Sulfate Ester Detergent Degradation in *Pseudomonas aeruginosa* Is Subject to both Positive and Negative Regulation

Gianna Panasia,^a Sylvia Oetermann,^a Alexander Steinbüchel,^{a,b} Bodo Philipp^a

^aWestfälische Wilhelms-Universität Münster, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany

^bEnvironmental Sciences Department, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT Bacteria using toxic chemicals, such as detergents, as growth substrates face the challenge of exposing themselves to cell-damaging effects that require protection mechanisms, which demand energy delivered from catabolism of the toxic compound. Thus, adaptations are necessary for ensuring the rapid onset of substrate degradation and the integrity of the cells. *Pseudomonas aeruginosa* strain PAO1 can use the toxic detergent sodium dodecyl sulfate (SDS) as a growth substrate and employs, among others, cell aggregation as a protection mechanism. The degradation itself is also a protection mechanism and has to be rapidly induced upon contact to SDS. In this study, gene regulation of the enzymes initiating SDS degradation in strain PAO1 was studied. The gene and an atypical DNA-binding site of the LysR-type regulator SdsB1 were identified and shown to activate expression of the alkyl-sulfatase SdsA1 initiating SDS degradation. Further degradation of the resulting 1-dodecanol is catalyzed by enzymes encoded by *laoCBA*, which were shown to form an operon. Expression of this operon is regulated by the TetR-type repressor LaoR. Studies with purified LaoR identified its DNA-binding site and 1-dodecanoyl coenzyme A as the ligand causing detachment of LaoR from the DNA. Transcriptional studies revealed that the sulfate ester detergent sodium lauryl ether sulfate (SLES) induced expression of *sdsA1* and the *lao* operon. Growth experiments revealed an essential involvement of the alkylsulfatase SdsA1 for SLES degradation. This study revealed that the genes for the enzymes initiating the degradation of toxic sulfate-ester detergents are induced stepwise by a positive and a negative regulator in *P. aeruginosa* strain PAO1.

IMPORTANCE Bacterial degradation of toxic compounds is important not only for bioremediation but also for the colonization of hostile anthropogenic environments in which biocides are being used. This study with *Pseudomonas aeruginosa* expands our knowledge of gene regulation of the enzymes initiating degradation of sulfate ester detergents, which occurs in many hygiene and household products and, consequently, also in wastewater. As an opportunistic pathogen, *P. aeruginosa* causes severe hygienic problems because of its pronounced biocide resistance and its metabolic versatility, often combined with its pronounced biofilm formation. Knowledge about the regulation of detergent degradation, especially regarding the ligands of DNA-binding regulators, may lead to the rational development of specific inhibitors for restricting growth and biofilm formation of *P. aeruginosa* in hygienic settings. In addition, it may also contribute to optimizing bioremediation strategies not only for detergents but also for alkanes, which when degraded merge with sulfate ester degradation at the level of long-chain alcohols.

KEYWORDS alkylsulfatase, LaoABC long-chain alcohol oxidation, LysR-type regulator, *Pseudomonas*, LaoABC, *Pseudomonas aeruginosa*, SDS, SLES, TetR-type regulator

Citation Panasia G, Oetermann S, Steinbüchel A, Philipp B. 2019. Sulfate ester detergent degradation in *Pseudomonas aeruginosa* is subject to both positive and negative regulation. *Appl Environ Microbiol* 85:e01352-19. <https://doi.org/10.1128/AEM.01352-19>.

Editor M. Julia Pettinari, University of Buenos Aires

Copyright © 2019 American Society for Microbiology. All Rights Reserved.

Address correspondence to Bodo Philipp, bodo.philipp@uni-muenster.de.

Received 14 June 2019

Accepted 14 September 2019

Accepted manuscript posted online 20 September 2019

Published 14 November 2019

Bioremediation processes such as wastewater treatment and decontamination of soils rely on the ability of bacteria to use toxic organic compounds, such as solvents or detergents, as growth substrates. The presence of such toxic compounds is generally a challenge for bacteria and requires energy-consuming protection mechanisms such as efflux pumps (1) and chaperones (2). If the toxic compound is the only source of energy and carbon, this challenge is increased because the bacterial cells have to take up and expose their cell membrane and cytosol to these chemicals. Since the energy needed for protection against this chemical stress has to originate from the catabolism of the toxic substrate, this process requires elaborated adaptations for providing a well-balanced regulation of catabolism and protection mechanisms to avoid detrimental cell damage.

Degradation of the anionic detergent sodium dodecyl sulfate (SDS) by *Pseudomonas aeruginosa* is a suitable paradigm for studying how bacteria cope to grow with a toxic compound as a carbon and energy source. In the past we have shown that a sufficient energy status of the cells is crucial for survival, e.g., for operating proton-motif-force-dependent efflux-pumps such as MexEF-OprM (3–5). In addition, we were able to show that cell aggregation serves as a preadaptive survival strategy of *P. aeruginosa* for growth with SDS (6). Cell aggregation is induced via a signal transduction system encoded by *siaABCD* and increases the survival rate upon exposure to SDS-related stress several hundredfold compared to suspended single cells (4, 7). Since the degradation of the toxic compounds also contributes to minimizing the toxic effects, the rapid induction of synthesis of the respective enzymes is crucial. This calls attention to the genetic regulation of the enzymes that initiate SDS degradation in *P. aeruginosa*. While the ability of SDS degradation in pseudomonads has been known for many years (8–12), the regulation of the involved enzymes and stimulus perception is poorly understood.

The initial step in SDS degradation by *P. aeruginosa* strain PAO1 starts with the hydrolysis of the sulfate ester group catalyzed by the well-studied alkylsulfatase SdsA1 (PA0740) (13) (Fig. 1, left panel). We recently identified the Lao system to be involved in the following oxidation steps of the first intermediate, the long-chain primary alcohol 1-dodecanol, and the emerging aldehyde 1-dodecanal (14). In particular, LaoA and LaoB (PA0364–65) were found to be responsible but not essential for the 1-dodecanol oxidation. The respective genes are located together with *laoC* (PA0366), encoding an aldehyde dehydrogenase, in one gene cluster. In addition, the Lao system has been shown to participate in the oxidation of long-chain alcohols originating from alkane degradation (14). Thus, the Lao system is apparently disconnected from the initial SDS degradation step not only by its genomic localization but also by its substrate specificity. Accordingly, different regulatory pathways are required to enable the expression of the Lao system in the absence of SDS. Thus far, the LysR-type regulator SdsB for the alkylsulfatase SdsA in *Pseudomonas* sp. strain ATCC 19151 has been specified and analyzed. This regulator gene has 54% identity to PA0739 (15, 16), which is located directly adjacent to *sdsA1* in an opposite direction on the *P. aeruginosa* PAO1 genome (Fig. 1, right panel). In addition, we have already shown that the TetR-type regulator LaoR (formerly PA0367), the gene of which is located directly adjacent to *laoCBA* in the opposite direction (Fig. 1, right panel), regulates the genes encoding the Lao enzymes (14).

The goal of our study was to study the question how *P. aeruginosa* copes to grow with SDS as a carbon and energy source from the point of genetic regulation. In particular, we sought to identify the regulator for *sdsA1*, as well as inducers for expression of the initiating enzymes in the SDS degradation.

RESULTS

Identification of the regulator SdsB1. For analyzing whether the gene PA0739 is involved in the SDS metabolism of *P. aeruginosa* strain PAO1, a chromosomal gene deletion strain was constructed. Growth of the wild-type strain PAO1 was compared to that of the PAO1 Δ *sdsB1* deletion mutant strain and the complemented mutant. During

FIG 1 Overview of SDS degradation by *P. aeruginosa* strain PAO1. (Left) Initial steps of metabolic pathway. (Right) Genetic loci for enzymes catalyzing the initial steps and the respective regulatory proteins SdsB1 and LaoR.

growth with succinate, no differences between strain PAO1 and the PAO1 $\Delta sdsB1$ deletion mutant strain were observable (data not shown). With SDS as the sole carbon and energy source (Fig. 2), the PAO1 $\Delta sdsB1$ deletion mutant strain exhibited almost no growth, as well as no SDS degradation (Fig. 2A). Growth could be restored to wild-type level by providing an intact copy of PA0739 on the plasmid pUCP18 (Fig. 2B). These results demonstrated that PA0739 constituted the respective regulator for *sdsA1*, which encodes the essential alkylsulfatase for growth with SDS (13, 16). Hence, PA0739 was named *sdsB1*.

