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Characterization of SotA and SotB, Two *Erwinia chrysanthemi* Proteins Which Modify Isopropyl-β-D-Thiogalactopyranoside and Lactose Induction of the *Escherichia coli lac* Promoter

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The expression, in *Escherichia coli*, of variants of the *Erwinia chrysanthemi* secretion genes *outB* and *outS* under the P*tac* promoter is toxic to the cells. During attempts to clone *E. chrysanthemi* genes able to suppress this toxicity, I identified two genes, *sotA* and *sotB*, whose products are able to reduce the isopropyl-β-D-thiogalactopyranoside (IPTG) induction of the *E. coli lac* promoter. SotA and SotB belong to two different families of the major facilitator superfamily. SotA is a member of the sugar efflux transporter family, while SotB belongs to the multidrug efflux family. The results presented here suggest that SotA and SotB are sugar efflux pumps. SotA reduces the intracellular concentration of IPTG, lactose, and arabinose. SotB reduces the concentration of IPTG, lactose, and melibiose. Expression of *sotA* and *sotB* is not regulated by their substrates, but *sotA* is activated by the cyclic AMP receptor protein (CRP), while *sotB* is repressed by CRP. Lactose is weakly toxic for *E. chrysanthemi*. This toxicity is increased in a *sotB* mutant which cannot efficiently efflux lactose. This first evidence for a physiological role of sugar efflux proteins suggests that their function could be to reduce the intracellular concentration of toxic sugars or sugar metabolites.

Active efflux has been shown to be the cause of the drug resistance of many bacteria. Some of the proteins performing this efflux can deal with one type or a narrow range of structurally related compounds, like the *Escherichia coli* tetracycline exporter TetB (20). Other proteins are able to extrude from the cell a broad variety of unrelated compounds (antibiotics, quaternary amines, and basic dyes). The resistance conferred by these proteins is called multidrug resistance (MDR). The activity of these pumps gives bacterial pathogens a significant resistance to widely used antibiotics. The gram-negative MDR proteins belong to four different families of transport protein (23, 26). (i) The first is the major facilitator superfamily (MFS). This large group of proteins, which contains membrane transport proteins from bacteria to higher eukaryotes, is involved in the symport, antiport, or uniport of various substrates (25). It includes the well-studied MDR transporters QacA of *Staphylococcus aureus* and Bmr of *Bacillus subtilis*. These proteins contain 12 or 14 transmembrane segments. (ii) The second is the resistance nodulation division family (RND). Proteins of this family contain 12 transmembrane helices. The *E. coli* AcrB and the *Pseudomonas aeruginosa* MexB proteins belong to this group (19, 27). (iii) The third, the Smr family, exemplified by the *S. aureus* Smr protein, contains proteins smaller than those of the former groups, which possess four transmembrane segments (11). (iv) The fourth, the recently described MATE family, is exemplified by the *Vibrio parahaemolyticus* NorM protein (6).

The specificity of these MDR proteins is generally very broad, and a large spectrum of compounds can be effluxed (10). However, their substrates have been sought among antibiotics and drugs (basic dyes, sodium dodecyl sulfate [SDS], quaternary ammonium compounds, carbonyl cyanide *m*-chlorophenylhydrazone, etc.). Recent experiments have shown that other types of molecules, such as sugars, can be pumped out of bacteria. The CmlA efflux pump, which can export a wide variety of antibiotics from different families, can efflux the galactoside isopropyl-β-D-thiogalactopyranoside (IPTG), a gratuitous inducer of genes controlled by the LacI repressor (3). Induction-based suppression of toxic effects has led to the discovery of two *E. coli* genes, belonging to two different families, encoding sugar efflux proteins. The first of these genes, *ydeA*, is a member of the MFS with 12 transmembrane segments (5, 7). Its expression prevents accumulation of arabinose and IPTG in the cytoplasm. The second one, *setA* (*yabM*), belongs to a new subfamily of the MFS, the SET (sugar efflux transporter) family (18), which includes two other *E. coli* proteins (YieO and YicK), a protein from *Yersinia pestis*, and one from *Deinococcus radiodurans*. SetA is able to prevent the accumulation of lactose and IPTG in *E. coli* cells. Thus, sugars and glycosides can both be substrates of efflux pumps.

