Molecular characterization of linear alkylbenzene sulphonate degrading *Pseudomonas nitroreducens* (MTCC 10463) and *P. aeruginosa* (MTCC 10462)

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Surfactants are surface active chemical compounds, which are extensively used in various industrial and household formulations. Now-a-days, linear alkylbenzene sulphonate (LAS) is the most important anionic surfactant in use. The biodegradability of LAS is the main reason for its acceptance as a major chemical in industrial applications. But the huge amount discharged upsets the efficient removal by biodegradation and its acute exposure pose harm to the environment. Studies on indigenous isolates capable of efficient LAS degradation were conducted and two strains of the genus *Pseudomonas* showing about 81% of LAS degradation were selected. The biochemical and molecular characterization of the isolates were done in order to identify them. The selected isolates were identified as *P. nitroreducens* (L9) (MTCC 10463) and *P. aeruginosa* (L12) (MTCC 10462). The sequences generated in the 16S rDNA analyses were deposited in the NCBI GenBank under the accession numbers HQ271083 (L9) and HQ271084 (L12). The role of plasmid in biodegradation was checked and it was found that genomic DNA and plasmid together code for the degradation capacity of the selected strains. Though the colony count was reduced at high LAS concentrations, selected isolates were able to withstand very high concentrations of LAS (12000 ppm). In the presence of an alternate carbon source, such as, dextrose, the isolate showed diauxic growth. The selected strains were found to be two promising candidates for the bioremediation of the anionic surfactant LAS.

**Keywords**: Alkali lysis method, diauxic growth, methylene blue active substrate assay, phylogenetic analysis

**Introduction**

Surfactants (surface active compounds) are extensively used in day to day life. Chemically synthesized surfactants are widely accepted due to its low cost and high efficiency. Linear alkylbenzene sulphonate (LAS) is a widely used anionic surfactant. LAS are nonvolatile compounds produced by sulphonation of linear alkylbenzene. The LAS molecule contains an aromatic ring sulfonated at the para-position and attached to a linear alkyl chain at any position except the terminal carbons. Frequently used as sodium salt, LAS finds its application as sole surfactant or in combination with other surfactants in detergent formulations. The use and disposal of LAS is in an increasing ladder. The release of these surfactants into the environment by waste water is a threat to the sustainability of the environments. These compounds can act on biological wastewater treatment processes and cause problems in sewage aeration and treatment facilities due to their high foaming and lower oxygenation potentials\(^1\). The presence of LAS in sludge depends up on its use in industrial and domestic formulations, and also on the type of sewage treatment. Sewage sludge that is aerobically digested may contain LAS concentrations of about 100-500 mg/kg dry wt, considerably lower than those found in anaerobically treated sludge (5,000-15,000 mg/kg dry wt). Therefore, the extent of LAS contamination of sewage is generally dependent upon the individual waste water treatment plant (WWTP) and the method of sludge digestion employed\(^2\).

The analysis of toxicity data has shown that LAS is causing acute toxicity to the terrestrial and aquatic habitats. The increased awareness of the ill effects of these compounds has led to the intensified research on various strategies that may be employed to clean up the environment. The potential of various microorganisms as an alternative to costly chemical waste water treatment has been realized\(^3\). Microbial processes play a major role in the removal of recalcitrant compounds taking advantage of the
astonishing catabolic versatility of microorganisms to degrade or transform such compounds. As much as the diversity in sources and chemical complexities in organic pollutants exists, there is probably more diversity in microbial members and their capabilities to synthesize or degrade organic compounds. The biodegradation of surfactants by indigenous microorganisms is important since those organisms could easily adapt to the environment.

The present study was carried out to isolate and identify LAS degrading bacteria from detergent contaminated soil. The LAS degradation efficiency of the isolates was assessed and the best isolates were subjected to molecular characterization.

**Materials and Methods**

**Substrate and Chemicals**

Sodium dodecylbenzene sulphonate (C<sub>18</sub>H<sub>35</sub>NaO<sub>4</sub>S) having mol wt 348.48, supplied by Sigma-Aldrich were used for carrying out the study. All other inorganic chemicals were of analytical grade and obtained from MERK Research Laboratories Pvt. Ltd. and HiMedia Pvt. Ltd., Mumbai, India.

