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Transcription factor based whole-cell biosensor for specific and sensitive detection of Sodium Dodecyl Sulphate

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Abstract

Extensive use of Sodium Dodecyl Sulfate (SDS) in households, agricultural operations, and industries is leading to its subsequent disposal in waterways. There is an apprehension of the adverse effect of such detergents on various living organisms. Thus, an efficient, specific, and simple detection method to monitor SDS reliably in the environment is needed. We used sdsB1 activator protein and SDS-responsive promoter of sdsA1 gene along with Green Fluorescent Protein (GFP) to construct a novel SDS biosensor in *Pseudomonas aeruginosa* chassis. The GFP intensity of the biosensor showed a linear relationship ($R^2 = 0.99$) from 0.4 to 62.5 ppm of SDS with a detection limit of 0.1 ppm. This biosensor is highly specific for SDS and has minimal interference from other detergents, metals, and inorganic ions. The biosensor showed a satisfactory and reproducible recovery rate for the detection of SDS in real samples. Overall, this is a low cost, easy-to-use, selective, and reliable biosensor for monitoring SDS in the environment.

Keywords: *Pseudomonas aeruginosa* PAO1, sdsB1 activator protein, sodium dodecyl sulphate, bacterial biosensor, fluorescence-based assay.

1. Introduction

Sodium Dodecyl Sulfate (SDS) is the most commonly used anionic detergent in household products, cosmetics, and commercial surfactants (Cowan-Ellsberry et al., 2014; Singer and Tjeerdema, 1993). SDS has diverse applications in the industrial sector as an emulsifier, food processing agent, stabilizer, leather softening, foaming, flocculating, and cleaning agent, due to which it has been referred to as the “industrial monosodium glutamate” (Wen et al., 2013). According to contemporary toxicity classifications, anionic surfactants are regarded as harmful chemicals (Liwarska-Bizukojc et al., 2005). SDS in the domestic discharges and industrial effluents is one of the primary causes of pollution in aquatic ecosystems and potable water (Stavskaya et al., 1988). The constant deterioration of the quality of drinking water and the harm caused to marine life are some of the major concerns. Several studies have reported that SDS has harmful effects on the survival and breeding of organisms in the aquatic ecosystem, hampering biological processes such as solubilization of phosphate, reduction of ammonia, nitrogen fixation and photosynthesis (Martinez-Jeronimo and Munoz-Mejia, 2007; Rebello et al., 2014; Rosety et al., 2001; Sandbacka et al., 2000). SDS is also reported to cause oxidative stress on mouse hepatocytes (Wang et al., 2016). Detergents can cause dermal (Lee et al., 2000) and ocular irritation (North-Root et al., 1982), cardiac anomaly, hemolysis, tachycardia, kidney failure, and even death (Ago et al., 2003; Okumura et al., 2000). Due to high foaming properties, SDS can disrupt

biological wastewater treatment processes and cause problems in sewage aeration and treatment facilities. Efficient and rapid techniques need to be developed for the reliable detection of these surfactants in the water bodies at environmentally relevant concentrations. Specific biosensors for the SDS detection are lacking. The conventional techniques for anionic surfactant detection include GC/LC-MS (Trehly et al., 1990), paper-based colorimetric assays (Barui et al., 2012), dye-based techniques (Hayashi, 1975), an amperometric method based on oxygen consumption by biodegrader strains (Taranova et al., 2002), gold nanoparticle-based assays (Kumar et al., 2016) and other analytical techniques which are laborious, costly, time-consuming, use toxic chemicals, require skilled workers and have a limited linear range of detection. Most of the existing detection techniques cannot distinguish between the two most commonly used anionic detergents- SDS and SDBS (An et al., 2011; Gao et al., 2017; Qian et al., 2009). Two sensors based on eosin Y- Polyethyleneimine and glutathione stabilized gold nanocluster-poly diallyldimethylammonium chloride have been reported for specific detection of SDS (Wan et al., 2019; Zheng et al., 2014). However, both of the techniques had weak fluorescence variations for SDBS at 4 ppm and 8 ppm respectively. Moreover, these sensors either require conjugated polymers and the additional dye or nanoparticle components, which are challenging to synthesize. All these factors make these analytical techniques cumbersome and difficult to use. None of the techniques have explored the possibility of amalgamating the extraordinarily selective and reliable transcription factor-based detection using whole-cell biosensors. Such biosensors have garnered immense popularity in heavy metal and xenobiotic detection (Huang et al., 2015; Jia et al., 2019; Kim et al., 2016; Wan et al., 2019).

