Abstract:

The widespread use of sodium dodecyl sulfate (SDS) in a variety of products during recent decades has caused an increase in contamination of aquatic ecosystems. As the concentration of SDS builds to higher and higher levels in the ponds, lakes, estuaries and other such ecosystems, it has begun to have an observable effect on the flora and fauna within. This has driven increased research into the field of bioremediation, not only in the removal of SDS from contaminated waters, but now also in the treatment of sewage and runoff before it has the opportunity to accrue in such locations. Enriching for bacteria that naturally possess the ability to degrade SDS has uncovered *Pseudomonas*, a genus known for its catabolic diversity, as a likely target for exploitation in these efforts. The SdsA1 alkyl sulfatase of *Pseudomonas* was discovered by a group looking for a bioremediation solution for SDS. Divergently transcribed from the *sdsA1* gene is the predicted LysR family transcriptional regulator, *PA0739*. Results from β-Galactosidase induction assays indicate that PA0739 may act as a transcriptional repressor of the *sdsA1* gene. Further experimentation has revealed that survivability in SDS, as a component of Luria Broth, is unaffected in the *sdsA1* transposon mutant, which is able to grow unhindered through 10% SDS, indicating that other mechanisms for tolerating SDS are at play and that further research should be pursued into *Pseudomonas'* ability to tolerate and degrade SDS.

Introduction:

The use of synthetically derived detergents has become commonplace over

the course of the last half-century. Anionic detergents, sodium dodecyl sulfate (SDS) in particular, are used heavily in both industry and the household. SDS is a key component in engine degreaser, floor cleaner and car wash soap as well as shampoo, toothpaste and shaving cream (Rebello et al, 2012). It is no great surprise that it has become a pollutant in many terrestrial and aquatic ecosystems. Toxic effects on aquatic fauna and flora have been observed with minute concentrations of SDS (Rebello et al, 2012). Likewise SDS has adverse effects on the growth and motility of algae and inflammatory effects on the mucous layer of fish, predisposing the fish to infection by microorganisms (Rebello et al, 2012). Of increasing importance is the ability to remove SDS from these systems. The biodegradation of detergents in wastewater and in wastewater treatment in particular is well known, the use of microorganisms in bioremediation is thus not a novel idea (Davison et al, 1992, Jovcic et al, 2010, Rebello et al, 2012). Rapid removal of pollutants is important to avoid long-term consequences and make safer use of agents such as SDS (Jovcic et al, 2010).

Pseudomonas is a genus of Gram-negative bacteria widely noted for its catabolic diversity, which has been discovered in ponds and other aquatic ecosystems with heavy detergent contamination (Jovcic et al, 2010, Römling et al, 1994). Growth in surfactant by *Pseudomonas aeruginosa* is well characterized; *Pseudomonas'* ability to thrive in lung surfactant contributes to its role as an opportunistic pathogen in the lungs of cystic fibrosis patients (Römling et al, 1994). Its ability to persist in an environment where other genera of bacteria cannot has made it a candidate for use in bioremediation. Interestingly, *Pseudomonas* has been shown not only to be able to tolerate the presence of SDS

but also capable of utilizing the SDS as a sole carbon source (Davison et al, 1992). Better understanding of the regulation of the expression of the genes that contribute to this ability of *Pseudomonas* could lead to more efficient exploitation of the organism in bioremediation.

SdsA1 is the name given to the gene that encodes a secreted alkyl-sulfatase, which is at least partially responsible for the degradation of SDS as observed in *Pseudomonas aeruginosa* (Hagelueken et al, 2006*)* although SdsA1 can be expressed with up to five other alkyl- or arylsulftases in *Pseudomonas aeruginosa*, SdsA1 was shown to be essential for the growth of *Pseudomonas aeruginosa* on minimal media with SDS as a sole carbon or sulfur source (Hagelueken et al, 2006). A divergently transcribed gene, *PA0739*, codes for a predicted LysR type transcriptional regulator (LTTR) (Winsor et al, 2011).