**Isolation, Screening and Identification of LAS Degrading Bacteria**

A minimal medium was used to isolate the surfactant degrading organisms with LAS as the sole carbon source. The mineral salt medium (MSSM) contained (L<sup>−1</sup>): Na<sub>2</sub>HPO<sub>4</sub> (1.6 g), KH<sub>2</sub>PO<sub>4</sub> (1.0 g), NH<sub>4</sub>Cl (0.5 g), K<sub>2</sub>SO<sub>4</sub> (0.06 g), CaCl<sub>2</sub> (0.025 g), trace element solution (2.0 mL). The trace element solution contained (L<sup>−1</sup>): FeSO<sub>4</sub>·7H<sub>2</sub>O (0.1 g), MnCl<sub>2</sub>·4H<sub>2</sub>O (0.1 g) and ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.1 g).

Soil samples were collected from different parts of detergent-contaminated domestic laundry premises of Meenachil river basin, Kottayam, Kerala. Soil samples were enriched<sup>4</sup> and LAS utilizing organisms were isolated. Incubation was carried out at room temperature (28±2°C) for 24 h. The selected cultures were purified and were stored at −20°C in 50 mM KH<sub>2</sub>PO<sub>4</sub> buffer at pH 7.2 containing 20% glycerol. Working cultures were maintained by subculturing every 2 wk on mineral salt agar slants containing LAS.

The selected isolates were identified based on morphological, physiological and biochemical characteristics. The carbon source utilization pattern of the selected organisms was studied using an automated instrument BIOLOG using GN2 (Gram-negative identification test) plates.

**Biodegradation Studies**

Biodegradation studies were carried out in mineral salt medium with LAS as sole carbon source in 250 mL Erlenmeyer flasks containing 50 mL medium. Seed cultures were prepared by transferring single colony into 50 mL nutrient broth and incubated at 37°C for 12-24 h. After incubation, bacterial cells were harvested by centrifuging the culture medium at 10,000 rpm for 10 min in a cooling centrifuge maintained at 4°C. Washed bacterial pellet were resuspended in sterile saline water at a concentration of 1 unit A<sub>600</sub> (approx ×10<sup>7</sup> cells/mL). One per cent inoculum of these bacterial cells was used as mother inoculum. The flasks were maintained at 28±2°C temperature with 150 rpm/min shaking speed. The surfactant solution was prepared by dissolving 0.05 g/L of LAS in the medium. In biodegradation studies, uninoculated control was maintained in the same test conditions to check the possible abiotic loss of LAS.

**Molecular Characterization of Isolates**

Bacterial genomic DNA of the selected isolates was extracted using alkali lysis method. The purity of the isolated DNA was checked by agarose gel electrophoresis<sup>5</sup>. The Nano Drop® ND-1000 was used for DNA quantification. 16S rDNA analysis of the isolates was done using the following primers: FP: 5′-CAGGCCTAACACATGCAAGTC-3′; RP: 5′-GGGCGGAGTGTACAAGTC-3′. The polymerase chain reaction (PCR) products were size fractionated on 1.5% agarose gel and stained with ethidium bromide. The amplified PCR products were purified with PCR clean up kit-100 (Chromus Biotech, Bengaluru).

The sequencing of the amplified region was done using the automated DNA sequencing service provided by Chromus, Bangalore, India. The sequences were analysed using nucleotide basic local alignment search tool (BLAST) search algorithm BLASTN 2.2.24+ version (www.ncbi.nlm.nih.gov/BLAST)<sup>7</sup>. The sequence was deposited in the National Centre for Biotechnology Information (NCBI) GenBank data base and acquisition numbers were assigned (HQ271083 & HQ271084).

The phylogenetic analysis of the isolates was done by multiple sequence alignment. The multiple sequence alignment program ClustalW2 was accessed from the website: http://www.ebi.ac.uk/clustalw/. The phylogenetic tree was constructed by using the
MEGA 3.1 by neighbor joining method with *Escherichia coli* as the out-group in the phylogenetic tree. The aligned sequences were organized to reflect sequence homology and the evolutionary distance calculation.

**Random Amplification of Polymorphic DNA (RAPD) Fingerprinting of Selected Bacteria**

The total genomic DNA isolated from the two bacterial isolates was amplified in RAPD analysis. The RAPD data were scored and converted to quantitative data using NTSYS-pc version 2.02i software. Clustering of the data was done using unweighted pair group method with arithmetic mean (UPGMA). The similarities between the isolates were calculated using Jacquard coefficient.

