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The Sulfur-Regulated Arylsulfatase Gene Cluster of *Pseudomonas aeruginosa*, a New Member of the *cys* Regulon

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A gene cluster upstream of the arylsulfatase gene (*atsA*) in *Pseudomonas aeruginosa* was characterized and found to encode a putative ABC-type transporter, AtsRBC. Mutants with insertions in the *atsR* or *atsB* gene were unable to grow with hexyl-, octyl-, or nitrocatecholsulfate, although they grew normally with other sulfur sources, such as sulfate, methionine, and aliphatic sulfonates. AtsRBC therefore constitutes a general sulfate ester transport system, and desulfurization of aromatic and medium-chain-length aliphatic sulfate esters occurs in the cytoplasm. Expression of the *atsR* and *atsBCA* genes was repressed during growth with sulfate, cysteine, or thiocyanate. No expression of these genes was observed in the *cysB* mutant PAO-CB, and the *ats* genes therefore constitute an extension of the *cys* regulon in this species.

Sulfate esters make up a large proportion of the sulfur that is found in aerobic soils, and so it is not surprising that many soil microorganisms have evolved enzymes that catalyze the hydrolysis of these compounds, either to release the sulfate moiety as a sulfur source for growth, or as the first step in their mineralization. Bacterial sulfatases have been studied extensively in the past, with particular emphasis placed on those enzymes that lead to degradation of surfactants (5). Strains that are able to grow with alkylsulfates such as sodium dodecyl sulfate (SDS) as the source of carbon are widespread in the environment, even in samples isolated from uncontaminated sites (23). A variety of alkylsulfatases is responsible for the hydrolysis reaction, often even within one species. The best-studied such strain is *Pseudomonas* sp. strain $C_{12}B$ (reviewed in reference 5), which displays a broad substrate tolerance even though the enzymes it contains are relatively substrate specific in terms of chain length and stereospecificity. Synthesis of these enzymes is controlled by a complex network of substrate and product induction (5).

Hydrolysis of aromatic sulfate esters, in contrast, is controlled in bacteria exclusively by the supply of sulfur to the cell, and is catalyzed by enzymes of the arylsulfatase family. These enzymes are common soil enzymes and, because they are easy to assay, are often used as a measure of soil quality (16). Synthesis of arylsulfatase is repressed during growth with inorganic sulfate or cysteine as the sulfur source and upregulated under sulfate-limiting conditions (e.g., during growth with sulfonates, sulfate esters, sulfamates, or methionine) (7). In *Pseudomonas aeruginosa*, the repressive effect in vivo was recently traced to two independent effectors—sulfite and either sulfide or cysteine (7)—whereas in *Klebsiella pneumoniae*, sulfate and cysteine repress arylsulfatase synthesis, also independently of each other (12).

The regulation of arylsulfatase synthesis is correlated with that of a group of so-called sulfate starvation-induced proteins, which were identified by differential two-dimensional electrophoresis (7, 13), and we have therefore used arylsulfatase as a model system for the sulfate starvation response. In this report, we show that, in *P. aeruginosa*, arylsulfatase is encoded together with a general transport system for both aliphatic and aromatic sulfate esters, and expression of this gene cluster requires the LysR-type transcriptional activator CysB.

Cloning and sequence analysis of the *ats* gene cluster. Previous studies of arylsulfatase in *P. aeruginosa* led to the identification and characterization of the *atsA* gene (1), but complementation studies with a DNA fragment carrying only this gene were unsuccessful. We therefore cloned and sequenced a region upstream of the *atsA* gene, in order to identify the promoter region from which *atsA* is expressed. Screening of a cosmid bank of *P. aeruginosa* yielded two cosmid clones carrying parts of the desired locus, and these were subcloned onto pBluescript (Stratagene) to give a 7-kb fragment on the plasmid pME4326. Sequencing of this fragment led to the identification of three further open reading frames, *atsB*, *atsC*, and *atsR* (Fig. 1). The genes *atsB* and *atsC* were carried as part of a putative operon with *atsA*, and overlapped each other by four nucleotides, whereas a fourth gene, *atsR*, was identified on the complementary strand, oriented divergently from *atsBCA*. The overall G+C content of the coding regions was 68.2%, although this dropped to 50% in the intergenic region between *atsR* and *atsB*.

