulfate permeases, but it is lacking in other eukaryotic sulfate permeases and in all prokaryotic permeases. The current model predicts the presence of TM helices 13 and 14, whereas in most previous models a single TM helix was predicted. However, the previously proposed topology models disobey the so-called positive-inside rule (14, 47, 50, 58), while the prediction of TM helix 14 yields a topology with a charge distribution which is in better agreement with the positive-inside rule, as seen in Fig. 5.

Summarizing, P. chrysogenum contains two genes, designated suta and sutb, that encode putative sulfate transporters. Sutb is the system responsible for sulfate uptake in mycelium of P. chrysogenum, whereas the role of SutA remains to be determined. Future studies will address the regulation and expression of these systems in relation to the high demand for sulfur during penicillin biosynthesis.

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Old PROSITE-motif: P-x-Y-[GS]-L-V-[STAG](2)-x(4)-[LVMFY](3)-x(3)-[GSTA](2)-[S]-[KR]

New PROSITE-motif: D-[LVMFY](2)-[GS]-G-[ILV](x)-[PL]-x(4)-[YFIL]-x(10,11)-[GS]-L-[YFIL].

\( \text{TM1} \text{TM2} \text{TM3} \text{TM4} \)

<table>
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<tr>
<th>Gene</th>
<th>Amino Acid Sequence</th>
<th>Alignment Score</th>
</tr>
</thead>
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<tr>
<td>SUTA</td>
<td>GLIGDLVAGTLGVAVGIFCGNATLGLKLKLPKLPGYLSFFMVLITNFPSSCITGIPAVSWS</td>
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</tr>
<tr>
<td>SUTB</td>
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<td>SUL2</td>
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</table>

\( \text{--- Old motif------} \)

\( \underline{\text{- New motif--------------------------}} \)