Analysis of the DNA-binding site of SsdB1. To identify the DNA-binding site of SdsB1, we purified His-tagged SdsB1 and performed electrophoretic mobility shift

FIG 2 Growth (solid lines) of *P. aeruginosa* PAO1 (cyan diamond) and $\Delta sdsB1$ strains or a derivative strain (magenta circles) with SDS (dashed lines) as carbon and energy sources. (A) $\Delta sdsB1$ deletion mutant and PAO1 strains. (B) Deletion mutant strain ($\Delta sdsB1$) complemented with pUCP18[*sdsB1*] and strain PAO1. (C) Deletion mutant strain ($\Delta sdsB1$) with pUCP18 (vector control) and strain PAO1. The data show results from one of two representative independent experiments. Error bars indicate the standard deviations ($n = 3$).

FIG 3 Identification of the DNA-binding site of SdsB1 in *P. aeruginosa* strain PAO1. (A) Overview of the intergenic region and fragments used for the DNA-SdsB1 binding analysis. (B) EMSAs of SdsB1 with DNA fragments. (C) DNA sequences without (magenta "B") or with (magenta "B*" to "B***") nucleotide exchanges. Colored nucleotides in boxes indicate inverted repeats. Overlapping nucleotides of inverted repeats are shown in dark purple. Underlined sequences depict nucleotide exchanges. (D) EMSA with changed DNA sequences. The pink arrowhead indicates the height of a shifted band by protein-DNA complex formation.

assays (EMSAs) with different DNA fragments of the intergenic region between *sdsB1* and *sdsA1* (Fig. 3). These experiments revealed a binding within the first 40 bp of the intergenic region (Fig. 3B). This DNA fragment exhibits two inverted repeats (IRs), which partly overlap. The IRs were either solely or both exchanged by the nucleotide exchanges depicted in Fig. 3C. Changes in one of these IRs revealed a reduced SdsB1 DNA binding, while the change of both IRs completely abolished SdsB1 DNA binding (Fig. 3D). This result revealed binding of SdsB1 to an atypical LysR-type regulatory binding site (RBS) with a binding motif which is different from the classical and conserved T-N₁₁-A binding motif (17, 18).

Reverse transcriptase PCR analysis of the gene cluster *laoCBA*. The genes *laoCBA* are located in a gene cluster on the minus strand separated by only two 53- to 54-bp-long intergenic regions, suggesting an operon organization (Fig. 1). Reverse transcriptase PCR was used to identify such putative polycistronic mRNA in cells grown with 1-dodecanol compared to cells grown with succinate (Fig. 4). Succinate-grown cells have already been demonstrated to not exhibit 1-dodecanol oxidation activity by the Lao system (14). As shown in Fig. 4, polycistronic mRNA could be detected in cells grown with 1-dodecanol but not in succinate-grown cells. As a result, the *laoCBA* genes constitute an operon, which is induced during growth with 1-dodecanol.

Analysis of the DNA-binding site of LaoR. In the next step, we examined whether the TetR-type repressor LaoR binds to the intergenic region between *laoC* and *laoR*. For this purpose, His-tagged LaoR was purified and EMSAs with different DNA fragments of the 288-bp intergenic region were performed (Fig. 5). LaoR was found to bind within the central 88-bp fragment. Two inverted repeats (within fragment C₁ or C₂) of this central 88-bp fragment were assumed to constitute the possible binding sites (Fig. 5A and C). Both IRs, as well as exchanges of nucleotides within fragment C₁, were analyzed

FIG 4 Identification of a polycistronic transcript of the *laoABC* genes in *P. aeruginosa* strain PAO1. (A) Positions of primers used for the PCR analysis of *laoABC* genomic DNA and transcripts. (B) PCR analysis with genomic DNA (I), cDNA from succinate-grown cells (II), and cDNA from 1-dodecanol-grown cells (III). The blue arrowhead indicates the height of the PCR products. The data show results from one of three representative independent experiments.

by EMSAs (Fig. 5B and D). The IR of fragment C₁ was shown to be the actual binding site for LaoR.

Analysis of SdsB1 and LaoR binding sites by thermal shift assays. Thermal shift assays are used as a biochemical method for the detection of protein stability by

FIG 5 Identification of the DNA binding site of LaoR in *P. aeruginosa* strain PAO1. (A) Overview of the intergenic region and used fragments for the DNA-LaoR binding analysis. (B) EMSA of LaoR with DNA fragments. (C) DNA sequences without (blue "C1") or with nucleotide exchanges (blue "C1*" to "C1**"). Light blue nucleotides in boxes indicate inverted repeat. Underlined, dark blue sequences depict nucleotide exchanges. (D) EMSA with modified DNA sequences. The blue arrowhead indicates height of shifted band by protein-DNA complex formation.

FIG 6 Verification of the regulator-DNA binding interaction by thermal shift assay. (A) Determination of SdsB1 melting temperature (T_m) without DNA or in the presence of a DNA binding site (fragment B in Fig. 3A) and DNA without a binding site (fragment C in Fig. 3A). (B) Determination of LaoR melting temperature (T_m) without DNA or in the presence of a DNA binding site (fragment C in Fig. 5A) and DNA without a binding site (fragment D in Fig. 5A). Error bars indicate the standard deviations ($n = 3$). A Student t test was performed (*, significant at $P < 0.001$).

quantification of their melting temperature. Because DNA binding should have a stabilizing effect on regulator proteins, this method should be applicable to analyze DNA-binding sites but has, to our knowledge, not been reported thus far. The DNA-regulator binding activities for the regulators SdsB1 (Fig. 6A) and LaoR (Fig. 6B) showed an increased protein melting temperature in the presence of the specific DNA-binding sites identified by the EMSA analysis. Thus, the thermal shift assays could constitute an alternative and rapid method for analyzing DNA-binding sites of regulators.

Ligand analysis of LaoR. The classical TetR-type regulators are released from the DNA by ligand binding, which leads to the expression of their controlled genes (19). To identify the corresponding ligand for LaoR, EMSAs with SDS and intermediates of SDS degradation were performed (Fig. 7). Increasing SDS concentrations exhibited an increasing influence on the LaoR-DNA complex resulting in a detachment of LaoR from its DNA-binding site (Fig. 7A). This effect can probably be explained by the protein denaturing property of the toxic detergent SDS (20). The degradation intermediate 1-dodecanal also exhibited an influence on the LaoR-DNA complex, with a slight detachment of the regulator from the DNA binding site, which also might be due to denaturing effects on the protein since this is well known for reactive aldehydes (21). The SDS degradation intermediates 1-dodecanol and 1-dodecanoic acid, as well as coenzyme A (CoA) or a combination of 1-dodecanoic acid and CoA, exhibited no detachment. Strikingly, the degradation intermediate 1-dodecanoyl-CoA influenced the LaoR-DNA complex, resulting in a detachment detected by removal of the shifted band (Fig. 7B). Decanoyl-CoA caused detachment only at higher concentrations, while hexanoyl-CoA did not cause detachment even at the highest concentration. Accordingly, this experiment reveals specificity for long-chain acyl-CoA esters as ligands for LaoR.

FIG 7 Identification of ligands of LaoR causing its dissociation from its DNA-binding site within 5' JOE-labeled fragment C₁ (Fig. 5C). (A) EMSA analysis with SDS and SDS degradation intermediates as putative ligands. (B) EMSA analysis with acyl-CoA esters in various concentrations as putative ligands. Blue arrowheads indicate the height of shifted bands caused by protein-DNA complex formation.