Materials and Methods:

Cloning Experiments:

NEB-5-α *Escherchia coli* cells were used in the cloning of the suicide plasmid, pTJ01, for use in generation of the Δ *PA0739* PA14 strains. λ-pir *Escherchia coli* cells were electrotransformed with pTJ01 in order to be used in an experiment to conjugate the pTJ01 plasmid into the PA14 strains; *Pseudomonas aeruginosa* strains, PA14 wild-type and PA14Δ*gbdR,* were used to generate *PA0739* knockout strains. Strains were maintained on LB medium with 10μg/mL gentamicin. An auxotrophic strain of *Saccharomyces*, lacking the ability to synthesize uracil, was used to generate the *sdsA1-lacZYA* reporter plasmid. URA dropout plates were used to select for cells carrying the desired

plasmid.

β-Galactosidase Assays:

The PA14 strains (wt, Δ*gbdR*, Δ*PA0739*, Δ*gbdR* Δ*PA0739*) containing the *sdsA1-lacZ* reporter plasmid were grown in overnight cultures in MOPS minimal media supplemented with pyruvate and glucose. The cells were then treated with the selected agents for 6 hours (again in MOPS) before the β-Gal assay was conducted. Induced cells were suspended in Z-buffer, and then permeabilized by treatment with SDS and chloroform. Assays were conducted in Z-buffer containing 50 mmol β-mercaptoethanol (Miller 1972). Activities (changes in optical density at 420 nm per min) were normalized to the actual cell density (OD600nm).

Minimum Inhibitory Concentration:

Attempts to establish an MIC for the PA14 strains used were conducted in Luria Broth with SDS. LB/20% SDS (weight/volume) was prepared and diluted with LB to the concentrations used in the assay such that changes in growth could not be attributed to drops in the essential nutrient concentration of the media by dilution with SDS solution. The MIC assay was performed in both a 96 well dish on a rocker and glass test tubes in a rotary wheel.

Results:

The expression of the *sdsA1* gene was observed to be upregulated in a Δ*gbdR* strain of *Pseudomonas aeruginosa* grown in surfactant in previous microarray data produced by the Wargo lab. In order to verify the accuracy of this effect, a Miller Assay was performed using PA14 strains (wt, Δ*gbdR*,

Δ*PA0739*, and Δ*gbdR* Δ*PA0739*) containing the pTJ02 *sdsA1-LacZYA* reporter plasmid induced in surfactant. As seen in figure 1, there is no significant increase in the expression of the *sdsA1* gene in the Δ*gbdR* strains vs. wildtype, observed under these conditions. The experiment was performed in triplicate and the results presented as the average value, the error bars represent one standard deviation.

Next, we decided to investigate the response of *sdsA1* to other membrane disrupting compounds. Again, the Miller Assay was employed to determine the relative transcription of the *sdsA1* gene. The same conditions used in the first assay were used, however with only PA14 wt and Δ*PA0739* strains. Polymyxin B, surfactant, and sphingosine were used, as well as SDS, as inducing agents. The data from the SDS induction are omitted as they do not provide useful information (the concentration of the SDS used for induction was too high for the β-Galactosidase enzyme to be active). In figure 2 we observe that the effect that surfactant had on the induction of *sdsA1* is maintained in both the wildtype (Fig. 2A) and Δ*PA0739* (Fig. 2B) strains. The other inducers did not produce a significant effect upon the transcriptional activity of the *sdsA1* gene. The induction experiment was performed in triplicate and the results presented as an average value, the error bars represent one standard deviation.

We next attempted to establish a phenotype for growth in SDS. In hopes of accomplishing this, a MIC was sought among the PA14 strains (wt, Δ*PA0739*, and *sdsA1*::*TnM*) for growth in SDS. As presented in Figure 3, the strains tolerated the presence of 10 % SDS in LB media with no inhibition. The seemingly random pigmentation of wells on the plate was not understood, but stronger

growth in outside wells indicated a possible oxygen dependence for SDS degradation/survivability. As such, the strains were grown in 3 mL (LB + SDS) overnights on a roller drum, and no difference in growth between PA14 wt, Δ*PA0739*, and *sdsA1*::*TnM* was observed through 10% SDS.

Discussion:

The reason for our looking into the regulation of *sdsA1* was simple; the ~12 fold upregulation that was indicated in the group's previous research with a Δ*gbdR* strain of *Pseudomonas aeruginosa* made it as likely a target as any for research. However, this upregulation was not supported in the experimentation done to verify the microarray data. (Figure 1) As such, the use of the Δ*gbdR* strains of PA14 was discontinued in further experimentation.