Studies have shown field applicability of bacterial biosensors due to their simplicity, low cost, and portability (Belkin et al., 2017; Kabessa et al., 2016; Kylilis et al., 2019; Olaniran et al., 2011; Watstein and Styczynski, 2018).

Pseudomonads have an inherent capability to be used as an optimal destination chassis for synthetic biology applications. The selected species of *Pseudomonas* can be engineered to detect various xenobiotics based on their resilient nature to survive and adapt to harsh environmental conditions (Nikel et al., 2014). Recent research in the SDS regulation pathway has identified a regulatory protein sdsB1, which forms an activator complex with SDS and provides positive feedback for the production of alkyl sulfatase in *Pseudomonas aeruginosa* PAO1 during its SDS metabolic activity (Panasia et al., 2019). The sdsA1 promoter undergoes specific expression only in the presence of this complex which makes the activator-promoter pair an excellent combination for inducible protein expression. This detailed research on the pathway provided us with logical evidence to exploit the transcription factor-based machinery of the *P. aeruginosa* PAO1 strain for the detection of SDS in natural samples with high specificity and selectivity without any other chemical interference agents. Thus, the biosensor strain developed is the first report of a whole cell bacterial biosensor for the direct, specific and efficient detection of SDS without employing the assistance of any sample preparation steps, toxic chemicals, sophisticated polymers and sensor development steps.

2. Experimental

2.1. Media, plasmid, and bacterial strains

E. coli DH5 α and *Pseudomonas aeruginosa* PAO1 bacterial strains were used as routine base strains for transformation and biosensor studies. Strains were regularly maintained in the Luria Bertani (LB) medium. Growth media was supplemented with kanamycin 50 μ g/mL and 150 μ g/mL for plasmid maintenance as required for *E.coli* and *P. aeruginosa* PAO1, respectively. All the reagents, detergents, and minimal media were procured from Sigma-Aldrich, USA, or HiMedia, India. The plasmid vector pPROBE-NT used in this study was a kind gift from Prof. Steven Lindow, Berkeley University, USA. The primers used in this study were synthesized by Sigma - Aldrich, India.

2.2. Construction of biosensor module

The *sdsB1* genetic locus encoding the cognate transcriptional activator protein was PCR amplified from the PAO1 genome with its native promoter sequence along with the operator region. The PCR product was double digested with *HindIII*/*EcoRI* restriction enzymes and ligated in the same sites of the MCS region in the pPROBE-NT vector, upstream of the Green Fluorescent Protein (GFP) gene. The ligated mixture was transformed into *E.coli* DH5 α ultracompetent cells and selected on LB-plates supplemented with kanamycin. The final construct was retransformed in sucrose competent PAO1 cells by electroporation and selected on kanamycin plates as per established protocol (Smith and Iglewski, 1989).

2.3. Microtiter plate-based SDS detection assay

A single colony of PAO1-pSAN001 was inoculated in fresh LB medium supplemented with kanamycin and cultured overnight at 37 °C with shaking at 180 rpm. The primary culture was diluted in LB medium containing kanamycin in a 1:50 (v/v) ratio and incubated until OD₆₀₀ reached 0.4. The cells were harvested by centrifugation (10 min, 4°C, 4000 rpm). The supernatant was discarded and the pellet was resuspended in a final volume of 10 mL 1X minimal medium (1% (w/v) M9 Broth Powder, 0.8% glucose (w/v), 2 mM MgSO₄, 100 µM CaCl₂). 100 µL of the diluted cell culture was added to the clear bottom 96-well microtiter plate (Corning® 96 well opaque black plate, USA) corresponding to cells at 0.4 OD₆₀₀ in each well. Before the addition of the cells, SDS was added to the 96-well plate by serial dilution ranging from 0.12 ppm to 500 ppm. Finally, the plates were incubated at 37°C for 3h during which fluorescence (excitation 490 nm, emission 520 nm) and absorbance was measured at regular intervals of 30 min using the microplate reader (Synergy H1 multimode plate reader, Biotek, USA). Fluorescence values were normalized with the optical density of the bacterial cells to calculate the Relative Fluorescence Units (RFU) using the formula $RFU = \text{Fluorescence} / OD_{600}$.