During a search for *Erwinia chrysanthemi* genes whose products are able to suppress the toxic effect due to the expression of the *E. chrysanthemi outS* and *outB* genes cloned under the P*tac* promoter, I identified two genes able to reduce the intracellular IPTG concentration of the bacteria. This leads to a reduction in the toxicity resulting from the accumulation of OutS and OutB in the bacteria. I also showed that their products are able to prevent the accumulation of several sugars in the bacteria.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and drug resistance assays. The strains and plasmids used in this study are listed in Table 1. *E. chrysanthemi* and *E. coli* strains were grown at 30 and 37°C, respectively, unless otherwise stated. The media used were Luria broth (LB) or M63 minimal medium supplemented with a carbon source (0.2%), and for cloning experiments, the following antibiotics were used at the concentrations indicated: ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; chloramphenicol, 20 μg/ml; and tetracycline, 10 μg/ml. The resistance of *E. coli* strains containing cloned genes to drugs was tested by spotting 10 μl of a 10^5-fold dilution of an overnight culture on LB plus ampicillin plates containing

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source or reference
Strains		
E. chrysanthemi		
3937	Wild type	Laboratory collection
A350	*lmrT*(*Con*) *lacZ*	15
A2507	*lmrT*(*Con*) *lacZ crp*::Cm	29
A3145	*lmrT*(*Con*) *lacZ sotA*::*uidA*-kan	This work
A3501	*lmrT*(*Con*) *lacZ sotB*::*uidA*-kan	This work
E. coli		
NM522	*supE thi-1* Δ(*lac-proAB*) Δ(*mcrB-hsdSM*)*5* r_K^- m_K^- F′ *proAB lacI*^{q} Δ*lacZM15*	Stratagene
P4X	Hfr *metB relA1 spoT1*	Laboratory collection
NA1	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR uxaB*::Mu*d*(Ap^r *lac*)	14
1701	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR* (*rbs-lac*)op6.16	J. Beckwith
9665	F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR trp*::(*malK-lac*)1	1
Plasmids		
pACT3	Cm^r P*tac* ori p15A	9
pEXT20	Ap^r P*tac*	9
pACTPB	pACT3 expressing *pelB*	This work
pABSC3	pACT3 expressing *pelB*^{sp}-*outB*	G. Condemine and V. E. Shevchik
pABCT2	pACT3 expressing *outB*	G. Condemine and V. E. Shevchik
pACTS1	pACT3 expressing *pelB*^{sp}-*outS*	32
pBAD18	Ap^r P_{BAD}	12
pBADuid	*uidA*-kan cloned in the pBAD18 polylinker	This work
pBR322	Tet^r Ap^r	4
pBRS1	pBR322 expressing *sotA*	This work
pBRS6	pBR322 expressing *sotB*	This work
pSU9	Cm^r	2
pS1A	pSU9 expressing *sotA*	This work
pS6E	pSU9 expressing *sotB*	This work
pUC18	Ap^r	Biolabs
pUCS1	pUC18 expressing *sotA*	This work
pUCS6	pUC18 expressing *sotB*	This work
pULB110	Tet^r Ap^r (Mu 3A)	34

various dilutions of the tested compound. The ability of the strain to form single colonies was recorded after 24 h.

Genetic techniques. Transduction with phage ϕEC2 was performed as described by Résibois et al. (28). Genetic mapping was performed with pULB110 as described by van Gijsegem and Toussaint (34). Marker exchange recombinations were obtained after growth in a low-phosphate medium as described by Roeder and Collmer (30).

Enzyme assays. β-Glucuronidase assays were performed with toluenized cells grown to the exponential phase with *p*-nitrophenol-β-D-glucuronate as the substrate (24). β-Galactosidase assays were performed with toluenized cells grown to the exponential phase with *o*-nitrophenol-β-D-galactose as the substrate (21).

Recombinant DNA techniques. Preparations of chromosome and plasmid DNA, restriction digestions, ligations, DNA electrophoresis, transformations, and electroporations were carried out as described by Sambrook et al. (31). Sequencing was performed by Genome Express SA (Grenoble, France).