Transcriptional analysis of *sdsA1* and *laoCBA*. In the next step, we analyzed whether the SDS substrate analogues sodium lauryl ether sulfate (SLES) and dodecane sulfate would also induce the expression of *sdsA1* and the *lao* operon. For this purpose, transcriptional fusions of the intergenic regions between genes *sdsB1* and *sdsA1* and between genes *laoC* and *laoR* with the *lacZ* gene were constructed and analyzed by supplying suspensions of noninduced succinate-grown cells with the putative inducers (Fig. 8A). These experiments showed that gene expression of both systems is induced in the presence of the sulfate esters SDS and SLES. In contrast, dodecane sulfonate, in which the sulfur atom is directly attached to the alkyl chain by a C-S bond, did not induce gene expression. The expression of the *lao* operon was additionally induced in the presence of the LaoAB substrate 1-dodecanol. In the $\Delta sdsB1$ mutant, expression of *sdsA1* could not be induced (Fig. 8A), while in the $\Delta laoR$ mutant, expression of the *lao* operon was constitutively and strongly induced, reaching Miller unit values of about 2,000 even for succinate-grown cells (data not shown). Furthermore, we analyzed the mRNA levels of *sdsA1* and *laoA* in SDS-, SLES-, and 1-dodecanol-grown cells in comparison to succinate-grown cells by quantitative real-time PCR (qRT-PCR) (Fig. 8B). An induced expression of *sdsA1* in the presence of SDS and SLES could be confirmed (Fig. 8B). The reason for the large quantitative difference of induction between the *lacZ* reporter gene assay (~3.5-fold) and the quantitative real-time PCR measurement (~140-fold) is unknown. It might be due to the different experimental setups (induction

FIG 8 Transcriptional analysis of *sdsA1* and *laoABC* in *P. aeruginosa* strain PAO1. (A) Influence of different substrates on β -galactosidase activities of plasmid-based promoter-*lacZ* reporter gene fusion of the *sdsA1* promoter region (P_{sdsA1}) corresponding to the 102-bp intergenic region between *sdsB1* and *sdsA1* (Fig. 3, fragment A) or *laoCBA* promoter region (P_{laoCBA}) corresponding to the 288-bp intergenic region between *laoC* and *laoR* (Fig. 5, fragment A) compared to a vector control and to the Δ *sdsB1* deletion mutant. Error bars indicate standard deviations ($n = 3$). A Student *t* test was performed (*, significance at $P < 0.01$; **, significance at $P < 0.001$). (B) quantitative real-time PCR analysis of mRNA levels of *sdsA1* (I), *laoA* (II), *sdsB1* (III), and *laoR* (IV) in SDS-, SLES-, or 1-dodecanol-grown cells expressed as the fold change compared to the respective mRNA levels in succinate-grown cells. Error bars represent standard deviations ($n = 3$).

assays with cell suspensions and growth experiments, respectively) or to an influence of SDS on mRNA stability. The *lao* operon was shown to be induced with 1-dodecanol and SDS, while an induction by SLES was hardly detectable (mRNA fold change, 2.4 ± 0.9) (Fig. 8BII).

We also analyzed the mRNA levels of the regulators SdsB1 and LaoR themselves. While the mRNA level of *sdsB1* was not increased in SDS- and SLES-grown cells

FIG 9 Analysis of SLES degradation by *P. aeruginosa* strain PAO1. (A) Growth (solid lines) of PAO1 (black circles), $\Delta sdsB1$ (magenta circles), and $\Delta laoA$ (cyan circles) strains with SLES and growth (dashed lines) of $\Delta sdsB1$ pUCP18[*sdsB1*] and $\Delta laoA$ pUCP18[*laoA*] complemented strains. (B) SLES concentrations in the respective cultures shown in panel A. The same data set for strain PAO1 is shown in panels I and II. Error bars represent standard deviations ($n = 3$).

compared to succinate-grown cells, the mRNA level of *laoR* was increased in SDS- and 1-dodecanol-grown cells (Fig. 8BIII and IV), indicating an autoinduction of *laoR* expression.

Growth of *P. aeruginosa* with SLES. The inducing effects of SLES raised the question whether this detergent is also a growth substrate for strain PAO1 and, if so, whether SdsA1 and LaoABC are also involved in its degradation. In the respective growth experiments, the PAO1 $\Delta sdsB1$ mutant strain exhibited almost no growth in comparison to the wild-type and complemented strains (Fig. 9A), and analysis of the SLES concentration revealed no SLES degradation for the PAO1 $\Delta sdsB1$ mutant strain (Fig. 9BI). Thus, the alkylsulfatase SdsA1 was apparently essential for SLES degradation in *P. aeruginosa* PAO1. The growth rate of the PAO1 $\Delta laoA$ mutant strain ($\mu = 0.06 \text{ h}^{-1}$) was about 50% lower than that of the wild type ($\mu = 0.13 \text{ h}^{-1}$) during growth with SLES. In contrast, SLES degradation was similar to that of the wild type (Fig. 8BII). Cultures of strain PAO1 growing with SLES revealed a very similar formation of macroscopic cell aggregates, as has been described during growth with SDS (5).

DISCUSSION

The goal of this study was to elucidate how the expression of the enzymes catalyzing the initial reactions of SDS degradation, namely, of the alkylsulfatase SdsA1 and the long-chain alcohol-oxidizing enzymes encoded by *laoCBA*, in *P. aeruginosa* strain PAO1 is regulated. We could show that these reactions are induced in a two-step manner by a consecutive positive and negative regulation. In the first step, the transcription of *sdsA1* encoding the sulfatase converting SDS into sulfate and 1-dodecanol (13) is activated by the LysR-type regulator SdsB1 (PA0739), which we identified in this study. This activation was caused by SDS, as well as by SLES, another sulfate ester detergent and commonly used alternative for SDS. In the second step,

transcription of the *laoCBA* operon is induced by a detachment of the TetR-type repressor LaoR from its DNA binding site. Our analysis revealed that long-chain acyl-CoA esters can act as ligands, with dodecanoyl-CoA exhibiting the highest efficiency of LaoR detachment from its DNA-binding site. Thus, derepression of the *laoCBA* operon would require basal expression of the encoded enzymes and an as-yet-unknown acyl-CoA-ligase for the formation of this ligand. This two-step induction of SDS degradation could be the outcome of a strategy that ensures rapid inactivation of a toxic sulfate ester detergent, while the degradation of the resulting long-chain alcohol is only induced when it can sufficiently be further converted into a metabolite that signals the availability of an energy-rich substrate for β -oxidation. This derepression of the *laoCBA* operon would result in the increased formation of the inductor 1-dodecanoyl-CoA and thus in a positive regulatory feedback loop. Further degradation of the activated fatty acid by β -oxidation then enables both growth and the operation of protection mechanisms against SDS. The latter is particularly important for survival since we have previously concluded that dividing cells are more vulnerable to the damaging effects of SDS (5). The observation that the *laoR* gene is subject to positive autoregulation suggests that the *lao* operon can be rapidly repressed again when the inducer is depleted, thereby preventing undue expression. In this respect it is unlikely that SDS, although it caused detachment of LaoR in the EMSAs, is a specific inducer of the *lao* operon, which is also required for the degradation of alkanes that are much more abundant in nature than SDS (16).

EMSA analyses revealed that SdsB1 possesses an atypical DNA-binding site within the first 40-bp DNA fragment upstream of *sdsB1*, which is located in the intergenic region between *sdsB1* and *sdsA1* (Fig. 3B). LysR-type regulators often bind to an RBS and a distinct activation binding site (18). The RBS generally has a recognized LTTR (LysR-type transcriptional regulator) box with the conserved palindromic sequence T-N₁₁-A (18, 22) that was not identified within the fragment bound by SdsB1. Instead, we identified two IRs, gcat-N₁₆-atgc and aatg-N₁₃-catt, which partly overlap within the bound DNA fragment (Fig. 3). An *in silico* protein domain analysis (Interpro) (23) of SdsB1 exhibited a conserved CysB-like substrate binding domain (IPR 000847). Interestingly, complex DNA-binding with multiple overlapping DNA binding sites is also described for CysB from *Salmonella enterica* serovar Typhimurium (24, 25). Moreover, the binding site of the master regulator CysB from *P. aeruginosa* (26–28) contains two inverted repeats, which partly overlap (28). However, an alignment of SdsB1 and CysB from *P. aeruginosa*, as well as *S. Typhimurium*, revealed only 24% identity in each case (BLASTP) (29). Nevertheless, both CysB regulators share their involvement in metabolism of organic sulfur compounds with SdsB1 (30, 31). In addition, a recent study identified sulfate ions captured within the cleft of the crystallized C-terminal regulatory domain (substrate binding domain) of CysB from *P. aeruginosa*, pointing toward sulfate ions as potential ligands influencing the function of this regulator (27). Since sulfate was the sulfur source for *P. aeruginosa* in our experiments, it is unlikely to be the inducer for SdsB1. The expression of *sdsA1* via SdsB1 was induced only by substrates of SdsA1, namely, SDS and SLES, but not by the structural analogue dodecyl sulfonate, which cannot be cleaved by SdsA1. Thus, a sulfate group attached to a long-chain alcohol is apparently a decisive structural element for a ligand of SdsB1, thereby supporting the similarities between SdsB1 and CysB.