2.4.Evaluation of specificity and linearity

Different categories of detergents (cationic, non-ionic, and anionic) were tested to assess the specificity of the sensor towards SDS. Detergents like Cetyl Trimethyl Ammonium Bromide (CTAB), Tween 20, Tween 80, Triton X-100, N-LauroylSarcosine and Sodium Dodecyl Benzene Sulfonate (SDBS) were analyzed at a concentration of 4 ppm in 1X minimal medium. Apart from that, different monovalent and divalent metal

ions [including heavy metal ions like As (III) and Hg (II)], anionic radicals, and chelating agents like EDTA commonly found in aqueous samples were tested for sensor orthogonality at a concentration of 100 μ M. The linearity of the proposed biosensor was assessed at various concentrations ranging from 0.12 ppm to 125 ppm in 1X minimal medium. The fluorescence values were measured as described in Section 2.3. The RFU values were analyzed using the linear regression equation of GraphPad Prism Software (GraphPad, California, USA).

2.5. Fluorescence microscopy and flow cytometry analysis

The biosensor assay was set for 3 h to gain optimal fluorescence after SDS induction at a concentration of 10 ppm. The cells were harvested by centrifugation (4000 rpm, 10 min, 4°C) and washed twice with Dulbecco's 1X PBS (Phosphate Buffer Saline). The final pellet was resuspended in 20 μ L of 1X PBS and observed under the fluorescence microscope (Zeiss Fluorescence Microscope, USA). GFP expression was observed using a 480-500 band-pass excitation filter and long-pass 520 nm barrier filters under 1000X magnification. For FACS analysis, a set of cells treated for microscopy were analyzed using flow cytometry (BDFACSVerse; BD Biosciences, USA), and 10,000 events were recorded during the analysis. Gating was performed to avoid the debris and clumped cells. The photomultiplier tube (PMT) voltage settings used were as follows; for Forward Scatter (FSC), 581 V; for Side Scatter (SSC), 471V, and fluorescein isothiocyanate (FITC), 307 V.

2.6. SDS detection in real samples

Detection precision to quantify SDS by the whole-cell biosensor method was assessed by employing sewage and pond water samples. SDS detection was performed in the tertiary treated sewage water collected from North India Water Treatment Plant, Uttarakhand (NIWTP), and the pond water sample by spiking it with environmentally relevant concentrations of SDS ranging from 0.1 ppm to 62.5 ppm. A positive control was arranged where SDS was spiked in minimal media to confirm the biosensor's functionality under normal circumstances. All the experiments were conducted in triplicates. The RFU values obtained in the SDS spiked sewage and pond water were plotted against their respective SDS concentrations. The SDS detection in the spiked samples was also performed by a commercially available kit (SDS Detection Kit, GBiosciences, St. Louis, MO, USA) as per there given protocol.

2.7. Data Analysis and statistical calculations

Data analysis and statistical calculations were performed using GraphPad Prism Version 8, California, USA. A Hill equation was fitted for SDS based whole-cell biosensor using the RFU values with hill slope function: $Y = B_{max} * X^H / (K_d^H + X^H)$, where B_{max} is the maximum RFU, K_d is the concentration of ligand required to achieve half-maximum RFU; H is the Hill coefficient. The limit of detection (LOD) was calculated using the formula $LOD = LOB + 1.645 * SD_{(lower\ concentration\ sample)}$ (Wen et al., 2017). The limit of blank (LOB) was calculated using the equation $LOB = Mean_{(blank)} + 1.645 * SD_{(blank)}$. All the experiments were conducted in triplicates, and the results were plotted according to the standard deviation of the mean. The data were analyzed using a one-way analysis of variance (ANOVA) and significance analysis of the contrasting

parameters was conducted by covariance analysis wherever applicable. The p values of the differences between the slopes and intercepts were calculated from the software.

