UV Treatment Increases Hydrocarbon Degrading Potential of Bacillus spp. Isolated from Automobile Engines

Debjit Borah and R.N.S. Yadav

Centre for Studies in Biotechnology, Dibrugarh University, Dibrugarh-786004, India

Abstract: To investigate the presence of hydrocarbon degrading microorganisms in various parts of automobile engines and to enhance the degradation potential of the best isolate by exposing to UV irradiation. Hydrocarbon degrading microorganisms were isolated by inoculating hydrocarbon residues collected from various parts of automobile engines on BH medium. The potential of the best isolate could successfully further increased by exposing to UV irradiation and the mutation was confirmed my performing RAPD. The study shown *Bacillus* spp. with maximum hydrocarbon degrading potential followed by *Staphylococcus* spp. *Streptococcus* spp. and *Micrococcus* spp. The potential of the *Bacillus* isolate was further increased by UV irradiation and the RAPD analysis confirmed the mutant showing 2.8 units of linkage distance from the wild type. The results of the study are useful for developing eco friendly bioremediation strategies for soil contaminated with petroleum oil.

Key words: Bioremediation • Bacillus Spp • Staphylococcus Spp • Streptococcus Spp • Mutation

INTRODUCTION

Interest in the microbial biodegradation of pollutants has intensified in recent years as humanity strives to find sustainable ways to cleanup contaminated environments. These bioremediation and biotransformation methods endeavor to harness the astonishing, naturally occurring, ability of microbial xenobiotic metabolism to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radio nuclides and metals [1]. Soil contamination (soil pollution) is caused by the presence of xenobiotic (human-made) chemicals or other alteration in the natural soil environment. This type of contamination typically arises from the rupture of underground storage tanks, application of pesticides and percolation of contaminated surface water to subsurface strata, oil and fuel dumping, leaching of wastes from landfills or direct discharge of industrial wastes to the soil. An oil spill is the release of a liquid petroleum hydrocarbon into the environment due to human activity and is a form of pollution. The term often refers to marine oil spills, where oil is released into the ocean or coastal waters. The oil may be a variety of materials, including crude oil, refined petroleum products (such as gasoline or diesel fuel) or by-products, oily refuse or oil mixed in waste [2].

Bioremediation is a process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition [3]. In the present study, some of the indigenous hydrocarbon degrading microorganisms were isolated from various parts of automobile engines and an effort was made to enhance the hydrocarbon degradation potential of the best isolates by treating with UV irradiation.

MATERIALS AND METHODS

Isolation and Screening of Hydrocarbon Degrading Microorganisms: Hydrocarbon residues from various parts of automobile engine were collected and inoculated on BH (Bushnell and Haas) agar plates (composition g/lit: MgSO$_4$-0.2, CaCl$_2$-0.02, KH$_2$PO$_4$-1.0, K$_2$HPO$_4$-1.0, NH$_4$NO$_3$-1.0, FeCl$_3$-0.05, agar-agar-20.0, pH-7.0 at 25°C) and incubated (Sciegenics Biotech India (P) Ltd) at 36°C.
for 36 hrs. Bacterial isolates obtained were sub cultured and were again inoculated in BH broth supplemented with used engine oil as a sole carbon source, taken in 250 ml Erlenmeyer flask and allowed to incubate at 32°C for 72 hrs at 120 rpm on rotary shaker (Labtec Korea Ltd.). An increase in O.D. will indicate the increase in cell density. All the media were purchased from HiMedia India Pvt. Ltd.

**Identification of the Isolates:** The bacterial isolates were identified on the basis of various staining techniques and their biochemical characteristics prescribed by Bergey’s Manual of Systematic Bacteriology [4]. The preliminary identification of the bacterial isolates was done by performing various staining techniques and observing the cell morphology under light microscope (Leica ATC2700). Various biochemical tests were performed to identify the bacterial isolates using commercially available test kits (KB001 HiMViC biochemical test kits, KB002 Hi-Assorted biochemical test kits, KB004 HiStaph identification kits, KB005A HiStrep identification kits and KB013TM HiBacillus identification kits purchased from HiMedia Laboratories Pvt. Ltd. India).

**To Enhance the Degradation Potential of the Potential Isolates by Uv Irradiation:** The best isolates were exposed to UV lamp (16W-Phillips India Pvt. Ltd.) for different time duration: 10, 20 and 30 seconds at a distance of 30 cm and the culture plates were incubated at 36°C for 24 hrs. The mutant bacteria was identified by doing RAPD (randomly amplified polymorphic DNA) using a random primer OPA16 (5’ AGCCAGCGAA). RAPD amplification was performed in a final volume of 25 µl containing 10X PCR buffer, 2mM MgCl2, 100 µM each of dNTPs, 25 ng genomic DNA, 1.0 unit of Taq polymerase and 0.5 µM of primer. Reaction was carried out in a thermo cycler (Biored C1000 Touch™) with 45 cycles of amplification at 95°C for 5 min (preheating), template DNA denaturation at 94°C, primer annealing at 35°C for 1 min, primer extension at 72°C for 2 min and holding temperature at 4°C.

The mutant bacteria was inoculated in BH medium supplemented with used engine oil to see any possible changes in its hydrocarbon degradation potential. The entire chemicals used in the study were purchased from Merck Millipore India Pvt. Ltd.

**RESULTS**

Four types of hydrocarbon degrading microorganisms viz. *Streptococcus spp. Staphylococcus spp. Micrococcus spp.* and *Bacillus spp.* were isolated from various parts of automobile engines. An increase in cell density and CFU in BH medium supplemented with used engine oil as their sole carbon source indicates an increase in oil degradation [5]. *Bacillus spp.* was found to show maximum potential to degrade used engine oil followed by *Staphylococcus spp.* characterization of the degradation potential of the two isolates was done by allowing them to grow on the medium containing different concentration of used engine oil (Table 1 and Figure 1).
The growth of all the isolates was found to be increasing with the increase in the concentration of used engine oil as they are using it as their carbon source. However the growth declines after a certain concentration of used engine oil, which may be may be due to an increase in the toxicity caused with the increase in the concentration of the used engine oil. After inducing mutation by exposing to UV irradiation, an increase in hydrocarbon degradation potential was observed in case of the isolate CB₄ (Table 2), which may be due to the mutation caused at a critical site that may have caused an
increased synthesis of hydrocarbon degrading key enzymes (cathechol 2,3 dioxygenase (C23O) and monoxygenase etc.) [9].

In conclusion, though most of the literacy [2, 3, 6, 7] showed *Pseudomonas spp.* and *Acinetobacter spp.* having the best potential to degrade used engine oil but the present study, *Bacillus spp.* showed the maximum potential to degrade used engine oil and the mutation was also successful to increase its hydrocarbon degradation potential.

REFERENCES