**Plasmid Profile of Isolates**

Plasmid DNA was isolated by the alkali lysis method described by Sambrook *et al.*\(^6\). The approx size of the plasmid was determined by comparing the mobility with that of a DNA ladder. Plasmid curing was performed as described by Trevors\(^8\) in order to study the role of plasmid in LAS degradation. The curing of plasmid was carried out by applying intercalating dyes like ethidium bromide and acridine orange, anionic surfactant sodium dodecyl sulphate (SDS) (10%), as well as by applying elevated growth temperature. The concentration range of mutagen was 50 to 550 µg mL\(^{-1}\) (with increments of 50 µg) for ethidium bromide and 100 to 800 µg mL\(^{-1}\) (with increments of 100 µg) for acridine orange. For curing by elevated temperature, cultures were incubated at the desired temperature, *i.e.*, 42, 44 and 46°C overnight, reinoculated in fresh LB medium and incubated at the corresponding temperature until late log phase. Samples from culture flasks displayed observable turbidity were plated on nutrient agar and incubated overnight at 37°C. Colonies developed in the Nutrient agar mother plate were patch plated on ampicillin supplemented Muller-Hinton agar and incubated. Ampicillin sensitive colonies were selected and inoculated in mineral salt medium supplemented with LAS to check the ability of the cured cells to grow in the presence of LAS. A combination of acridine orange and elevated temperature was also given to the inoculated flasks for curing the plasmid. The curing efficiency was expressed as percentage of curing by counting number of colonies with cured phenotype per 100 colonies tested.

**Susceptibility of High Concentrations of LAS by Selected Isolates**

To check the toxicity of high concentrations of surfactant on the isolated bacteria, mineral salt medium was supplemented with LAS at mass range of 600 to 1200 ppm. After incubation, appropriate dilutions were plated on nutrient agar plates and colony forming units (CFU/mL) were determined.

**Analytical Methods**

**Effect of Different Carbon Source on LAS Degradation**

The effect of different carbon sources (dextrose, sucrose, maltose & mannitol) on LAS degradation by the selected isolates was also studied. Each carbon source was supplemented individually at final concentration of 0.2% as an additive to mineral salt medium containing LAS. The carbon source was sterilized separately and added to the medium before inoculation in order to prevent charring of sugar.

**Methylene Blue Active Substrate (MBAS) Assay**

The concentrations of anionic surfactant LAS was determined by a modified version of the methylene blue active substrate assay (MBAS) as described by Hayashi\(^9\). The method is based on the formation of a complex between the anionic surfactant and an excess of the cationic dye methylene blue, followed by extraction of the complex (but not excess dye) into chloroform and measurement of the absorbance of the blue chloroform layer.

Aliquots of 0.1 mL methylene blue, 0.4 mL of 0.825 mM phosphate buffer and 1 mL sample (all in triplicates) were mixed in an acid washed, optically matched glass tubes. Then 3 mL chloroform was added and vortexed. The tubes were allowed to stand at 4°C for 5 min, followed by centrifugation at 2000 rpm for 4 min. The tubes were allowed to warm to room temperature and the absorbance of the chloroform layer was measured at 655 nm against an appropriate blank using spectrophotometer. 0.05 g/L LAS was used as control.

In order to compare the degradation ability achieved between two different conditions, the percentage degradation (%) of LAS was calculated and compared to the corresponding control\(^10\).

**Percentage of degradation (%) =**

\[
100 - \frac{[A_{655\text{Experimental}} - A_{655\text{Blank}}]}{A_{655\text{Standard}}} \times 100
\]

Where, \(A_{655}\): Absorbance at 655 nm; Experimental: Mineral salt medium with LAS as sole carbon source
inoculated with bacterial isolates; Standard: Mineral salt media with LAS without bacterial isolates (abiotic control); Blank: Reagent blank.

**HPLC Analysis**

LAS concentration was measured by reverse-phase high performance liquid chromatography (HPLC) using a C18 column and SPD-20A UV-VIS detector (220 nm). Culture supernatant was acidified with HCl, saturated with sodium chloride and extracted three to five times with an equal volume of diethyl ether. The combined extracts were dried over anhydrous sodium sulphate, evaporated and 20 µL of sample was loaded to the column. The mobile phase was an aqueous solution of acetonitrile (80:20) buffered with 1 M sodium perchlorate. The flow rate was set to 1 mL/min. Retention time was 10 min. Data acquisition and processing were performed by using LC solution system (Shimadzu)\(^{11}\).