3. Results and discussion

3.1. Cloning of biosensing cassette

Lack of a specific bioreporter of SDS prompted us to search for probable SDS-metabolizing genetic loci in *Pseudomonas aeruginosa* genomes and the extant literature. We noticed that a genetic locus coding for the sdsB1 gene was recently identified as a transcriptional activator of the sdsA1 (alkyl sulfatase) gene in PAO1 (Panasia et al., 2019). The entire SDS-metabolizing and regulation pathway is arranged in a linear stretch in the PAO1 genome [PA0739, 805788 - 806702 (- strand), RefSeq NP_249430.1 and sdsA1, 806805 – 808781 (+strand), RefSeq NP_249431.1]. The intergenic region of 102 bp represents a unique bi-directional promoter for both activator and the sdsA1 protein (Jovcic et al., 2010). The genetic locus coding for sdsB1 and the corresponding promoter-operator sequence was cloned in the pPROBE-NT vector (Fig. S1, Table S1) and examined for SDS induced GFP expression.

3.2. Whole-cell biosensor assay

No response was observed with SDS when *E.coli* DH5 α was used as the biosensor chassis, which can be attributed to the low efficiency of the native (PAO1) promoter in *E.coli* (Hettwer et al., 1998). However, the construct pSAN001 when transformed in *P. aeruginosa* PAO1 chassis showed transcription factor-mediated GFP expression (Fig. 1A). The biosensor showed a concentration-dependent increase in fluorescence when

transformed into *P. aeruginosa* PAO1 cells (Fig 1C). The discovery of the SDS-responsive nature of the GFP-reporter led us to optimize the biosensor assay in terms of different parameters like response time (Fig. S2A), cell stage (Fig. S2B), and cell number (Fig. S2C). The biosensor strain showed dose-dependent induction of GFP with SDS with a gradual increase in OD₆₀₀ over time (Fig. S10). The temporal resolution of 3 h was found optimal for the biosensor response. Beyond 4 h, the linear characteristics of the fluorescence response showed a minor deviation from the standard curve probably due to the saturation kinetics of the fluorescent protein production. Apart from that, an independent assessment of the fluorescence production and cell density change was conducted at an SDS concentration of 4 ppm over 12 h (Fig. S8, Fig. S9). The observation of this study was in agreement with the previously recorded time-dependent spectrophotometric based results. Biosensor cells in the early log stage of growth showed a better response to SDS induction than the stationary phase, confirming that the metabolically actively growing cells are best suited for SDS response (Fig. S2B). Another important factor determining the optimal RFU determination was the primary inoculum of cells. Although the general trend for increasing fluorescence was the same in both cases, a lower cell number produced a better RFU response than the higher number of cells (Fig. S2C). The SDS molecules present in the assay medium were limited in each test condition that resulted in a similar fluorescence intensity (a.u.) value for both 0.4 and 1.0 OD₆₀₀ seeded inoculum. However, during normalization of the fluorescence intensity with OD₆₀₀, the latter produced a lower RFU as compared to the former test condition. After repeated experiments, the temporal resolution of the optimized biosensor assay for SDS detection was finalized to be 3h (Fig. 1C), which is

comparable to the response time of the recently developed whole-cell biosensors for other xenobiotics (Du et al., 2019; Huang et al., 2015; Jia et al., 2019; Kim et al., 2016; Wan et al., 2019). Another critical concern that could have hampered the biosensor response to SDS was the simultaneous expression of alkyl sulfatase through the activation of *sdsA1* gene. Real-Time Quantitative Reverse Transcription PCR (qRT-PCR) study (method listed in supplementary note 2.4) showed that there was subsequent activation of the gene (~33 fold) in presence of SDS in the *Pseudomonas aeruginosa* PAO1 strain as compared to the control (Fig. 1D). Therefore, to assess whether the biorecognition element would perform better in the absence of *sdsA1* gene, pSAN001 was transformed into both wild type *Pseudomonas aeruginosa* PAO1 and transposon mutant strain PW2345 (genotype *sdsA1*-F05::IS *lacZ*/hah). Both the biosensor chassis were then evaluated in terms of GFP fold induction during the SDS detection assay. The biosensor plasmid in mutant strain PW2345 did not produce any better response in terms of relative fold induction in the presence of SDS as compared to the former chassis. There was high background GFP production in the mutant strain which led to low relative fold GFP expression (Fig. S6). Therefore, the wild type strain was decided to be the optimal biosensor chassis for our system.

