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Research Paper

Isolation of Sodium Dodecyl Sulphate Degrader from the Contaminated Water Sample and its Bioassay

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A B S T R A C T

Detergents one of the most commonly used chemicals in the household, pharmaceuticals, cosmetics, and in agriculture- finding its success in many ways. However, with increasing use, it is increasingly concentrating in the environment and now grouped in significant pollutant. Hence it is essential to degrade the accumulated detergent by biological means. In this study, detergent contaminated water sample found to be positive for bacterial species able to degrade sodium dodecyl sulphate (SDS). We identified those species as *Staphylococcus aureus* SS1 and *Pseudomonas aeruginosa* SS2 and appeared to be efficient degrader of SDS within ten days at high concentration (about 1% of total volume). As per Fourier Transform InfraRed spectroscopy and Thin Layer Chromatography, SDS is degraded to dodecanoyl by these bacterial species and exhibit exceptional capabilities to become resistant and further breaks SDS, and hence those are considered as a source for biotechnological tools to bring about cheap bioremediation for industrial applications in coming time.

***Key words*:** Detergent, Degradation, Sodium dodecyl sulphate, Bacteria

Surfactants represent their uniqueness of amphiphilic nature. This property makes them capable- interface between water and oil, air and water and thereby lowers the surface tension. Once present in aqueous solution, surfactants represent its anionic, non-ionic, cationic or amphoteric classes [1]. The surfactants being anionic represent low price and widely used in pharmaceuticals, cosmetics, agriculture, household properties. Since they are now increasing in use resultant getting accumulated in aquatic and terrestrial environments, becoming toxic to the living organisms [2]. The real problem of surfactants is its large quantity accumulating in sewage treatment plants, which influences physiological and biological processes in water purification [3]. Detergents can very easily interact with intracellular components of living organisms by bringing about electrostatic or hydrophobic interactions, making them toxic [4]. Even though we know detergents are toxic, still they prominently used in increasing water solubility, and for bioavailability of xenobiotics. In this content, SDS is widely used in soil bioremediation [5-6].

Numerous reports are mentioning the bacterial role in degrading surfactants, for example, *Klebsiella oxytoca* [7]; *Pseudomonas strains* [8-10]. These microorganisms are prominently isolated from the detergent contaminated environment and reported to be a potential source for them. In the present study, we have sampled the detergent contaminated water, assuming that microorganisms inhabiting this water should contain features to degrade detergents. Further, we reported the potential degradation of SDS and bio-products formed from them.

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MATERIALS AND METHODS

Sampling of bacterial species

Since the detergent stands one of the pollutants, its degrader biological species isolation (Bacteria) carried out from the contaminated water source, Pune India. The water sample immediately processed for bacterial species isolation.

Enrichment

Collected 5 ml of water sample inoculated to sterile 50 ml basal medium broth containing 1% detergent mainly in 250 ml Erlenmeyer flask. For 1% detergent, different makes of it utilized namely (brand name) Sodium dodecyl sulphate, Wheel, Rin, Nirma and Ghadi. Once the inoculation made, flask kept static for 48 hours for the enrichment of culture in the presence of detergent while maintained at 30°C.

Isolation of Detergent degrader

The sample from enrichment broth inoculated on the sterile basal plates supplemented with given detergent in 1% concentration. After that by 48hrs of inoculation, plates were flooded with Lugol's iodine solution, and colonies were recorded for the formed zone of degradation around the colonies.

Morphological and biochemical analysis

Selected isolates then Gram-stained, and biochemically tested with Gelatin hydrolysis, Catalase test, Oxidase test, Methyl red, Voges Proskaure's test, Urease test, Nitrate

reduction, SIM test and Sugar fermentation (Glucose, Maltose, Mannitol, Lactose). Species further identified by 16S rRNA gene sequencing (data not shown).

Detection of anionic surfactant production

The ability to degrade surfactant (detergent) by the bacterial isolates recorded previously as positive for the degradation of several surfactants confirmed by MBAS assay.

In a protocol, three sets were prepared as follows:

Set 1: In a blank set, inoculum 1 ml (0.5 O.D.) added to the 100 ml basal medium broth without any surfactant.