**Results**

From detergent contaminated soil, 20 bacterial strains were isolated by soil enrichment method. The organisms were selected based on their ability to utilise LAS as sole carbon source. In order to compare the degradation efficiency, the percentage of anionic surfactant degradation was calculated and, on this basis, selection of strains was done. These bacterial isolates obtained by soil enrichment and plating methods were screened using MBAS assay (Table 1). The initial concentration of LAS was 0.05 g/L. Abiotic control was maintained throughout the study period. After 7 d, samples were taken from the control and tests and MBAS assay was done. Of 20 isolates, 5 showed more than 65% degradation efficiency and were selected. Hence, the isolates L4 (68.32±0.5), L9 (81.33±0.7), L12 (81.81±0.8), L14 (72.7±0.2) and L15 (75.5±0.2) were selected for secondary screening using HPLC (Fig. 1). The control LAS concentration was 0.05 g/L and the optical density of control was used as the standard to compare degradation efficiency of the isolates. Using HPLC, LAS was identified based on its retention time, by comparing the fingerprint areas of the standard LAS obtained from Sigma chemicals. Since LAS is a mixture of closely related homologues and isomers, the chromatogram showed the presence of multiple peaks. The quantitative calculation was carried out on the basis of peak area\(^{12}\). After secondary screening, 2 most efficient LAS degrading isolates, namely L9 and L12, were selected.

The identification of selected isolates, L9 and L12, was carried out based on cultural, morphological, physiological and biochemical characteristics. Both the organisms were Gram-negative bacilli. In nutrient agar plates, L9 formed round, convex, opaque colonies with a cream colour pigmentation; whereas L12 formed irregular, convex, and opaque colonies with red colour pigmentation. In King’s B agar, after incubating for 48 h, L12 was found to produce green fluorescent pigment (Fig. 2). Both the isolates were motile and non-spore producers. The isolates were able to ferment lactose and utilize citrate. L9 is nitrate reducer and L12 was found to produce H\(_2\)S and liquefies gelatin. Based on the results, L9 was identified as *Pseudomonas nitroreducens* and L12 as *P. aeruginosa*.

The identification of isolates was confirmed by Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India and the cultures were deposited with accession numbers MTCC 10463 (L9) and MTCC 10462 (L12). The 16S rDNA sequences of the isolates were deposited in GenBank, NCBI and were assigned the accession numbers HQ271083 (L9) and HQ271084 (L12). Phylogenetic analysis of the

<table>
<thead>
<tr>
<th>No.</th>
<th>Isolates</th>
<th>% of anionic surfactant degraded*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L1</td>
<td>54.85±1.80</td>
</tr>
<tr>
<td>2</td>
<td>L2</td>
<td>27.57±0.40</td>
</tr>
<tr>
<td>3</td>
<td>L3</td>
<td>18.10±0.70</td>
</tr>
<tr>
<td>4</td>
<td>L4</td>
<td>68.32±0.5</td>
</tr>
<tr>
<td>5</td>
<td>L5</td>
<td>24.98±3.7</td>
</tr>
<tr>
<td>6</td>
<td>L6</td>
<td>13.01±0.10</td>
</tr>
<tr>
<td>7</td>
<td>L7</td>
<td>35.92±20</td>
</tr>
<tr>
<td>8</td>
<td>L8</td>
<td>64.42±0.40</td>
</tr>
<tr>
<td>9</td>
<td>L9</td>
<td>81.33±0.70</td>
</tr>
<tr>
<td>10</td>
<td>L10</td>
<td>42.05±0.10</td>
</tr>
<tr>
<td>11</td>
<td>L11</td>
<td>28.42±0.30</td>
</tr>
<tr>
<td>12</td>
<td>L12</td>
<td>81.81±0.80</td>
</tr>
<tr>
<td>13</td>
<td>L13</td>
<td>41.50±0.30</td>
</tr>
<tr>
<td>14</td>
<td>L14</td>
<td>72.70±0.28</td>
</tr>
<tr>
<td>15</td>
<td>L15</td>
<td>75.25±0.24</td>
</tr>
<tr>
<td>16</td>
<td>L16</td>
<td>59.42±0.45</td>
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<td>17</td>
<td>L17</td>
<td>40.51±0.40</td>
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<td>L18</td>
<td>23.99±0.40</td>
</tr>
<tr>
<td>19</td>
<td>L19</td>
<td>58.12±0.20</td>
</tr>
<tr>
<td>20</td>
<td>L20</td>
<td>64.17±0.30</td>
</tr>
</tbody>
</table>