3.3. Dynamic range and specificity of the biosensor

The biosensor has a dynamic range extending from 0.12 ppm to 125 ppm (Fig. 2A). It is also evident that an excellent linear correlation exists ($R^2=0.99$) between the RFU and concentration of SDS ranging from 0.48 ppm to 62.5 ppm (Fig. 2A (inset)), which represents a wider response range than the previously developed SDS specific sensors

(Table S3). The biosensor was subjected to higher concentrations of SDS beyond 125 ppm to estimate its tolerance range (Fig. S11). This helped us determine that beyond 500 ppm of SDS the cell viability was compromised thus limiting the SDS biosensing. The SDS detection limit was calculated to be 0.1 ppm, which corresponds to the environmentally relevant concentrations of SDS (Nomura et al., 1998; Singer et al., 1993.)

To assess the orthogonality of the biosensor, the fluorescence response of the cells was tested in the presence of the different categories of competitive surfactants and metals expected to be present in the environment. These included cationic, non-ionic, and anionic surfactants, metal ions, and anionic radicals under environmentally relevant concentrations. As shown in Figure 2B, except for SDS, no other detergent, metal ions and anionic radicals were able to induce significant fold change at concentrations of 4 ppm (for detergents) and 100 μ M (for other ions) used. Despite possessing only an extra benzene ring, a highly related anionic surfactant SDBS could not induce GFP fluorescence under various assay conditions (Fig. 1B, Fig. 2B). The specific induction of the sensor by SDS showed the highly selective nature of the sdsB1-SDS activator complex towards the activation of the sdsA1 promoter even at an extremely low concentration of 0.5 ppm (Fig. S12). The analytical features of the SDS-biosensor were compared and validated with the SDS-detection kit available commercially (Table S4). This kit cannot differentiate between SDS and closely related anionic detergent (SDBS). The parameters like dynamic range, repeatability, and sensitivity of the method are

comparable to the commercially available kit. The results suggest that the SDS-biosensor is robust and may be suitable for on-site SDS detection.

3.4. Fluorescence imaging and flow-cytometry analysis

As shown in Figure 3A, the cells induced with 10 ppm of SDS expressed sufficient GFP production after 3 h as compared to the uninduced PAO1 cells harboring pSAN001. The negligible background noise in the uninduced cells confirmed that the *sdsA1* promoter is tightly regulated, and GFP production is subject to the presence of SDS in the system only. The flow cytometry analysis provided a quantitative estimation of SDS induced fluorescence in terms of the increased fluorescent intensity after induction with SDS at a concentration of 10 ppm (Fig. 3B). To provide additional validation of the biosensor performance even at a lower range of SDS induction (4 ppm), the assay was continued for 12 h. During this period, at every 3 h interval, the cells were collected and subjected to fluorescence microscopy and flow cytometry analysis. Both the results confirmed the spectrophotometric observations obtained from the biosensor during SDS detection were sufficient and significant after the incubation time of 3 h (Fig. S4, Fig.S5). Additional incubation beyond 3 h was not required for better RFU production.

3.5. Cooperativity analysis of the biosensor performance

The hill coefficient scale reflects the cooperativity of the transcription based genetic system (Rogers et al., 2015). Transcription factor-based biosensors that employ activation mechanisms usually show non-cooperativity like the AraC (Madar et al., 2011) and CdaR (Rogers et al., 2015). A dose-responsive curve was fitted to the Hill equation to understand the role of *sdsB1* regulator in transcriptional activation of the *sdsA1*

promoter. The curve fitting model demonstrated that the R^2 , K_d , and hill Coefficient (H) as 0.98, 30, and 1.08 respectively (Fig. 3C). The hill coefficient of 1.08 leads to the conclusion that the activation-based regulation is non-cooperative. The lack of cooperativity either may be a result of sdsB1 protein acting as a monomer, or the dual expression of the regulator from both the plasmid and the genomic counterpart is creating feedback between biosensor activation and its expression (Rogers et al., 2015).