Set 2: In a standard set, 100 ml basal medium supplemented with 1% surfactant without any bacterial load.

Set3 : In an experimental set, 100 ml basal medium supplemented with 1% surfactant plus 0.5 O.D. 1 ml bacterial inoculum. All the flasks kept static and incubated for 24-240 hours (24 h interval). Upon every incubation (24 h), sample centrifuged at 10000 rpm and cell-free supernatant collected. To the three ml of a supernatant equivalent volume of methylene blue and chloroform added. The sample then mixed vigorously and incubated for 20 minutes at 30°C on the shaker. The sample allowed to settle at static condition. Obtained chloroform layer then is drawn off in fresh tube for analysis.

During analysis absorbance recorded at 652 nm. The calculation of degradation carried out by the equation:

$$\% \text{ Degradation} = 100 - \frac{[\text{Absorption of test} - \text{Absorption of blank}] \times 100}{\text{Absorption of standard}}$$

Detection of degraded product

Thin layer chromatography

The ability of the bacterial isolate to degrade detergent confirmed via Thin Layer Chromatography protocol as reported by Cai, (2014). Here the solvent system used as Chloroform: Methanol (9:1) and developer as potassium permagnet solution ($KMnO_3$).

Fourier transform infrared spectrophotometer analysis

As per protocol [11] frequency data recorded for wave numbers typically over the range 4000-600 cm^{-1} and noted for sets as:

a) Standard (broth + SDS) and b) experimental sets (broth + SDS + inoculum).

RESULTS AND DISCUSSION

Identification of detergent degrading bacteria

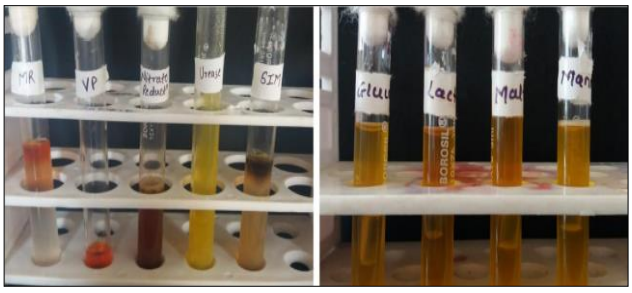

Fig 3a Biochemical profile of *S. aureus* SS1

Detection of anionic surfactant production

The ability to degrade detergent strongly studied in an equivalent assay MBAS. Here the degradation of methylene blue by producing anionic surfactant by the isolates SS1 and SS2 confirmed up to 240 hours of analysis. As per result once compared to blank and standard sets, experimental sets

The prominent isolates named as SS1 and SS2 found to be growing on basal medium added with various 1% detergents. The better growth and ability to grow in the presence of detergent noted them potential degrader in an initial study since detergent is the only carbon source given to them as nutrient as shown in (Fig 1).

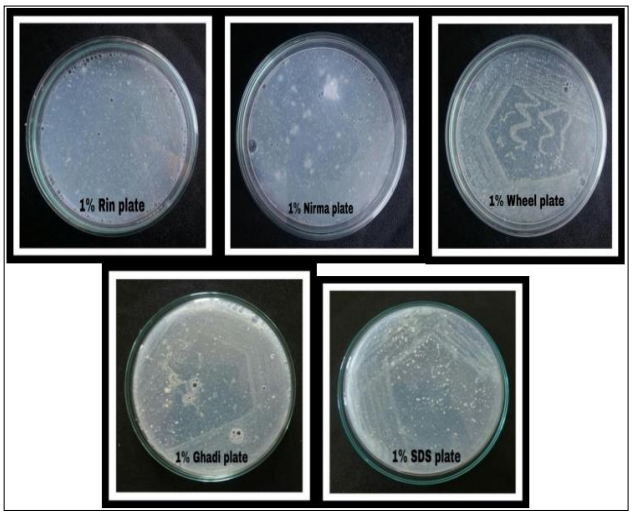

Fig 1 The colonies of isolate SS1 showing prominent growth on basal medium added with 1% detergent

Detergent degrading isolates SS1 and SS2 Gram-stained as Gram-positive and Gram-negative bacteria with cocci and rod-shaped morphology, respectively as in (Fig 2).