*Values are average of three replications. Results represented as Mean ± SD.
Fig. 1 (a-f)—Secondary screening of the LAS degrading isolates using HPLC analysis: HPLC chromatogram of isolates L4 (a), L9 (b), L12 (c), L14 (d), L15 (e) and LAS control (f) after 7 d of incubation.
isolates was done based on the sequence generated in the present study. The selected isolates L9 and L12 were separated by a distance of 0.0112 bp. They were closely related to *P. aeruginosa* strain RM3 with a distance of 0.0242. Nearest neighbor of this group is *P. fulva* strain Hb-0202 by a distance 0.02695 (Fig. 3). The sequence identity of the isolates substantiates the biochemical identification results. In RAPD analysis, amplification of total genomic DNA from the both isolates showed distinct profiles. L9 formed 4 bands, while L12 formed 9 bands, of which 5 bands were distinct for L12. It was observed that both the isolates showed 50% similarity (Fig. 4).

The plasmids of the selected isolates were found to be about 8-10 kb in size (Fig. 5). To ascertain the role of plasmids in LAS degradation, different curing treatments were performed and curing frequency of plasmids was calculated (data not shown). The results show that application of acridine orange, an intercalating dye, along with elevated temperature of incubation (44°C) was effective in curing the plasmids of isolates.
After curing, the cured cells were selected by patch plate method. The plasmid curing was confirmed by agarose gel electrophoresis. The cured cells were plated on mineral salt agar with LAS as the carbon source. Even though the cells were able to grow in mineral salt with LAS, the colonies formed were very small compared to the mother colonies and required a longer incubation period for colony development.

In growth pattern analysis using LAS as sole source of carbon, the selected isolates showed a zigzag pattern of growth. When LAS degradation was analyzed continuously for 10 d, the maximum degradation was showed on 8th d by L9 and on 9th d by L12 (Fig. 6). However, when both the isolates were coinoculated as a consortium, the isolates were found incompatible and showed a low rate of degradation of LAS. Isolates L9 and L12 independently showed about 81% degradation of LAS but they showed only about 50% degradation on coinoculation. The HPLC analysis of the culture supernatant was done and it was found that isolates L9 and L12 showed about 99% reduction in selected peak area compared to control by the 15th d. At the same time, the longer carbon chain isomers were found to be resistant to degradation.

LAS at mass range of 600-12000 ppm were supplemented to the cultures of individual strain growing in broth medium in order to investigate the toxic effects of high surfactant concentration on bacterial growth. The growth expressed as CFU/mL was found to be proportionally suppressed with increase in the amount of surfactant present in culture (Fig. 7). At concentration 600 ppm L9 and L12 formed $3\times 10^6$ and $22.8\times 10^5$ CFU/mL, whereas the count reduced to $6\times 10^5$ and $11\times 10^5$ CFU/mL when the LAS concentration was increased to 12000 ppm. Though the count was reduced at high LAS concentrations, both the isolates were able to withstand very high concentrations of LAS.

In the presence of an alternate carbon source, such as, dextrose, sucrose, maltose and mannitol, both the selected isolates were found to display a diauxic growth and this in turn reduces the efficiency of LAS degradation (Fig. 8). The LAS degradation was found to reduce below 60% when an alternate carbon source was present in the growth media.

**Discussion**

Synthetic surfactants have become an inevitable ingredient both in household and personal hygiene products. Since anionic surfactants are essential as cleaning and processing chemicals, they are produced in large quantities and are mostly discharged after use into waste water treatment plants, or into coastal regions, estuaries, agricultural lands, seas and oceans. Thus they become a great hazard to the environment.

In the present study, 2 isolates were selected for LAS degradation and identified by employing biochemical and molecular biology techniques.
Both the isolates were found to be from the genus *Pseudomonas*. The isolate L9 was identified as *P. nitroreducens* (MTCC 10463) and the isolate L12 was identified as *P. aeruginosa* (MTCC 10462). Prats et al\textsuperscript{13} have reported that *Pseudomonas* was the most frequently used genus for LAS and alcohol etoxylate biodegradation studies.