SDS-PAGE and Western blotting. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (17). After electrophoresis, proteins were electroblotted onto nitrocellulose in a semidry apparatus at 1 mA/cm^2 for 1 h in transfer buffer containing 40 mM glycine, 50 mM Tris, 0.4% SDS, and 10% methanol. The nitrocellulose was then saturated and incubated with antibodies and developed with the ECL enhanced chemiluminescence detection kit (Pharmacia-Amersham Biotech).

Nucleotide sequence accession number. The complete DNA sequences of *sotA* and *sotB* have been deposited in the EMBL database under accession no. AJ249180 and AJ249181, respectively.

RESULTS

Isolation of two genes suppressing the toxicity of PelB^{sp}-OutS and PelB^{sp}-OutB. OutS and OutB are two components of the *E. chrysanthemi* Out secretion machinery, involved in the secretion of pectinases in the external medium (8). OutS, an outer membrane lipoprotein, is a chaperone of the secretin OutD (32, 33). OutB is an inner membrane protein that interacts with OutD (G. Condemine and V. E. Shevchik, unpublished data). We have constructed variants of *outS* and *outB* in which the region coding for their N-terminal anchors has been replaced by the region coding for the PelB signal sequence (32; G. Condemine and V. E. Shevchik, unpublished data). These constructs have been cloned under the P*tac* promoter of plasmid pACT3. When *E. coli* cells containing these plasmids were grown in the absence of IPTG at 30°C, the resulting proteins (PelB^{sp}-OutS and PelB^{sp}-OutB) were correctly synthesized, processed, and accumulated in the periplasm. When grown at 37°C in the presence of IPTG, the cells were unable to form colonies on plates and were lysed in liquid medium. To identify proteins interacting with OutS and OutB, I tried to isolate *E. chrysanthemi* genes which, expressed on a multicopy plasmid, could suppress the toxic effect due to the overexpression of PelB^{sp}-OutS and PelB^{sp}-OutB. An *E. chrysanthemi* gene library constructed in plasmid pUC18 was introduced into *E. coli* strain NM522 containing plasmid pACTS1 (*pelB*^{sp}-*outS*). A total of 6×10^4 transformants were spread onto LB-ampicillin-chloramphenicol-IPTG plates that were incubated overnight at 37°C. Six transformants were able to grow on this medium. Restriction mapping of the six plasmids suppressing the toxicity of PelB^{sp}-OutS showed that they fall into two

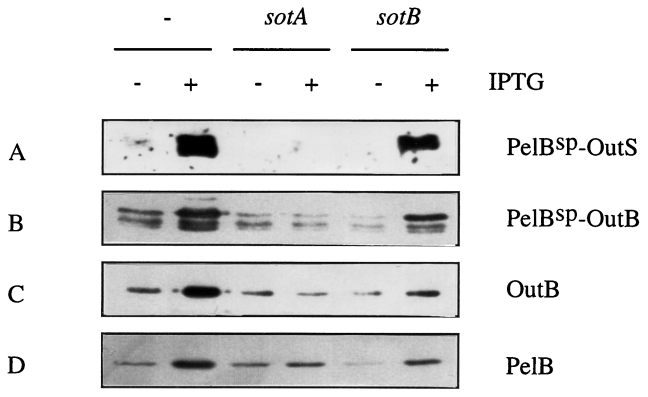

FIG. 1. Effect of the presence of *sotA* and *sotB* on the expression of genes cloned into plasmid pACT3. *E. coli* NM522 cells containing plasmids pACTS1 (*pelBsp-outS*) (A), pABSC3 (*pelBsp-outB*) (B), pABTC2 (*outB*) (C), and pACTPB (*pelB*) (D) were transformed with plasmids pUC18, pUCS1 (*sotA*), and pUCS6 (*sotB*). Cells were grown for 3 h in the absence or presence of 10^{-4} M IPTG. Aliquots were loaded onto SDS-PAGE gel and immunoblotted with anti-OutS (A), anti-OutB (B and C), and anti-PelB (D) antibodies.

groups. Five contained a common region, and one had a totally different restriction map. One plasmid of each type was retained for further studies. The two genes suppressing toxicity were named *sotA* and *sotB* (for suppressor of Out toxicity). The plasmids bearing *sotA* and *sotB* were introduced into NM522 containing plasmid pABSC3 (*pelB*^{sp}*-outB*). Both transformants were able to grow at 37°C in the presence of IPTG, indicating that the *sotA* and *sotB* genes are also able to suppress the toxicity induced by PelB^{sp}-OutB.