A typical DNA-binding site was identified to be occupied by the TetR-type regulator LaoR. Ligands of TetR-type regulators comprise a remarkable variety of compounds, including antibiotics, bile acids, cell-cell signaling molecules, proteins, metal ions, or fatty acids (19, 32). Also, acyl-CoA esters such as oleoyl-CoA and palmitoyl-CoA for the regulator DesT, which is involved in lipid metabolism of *P. aeruginosa*, are known as ligands (33). One example very similar to our proposed model for the positive-feedback regulation for LaoR is the TetR regulator AlkX from *Dietzia* sp. strain DQ12-45-1b (34). This regulator controls the expression of genes involved in the degradation of alkanes and binds long-chain fatty acids derived from alkane degradation as ligands. The relatively high concentration of dodecanoyl-CoA required for the detachment of LaoR

from its DNA-binding site might indicate that a threshold concentration is required for inducing the postulated positive-feedback regulation. However, since acyl-CoA esters are already unstable in aqueous solution at neutral pH values, the actual concentration of this ligand might have been overestimated in the respective experiments.

The finding that SLES serves as an inducer for the SdsB1-mediated *sdsA1* transcription prompted us to test whether this sulfate ester detergent could also serve as a carbon and energy source. To the best of our knowledge, the growth of *P. aeruginosa* strain PAO1 with SLES has not yet been described. This milder detergent often replaces SDS in cosmetic and hygienic products due to its reduced skin irritation properties (35–37). Since SLES also induced cell aggregation in *P. aeruginosa*, this sulfate ester detergent apparently also activates the Sia system, indicating that it presumably has toxic effects similar to those of SDS. Generally, SLES degradation has been observed in other pseudomonads, but the degradation pathway is not completely understood. Metabolite analysis in *Pseudomonas* sp. strain Des1 (38, 39), *Pseudomonas* sp. strain SC25A (40), and *P. nitroreducens* (41) revealed that degradation starts with cleavage of the ether bonds, but cleavage of the sulfate ester bond has also been shown to occur concomitantly to ether cleavage in some of these strains (41, 42). However, in strain PAO1, the *sdsB1* deletion mutant, which does not express the alkylsulfatase SdsA1, could not no longer grow with SLES, indicating that SLES degradation is compulsively initiated by sulfate ester hydrolysis. This conclusion is supported by the fact that the stains-all assay (see Materials and Methods) used to quantify sulfate ester detergents did not indicate a decrease in SLES concentration. While SdsA1 apparently had an essential role in SLES metabolism, the Lao system is clearly involved but not essential. The reduced growth rate of the PAO1 Δ *laoA* strain with SLES could plausibly be explained by the presumptive formation of 1-dodecanol after cleavage of the ethoxy units from the SLES molecule. There are many different ether-cleaving reactions known among bacteria (43), but they are unknown for *P. aeruginosa* and are therefore under investigation in our laboratory.

In conclusion, our study is a further step toward the understanding of how *P. aeruginosa* copes to grow with toxic detergent SDS as a carbon and energy source. The next steps for pursuing this research question further involve analysis of how expression of the metabolic pathway and the protection mechanisms, especially cell aggregation and efflux pumps, are coordinated.

MATERIALS AND METHODS

Bacterial strains and growth experiments. Bacterial strains and plasmids used in this study are described in Table 1. Cells were grown in lysogeny broth (LB) medium or in modified M9 mineral medium (5, 44) with variable carbon and energy sources, which were supplied at the following concentrations (the percent purity is indicated in parentheses): 10 mM succinate ($\geq 99\%$), 3.5 mM SDS ($\geq 99.5\%$), and 3.5 mM 1-dodecanol ($\geq 98.0\%$) (Sigma-Aldrich, St. Louis, MO). A 27% SLES aqueous solution was purchased from Caelo (Hilden, Germany) with an average M_w of 382 g mol^{-1} , an average of two degrees of ethoxylation ($n = 2$), and an average alkyl chain length of 12 carbons (information given by the manufacturer). *Escherichia coli* strains harboring plasmids were maintained and selected on LB agar plates (1.5% [wt/vol]) with $100 \mu\text{g ml}^{-1}$ carbenicillin (Carl Roth GmbH, Karlsruhe, Germany). For *E. coli* ST18, media were supplemented with $50 \mu\text{g ml}^{-1}$ 5-aminolevulinic acid (Sigma-Aldrich). Plasmid-harboring *P. aeruginosa* strains were maintained and selected on LB agar plates (1.5% [wt/vol]) with $200 \mu\text{g ml}^{-1}$ carbenicillin. Growth was monitored as the optical density at 600 nm (OD_{600}) with the UV-mini 1240 photometer (Shimadzu, Kyoto, Japan). Overnight precultures in 10-ml test tubes with 3 ml of LB were inoculated from LB agar plates and incubated at 30°C and 200 rpm in a rotary shaker (INFORS HT; Minitron, GmbH, Bottmingen, Switzerland) for 10 to 16 h. Cells for experiments were centrifuged for 1.5 min at $10,000 \times g$ from precultures and resuspended in M9 medium without a C source. For growth experiments with SDS or SLES, C-source volumes of 75 ml of M9 medium in 500 ml in Erlenmeyer flasks without baffles were used. Main cultures were inoculated with cells from precultures to an OD_{600} of 0.01 and cultivated at 30°C and 200 rpm. Immediately after inoculation and at several time points thereafter, samples from the cultures, avoiding the macroscopic cell aggregates as described previously (5), were taken to measure the OD_{600} and quantify the substrate concentrations. Samples for substrate quantification were centrifuged at $18,500 \times g$ for 10 min at room temperature. Supernatants were transferred to new plastic tubes and stored at -20°C .

Construction of unmarked *P. aeruginosa* deletion mutant and complementation/overexpression/reporter gene plasmids. The PAO1 Δ *sdsB1* deletion strain was generated with primers 1 to 4 (Table 2) as already described (14). Selected mutants were checked for correct deletion by colony PCR

TABLE 1 Strains and plasmids used in this study

Strain or plasmid	Relevant characteristics ^a	Source or reference
Strains		
<i>Pseudomonas aeruginosa</i>		
PAO1	PAO1 Nottingham wild-type strain	Holloway collection
PAO1 $\Delta sdsB1$	<i>sdsB1</i> deletion mutant	This study
PAO1 $\Delta sdsB1$ /pUCP18[<i>sdsB1</i>]	<i>sdsB1</i> deletion mutant complementation of <i>sdsB1</i>	This study
PAO1 $\Delta sdsB1$ /pUCP18	<i>sdsB1</i> deletion mutant pUCP18 (empty vector control)	This study
<i>Escherichia coli</i>		
DH5 α	<i>recA1 endA1 hsdR17 thi-1 supE44 gyrA96 relA1 deoR</i> $\Delta(lacZYA-argF)U196$ ($\phi 80lacZ\Delta M15$)	55
ST18	<i>pro thi hsdR⁺ Tp^r Sm^r</i> ; chromosome::RP4-2 Tc::Mu-Kan::Tn7/ λ pir $\Delta hema$	56
C41(DE3)	F ⁻ <i>ompT gal dcm hsdSB[r_B⁻ m_B⁻]</i>	57
C41(DE3)/pET23a[his- <i>sdsB1</i>]	Overexpression of his- <i>sdsB1</i>	This study
C41/pET23a[<i>laoR</i>]	Overexpression of <i>laoR</i>	This study
Plasmids		
pEX18Ap	Gene replacement vector; Ap ^r , <i>sacB</i>	58
pUCP18	<i>Escherichia-Pseudomonas</i> shuttle vector; Ap ^r	59
pBBR1MCS2Y[<i>lacZ</i>]	pBBR1MCS2 vector backbone modified as reporter gene vector with <i>lacZ</i> (without promoter); Ap ^r	M. Czieborowski et al., unpublished data
pET23a	Overexpression vector; Ap ^r , C-terminal His tag	Merck Millipore
pET23a[his- <i>sdsB1</i>]	Vector with his- <i>sdsB1</i> for purification	This study
pET23a[<i>laoR</i>]	Vector with <i>laoR</i> for purification	This study
pUCP18[<i>sdsB1</i>]	Vector for <i>sdsB1</i> complementation	This study
pBBR1MCS2[<i>P_{sdsA1}</i> - <i>lacZ</i>]	Gene fusion of intergenic <i>sdsB1/sdsA1</i> region with <i>lacZ</i>	This study
pBBR1MCS2[<i>P_{laoCBA}</i> - <i>lacZ</i>]	Gene fusion of intergenic <i>laoR/laoCBA</i> region with <i>lacZ</i>	This study

^aAp^r, ampicillin resistance; Sm^r, streptomycin resistance; Tp^r, trimethoprim resistance.

amplifying the region of interest with primers 5 and 6 (Table 2). For complementation, the gene of interest was amplified from the genomic DNA of strain PAO1 (primers 5 and 6, Table 2) and cloned into the vector pUCP18 (Table 1) into the XbaI and HindIII restriction sites. For overexpression of *sdsB1* and *laoR*, the genes were amplified with primers 7 to 10 (Table 2) from the genomic DNA of strain PAO1 and cloned into the NdeI and HindIII restriction sites into vector pET23a (Table 1). For reporter gene assay of the putative promoter regions of *sdsA1* and *laoCBA*, the respective intergenic regions were amplified with primers 11 to 14 (Table 2) from genomic DNA of strain PAO1 and cloned into the BamHI and XhoI restriction sites into vector pBBR1MCS2::*lacZ* (Table 1). Plasmids for complementation, overexpression, or reporter genes (Table 1) were transferred into the respective strains by transformation, and the strains were checked by colony PCR amplifying the multiple cloning site of the vector (primers 15 to 20, Table 2). All plasmids were verified by DNA sequencing prior to transformation (Microsynth Seqlab GmbH, Göttingen, Germany).