3.6. Detection of SDS in real samples

The results obtained with SDS-biosensor in the laboratory scale prompted us to assess the performance of SDS-biosensor in field samples. The challenging aspect that required further analysis was whether the variety of interfering agents present in water bodies could hinder the performance of the biosensor. The viability of the PAO1 pSAN001 biosensor was primarily assessed over 24 h under different treatment conditions like the presence of a solvent, multiple concentrations of SDS dissolved in distilled water and real samples by observing the Colony Forming Units (CFU). This assessment helped us determine that the viability of the biosensor was not affected in the presence of real samples and was similar to the control samples (Fig. S7). Treated sewage water from NIWTP, Uttarakhand, and pond water from IIT Roorkee, Uttarakhand was collected and used for the assay after filtration. The collected samples were spiked with SDS ranging from 0.12 ppm to 62.5 ppm. We observed significant fluorescence enhancement after 3 h of incubation. The SDS-biosensor could detect SDS in a concentration-dependent manner in spiked wastewater treatment plant

effluent and pond water with minor fluctuations (Fig. S3A, Fig. S3B). The recovery rate determined from the standard curve accounted for 98.83% and 99.18% for sewage water and pond water at 7.8 ppm of SDS induction, respectively (Table S2), indicating the reliability and practicality of the system.

4. Conclusions

In this work, we report a transcription-factor based whole-cell biosensor for specific detection of anionic detergent sodium dodecyl sulfate. To date, there is no biosensor available for specific detection of SDS. The biosensing machinery was planted in *Pseudomonas aeruginosa* chassis to make it suitable for environmental applications. We show high sensitivity, linearity, and an impressive limit of detection for the biosensor, which are suited to the presence of SDS in the environment. The biosensor is specific to SDS and can discriminate between two closely related anionic detergents, SDS and SDBS. This biosensor overcomes limitations of sample processing, sophisticated instrumentation, hazardous chemical use, and synthesis of specialized polymers or nanomaterials. Nevertheless, this novel biosensor achieves remarkable detection of this anionic detergent of extreme importance. We believe this proposed biosensing method holds great potential for point-of-care and on-site detection of this xenobiotic and complements the extant methods of environmental monitoring.

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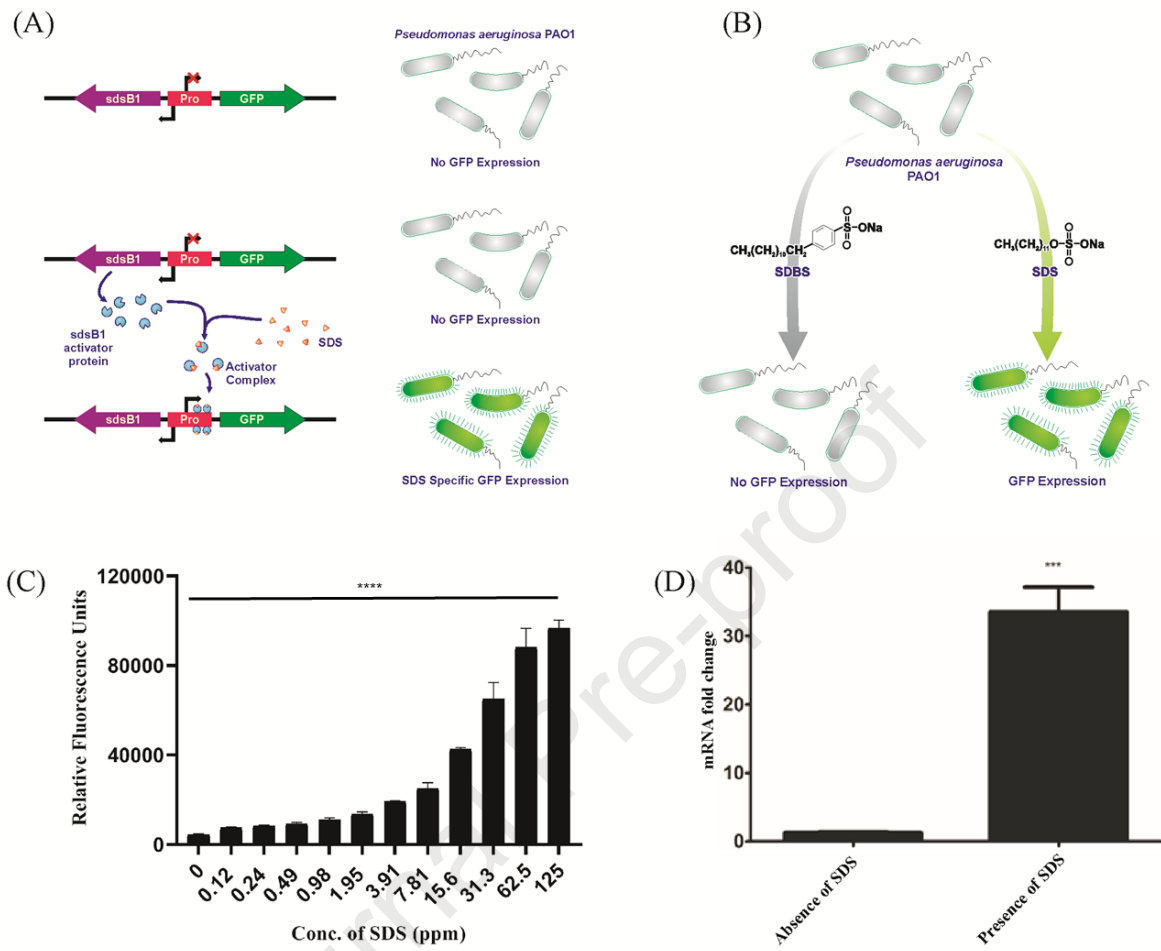
Figure legends