RAPD analysis has been of great use in revealing intraspecific variations in bacteria and fungi\textsuperscript{14}. The RAPD technique utilized in the present investigation differed from the conventional RAPD in use of high annealing temperature and a 20-mer primer with codon degeneracy at several positions. This helped to generate more specific RAPD profile due to the highly specific binding of the primer sequence. In the present study, RAPD profile has shown a clear demarcation between the two isolates at molecular level. The quantitative analysis of the RAPD data using NT sys 2.02i software and the similarity study (using Jacquard coefficient) showed that selected isolates L9 and L12 were sharing 50% similarity at molecular level.

Plasmids are widely reported from xenobiotic degrading bacteria. Moreover, the significant role played by plasmids in detergent degradation has also been well recognized\textsuperscript{15}. Therefore, plasmid profile of the selected isolates was studied and they were found to harbour plasmids. Breen et al\textsuperscript{16} reported that the heterotrophs from waste water system showed twice the incidence of plasmids compared to the isolates from uncontaminated pond system. They also reported that isolates grown on LAS showed a higher incidence of plasmids (44-46%) than the overall heterotrophic populations.

To prove the role of plasmids in LAS degradation, the selected isolates were subjected to plasmid curing. These isolates produced pinhead colonies in mineral salt agar with LAS and showed less vigour. This shows that the genes encoded in the plasmid DNA may not be the exclusive reason for the surfactant utilization character of the isolates. In other words, as reported by Ojo and Oso\textsuperscript{17}, the genetic information for detergent hydrocarbon utilization may not be absolutely responsible for the surfactant biodegradation trait. In a similar study, Kostal et al\textsuperscript{18} reported that the plasmid cured strains of *Pseudomonas* C12 were capable of slow feeble growth on longer alkyl chains of 12-16 carbons, whereas they could not grow in liquid medium. These reports supported the view that genomic and plasmid DNAs together are involved in LAS biodegradation. This is in contradiction to the findings of Yeldho et al\textsuperscript{19}. According to them, the SDS degrading bacteria *P. aeruginosa* S7 harbours a single 6 kb plasmid, which is responsible for the SDS degradation.

Microbial consortium has been found to be a better surfactant degrader. Sigoillot et al\textsuperscript{20} have reported that mixed cultures of different bacteria could dramatically improve the biodegradation potential. However, in the present study, co-culture of the isolates L9 and L12 resulted in reduction in the rate of LAS degradation. This may be because of the incompatibility of the strains. According to Hosseinia et al\textsuperscript{21}, no significant changes were observed in the biodegradation of SDS when *P. betelli* and *Acinetobacter johnsoni* strains were co-inoculated.

Surfactants are the principal ingredient of detergents and, hence, their concentration in the detergents remains comparatively high. In the present study, ability of the selected isolates to withstand higher concentration of LAS was tested by selecting a range of concentrations between 600 to 12000 ppm. The selected isolates showed resistance to high LAS concentrations even though the colony count was comparatively low. However, results contradictory to the present has also been reported earlier. A mixed culture of *A. calcoaceticus* and *Pantoea agglomerans* showed better resistance to 700 ppm LAS compared to either bacterial strain alone\textsuperscript{22}. They also reported that mixed culture could degrade higher concentration of SDS (3000-8000 ppm), even though there is an inverse relationship between detergent concentration and extent of degradation.

The LAS degradation efficiency of the selected isolates was studied in presence of an easily available carbon. In carbon excess condition, the alternate carbon source repressed LAS degradation resulting in diauxic growth; whereas under carbon limited condition, the LAS utilisation was high. It may be because the assimilation of dextrose prior to LAS inhibited the production of relevant enzymes for LAS degradation. Under optimal conditions, LAS degrading organisms were reported to exhibit a diauxic or diphasic growth in a medium where acetate and LAS were present\textsuperscript{23}. Wong et al\textsuperscript{24} have also observed that increasing concentration of glucose although showed better growth rate
of *Burkholderia cocovenanens* but reduced the degradation of phenantherene significantly. Bucheli-Witschel *et al.* have also reported that the presence of succinate repressed benzene consumption by *Ralstonia pickettii*. Similar results were also obtained in the present study.

*Pseudomonas* spp., ubiquitous in soil and water, are of considerable scientific and technological importance and comprise a taxon of metabolically versatile organisms capable of utilizing a wide range of simple and complex organic compounds. They are known to be involved in biodegradation of natural or man-made toxic chemical compounds. The characterization of such xenobiotic degrading organisms is of great application in developing green technology for pollutant cleanup.

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**References**