***sotA* and *sotB* modify IPTG induction of the P*tac* promoter.** To determine the mode of action of SotA and SotB, I estimated the amount of PelB^{sp}-OutS and PelB^{sp}-OutB in cells expressing, or not, SotA and SotB in the absence or presence of IPTG. While in the absence of IPTG no significant variation was observed, the amounts of both PelB^{sp}-OutS and PelB^{sp}-OutB were reduced with either SotA or SotB. This effect was more pronounced when SotA was present (Fig. 1). To test whether the effect of *sotA* and *sotB* was specific to PelB^{sp}-OutS and PelB^{sp}-OutB, I quantified the products of two other genes (*outB* and *pelB*) cloned under the P*tac* promoter of plasmid pACT3 and expressed under the same conditions. Overexpression of wild-type OutB or PelB from pACT3 is not toxic for the bacteria. The amount of OutB or PelB was also reduced when produced with SotA or SotB (Fig. 1). This reduction could result from a decrease in the copy number of plasmid pACT3, from a reduced induction of the P*tac* promoter, or from an increased degradation of mRNAs or proteins. I first checked that the copy number of pACT3 was unchanged in the presence of *sotA* or *sotB* (data not shown). To test the other hypotheses, we expressed OutB under its own promoter in plasmid pBR322 and under the *tac* promoter of plasmid pEXT20. pEXT20 is a pBR322 derivative that contains the expression cassette comprising the P*tac* promoter of plasmid pACT3. While expression of OutB from pBR322 was not modified by the presence of SotA and SotB, even in the presence of IPTG, expression from pEXT20 was reduced (data not shown). This result indicates that SotA and SotB do not modify the stability of RNAs or proteins, but they do influence the IPTG induction of the P*tac* promoter of pEXT20 or pACT3. Induction of *pelB* cloned into pACT3 was tested by using increasing amounts of IPTG. Induction of PelB synthesis was observable with concentrations of IPTG as low as 10^{-6} M in the control strain. A 10^{-5} M concentration of IPTG was required to induce PelB synthesis when *sotB* was present, and 10^{-4} M IPTG was required when *sotA* was present (Fig. 2). Thus, SotA and SotB could decrease the sensitivity of P*tac* to IPTG or reduce the intracellular concentration of IPTG.

The products of *sotA* and *sotB* are putative membrane transport proteins. The DNA fragment containing the *sotA* gene, reduced to 1.6 kb by subcloning, was sequenced. It contains one putative open reading frame of 1,179 nucleotides, encoding a protein of 42,433 Da with 12 putative transmembrane domains. A similarity search in data banks revealed that it belongs to the recently identified sugar efflux transporter family, a subgroup of the MFS of membrane transporters. A strong homology exists with the proteins SetB (YeiO) (68% identity), YicK (61% identity), and SetA (YabM) (53% identity) of *E. coli*. SetA and SetB can efflux lactose and/or IPTG from *E. coli* cells.

The *sotB* gene has been localized by subcloning on a 2.1-kb DNA fragment which has been sequenced. It contains one putative complete open reading frame of 1,185 nucleotides, encoding a protein of 41,960 Da with 12 putative transmembrane domains. SotB belongs to another subfamily of the MFS which contains MDR proteins. SotB presents homology with the chloramphenicol resistance protein of *Streptomyces lividans* (CmlR) which exports the drug, the AraJ protein of *E. coli*, and the YdeA proteins of *E. coli* and *Haemophilus influenzae*. The highest identity was observed with YdeA of *E. coli* (61%). The *E. coli* YdeA protein is able to export arabinose and IPTG from the bacteria.