Sequence analysis of the deduced AtsRBC proteins suggested that they represented an ABC-type transporter of unknown specificity. *atsB* encoded a 57.8-kDa polypeptide with 30 to 40% identity to known bacterial permeases. Hydrophobicity analysis with the program TMpred (6) predicted the presence of 12 membrane-spanning domains, and since the predicted AtsB protein is twice the size of related permeases (e.g., TauC of *Escherichia coli*, encoding the putative taurine permease [20], is 30 kDa in size), AtsB therefore corresponds as a monomer to the dimeric form of other permeases. The deduced AtsC protein was 31 kDa in size and was related (40 to 44% amino acid identity) to ATP-binding proteins of ABC-type transporters. The two Walker motifs which are characteristic of proteins of this family were present (GASGCGKST and LLLLDEPF [consensus residues underlined]), as was the so-called ABC signature, LSGG (11). The third open reading frame identified, *atsR*, encoded a 34-kDa protein carrying a

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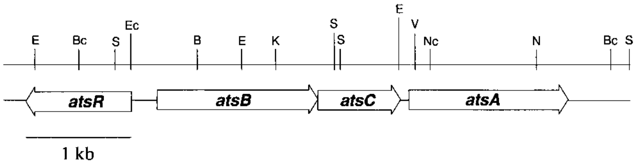

FIG. 1. Genetic organization of the *ats* locus of *P. aeruginosa*. Selected restriction sites are shown: B, *Bam*HI; Bc, *Bcl*I; E, *Eco*RI; Ec, *Eco*47III; K, *Kpn*I; N, *Not*I; Nc, *Nco*I; S, *Sal*I; V, *Eco*RV.

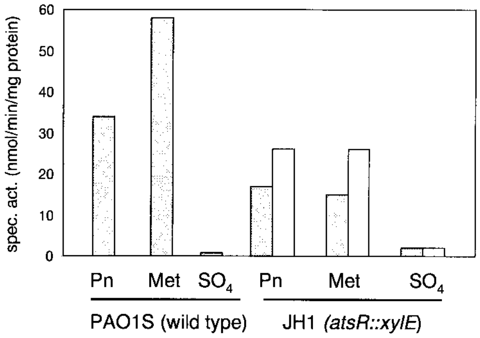

FIG. 2. Arylsulfatase and catechol oxygenase activities in strain JH1. Cells were grown in succinate-minimal medium with pentanesulfonate (Pn), methionine (Met), or sulfate (SO_4) as the sulfur source (250 μM), and were harvested in the mid-exponential phase. □, arylsulfatase activity; ▨, catechol oxygenase activity. Spec. act., specific activity.

putative N-terminal signal peptide. The AtsR protein was 25 to 42% identical to periplasmic substrate binding proteins involved in uptake of arylsulfatases or aliphatic sulfonates (21). These proteins are sufficiently similar that they have been proposed to form an independent family of binding proteins (21), adding to those previously defined by Tam and Saier (17).

AtsRBC proteins constitute a general sulfate ester transport system. To further characterize the AtsRBC transporter, mutations were introduced into the *atsR* and *atsB* genes. The promoterless *xylE*::*Gm* cassette from the plasmid pX1918GT (15) was ligated into the *Eco*47III site in *atsR* (nucleotide 8 of *atsR*) and between the *Bam*HI and *Eco*RI sites in *atsB*, respectively (Fig. 1). The resulting constructs were subcloned onto the suicide plasmid pME3087 (22) and transferred onto the *P. aeruginosa* PAO1 chromosome by homologous recombination. This yielded strains JH1 (*atsR*::*xylE*/*Gm*), in which the *xylE* was present as a transcriptional fusion to the *atsR* gene, and JH3 (*atsB*::*Gm*), where unfortunately we were only able to generate the construct with *xylE* in the reverse orientation to *atsB*. These strains, and the previously described strain, ATS1, which carries an 832-bp deletion in *atsA* (4), were grown in a succinate-mineral medium (1) with various sulfur sources (Table 1). Strain ATS1 was found to be defective only in growth with nitrocatechol sulfate, but strains JH1 and JH3 had lost the ability to grow with aromatic sulfates or medium-chain-length alkyl sulfates as the sulfur source. Complementation of these strains with the entire gene cluster on the broad-host-range vector pBBRMCS-3 (9) led to restoration of the wild-type phenotype (data not shown). These results demonstrate that the AtsRBC proteins constitute a general sulfate ester transporter which is involved in the uptake of both aromatic and aliphatic sulfate esters and also confirm the previous conclusion that the arylsulfatase is not involved in alkylsulfatase metabolism (1). Interestingly, JH1 and JH3 retained the ability to grow with SDS. This finding is consistent with previous studies of SDS degradation as a carbon source, which showed that the SDS sulfatase is periplasmically located (2, 5). In contrast to SDS sulfatase, however, the medium-chain-length sulfatase appears to be localized in the cytoplasm in *P. aeruginosa*, as is arylsulfatase (1).

