ORIGINAL ARTICLE

Isolation and Characterization of Diesel Oil-Degrading *Pseudomonas aeruginosa*

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Abstract

Objective: Diesel oil-degrading bacterium *Pseudomonas aeruginosa* was isolated from diesel oil-polluted soil sample from various locations of diesel loco sheds, Vijayawada City, Andhra Pradesh, India. **Materials and Methods:** Two different strains having specific ability to degrade and utilize diesel oil for their growth were isolated. The isolated bacterium was identified as *P. aeruginosa* based on its 16S rRNA typing as well as various morphological and physiological characteristics. **Results:** On the basis of molecular analysis, these strains were belonged to genus *Acinetobacter*. Antibiotic sensitivity of the isolates was also checked against different antibiotics of which they showed resistance against amoxicillin, levofloxacin, minocycline, chloramphenicol, and sulfadiazine. **Conclusion:** This study might be an important step toward the bioremediation of diesel oil contaminated soil and waste water.

Keywords: *Acinetobacter*, loco sheds, diesel oil, amoxicillin, minocycline, sulfadiazine

INTRODUCTION

Hydrocarbon contamination happens in various ways such as accidents during fuel transportation by trucks and ships, leakage of oil from underground storage tanks, or during extraction and processing of oil.[1] These contaminations can be treated by several methods including physical, chemical, and biological treatment. During biological cleaning up, hydrocarbon-degrading bacteria emulsifying hydrocarbons by producing biosurfactants are used.[2] Hence, isolation and identification of biosurfactant producing and hydrocarbon-degrading bacteria are pivotal for effective bioremediation of hydrocarbon contaminated surface waters. Hydrocarbon contamination is one of the most important pollution sources around the world.[3] Due to oil spills, hydrocarbon pollution becomes a global problem, especially in developing and industrialized countries.[4] Petroleum is used as a conventional energy source even though it has significance as a global environmental pollutant. Diesel oil is a mixture of low-molecular-weight compounds, which are usually more toxic than long-chained hydrocarbons, and more bioavailable and more soluble than the long-chained hydrocarbons.[5] Because of its harmful effects, diesel oil should be treated and removed from the environment. Bioremediation of petroleum hydrocarbons has been proposed as an effective, economic, and environmentally friendly technology.[6] Microorganisms degrading the components of petroleum hydrocarbons can be isolated from oil contaminated sites.[7] It was found that wide range of bacteria and fungi can use n-alkanes as sole carbon and energy source. However, bacteria have the major share in hydrocarbon degradation.[8]

MATERIALS AND METHODS

Collection and samples processing

All soil samples as listed in Table 1 are collected from different areas located in loco sheds, Vijayawada City, Andhra Pradesh, India, and they were sealed carefully with zip lock covers to avoid contaminants and dust.[9]

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Bacterial isolation

Bacterial isolation started by 9 mL of Luria Broth (LB) medium (HiMedia) added with soil sample as oil source. Samples were incubated at 37°C in an orbital shaker. After 24 h, 1 mL of the first growth was transferred to test tubes containing 9 mL of LB medium and incubated under the same conditions described above. To obtain pure colonies, the highest dilution was placed on LB agar (LB medium with agar 1.5%) and incubated at 37°C for 24 h. Subsequently, each colony was used to inoculate three test tubes containing 9 mL of LB medium, which were monitored for oil degradation. *Pseudomonas desmolyticum* NCIM 2112 was used as positive control. Nine milliliters of LB medium without bacteria were incubated under the same conditions to evaluate the diesel oil degradation background.[10]

Antimicrobial activity

Antimicrobial activity of *Pseudomonas aeruginosa* strains isolated from four different locations against amoxicillin, levofloxacin, minocycline, chloramphenicol, and sulfadiazine antibiotics[11] was tested [Table 2].

16S rRNA typing

Genomic DNA was extracted from overnight incubated bacterial cultures in Luria Bertani LB-broth at 37°C with 120 rpm. Genus identification for bacteria exhibiting oil-degrading activity was done by polymerase chain reaction (PCR) amplification and sequencing of the 16S rRNA gene.[12] The product was purified using GeneJET extraction kit (Fermentas) and sequenced using 27f and 1522r as forward and reverse primer, respectively (HiMedia). The amplified products were applied into 1% agarose gel and submitted to electrophoresis. DNA was stained with ethidium bromide and bands visualized with an imaging system (UVI-TECH; Figure 1).

Table 1: Summary of soils located at loco sheds, Vijayawada

S. No.	Type of soil	Location	pH
1	Black cotton soils	Diesel works	5.5
2	Sandy clay loams	Power systems	6.5
3	Red loamy soils	Lubricants house	4.5

Sequence analysis

16S rRNA gene sequences were assembled with the CAP3 Sequence Assembly Program. DNA sequences were analyzed by basic local alignment search tool. Species identification was based on maximum score, identity, and coverage values [Table 3]. Sanger's dideoxy sequencing was performed for the Act2 sample [Figure 2].[13]

RESULTS AND DISCUSSION

Black cotton soils and sandy clay loams have highest pH value and red loamy soils show lowest pH value, respectively.

Minocycline antibiotic in sandy clay loam soil showing maximum inhibitory activity by Act1 strain followed by amoxicillin antibiotic and sulfadiazine in sandy clay loamy soil in Act1 and Act2, respectively.