SotA and SotB can export several sugars. The reduced induction of IPTG of the P*tac* promoter in the presence of SotA and SotB and the homology of these proteins with sugar exporters suggested that SotA and SotB are sugar efflux pumps. To test this hypothesis and to determine the substrates of these pumps, we measured whether the presence of SotA and SotB on a plasmid in *E. coli* modifies the induction of sugar utilization operons by their substrate. Plasmids bearing *sotA* and *sotB* were introduced into *E. coli* P4X, and *lacZ* induction was monitored. A reduction in the induction level of the *lacZ* gene by IPTG was observed in the presence of SotA or SotB (Fig. 3A and B). However, the concentration at which this effect was observable was 10-fold lower with SotB than with SotA (3×10^{-6} M versus 4×10^{-5} M). The *lac* operon is also inducible by lactose and melibiose (21). While induction of *lacZ* by these compounds was barely modified in the presence of SotA, it was clearly diminished by SotB (Fig. 3C, D, and E). Efflux of galacturonate, ribose, and maltose was tested by measuring the induction of an *uxaB-lacZ* fusion, an *rbs-lacZ* fusion, and a *malK-lacZ* fusion in the presence of *sotA* and *sotB*. No modi-

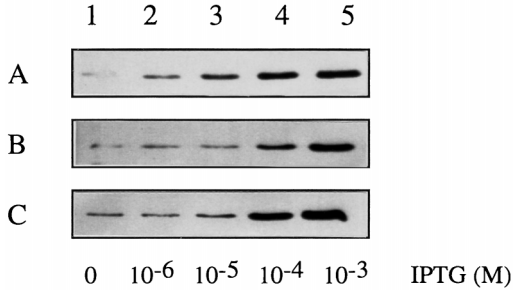

FIG. 2. Effect of the presence of *sotA* and *sotB* on the IPTG induction of *pelB* cloned into pACT3. *E. coli* NM522 cells containing pACTPB (*pelB*) and pUC18 (A), pUCS1 (*sotA*) (B), or pUCS6 (*sotB*) (C) were grown in LB medium with 0 (lane 1), 10^{-6} (lane 2), 10^{-5} (lane 3), 10^{-4} (lane 4), or 10^{-3} M IPTG (lane 5). Aliquots were loaded onto an SDS-PAGE gel (10% polyacrylamide) and immunoblotted with anti-PelB antibody.