TABLE 1. Growth of *P. aeruginosa* strains with different sulfur sources

Sulfur source	Relative growth of strain:			
	PAO1S (wild type)[a]	JH1 (*atsR*::*xylE*)	JH3 (*atsB*::*Gm*)	ATS1 (Δ*atsA*)
Sulfate	+++	+++	+++	+++
Nitrocatechol sulfate	+++	−	−	−
Hexyl sulfate	+++	−	−	+++
Octyl sulfate	+++	−	−	+++
Dodecyl sulfate	++	++	++	++
Pentanesulfonate	+++	+++	+++	+++
Methionine	+++	+++	+++	+++

[a] Strain PAO1S is a spontaneously streptomycin-resistant derivative of *P. aeruginosa* PAO1 (1).

Arylsulfatase and catechol oxygenase activities in strain JH1 were measured by standard methods (1, 8) and showed that expression of the *atsR* gene was repressed during growth with sulfate, and upregulated during growth with organosulfur sources such as pentanesulfonate or methionine (Fig. 2). Arylsulfatase synthesis was also regulated in the same way, although the arylsulfatase levels were not as high as in the wild-type strain (Fig. 2). As expected, no arylsulfatase activity was seen in strain JH3, due to the polar effect of the *atsB*::*Gm* insertion on transcription of the *atsA* gene.

Expression of *atsR* and *atsBCA* is controlled by CysB in *P. aeruginosa*. Expression of the *atsR* and *atsB* genes was now examined in the wild-type strain PAO1, by using transcriptional *lacZ* fusions constructed by cloning the *atsR*-*atsB* intergenic region in both orientations into the promoter probe plasmid pQF120 (14), to yield the plasmids pME4334 (*atsR*::*lacZ*) and pME4337 (*atsB*::*lacZ*). β-Galactosidase activities in mid-exponential-phase cells during growth with various sulfur sources were measured with *o*-nitrophenylgalactopyranoside (ONPG) as a substrate and are shown in Fig. 3. Both *atsR* and *atsB* were upregulated during growth with organosulfur sources, and repressed during growth with inorganic sulfate, even when the latter was combined with an organosulfur compound. The degree of downregulation in the presence of sulfate was consistent with that previously observed with the chromosomal *atsR*::*xylE* fusion (Fig. 2), demonstrating that copy number did not have an effect on the regulation. This suggested that expression might not be regulated by a direct repressor (there was no evidence for titration of a repressor protein in the presence of a high-copy-number reporter plasmid), but might be mediated by a positive regulator, such as the CysB protein. CysB is a LysR-type transcriptional activator which has been well characterized in enteric bacteria (10), where it activates transcription of the *cys* biosynthetic genes in the presence of the coinducer *N*-acetylserine, and during sulfur limitation. It has recently also been reported in *P. aeruginosa*, where it plays a role in *algD* expression (3), and is required for growth with a variety of organosulfur compounds (8). We therefore tested expression of the *atsB*::*lacZ* and *atsR*::*lacZ* fusion constructs in the *cysB*::*Gm* mutant strain PAO-CB (8).

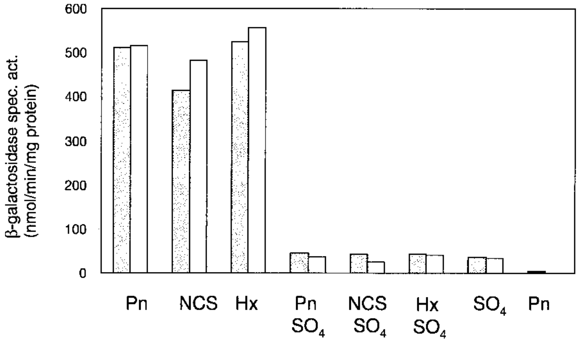

FIG. 3. Regulation of *atsR*::*lacZ* and *atsB*::*lacZ* expression in *P. aeruginosa* PAO1S. Cells were grown in succinate-minimal medium with pentanesulfonate (Pn), methionine (Met), nitrocatechol sulfate (NCS), hexyl sulfate (Hx), or sulfate (SO$_4$) as the sulfur source and were harvested in the mid-exponential phase. β-Galactosidase specific activity (spec. act.) was measured with ONPG as the substrate. ▨, *atsR*::*lacZ* (pME4334); □, *atsB*::*lacZ* (pME4337); ■, vector control (pQF120).