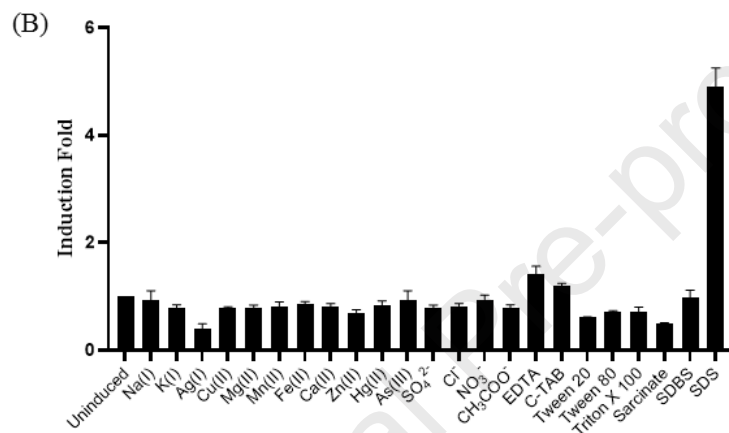
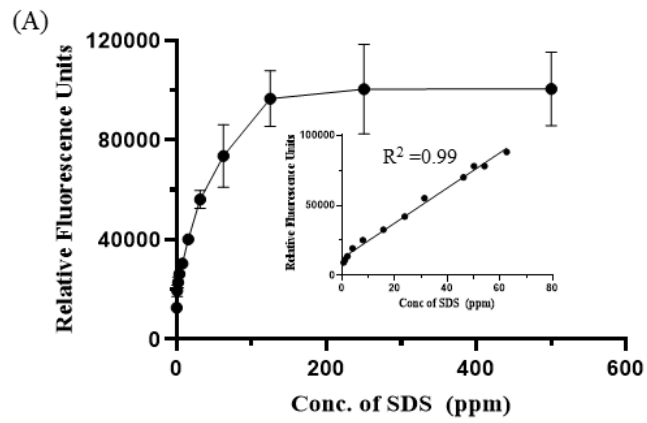
Fig. 1 (A) Schematic representation of the sdsB1 activator based biosensor for SDS (B) Schematic representation of the selectivity of the biosensor for SDS and SDBS (a related anionic detergent) (C) The Relative Fluorescence Units (RFU) of concentration-dependent expression of GFP induced by SDS. The concentration values range from 0 ppm to 125 ppm of SDS with a linear trend increase in their respective RFU values. The error bars represent standard deviation based on three independent measurements ($p < 0.0001$). (D) qRT-PCR analysis of the sdsA1 gene expression of *Pseudomonas aeruginosa* PAO1 in presence of SDS as compared to the uninduced control. The former condition shows an upregulation (~33 fold) in the mRNA expression of the sdsA1 gene ($p < 0.001$).