SDS and SLES quantification. Quantifications of the anionic detergents SDS and SLES were performed by a modified “stains-all” assay (14) as previously described. Briefly, stains-all solution was supplied to culture supernatants, and quantification of the absorption at an 438-nm wavelength was measured using a UV-VIS spectrophotometer (UV-2600; Shimadzu, Kyoto, Japan). Absorption spectra recorded with a double-beam spectrophotometer revealed that SLES and SDS reacted similarly with the stains-all solution (45), and calibration curves with SLES revealed linearity within the range of 0 to 20 μ M.

Purification of regulatory proteins. *E. coli* C41(DE3) was transformed with either pET23a[*laoR*] or pET23a[his-*sdsB1*]. For the expression of *laoR* and *sdsB1*, the cells were incubated at 30°C in liquid LB containing 100 μ g ml⁻¹ ampicillin. At an OD₆₀₀ of 0.4, the expression was induced by addition of 0.4 mM IPTG (isopropyl- β -D-thiogalactopyranoside), and the temperature was reduced to 20°C. After overnight incubation, the cells were harvested and disrupted using a French press (American Instrument Company, Silver Spring, MD). The soluble fraction was used for further purification steps. Affinity chromatography with a 1-ml HisTrap Fast Flow column (GE Healthcare, Chicago, IL) was performed to purify LaoR and SdsB1 containing C-terminal and N-terminal His₆ tags, respectively. The column was equilibrated with binding buffer (100 mM Tris, 500 mM NaCl, 40 mM imidazole [pH 7.4]), and the soluble fraction was loaded into the column and washed with binding buffer. Elution was performed with buffer containing 500 mM imidazole. Protein concentrations were quantified by a bicinchoninic acid assay (Pierce/Thermo Scientific, Rockford, IL) with bovine serum albumin as the standard. Purification results were verified by SDS-PAGE as described previously (14).

Electrophoretic mobility shift assay. Binding of SdsB1 and LaoR on the intergenic DNA sequences was analyzed using EMSAs. Putative binding regions were amplified by PCR with primers 21 and 22 and primers 35 to 40 (Table 2). DNA at 0.2 μ M was incubated with 0.5 μ M protein in a 10- μ l total volume in binding buffer (1 mM EDTA, 10 mM Tris/HCl [pH 7.0], 80 mM NaCl, 10 mM β -mercaptoethanol, and 5% [wt/vol] glycerol) (46). For fine localization of the binding sequences, oligonucleotides 23 to 34 and 41 to 48 (Table 2) were annealed within a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA) according to the following modified program: 2 min at 94°C, a rapid ramp down to 70°C at maximal cycler speed (3°C/s), followed by slow (0.1°C/min) cooling to 12°C (47). The obtained DNA fragments were tested at

TABLE 2 Oligonucleotides used in this study

No.	Oligonucleotide	Sequence ^a
1	sdsB1_Up_fw	tata <u>ctaga</u> CCGCTGTCGCCGCGATGAAG
2	sdsB1_Up_rev	GCGGACTCCGCATCGAATG
3	sdsB1_Dn_fw	CATTCGATGCGGAGTCCGCGGCTCAGCGCCAGCGAGCG
4	sdsB1_Dn_rev	tataaagcttGTCAACCTGGCCTACCCGC
5	sdsB1_fw	tata <u>ctaga</u> ATGAGCGATCTACGCCAGTTC
6	sdsB1_rev	tataaagcttTCAGGTGGCGGCGAAGC
7	his-sdsB1_fw	tata <u>catatg</u> caccaccaccaccacAGCGATCTACGCCAGTTC
8	his-sdsB1_rev	tataaagcttTCAGGTGGCGGCGAAG
9	laoR-his_fw	tata <u>catatg</u> GCACCACGCATCAAGACCC
10	laoR-his_rev	tataaagcttGAGCGGCACGTAGAGCCGC
11	P _{sdsA1} -fw	tatagga <u>tcc</u> GCGGACTCCGCATCGAATGTG
12	P _{sdsA1} -rev	tata <u>ctcga</u> CGGATACTCCGTTTTATTGTTGTGGG
13	P _{laoCBA} -fw	tatagga <u>tcc</u> GCGGACGACGGGCTCGTTCCG
14	P _{laoCBA} -rev	tata <u>ctcga</u> GGGCTGGCTCCTCGGCGAAG
15	pUCP18_fw	GAGCGATAACAATTTCACACAGG
16	pUCP18_rev	AGGGTTTTCCAGTCACGACGTT
17	pET23a_fw	GGAGACCACAACGGTTTCCC
18	pET23a_rev	TTGTTAGCAGCCGGATCTCA
19	pBBR1_fw	TCACACTGCTTCGGTAGTC
20	lacZ_rev	TCCGTGGGAACAACGGCGG
21	SdsB1_A_fw	GCGGACTCCGCATCGAATG
22	SdsB1_A_rev	CGGATACTCCGTTTTATTG
23	SdsB1_B_fw	GCGGACTCCGCATCGAATGTGGACCTGGAATGCATTGGAA
24	SdsB1_B_rev	TTCCAATGCATTCCAGGTCCACATTCGATGCGGAGTCCGC
25	SdsB1_C_fw	TTATTGCCGTGCCGCTCGGCATAGTGCCAGCCGAAGCCCA
26	SdsB1_C_rev	TGGGCTTCGGCTGGCACTATGCCGAGCGGCACGGCAATAA
27	SdsB1_D_fw	CAACAATAAAACGGAGTATCCG
28	SdsB1_D_rev	CGGATACTCCGTTTTATTGTTG
29	SdsB1_B*fw	GCGGACTCC ATGCC GAATGTGGACCTGGAATGCATTGGAA
30	SdsB1_B*_rev	TTCCAATGCATTCCAGGTCCACATTC GGCAT GGAGTCCGC
31	SdsB1_B**fw	GCGGACTCCGCATCG GGCAT GGACCTGGAATGCATTGGAA
32	SdsB1_B**rev	TTCCAATGCATTCCAGGTCCAT TGCC CGATGCGGAGTCCGC
33	SdsB1_B***fw	GCGGACTCC ATGCCGGGCAT GGACCTGGAATGCATTGGAA
34	SdsB1_B***rev	TTCCAATGCATTCCAGGTCCAT TGCCGGCAT GGAGTCCGC
35	LaoR_A_fw	tataGGGCTGGCTCCTCGGCGAAG
36	LaoR_A_rev	tataGGGCTCGTTCCGGTAACAGT
37	LaoR_B_fw	tataGGGCTGGCTCCTCGGCGAAG
38	LaoR_B_rev	tataAATCCAGCGACGACGCGAAA
39	LaoR_C_fw	tataTCTAGAGTAAATACTCTAAAG
40	LaoR_C_rev	tataGACCGGCCCGCCTTTCGTTG
41	LaoR_C1_fw	TTTCGCTGCGTCGCTGGATTCTAGAGTAAATACTCTAAAGTGTCAAACA
42	LaoR_C1_rev	TGTTTGACACTTTAGAGTATTTACTCTAGAAATCCAGCGACGACGCGAAA
43	LaoR_C1*fw	TTTCGCTGCGTCGCTGGATTCTAGAGTAAAC GTCTCGA AGTGTCAAACA
44	LaoR_C1*_rev	TGTTTGACACTT CGAGACG TTTACTCTAGAAATCCAGCGACGACGCGAAA
45	LaoR_C1**fw	TTTCGCTGCGTCGCTGGATTCT CGAGACGAACTCTCGA AGTGTCAAACA
46	LaoR_C1**rev	TGTTTGACACTT CGAGACGTTCTCTCGA ATCCAGCGACGACGCGAAA
47	LaoR_C2_fw	GCATGCCTGCCAGCCCGGCGCTTGCGTACCCAACGAAAGCGGGCGGTC
48	LaoR_C2_rev	GACCGGCCCGCCTTTCGTTGGGTACGCAAGCGCGGGCTGGCAGGCATGC
49	LaoR_C_fw_JOE	JOE-TTTCGCTGCGTCGCTGGATTCTAGAGTAAATACTCTAAAGTGTCAAACA
50	Lao_operon_fw	GGTCGTCGTCACGGCGCAG
51	Lao_operon_rev	GGCCAAGGGCGTGTTCAGCAAG
52	qRT_rpoS_fw	CTCCCCGGGCAACTCCAAAAG
53	qRT_rpoS_rev	CGATCATCCGCTTCCGACCCAG
54	qRT_sdsB1_fw	CACGCGTGCGCGTCTGACTG
55	qRT_sdsB1_rev	GGATGTCCGGGGCGGCAGAA
56	qRT_sdsA1_fw	CAATCCCAGCCTGCAACGCCA
57	qRT_sdsA1_rev	GTACGGATCGGCCTCTCGCCC
58	qRT_laoR_fw	CCTGCTGGAAGCGACCCGGA
59	qRT_laoR_rev	GGAAGCGCACCCAGGAGGTGA
60	qRT_laoA_fw	GCGGCACCAACCGGAAATC
61	qRT_laoA_rev	GGTGATGGCGCCGTCCTTG