Because this strain is auxotrophic for cysteine, we were unable to use sulfate as a repressing growth substrate, and we substituted it with thiocyanate, which also represses arylsulfatase expression in this species (7). No expression of the *atsB* or *atsR* gene was observed under derepressing conditions in the absence of a functional CysB protein (Fig. 4). We therefore conclude that the *atsR* and *atsBCA* genes are new members of the *cys* regulon in *P. aeruginosa*, and the CysB protein clearly controls not just cysteine biosynthesis, but also the cleavage of organosulfur compounds to release inorganic sulfur for cysteine biosynthesis. However, when the *atsRBCA* cluster was introduced into *E. coli*, no synthesis of arylsulfatase was observed, and the cells were unable to grow with aromatic sulfates, despite the presence of an active *E. coli cysB* gene. It is not yet clear whether this effect is due to specificity of the *P. aeruginosa* CysB protein, but not the *E. coli* CysB protein, for binding sites in the *atsR-atsB* intergenic region, or whether additional species-specific factors are required for expression of the *ats* genes. Such factors are known for the sulfur-regulated sulfonatase systems *asf* in *Pseudomonas putida* (21) and *tau*/*ssu* in *E. coli* (18, 19), which in addition to CysB also require the LysR-type regulators AsfR and Cbl, respectively, for expression. Further work to elucidate this is ongoing in our laboratory.

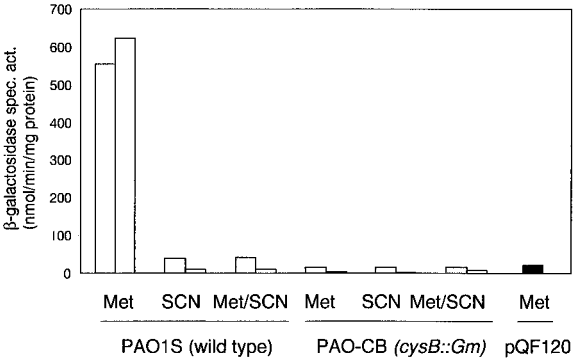

FIG. 4. Effect of *cysB* on *atsB* and *atsR* expression in *P. aeruginosa*. Cells were grown in succinate-minimal medium with methionine (Met), thiocyanate (SCN), or both as the sulfur sources and were harvested in the mid-exponential phase. Expression of *atsB*::*lacZ* and *atsR*::*lacZ* was measured as β-galactosidase activity in the wild-type strain PAO1S or in the *cysB* mutant PAO-CB. ▨, *atsR*::*lacZ* (pME4333); □, *atsB*::*lacZ* (pME4337); ■, vector control PAO1S(pQF120).

Nucleotide sequence accession number. The nucleotide sequence reported here has been included with the previously published *atsA* sequence (1) and is available under accession no. Z48540.