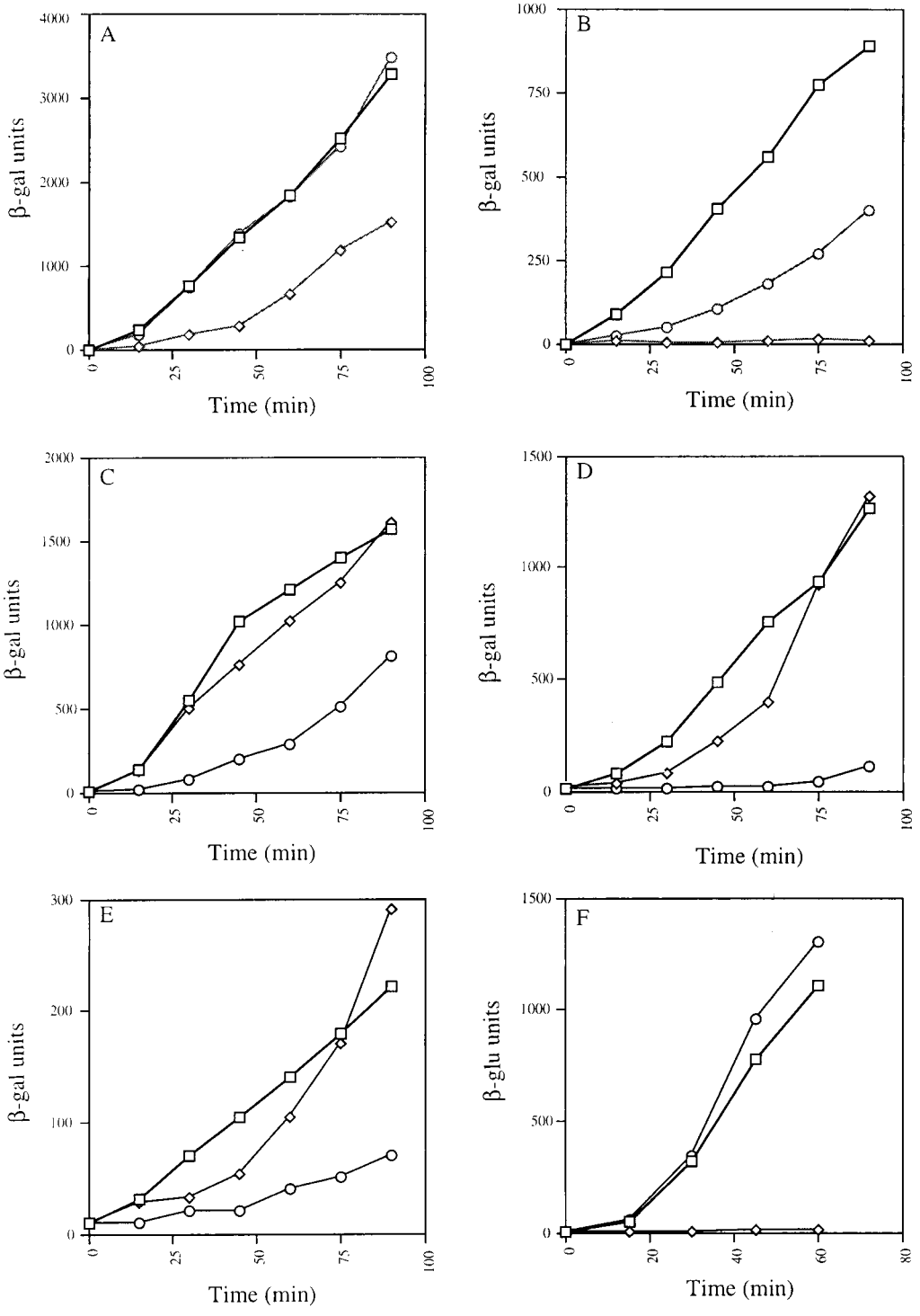

FIG. 3. Effect of the presence of *sotA* and *sotB* on the induction of the *lac* and *ara* promoter in *E. coli* by various compounds. Strains P4X (induction by IPTG, lactose, or melibiose) containing plasmid pBR322 (□), pBRS1 (*sotA*) (◇), or pBRS6 (*sotB*) (○), or NM522/pBADuid (induction by arabinose) carrying plasmid pSU9 (□), pS1A (*sotA*) (◇), or pS6E (*sotB*) (○) were grown in LB medium to an optical density at 600 nm of 0.5. Inducer was added at the concentration indicated, and the β-galactosidase (β-gal) or β-glucuronidase (β-glu) activity of the culture was measured over time. (A) IPTG (4×10^{-5} M). (B) IPTG (3×10^{-6} M). (C) Lactose (20 mg/liter). (D) Lactose (5 mg/liter). (E) Melibiose (40 mg/liter). (F) Arabinose (4 mg/liter).

fication of the induction rate of these fusions was observed, indicating that these sugars are not substrates of SotA or SotB. Efflux of arabinose was tested by measuring the induction of β-glucuronidase encoded by the *uidA* gene, which was cloned under the P*ara* promoter of plasmid pBAD18. The presence of SotA, but not of SotB, was able to prevent, very efficiently, induction by arabinose of *uidA* expression (Fig. 3F).

The multidrug transporter CmlA is able to confer resistance to a wide range of drugs and also to efflux IPTG (3). To test whether SotA and SotB could also efflux drugs, I compared the

TABLE 2. Expression of *sotA-uidA* and *sotB-uidA* fusions in the presence of various sugars

Growth condition[a]	Additional mutation	β-Glucuronidase activity[b]	
		sotA-uidA	*sotB-uidA*
Glycerol	—[c]	141 ± 5	16 ± 2
Glycerol + lactose	—	135 ± 7	18 ± 2
Glycerol + IPTG	—	141 ± 14	12 ± 2
Glycerol + melibiose	—	140 ± 9	49 ± 14
Glycerol + arabinose	—	104 ± 8	38 ± 12
Glycerol + galacturonate	—	216 ± 8	48 ± 6
Glycerol + rhamnose	—	147 ± 9	15 ± 2
Glycerol + maltose	—	152 ± 6	13 ± 1
Glucose	—	99 ± 7	62 ± 6
Glucose	*crp*	72 ± 6	125 ± 6

[a] Bacteria were grown in M63 medium with 0.2% glycerol or glucose as the carbon source and 0.2% of the sugars tested or 1 mM IPTG.