^aRestriction sites or nucleotides complementary to a vector are underlined. Lowercase letters indicate bases that are not complementary to the respective genome or vector sequence. Nucleotide exchanges are indicated in boldface.

concentrations of 0.5 and 0.1 μM with 2.5 μM SdsB1 and 0.4 μM LaoR, respectively. For ligand analysis, 5' JOE (6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluoresceine)-labeled oligonucleotides (Eurofins Genomics Germany GmbH, Ebersberg, Germany) (oligonucleotides 48 and 42 in Table 2) were used to achieve a more sensitive detection, and samples were prepared as described previously with the addition of the putative ligand. In general, after 30 min of incubation at room temperature, the samples were mixed with 2 μl of loading dye (25% [wt/vol] sucrose and 0.05% [wt/vol] bromophenol blue in 20 mM Tris/HCl [pH 8.0]) and separated in a native 8% (wt/vol) polyacrylamide gel containing TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM $\text{Na}_2\text{-EDTA}$) (46). Gels were run at 10 V cm^{-1} on ice in TBE buffer, and DNA was detected either with ethidium bromide or, in the case of labeled DNA visualization, with "Alexa₄₈₈" since an excitation/emission method at a ChemiDoc MP imaging system (Bio-Rad) was used.

Thermal shift assay. To investigate the thermal stability of the regulator SdsB1 or LaoR alone and in the presence of DNA fragments containing the putative binding site or not, thermal shift assays using a Sypro Orange dye (50 $\times$; Sigma-Aldrich, St. Louis, MO) were performed (48, 49). Briefly, 20- μl reaction mixtures containing 2 μM regulator protein, 1 $\times$ Sypro Orange dye, 1 μM DNA fragment, and 1 $\times$ binding buffer from EMSAs (as described above) were heated from 4 to 99°C in 0.5°C steps using a CFX96 Touch real-time PCR detection system (Bio-Rad). The fluorescence intensity was measured at excitation/emission wavelengths of 490/575 nm. The midpoint temperature of transition between folded and unfolded states, also known as the melting temperature (T_m), was calculated by using CFX Manager software (Bio-Rad).

β -Galactosidase assay. To analyze β -galactosidase expression from the putative *sdsA1* and the *lao* gene promoter, precultures of strain PAO1 harboring the respective reporter gene *lacZ* fusion plasmids (Table 1) were incubated in 3 ml of M9 medium with 10 mM succinate containing 200 $\mu\text{g ml}^{-1}$ carbenicillin for 6 to 7 h at 30°C and 200 rpm. Main cultures containing 50 ml of fresh M9 medium with 10 mM succinate and 200 $\mu\text{g ml}^{-1}$ carbenicillin were inoculated to an OD₆₀₀ of 0.001, followed by incubation for 10 h under the same conditions. The cells were harvested in their exponential phase and washed with M9 medium without a carbon source, and cell suspensions at an OD₆₀₀ of 0.3 to 0.4 were prepared with M9 medium without a carbon source. A 1-ml portion of cell suspension in a 1.5-ml reaction vessel was then supplied with 1 mM putative inducer substrate and incubated for 3 h at 30°C and 200 rpm. The OD₆₀₀ values of the culture samples were measured, and the cells were then treated as previously described (50). Samples with a volume of 200 μl were resuspended in 800 μl of buffer Z (50). After the addition of 50 μl of SDS (0.1% [wt/vol]) and 25 μl of chloroform, the samples were vortexed and incubated on ice for 20 min. After incubation at 30°C for 5 min, 200 μl of *o*-nitrophenyl- β -D-galactopyranoside (ONPG; 4 mg ml^{-1} , freshly prepared) was added. After 5 to 30 min of incubation, the reaction was stopped by the addition of 500 μl of 1 M Na_2CO_3 . The samples were then centrifuged at maximum speed for 10 min before the absorption at 405 nm was measured. The β -galactosidase activities were calculated as described previously (50).

Reverse transcriptase PCR. For reverse transcriptase PCR assays, RNA was extracted from 3 ml of exponentially growing *P. aeruginosa* strain PAO1 cultures with 3.5 mM 1-dodecanol or 10 mM succinate as the substrate. Cells were mixed with 1 ml of RNA_{later} stabilization solution (Thermo Scientific, Waltham, MA), and RNA was extracted using a PureLink RNA minikit (Thermo Scientific) according to the manufacturer's protocol. RNA was then treated with a Turbo DNA-Free kit (Invitrogen, Waltham, MA), and a PCR analysis with the primer pair 44/45 (Table 2) was performed with wild-type DNA as a positive control to check for contaminating chromosomal DNA. The RNA amounts were then adjusted and utilized for cDNA synthesis using a RevertAid H Minus first-strand cDNA synthesis kit (Thermo Scientific) with random hexamer primers according to the manufacturer's instructions. Reverse transcriptase PCR was then performed with 5 μl of cDNA and Q5 polymerase and the primer pair 50/51 (Table 2) for a transcript spanning *laoABC* (see Fig. 5A). As a positive control, 20 ng of chromosomal DNA was utilized. After 30 s of denaturation at 98°C, the following reaction regimen was used for 30 cycles: 98°C for 10 s, 72°C for 15 s, and 72°C for 50 s. The PCR products were detected on a 1% (wt/vol) agarose gel and stained with ethidium bromide. Experiments were performed in biological triplicates.

Quantitative real-time PCR. For quantitative real-time PCR (qRT-PCR) analysis, 3-ml portions of exponentially growing *P. aeruginosa* strain PAO1 cultures with 10 mM succinate or 3.5 mM 1-dodecanol as the substrate were extracted. As cells grown with 3.5 mM SDS or SLES formed macroscopic cell aggregates (6), 4-ml portions of nonaggregating, freely suspended cells from exponentially growing strain PAO1 cultures were extracted when these sulfate ester detergents were used as the substrates. RNA extraction was performed as described for reverse transcriptase PCR. RNA (500 ng) was treated with DNase I (using a Turbo DNA-Free kit) according to the manufacturer's instructions, and DNA contamination was checked by quantitative real-time PCR using 40 ng of RNA and the reference gene primer pair, as described above. Synthesis of cDNA was performed as described for reverse transcriptase PCR. Gene-specific primers were designed using Primer-BLAST software (NCBI Primer-BLAST) (51) to avoid nonspecific amplification of *P. aeruginosa* DNA. The well-established reference gene primer pair for *rpoS* was used (52, 53) (Table 2). For every sample, an adjusted amount of cDNA (50 ng) was amplified with gene-specific primers 54 to 61 (Table 2) and the iTaq Universal SYBR green Supermix (Bio-Rad) within the CFX96 Touch real-time PCR detection system (Bio-Rad). After 3 min of denaturation at 95°C, the following reaction cycle was used for 30 cycles: 95°C for 10 s, 60°C for 5 s, and 72°C for 10 s with data acquisition. Melting-curve analysis was performed in the range from 55 to 95°C at 0.5°C intervals to confirm specific amplification of single PCR products. Cycle threshold (C_T) values were generated by using CFX Manager software (Bio-Rad) and used to quantify the relative levels of gene expression by the $2^{-\Delta\Delta C_T}$ method with

the calculated reaction efficiencies of the respective primer pair and the *rpoS* gene as an internal control (54).