The 16S rRNA gene sequences from the two diesel oil-degrading isolates Act1 (JQ345720) and Act2 (JQ345721) were similar to the 16S rRNA genes from *P. aeruginosa baumannii* and *P. aeruginosa lwoffii*, respectively.

Separation patterns of PCR-amplified portions of 16S rRNA genes on ethidium bromide-stained agarose gels. A mixture of PCR products derived from two *P. aeruginosa* strains was applied on each gel as a standard to allow gel-to-gel comparisons.

Direct 16S rRNA gene sequencing of polymicrobial samples results in mixed chromatograms containing two or more fluorescent signals in positions where the 16S rRNA genes differ for the bacteria present in the sample.

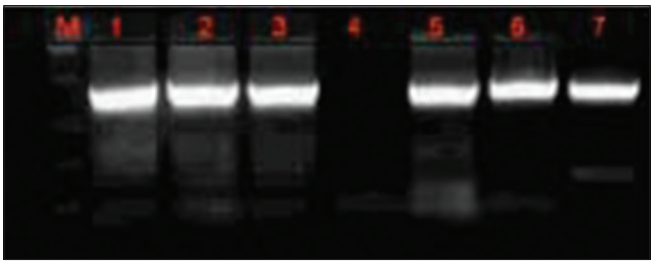

Figure 1: Polymerase chain reaction amplification of 16S rDNA of Act1 and Act2. M: Marker; 1, 2, and 3: Act1; 5, 6, and 7: Act2

Table 2: Antimicrobial activity of bacterial strains isolated from three different soil samples against amoxicillin (A), levofloxacin (L), minocycline (M), chloramphenicol (C), and sulfadiazine (S) antibiotics

Strain name	Black cotton soils					Sandy clay loams					Red loamy soils				
	A	L	M	C	S	A	L	M	C	S	A	L	M	C	S
Act1	15	16	18	22	18	20	18	22	15	16	12	15	18	16	13
Act2	14	13	17	16	15	22	15	24	17	19	13	11	15	12	14

Table 3: Local alignment results for oil-degrading bacterial isolates 16S rRNA sequences. Sequences were compared to those deposited at the National Center for Biotechnology Information using the regular BlastN algorithm available at http://www.ncbi.nlm.nih.gov/BLAST

Isolate (acc. no.)	Species/strain (accession no.)	Query coverage	Gaps	Max identity (%)
Act1 (JQ345720)	*Pseudomonas aeruginosa baumannii*	100%	18/245 (2%)	825/845 (97%)
Act2 (JQ345721)	*Pseudomonas aeruginosa lwoffii*	100%	18/245 (2%)	810/845 (96%)

Organisms with sequences similar to those of isolates Act 1 (JQ345720) and Act 2 (JQ345721) are listed on the second column. Values for the parameters total score, query coverage, and maximum identity and GenBank access codes are displayed in parentheses

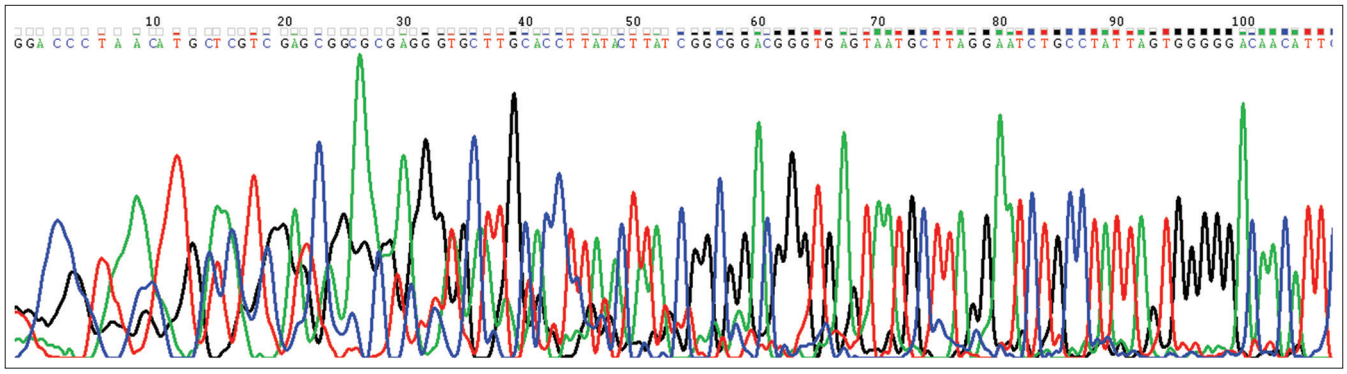

Figure 2: The chromatogram was obtained by direct 16S rRNA gene sequencing of *Pseudomonas aeruginosa lwoffii*

CONCLUSION

Direct 16S rRNA gene sequencing has several advantages to analyze chromatograms from samples containing different bacteria, could increase its usefulness, and make it relevant for a broader range of infections and samples**.** Because of the need to perform susceptibility testing, nucleic acid-based identification cannot replace cultivation. Cultivation is also more sensitive than PCR-based detection when samples contain viable bacteria. In sequencing, the sensitivity is limited by the number of cycles that can be run in the first PCR before contaminant DNA in the reagents gives a false-positive result. Our present study follows the isolation of diesel oil-degrading bacteria from the contaminated soil and was screened for their oil-degrading capacity and antibiotic susceptibility.

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CONFLICTS OF INTEREST

Authors do not have any conflicts of interest.

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