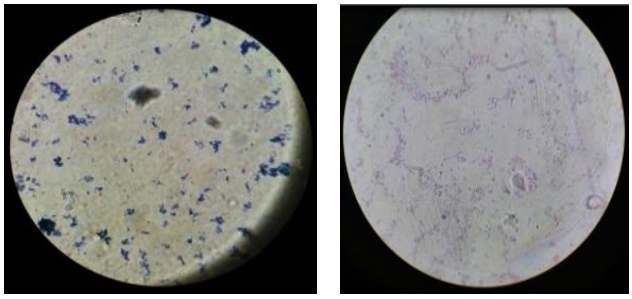

Fig 2 Gram nature of Isolate SS1 (Gram positive cocci) and Isolate SS2 (Gram negative Rod)

Morphology and biochemical features

As per Bergey's manual of bacteriology for biochemical and 16S rRNA gene sequencing isolate SS1 identified as *Staphylococcus aureus* and isolate SS2 as *Pseudomonas aeruginosa* once the biochemical and sequence information matched as in (Table 1, Fig 3).

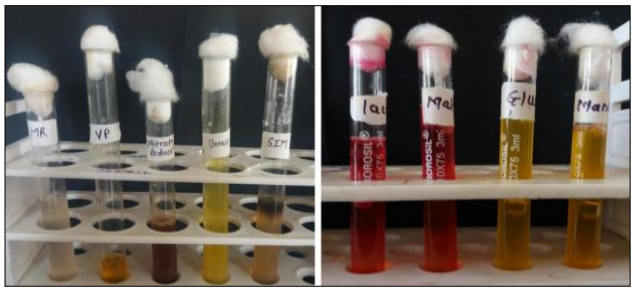

Fig 3b Biochemical profile of *P. aeruginosa* SS2

(isolate SS1 and SS2) registered profound dye degrading ability. Here from day three more than 40% degradation noted in both experimental sets which remained progressive as the time elapsed. On the 10th day, *S. aureus* SS1 isolate registered 96.41% dye degradation while *Pseudomonas aeruginosa* on the same day recorded with 88.18% degradation rate as given in (Fig 4, Table 2).

Table 1 Biochemical features of the *S. aureus* SS1 and *P. aeruginosa* SS2

Test	Isolate 1	Isolate 2
Oxidase	Positive	Positive
Catalase	Positive	Positive
Methyl red	Positive	Negative
Voges Proskauer's	Positive	Negative
Urease	Positive	Negative
Nitrate reduction	Positive	Positive
SIM test	Positive	Positive
Glucose	Positive	Positive
Maltose	Positive	Negative
Mannitol	Positive	Positive
Lactose	Positive	Negative
gelatinase	Positive	Negative

Table 2 Increasing percentage degradation of Methylene blue recorded in experimental sets inoculated with *S. aureus* SS1 and *P. aeruginosa* SS2

Days	% degradation by *Staphylococcus aureus*	% degradation by *Pseudomonas aeruginosa*
3	49.24%	43.45%
5	78.28%	65.34%
7	88.20%	97.33%
9	96.86%	98.52%
10	96.41%	88.18%

Degradation of detergent

TLC analysis: The ability of S. aureus SS1 and P. aeruginosa SS2 to degrade SDS has been confirmed by TLC analysis once hydroxyl group compound prominently recorded by both experimental sets with given retardation factors as given in (Table 3) once recorded with solvent (Chloroform: Methanol) (9:1) and developer as potassium permanganate.

Blank Standard Test

Fig 4 As per MBAS assay no discolouration recorded in blank, standard sets instead of test samples indicative of degradation in experimental set (Left). The chloroform layer representing high content of methylene blue in blank (right) and standard set (left) while it has been reduced in experimental set (Middle)

Table 3 TLC analysis of degraded SDS fraction

S. No.	Organism	Medium used	Solvent system	Developer	Observation	Rf value
1	*Staphylococcus aureus*	Basal medium broth	Chloroform:Methanol (9:1)	potassium permagnet ($KMnO_3$)		0.54 0.72
2	*Pseudomonas aeruginosa*	Basal medium broth	Chloroform:Methanol (9:1)	potassium permagnet ($KMnO_3$)		0.48 0.66

FTIR analysis

The chloroform extracted the degraded product of SDS once catalyzed by *S. aureus* SS1 and *P. aeruginosa* SS2 defined changes in FTIR vibration once compared with SDS FTIR pattern (Fig 5a). The vibrational changes recorded in both experimental sets and FTIR pattern found to be matching with dodecanol and nominated as degradation derivatives as noted in (Fig 5b-c).