ACKNOWLEDGMENTS

We thank Karin Niermann for experimental support and Michael Czieborowski for providing the reporter gene vector as well as for support in the preparation of some of the figures.

This study was funded by grants from the German Federal Ministry for Economic Affairs and Energy (BMWi) through its Central Innovation Program for SMEs (ZIM) and by the German Research Foundation (DFG) to B.P. (grants 16KNO13527 PakuNaS and PH71/3-2, respectively).

REFERENCES

- Nikaido H, Pagès J-M. 2012. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. *FEMS Microbiol Rev* 36:340–363. <https://doi.org/10.1111/j.1574-6976.2011.00290.x>.
- Rajagopal S, Sudarsan N, Nickerson KW. 2002. Sodium dodecyl sulfate hypersensitivity of *clpP* and *clpB* mutants of *Escherichia coli*. *Appl Environ Microbiol* 68:4117–4121. <https://doi.org/10.1128/aem.68.8.4117-4121.2002>.
- Li X-Z, Barré N, Poole K. 2000. Influence of the MexA-MexB-OprM multidrug efflux system on expression of the MexC-MexD-OprJ and MexE-MexF-OprN multidrug efflux systems in *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 46:885–893. <https://doi.org/10.1093/jac/46.6.885>.
- Klebensberger J, Birkenmaier A, Geffers R, Kjelleberg S, Philipp B. 2009. SiaA and SiaD are essential for inducing autoaggregation as a specific response to detergent stress in *Pseudomonas aeruginosa*. *Environ Microbiol* 11:3073–3086. <https://doi.org/10.1111/j.1462-2920.2009.02012.x>.
- Klebensberger J, Rui O, Fritz E, Schink B, Philipp B. 2006. Cell aggregation of *Pseudomonas aeruginosa* strain PAO1 as an energy-dependent stress response during growth with sodium dodecyl sulfate. *Arch Microbiol* 185:417–427. <https://doi.org/10.1007/s00203-006-0111-y>.
- Klebensberger J, Lautenschlager K, Bressler D, Wingender J, Philipp B. 2007. Detergent-induced cell aggregation in subpopulations of *Pseudomonas aeruginosa* as a preadaptive survival strategy. *Environ Microbiol* 9:2247–2259. <https://doi.org/10.1111/j.1462-2920.2007.01339.x>.
- Colley B, Dederer V, Carnell M, Kjelleberg S, Rice SA, Klebensberger J. 2016. SiaA/D interconnects c-di-GMP and RsmA signaling to coordinate cellular aggregation of *Pseudomonas aeruginosa* in response to environmental conditions. *Front Microbiol* 7:179. <https://doi.org/10.3389/fmicb.2016.00179>.
- Payne WJ, Feisal VE. 1963. Bacterial utilization of dodecyl sulfate and dodecyl benzene sulfonate. *Appl Microbiol* 11:339–344.
- Hsu Y-C. 1965. Detergent-splitting enzyme from *Pseudomonas*. *Nature* 207:385–388. <https://doi.org/10.1038/207385a0>.
- Marchesi JR, Owen SA, White GF, House WA, Russell NJ. 1994. SDS-degrading bacteria attach to riverine sediment in response to the surfactant or its primary biodegradation product dodecan-1-ol. *Microbiology* 140:2999–3006. <https://doi.org/10.1099/13500872-140-11-2999>.
- Thomas OR, White GF. 1989. Metabolic pathway for the biodegradation of sodium dodecyl sulfate by *Pseudomonas* sp. C12B. *Biotechnol Appl Biochem* 11:318–327.
- Furmanczyk EM, Kaminski MA, Spolnik G, Sojka M, Danikiewicz W, Dziembowski A, Lipinski L, Sobczak A. 2017. Isolation and characterization of *Pseudomonas* spp. strains that efficiently decompose sodium dodecyl sulfate. *Front Microbiol* 8:1872. <https://doi.org/10.3389/fmicb.2017.01872>.
- Hagelueken G, Adams TM, Wiehlmann L, Widow U, Kolmar H, Tümmler B, Heinz DW, Schubert W-D. 2006. The crystal structure of SdsA1, an alkylsulfatase from *Pseudomonas aeruginosa*, defines a third class of sulfatases. *Proc Natl Acad Sci U S A* 103:7631–7636. <https://doi.org/10.1073/pnas.0510501103>.
- Panasia G, Philipp B. 2018. LaoABCR, a novel system for oxidation of long-chain alcohols derived from SDS and alkane degradation in *Pseudomonas aeruginosa*. *Appl Environ Microbiol* 84:e00626-18. <https://doi.org/10.1128/AEM.00626-18>.
- Davison J, Brunel F, Phanopoulos A, Prozzi D, Terpstra P. 1992. Cloning and sequencing of *Pseudomonas* genes determining sodium dodecyl sulfate biodegradation. *Gene* 114:19–24. [https://doi.org/10.1016/0378-1119\(92\)90702-Q](https://doi.org/10.1016/0378-1119(92)90702-Q).
- Jovcic B, Venturi V, Davison J, Topisirovic L, Kojic M. 2010. Regulation of the *sdsA* alkyl sulfatase of *Pseudomonas* sp. ATCC19151 and its involvement in degradation of anionic surfactants. *J Appl Microbiol* 109:1076–1083. <https://doi.org/10.1111/j.1365-2672.2010.04738.x>.
- Schell MA. 1993. Molecular biology of the LysR family of transcriptional regulators. *Annu Rev Microbiol* 47:597–626. <https://doi.org/10.1146/annurev.mi.47.100193.003121>.
- Maddocks SE, Oyston P. 2008. Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. *Microbiology (SGM)* 154:3609–3623. <https://doi.org/10.1099/mic.0.2008/022772-0>.
- Cuthbertson L, Nodwell JR. 2013. The TetR family of regulators. *Microbiol Mol Biol Rev* 77:440–475. <https://doi.org/10.1128/MMBR.00018-13>.
- Helenius A, Simons K. 1975. Solubilization of membranes by detergents. *Biochim Biophys Acta* 415:29–79. [https://doi.org/10.1016/0304-4157\(75\)90016-7](https://doi.org/10.1016/0304-4157(75)90016-7).
- Fantl WJ, Stevens VJ, Peterson CM. 1982. Reactions of biologic aldehydes with proteins. *Diabetes* 31:15–21. <https://doi.org/10.2337/diab.31.3.515>.
- Parsek MR, Ye RW, Pun P, Chakrabarty AM. 1994. Critical nucleotides in the interaction of a LysR-type regulator with its target promoter region: *catBC* promoter activation by CatR. *J Biol Chem* 269:11279–11284.
- Finn RD, Attwood TK, Babbitt PC, Bateman A, Bork P, Bridge AJ, Chang HY, Dosztanyi Z, El-Gebali S, Fraser M, Gough J, Haft D, Holliday GL, Huang H, Huang X, Letunic I, Lopez R, Lu S, Marchler-Bauer A, Mi H, Mistry J, Natale DA, Necci M, Nuka G, Orengo CA, Park Y, Pesseat S, Piovesan D, Potter SC, Rawlings ND, Redaschi N, Richardson L, Rivoire C, Sangrador-Vegas A, Sigrist C, Sillitoe I, Smithers B, Squizzato S, Sutton G, Thanki N, Thomas PD, Tosatto SCE, Wu CH, Xenarios I, Yeh LS, Young SY, Mitchell AL. 2017. InterPro in 2017-beyond protein family and domain annotations. *Nucleic Acids Res* 45:D190–D199. <https://doi.org/10.1093/nar/gkw1107>.
- Hryniewicz MM, Kredich NM. 1994. Stoichiometry of binding of CysB to the *cysJH*, *cysK*, and *cysP* promoter regions of *Salmonella* Typhimurium. *J Bacteriol* 176:3673–3682. <https://doi.org/10.1128/jb.176.12.3673-3682.1994>.
- Hryniewicz MM, Kredich NM. 1995. Hydroxyl radical footprints and half-site arrangements of binding sites for the CysB transcriptional activator of *Salmonella* Typhimurium. *J Bacteriol* 177:2343–2353. <https://doi.org/10.1128/jb.177.9.2343-2353.1995>.
- Imperi F, Tiburzi F, Fimia GM, Visca P. 2010. Transcriptional control of the *pvdS* iron starvation sigma factor gene by the master regulator of sulfur metabolism CysB in *Pseudomonas aeruginosa*. *Environ Microbiol* 12:1630–1642. <https://doi.org/10.1111/j.1462-2920.2010.02210.x>.
- Song Y, Yang C, Chen G, Zhang Y, Seng Z, Cai Z, Zhang C, Yang L, Gan J, Liang H. 2019. Molecular insights into the master regulator CysB-mediated bacterial virulence in *Pseudomonas aeruginosa*. *Mol Microbiol* 111:1195–1210. <https://doi.org/10.1111/mmi.14200>.
- Kredich NM. 1992. The molecular basis for positive regulation of *cys* promoters in *Salmonella* Typhimurium and *Escherichia coli*. *Mol Microbiol* 6:2747–2753. <https://doi.org/10.1111/j.1365-2958.1992.tb01453.x>.
- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402. <https://doi.org/10.1093/nar/25.17.3389>.
- Tyrrell R, Verschueren KHG, Dodson EJ, Murshudov GN, Addy C, Wilkinson AJ. 1997. The structure of the cofactor-binding fragment of the LysR family member, CysB: a familiar fold with a surprising subunit arrange-