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REFERENCES

1. **Beil, S., H. Kehrli, P. James, W. Staudenmann, A. M. Cook, T. Leisinger, and M. A. Kertesz.** 1995. Purification and characterization of the arylsulfatase synthesized by *Pseudomonas aeruginosa* PAO during growth in sulfate-free medium and cloning of the arylsulfatase gene (*atsA*). Eur. J. Biochem. **229:**385–394.
2. **Davison, J., F. Brunel, A. Phanopoulos, D. Prozzi, and P. Terpstra.** 1992. Cloning and sequencing of *Pseudomonas* genes determining sodium dodecyl sulfate biodegradation. Gene **114:**19–24.
3. **Delic-Attree, I., B. Toussaint, J. Garin, and P. M. Vignais.** 1997. Cloning, sequence and mutagenesis of the structural gene of *Pseudomonas aeruginosa* CysB, which can activate *algD* transcription. Mol. Microbiol. **24:**1275–1284.
4. **Dierks, T., C. Miech, J. Hummerjohann, B. Schmidt, M. A. Kertesz, and K. von Figura.** 1998. Posttranslational formation of formylglycine in prokaryotic sulfatases by modification of either cysteine or serine. J. Biol. Chem. **273:** 25560–25564.
5. **Dodgson, K. S., G. F. White, and J. W. Fitzgerald.** 1982. Sulfatases of microbial origin. CRC Press, Boca Raton, Fla.
6. **Hofmann, K., and W. Stoffel.** 1993. TMBASE—a database of membrane spanning protein segments. Biol. Chem. Hoppe-Seyler **374:**166.
7. **Hummerjohann, J., E. Kuttel, M. Quadroni, J. Ragaller, T. Leisinger, and M. A. Kertesz.** 1998. Regulation of the sulfate starvation response in *Pseudomonas aeruginosa*: role of cysteine biosynthetic intermediates. Microbiology **144:**1375–1386.
8. **Kertesz, M. A., K. Schmidt-Larbig, and T. Wüest.** 1999. A novel reduced flavin mononucleotide-dependent methanesulfonate sulfonatase encoded by the sulfur-regulated *msu* operon of *Pseudomonas aeruginosa*. J. Bacteriol. **181:**1464–1473.
9. **Kovach, M. E., P. H. Elzer, D. S. Hill, G. T. Robertson, M. A. Farris, R. M. Roop II, and K. M. Peterson.** 1995. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. Gene **166:**175–176.
10. **Kredich, N. M.** 1996. Biosynthesis of cysteine, p. 514–527. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington, D.C.
11. **Linton, K. J., and C. F. Higgins.** 1998. The *Escherichia coli* ATP-binding cassette (ABC) proteins. Mol. Microbiol. **28:**5–13.
12. **Murooka, Y., T. Adachi, H. Okamura, and T. Harada.** 1977. Genetic control of arylsulfatase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **130:**74–81.
13. **Quadroni, M., P. James, P. Dainese-Hatt, and M. A. Kertesz.** 1999. Proteome mapping, mass spectrometric sequencing and reverse transcriptase-PCR for characterisation of the sulfate starvation-induced response in *Pseudomonas aeruginosa* PAO1. Eur. J. Biochem. **266:**986–996.
14. **Ronald, S. L., A. M. Kropinski, and M. A. Farinha.** 1990. Construction of broad-host-range vectors for the selection of divergent promoters. Gene **90:**145–148.
15. **Schweizer, H. P., and T. T. Hoang.** 1995. An improved system for gene replacement and *xylE* fusion analysis in *Pseudomonas aeruginosa*. Gene **158:** 15–22.
16. **Stott, D. E., and C. Hagedorn.** 1980. Interrelations between selected soil characteristics and arylsulfatase and urease activities. Commun. Soil Sci. Plant Anal. **11:**935–955.
17. **Tam, R., and M. H. Saier, Jr.** 1993. Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. Microbiol. Rev. **57:**320–346.
18. **van der Ploeg, J. R., R. Iwanicka-Nowicka, T. Bykowski, M. Hryniewicz, and T. Leisinger.** 1999. The Cbl-regulated *ssuEADCB* gene cluster is required for aliphatic sulfonate-sulfur utilization in *Escherichia coli*. J. Biol. Chem. **174:** 29358–29365.
19. **van der Ploeg, J. R., R. Iwanicka-Nowicka, M. A. Kertesz, T. Leisinger, and M. M. Hryniewicz.** 1997. Involvement of CysB and Cbl regulatory proteins in expression of the *tauABCD* operon and other sulfate starvation-inducible genes in *Escherichia coli*. J. Bacteriol. **179:**7671–7678.
20. **van der Ploeg, J. R., M. A. Weiss, E. Saller, H. Nashimoto, N. Saito, M. A. Kertesz, and T. Leisinger.** 1996. Identification of sulfate starvation-regulated genes in *Escherichia coli*: a gene cluster involved in the utilization of taurine

as a sulfur source. J. Bacteriol. **178:**5438–5446.

21. **Vermeij, P., C. Wietek, A. Kahnert, T. Wüest, and M. A. Kertesz.** 1999. Genetic organization of sulfur-controlled aryl desulfonation in *Pseudomonas putida* S-313. Mol. Microbiol. **32:**913–926.
22. **Voisard, C., C. T. Bull, C. Keel, J. Laville, M. Maurhofer, U. Schnider, G. Défago, and D. Haas.** 1994. Biocontrol of root diseases by *Pseudomonas fluorescens* CHA0: current concepts and experimental approaches, p. 67–89. *In* F. O'Gara, D. N. Dowling, and B. Boesten (ed.), Molecular ecology of rhizosphere microorganisms. VCH, Weinheim, Germany.
23. **White, G. F., N. J. Russell, and M. J. Day.** 1985. A survey of sodium dodecyl-sulfate (SDS) resistance and alkylsulfatase production in bacteria from clean and polluted river sites. Environ. Poll. Ser. Ecol. Biol. **37:**1–11.