Originally, we hypothesized that the wildtype and knockout strains would be able to be easily and rapidly differentiated based on their 'SDS growth' phenotype. Had the original hypothesis that the *PA0739* gene product (a predicted LTTR) functioned as a transcriptional activator in response to stimulation from SDS been correct, we would have expected growth in WT strains in higher concentrations of SDS than the Δ*PA0739*. This, however, is not the case. As evidenced in Figure 3, the attempts to establish a phenotypic difference based on ability to grow in the presence of SDS did not succeed. Not only is their no difference observed in survivability between the PA14 WT and Δ*PA0739* strains, but the *sdsA1* transposon mutant intended for use as a negative control was also able to tolerate concentrations of SDS up to 10%. Previous studies have identified six alkyl- or arylsulfatases that are expressed in *Pseudomonas*

aeruginosa (Hagelueken et al, 2006). These six, or perhaps other yet to be identified enzymes, are capable of allowing for growth even in the absence of a functional SdsA1 protein. The same group indicates that *sdsA1* is essential for growth on SDS as a sole carbon/sulfur source. In the future, a differential phenotype may be established based on this; our new hypothesis is that the ability of PA14 (Wt vs. Δ*PA0739*) to grow on minimal media with 0.1% SDS as the sole carbon source will be restricted based on the differential expression of *sdsA1*. As stated by Hagelueken et al, *sdsA1* is required for growth on SDS alone. The transposon mutant should be incapable of growing in such conditions and the expression of *sdsA1*, based on its *PA0739* status, should be immediately obvious.

Miller assays using different membrane disrupting agents as inducers were performed to test the hypothesis that *sdsA1* expression could be a response to membrane stress, as suggested by the response to surfactant. Polymyxin B, a cationic lipid oligopeptide, is an antimicrobial agent that is effective against Gram-negative bacteria because of its ability to disrupt the cell membrane (Zhai et al, 2013). Sphingosine as a cell membrane lipid was also tested as a means of observing response to membrane disruption. Neither of these compounds caused significant increase in the transcription of *sdsA1* (Figure 2). This indicates that the SdsA1 protein is likely not part of a broad response to membrane distress.

The results from the repeat treatment with surfactant are consistent with what was observed in the preliminary Miller assay (Figures 1 + 2). *sdsA1* transcription is increased dramatically in the *PA0739* knockout strain, indicating that the divergently transcribed transcription factor may act as a repressor. If *PA0739* acts as a negative regulator, constitutive expression of *sdsA1* would be

expected in the knockout, possibly difficult to assay with a simple growth curve and tricky to spot with a β-Gal assay. With that in mind, the use of the more sensitive qRT-PCR technique, using RT primers for the *sdsA1* mRNA, could be used to determine expression and compare the *PA0739* knockout strain to the PA14 wildtype strain. Succeeding in this, the next logical step would be to perform an electrophoretic mobility shift assay to demonstrate a physical interaction between the PA0739 transcription factor and the promoter region of the *sdsA1* gene.

Figures:

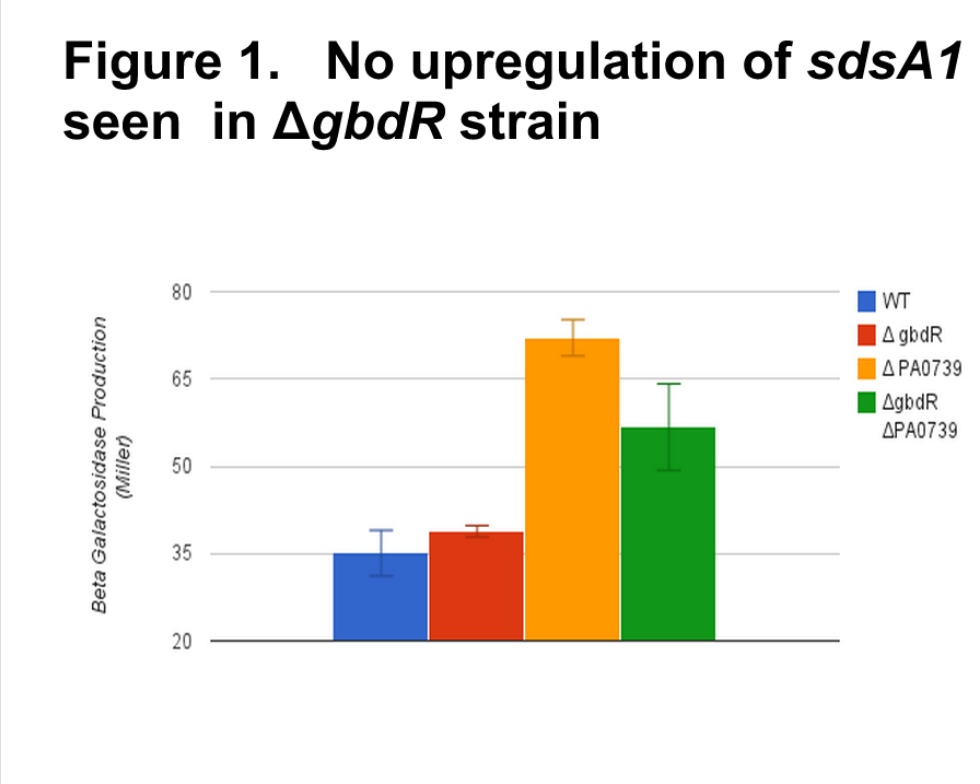

Figure 1: β-Galactosidase assay for induction of *sdsA1* in surfactant, in order to verify results seen in Wargo lab microarray data of *sdsA1* being upregulated in Δ*gbdR* mutant in surfactant. PA14 strains: wt, Δ*gbdR*, Δ*PA0739*, and Δ*gbdR* Δ*PA0739* grown in MOPS media plus lung surfactant. No upregulation of *sdsA1* in Δ*gbdR* knockout. The induction experiment was performed in triplicate and the results presented as an average value, error bars representing one standard deviation.

Figure 2A

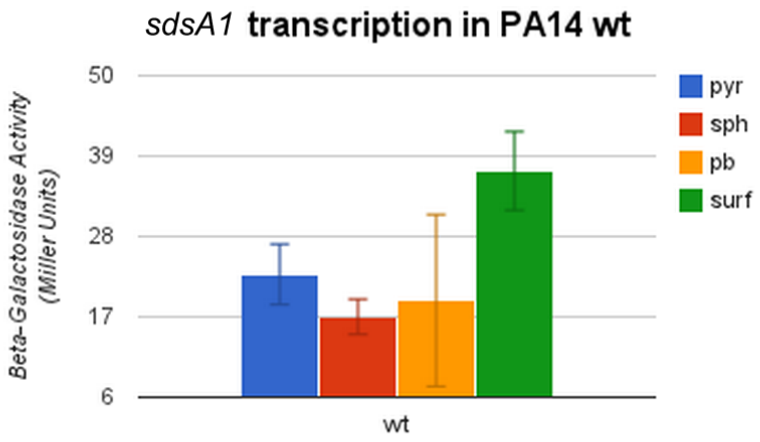

Figure 2B

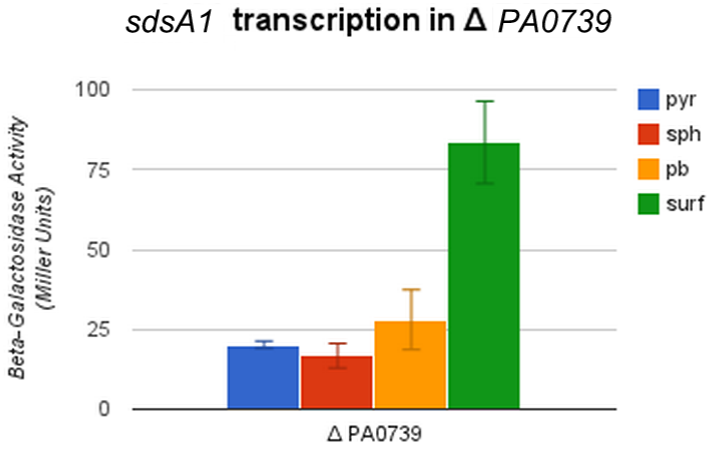

Figure 2: β-Galactosidase assay for induction of *sdsA1* in various membrane disrupting agents; Polymyxin B (pb), Sphingosine, Surfactant. Pyruvate used to compare. A) PA14 wt: Upregulation of *sdsA1* observed in only in surfactant. B) PA14 ΔPA*0739*: Upregulation of *sdsA1* observed in only in surfactant. The induction experiment was performed in triplicate and the results presented as an average value, error bars representing one standard deviation.