- ment. *Structure* 5:1017–1032. [https://doi.org/10.1016/S0969-2126\(97\)00254-2](https://doi.org/10.1016/S0969-2126(97)00254-2).
31. Kredich NM. 24 January 2008, posting date. Biosynthesis of cysteine. EcoSal Plus 2013 <https://doi.org/10.1128/ecosalplus.3.6.1.11>.
 32. Li D-F, Zhang N, Hou Y-J, Huang Y, Hu Y, Zhang Y, Liu S-J, Wang D-C. 2011. Crystal structures of the transcriptional repressor RolR reveals a novel recognition mechanism between inducer and regulator. *PLoS One* 6:e19529. <https://doi.org/10.1371/journal.pone.0019529>.
 33. Miller DJ, Zhang Y-M, Subramanian C, Rock CO, White SW. 2010. Structural basis for the transcriptional regulation of membrane lipid homeostasis. *Nat Struct Mol Biol* 17:971–975. <https://doi.org/10.1038/nsmb.1847>.
 34. Liang J-L, Nie Y, Wang M, Xiong G, Wang Y-P, Maser E, Wu X-L. 2016. Regulation of alkane degradation pathway by a TetR family repressor via an autoregulation positive feedback mechanism in a Gram-positive *Dietzia* bacterium. *Mol Microbiol* 99:338–359. <https://doi.org/10.1111/mmi.13232>.
 35. Tavass EA, Eigen E, Kligman AM. 1988. Anionic skin irritation and anionic pH rise of bovine serum albumin. *J Soc Cosmet Chem* 39:267–272.
 36. Löffler H, Happle R. 2003. Profile of irritant patch testing with detergents: sodium lauryl sulfate, sodium laureth sulfate and alkyl polyglucoside. *Contact Dermat* 48:26–32. <https://doi.org/10.1034/j.1600-0536.2003.480105.x>.
 37. Johnson DA, Tetu SG, Phillippy K, Chen J, Ren Q, Paulsen IT. 2008. High-throughput phenotypic characterization of *Pseudomonas aeruginosa* membrane transport genes. *PLoS Genet* 4:e1000211. <https://doi.org/10.1371/journal.pgen.1000211>.
 38. Hales SG, Dodgson KS, White GF, Jones N, Watson GK. 1982. Initial stages in the biodegradation of the surfactant sodium dodecyltriethoxy sulfate by *Pseudomonas* sp. strain DES1. *Appl Environ Microbiol* 44:790–800.
 39. Hales SG, Watson GK, Dodgson KS, White GF. 1986. A comparative study of the biodegradation of the surfactant sodium dodecyltriethoxy sulphate by four detergent-degrading bacteria. *Microbiology* 132:953–961. <https://doi.org/10.1099/00221287-132-4-953>.
 40. Tidswell EC, Russell NJ, White GF. 1996. Ether-bond scission in the biodegradation of alcohol ethoxylate nonionic surfactants by *Pseudomonas* sp. strain SC25A. *Microbiology* 142:1123–1131. <https://doi.org/10.1099/13500872-142-5-1123>.
 41. Paulo AMS, Aydin R, Dimitrov MR, Vreeling H, Cavaleiro AJ, García-Encina PA, Stams AJM, Plugge CM. 2017. Sodium lauryl ether sulfate (SLES) degradation by nitrate-reducing bacteria. *Appl Microbiol Biotechnol* 101:5163–5173. <https://doi.org/10.1007/s00253-017-8212-x>.
 42. Griffiths ET, Hales SG, Russell NJ, Watson GK, White GF. 1986. Metabolite production during the biodegradation of the surfactant sodium dodecyltriethoxy sulphate under mixed-culture die-away conditions. *J Gen Microbiol* 132:963–972. <https://doi.org/10.1099/00221287-132-4-963>.
 43. White GF, Russell NJ, Tidswell EC. 1996. Bacterial scission of ether bonds. *Microbiol Rev* 60:216–232.
 44. Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
 45. Rusconi F, Valton É, Nguyen R, Dufourc E. 2001. Quantification of sodium dodecyl sulfate in microliter-volume biochemical samples by visible light spectroscopy. *Anal Biochem* 295:31–37. <https://doi.org/10.1006/abio.2001.5164>.
 46. Oettermann S, Jongsma R, Coenen A, Keller J, Steinbüchel A. 2019. LcpRVH2: regulating the expression of latex-clearing proteins in *Gordonia polyisoprenivorans* VH2. *Microbiology* 165:343–354. <https://doi.org/10.1099/mic.0.000755>.
 47. Jullien N, Herman J-P. 2011. LUEGO: a cost and time saving gel shift procedure. *Biotechniques* 51:267–269. <https://doi.org/10.2144/000113751>.
 48. Pantoliano MW, Petrella EC, Kwasnoski JD, Lobanov VS, Myslik J, Graf E, Carver T, Asel E, Springer BA, Lane P, Salemme FR. 2001. High-density miniaturized thermal shift assays as a general strategy for drug discovery. *J Biomol Screen* 6:429–440. <https://doi.org/10.1177/108705710100600609>.
 49. Lo M-C, Aulabaugh A, Jin G, Cowling R, Bard J, Malamas M, Ellestad G. 2004. Evaluation of fluorescence-based thermal shift assays for hit identification in drug discovery. *Anal Biochem* 332:153–159. <https://doi.org/10.1016/j.ab.2004.04.031>.
 50. Miller J. 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
 51. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 13:134. <https://doi.org/10.1186/1471-2105-13-134>.
 52. Savli H, Karadenizli A, Kolayli F, Gundes S, Ozbek U, Vahaboglu H. 2003. Expression stability of six housekeeping genes: a proposal for resistance gene quantification studies of *Pseudomonas aeruginosa* by real-time quantitative RT-PCR. *J Med Microbiol* 52:403–408. <https://doi.org/10.1099/jmm.0.05132-0>.
 53. Alqarni B, Colley B, Klebensberger J, McDougald D, Rice SA. 2016. Expression stability of 13 housekeeping genes during carbon starvation of *Pseudomonas aeruginosa*. *J Microbiol Methods* 127:182–187. <https://doi.org/10.1016/j.mimet.2016.06.008>.
 54. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>.
 55. Sambrook J, Russell DW. 2001. *Molecular cloning: a laboratory manual*, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
 56. Thoma S, Schobert M. 2009. An improved *Escherichia coli* donor strain for diparental mating. *FEMS Microbiol Lett* 294:127–132. <https://doi.org/10.1111/j.1574-6968.2009.01556.x>.
 57. Miroux B, Walker JE. 1996. Over-production of proteins in *Escherichia coli*: mutant hosts that allow synthesis of some membrane proteins and globular proteins at high levels. *J Mol Biol* 260:289–298. <https://doi.org/10.1006/jmbi.1996.0399>.
 58. Hoang TT, Karkhoff-Schweizer RR, Kutchma AJ, Schweizer HP. 1998. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked *Pseudomonas aeruginosa* mutants. *Gene* 212:77–86. [https://doi.org/10.1016/S0378-1119\(98\)00130-9](https://doi.org/10.1016/S0378-1119(98)00130-9).
 59. West SE, Schweizer HP, Dall C, Sample AK, Runyen-Janecky LJ. 1994. Construction of improved *Escherichia-Pseudomonas* shuttle vectors derived from pUC18/19 and sequence of the region required for their replication in *Pseudomonas aeruginosa*. *Gene* 148:81–86. [https://doi.org/10.1016/0378-1119\(94\)90237-2](https://doi.org/10.1016/0378-1119(94)90237-2).