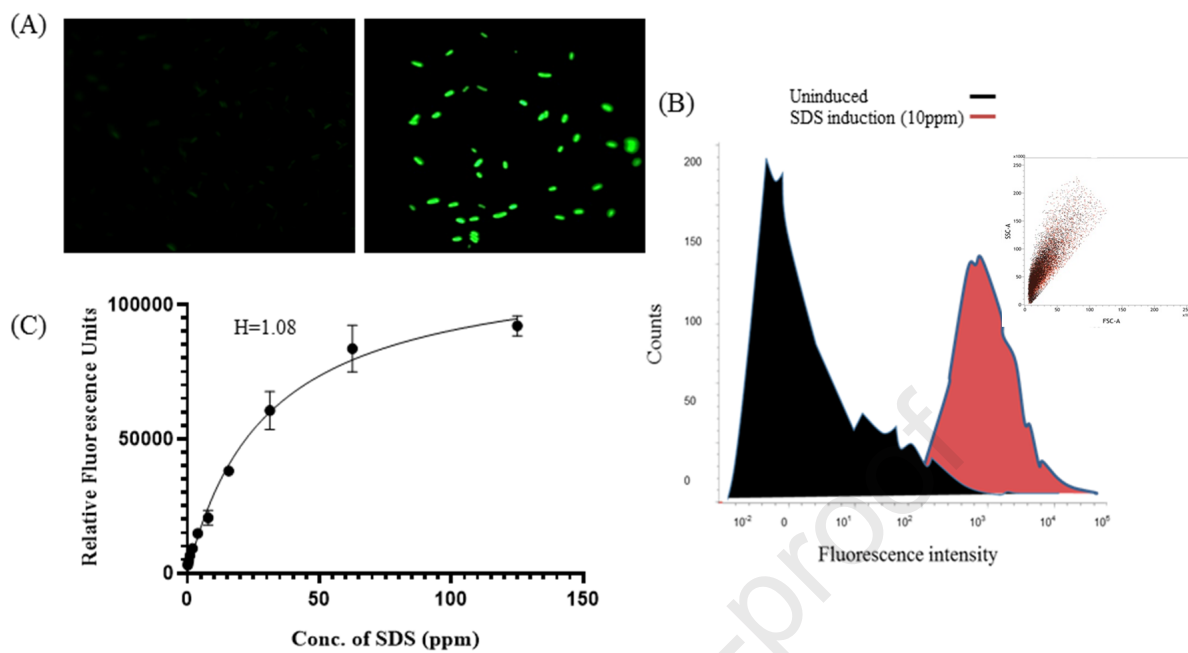
Fig. 2 Fluorescence expression of the biosensor in presence of SDS (A) Plot of SDS concentration vs Relative Fluorescence Units for quantification of SDS in minimal medium. The data from the experiment was used to compute LOD (see text). Inset shows the linearly fitted Relative Fluorescence Units against their respective concentrations of SDS. (B) The selectivity of the biosensor for SDS over other substances (all the metallic ions and anionic radicals were 100 μ M whereas the detergents were tested at a concentration of 4 ppm).

Fig. 3 Validation of the PAO1 pSAN001 based SDS biosensor by fluorescence microscopy and flow cytometry analysis. (A) Fluorescence microscopy of biosensor without SDS (left) and with 10 ppm SDS (right). (B) The behavior of biosensor in response to SDS was evaluated by flow cytometry. 10000 events were recorded for uninduced (black) and induced (pink) samples. Inset shows the overlaid dot plot of induced (in red) and uninduced (in black) biosensor cells after 3 h of incubation (C) The

457 Hill cooperativity analysis of the biosensor representing the RFU values vs
458 concentration of SDS (in ppm). The hill coefficient of 1.08 correlates to the non-
459 cooperativity of the transcription factor-mediated biosensor platform.







Highlights

- A novel, selective and robust transcription factor based whole cell biosensor was developed for sensitive detection of SDS in the fresh and polluted water resources
- The limit of detection of the whole cell biosensor is preferentially much lower than all other extant methods of SDS detection
- Biosensor could differentiate between two related detergents, SDS and SDBS reliably
- The biosensor does not require sample processing steps or involvement of any other chemicals for SDS detection