[b] β-Glucuronidase activity is expressed in nanomoles of *p*-nitrophenol formed per minute per milligram (dry weight) of bacteria. Each value is the average of four determinations.

[c] —, none.

resistance of strain NM522, with or without *sotA* and *sotB*, to 19 antibiotics and drugs (tetracycline, chloramphenicol, rifampin, nalidixic acid, novobiocin, kanamycin, spectinomycin, erythromycin, fusidic acid, trimethoprim, gentamicin, vancomycin, kasugamycin, crystal violet, SDS, ethidium bromide, oleic acid, coumaric acid, and ferrulic acid). The presence of SotA and SotB did not change the MICs of these compounds. Thus, SotA and SotB seem to efflux specifically sugars.

Analysis of *sotA* and *sotB* transcription. *sotA* and *sotB* mutants were constructed by reverse genetics. A *uidA*-kan cassette was inserted into the unique *Sma*I site of *sotA* and into the unique *Bam*HI site of *sotB*. Constructs in which the *uidA* gene was in the same orientation as *sotA* or *sotB* were retained, introduced into *E. chrysanthemi*, and recombined into the chromosome. The operon fusions obtained were used to study *sotA* and *sotB* transcription. Synthesis of multidrug transporters is often induced by one of the drugs they expel (22). Thus, we measured the expression of the *sotA-uidA* and *sotB-uidA* fusions in the presence of various sugars (Table 2). Expression of *sotA* was not induced by its substrates, IPTG and arabinose. Among the other sugars tested, only galacturonate gave a 1.5-fold induction. The presence of a *crp* mutation reduced by twofold the expression of *sotA*. The expression of *sotB* was not induced by lactose or IPTG, but increased fourfold in the presence of glucose. It was also induced to a lesser level by other metabolizable sugars, such as galacturonate, arabinose, and melibiose (Table 2). The fusion was expressed at an eightfold-higher level in a *crp* mutant. This suggested a repression of *sotB* by cyclic AMP receptor protein (CRP). A CRP binding site, which contains 9 out of the 10 nucleotides of the consensus ($TGTGAN_6TCGCA$), can be found at position 533 to 548, 65 bases upstream of the *sotB* initiation codon.

Analysis of *E. chrysanthemi sotA* and *sotB* mutants. The presence of lactose in the growth medium was detrimental to the growth of *E. chrysanthemi* A350, the strain used in this study (Fig. 4). It has been designed for the construction of *lacZ* gene fusion (15). Lactose uptake has been increased by the derepression of the expression of the broad-substrate-specificity transporter *lmrT* (involved in lactose, melibiose, and raffinose entry), and the *lacZ* gene has been inactivated. Thus, this strain is unable to metabolize lactose. The inhibitory effect of lactose on the growth of the *sotA* and *sotB* mutant was compared with that observed with A350. While the growth of the *sotA* mutant was equivalent to that of A350, the *sotB* mutant showed reduced growth, indicating that the presence of an efficient lactose efflux pump, SotB, is sufficient to reduce, at least partially, the lactose toxicity toward *E. chrysanthemi*.

sotA and *sotB* have been mapped on the *E. chrysanthemi* chromosome. Chromosomal mobilization mediated by the plasmid pULB110 was used for conjugation with various polyauxotrophic recipient strains. Both genes were localized between the *his-1* and *gal-1* markers (16). *sotA* and *sotB* are cotransducible by phage ϕEC2 with a frequency of 63%, indicating that they are probably located within 20 kb of each other.

DISCUSSION

The multicopy suppression strategy I used, which involved looking for genes whose products could suppress the toxicity of $PelB^{sp}$-OutS or $PelB^{sp}$-OutB, was aimed at finding proteins that interact with OutS and OutB. This approach led to the identification of two genes that are not related to the Out secretion system, but whose products are able to reduce the intracellular concentration of the *lac* gene inducer, IPTG. The presence of *sotA* or *sotB* reduces the expression of any gene cloned under the P*tac* promoter of plasmid pACT3 by lowering the intracellular concentration of IPTG. The results presented here suggest that SotA and SotB are sugar efflux proteins. The other known sugar efflux proteins have been isolated fortuitously by similar cloning strategies: *cmlA* and *setA* reduce induction by IPTG of toxic genes cloned under the P*tac* promoter (3, 18), and *ydeA* prevents the arabinose-dependent expression of a toxic protein expressed under the control of the arabinose P_{BAD} promoter (5, 7). Thus, the use of a multicopy suppression strategy when the toxic protein synthesis is under the control of an inducible promoter often leads to the identification of genes able to expel the inducer. New sugar efflux transporters could be identified, by the same strategy, by cloning toxic genes in other sugar-inducible promoter-based expression vectors.