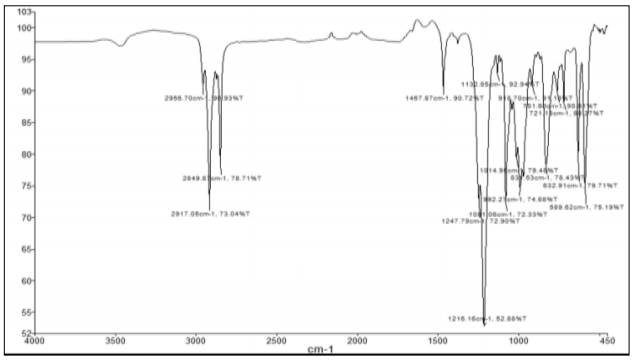

Fig 5a FTIR of sodium dodecyl sulphate

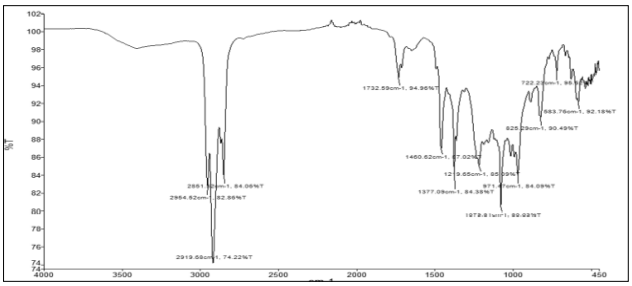

Fig 5b FTIR of extracted compound by using *Staphylococcus aureus* SS1

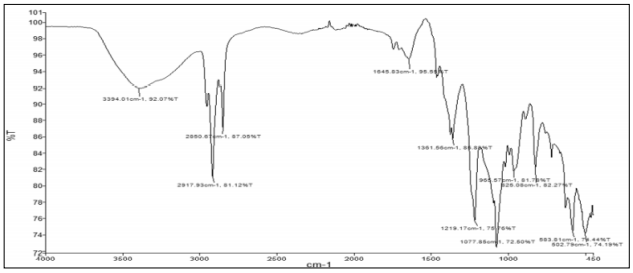

Fig 5c FTIR of extracted compound by using *Pseudomonas aeruginosa* SS2

The pollutant effect of detergents has been well noted by the number of researchers affecting air, water and soil-based organisms [12-15]. In a remedy point of view, use of bacterial species capable of degrading detergents finding a new ray of hope to control ever-increasing pollution [16-18].

In the present study, detergent contaminated water sample found to be prominent for detergent sustaining bacterial flora once confirmed by *in vitro* growth analysis. Based on the study, isolation of promising *S. aureus* SS1 and *P. aeruginosa* SS2 noted to be proficient in degrading detergents of various make.

The both of the isolates (*S. aureus* and *P. aeruginosa*) found to be degrading SDS completely upon ten days of incubation under aerobic with static conditions and able to give the product as dodecanol. Similar to the present study, *Pseudomonas aeruginosa* previously noted to degrade SDS to dodecanol by oxidation [19]. The formation of dodecanol once SDS undergoes photoelectrochemical degradation [20]. In a similar manner number of reports confirmed the formation of dodecanol as a byproduct while degrading SDS via microbial pathways [21-22].

CONCLUSIONS

In this study, we reported the presence of bacterial species in detergent contaminated water able to degrade a variety of detergent using them as the sole carbon source. Two isolates appear to be a close taxonomic link with *S. aureus*, and *P. aeruginosa* appeared to be the most efficient SDS degraders, able to decompose 80% to 100% of the SDS with ten days of treatment at 1% concentration. The detergent metabolism by these organisms confirmed the formation of biproduct- dodecanoyl once SDS given as a carbon source. Further work will open the molecular basis of these SDS degradation pathways and can be modified to enhance its rate of degradation to make the process more efficient.

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