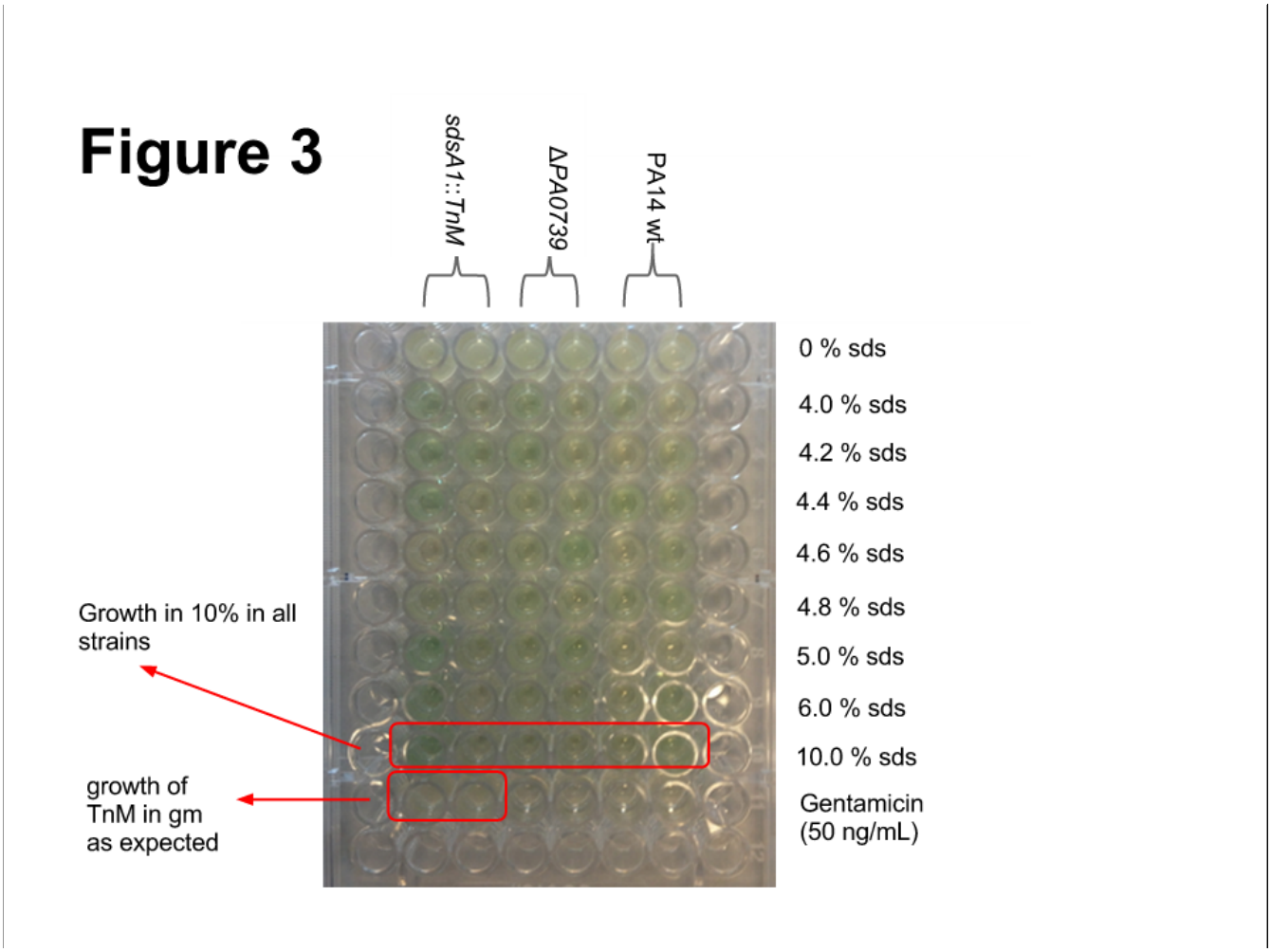

Figure 3: No inhibition observed in SDS containing LB media through 10% SDS. No phenotypic difference in SDS survivability observed between PA14 strains tested (wt, Δ*PA0739*, *sdsA1*::*TnM*). Results verified in secondary growth of strains in 3 mL overnights in rotary wheel (data not shown).

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