Up to now, no physiological function has been assigned to

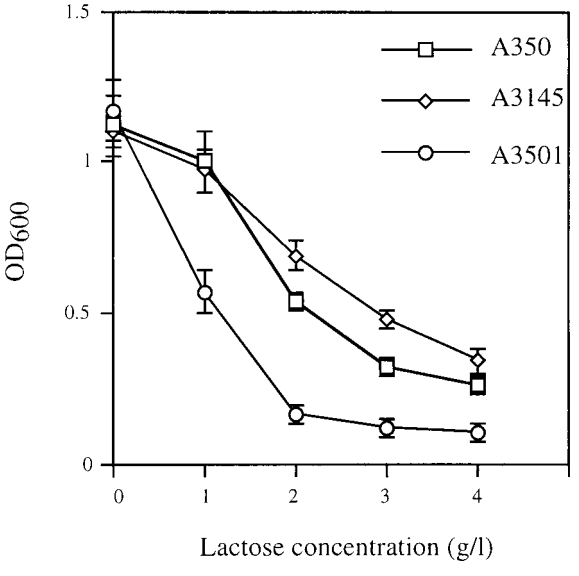

FIG. 4. Growth of strain A350 and of *sotA* and *sotB* mutants in the presence of lactose. M63 medium containing 0.2% glycerol and various concentrations of lactose was inoculated at a 1:100 dilution with a culture of strain A350, A3156, or A3501. After 16 h of growth, the optical density at 600 nm (OD_{600}) was measured.

sugar efflux pumps. The results presented here show that one of their functions could be to remove toxic sugars from the cytoplasm. Although the cause of lactose toxicity in *E. chrysanthemi* is not known, it is clear that the absence of an efflux system able to reduce the intracellular concentration of this sugar has a detrimental effect on the growth of the bacteria. Accumulation of nonmetabolizable sugars or of sugar metabolites, such as sugar phosphates, can have growth-inhibitory effects. The sugar efflux transporters could participate in the regulation of the intracellular concentration of these toxic compounds. The presence of three SET members in *E. coli* could indicate that this strategy of detoxification is widely used by bacteria. Huber et al. (13) have shown that when *E. coli* cells are grown in the presence of lactose, a large proportion of the β-galactosidase products are found in the culture medium. Sugar efflux pumps could help maintain an intracellular concentration of sugar within a range compatible with the biochemical and regulatory metabolism of the bacteria. Recently, amino acid efflux pumps have been described (35). It would be interesting to investigate whether the intracellular concentrations of other compounds could also be regulated by efflux pumps.

Initially, SotA and SotB may seem redundant, but the specificity of the two transporters appears different. IPTG, melibiose (6-O-α-D-galactopyranosyl-D-glucose), and lactose [β-D-galactose (1-4)-α-glucose] are apparently good substrates for SotB. Thus, SotB can be considered as an exporter of β-galactosides. The specificity of SotA seems to be wider, since it can apparently efflux IPTG, lactose, and arabinose, a pentose. The differences in substrate specificity may contribute as well to differences in the respective roles of SotA and SotB. Screening of other sugar efflux activities by SotA and SotB will show if they have a narrow specificity, recognizing only one type of glycoside, or if they are multisugar efflux pumps, with a specificity as broad as that of some multidrug efflux pumps. The absence of a specific regulation of *sotA* and *sotB* expression by lactose or arabinose and their control by the global regulator CRP, the homology of SotB with MDR proteins, suggest that they could have a broad specificity, contributing to the efflux of many sugars from the cytoplasm. However, in contrast to CmlA, they do not seem to be able to export drugs and antibiotics.

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