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BIODEGRADATION OF THE HYDROCARBON COMPONENTS OF THE CONTAMINATED SOIL BY VARIOUS *BACILLUS* SPECIES

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ABSTRACT

In environmental biotechnology, evaluation of microbial communities relevant to their genetics and ecological perspectives has significant concern. Microorganisms, when exposed to unusual factors in their micro environment, become able to adapt these unusual factors or components by manipulating the specific genes and metabolic pathways. Environment polluted with hydrocarbons, allows bacteria to enhance their metabolic capabilities by horizontal/vertical gene transfer, mutation and adaptation to ecological stresses. So, this property of microorganisms can be employed to make pollutant-free environment. The basis of this research is bioremediation of hydrocarbons in contaminated soil. For this purpose, quantitative analysis was carried out in order to examine the potential of isolated *Bacillus* species for hydrocarbon consumption. Among all strains, HRA-1 and HRA-4 exhibited hydrocarbon absorption ability. Isolate HRA-5 displayed xylene degradation while HRA-1 took up benzene efficiently. This study explores the importance of hydrocarbon utilizing bacteria (HUB) that could be suggested for bioremediation purposes.

Keywords: *Bacillus*, Biodegradation, Diesel, Hydrocarbon, Oil.

INTRODUCTION

One of the major ecological problems is hydrocarbon pollution which is linked with petrochemical business. Accidental oil spills and unusual exposure of other petroleum items to environment has become global concern especially in industrially revolutionized countries. A great attention of environmental biologists has been raised towards marine environment, as Seas and Oceans are most commonly affected sites of dramatic disposal of hydrocarbon based petroleum products, which is the crucial threat for marine life (Mithal and Singh, 2009). On the other hand, activities associated with petrol pumps and mechanic shops also lead to the oil spillage in soil, thus also contaminating soil environment and ground water with hydrocarbons. Petroleum items vary from petrol, lubricants (engine oil) to diesel. These products are mainly comprised of metals and heavy polycyclic aromatic hydrocarbons (PAHs) which could promote health hazards like carcinogenicity, neurotoxicity and mutagenicity (Daniel *et al*., 2017).

A wide range of mechanical techniques are available for the treatment of hydrocarbon contaminated ecological niches, in order to expel out hydrocarbons. These methods include dumping, safe landfilling, vapor and solvent extraction, soil flushing and washing, stabilization and solidification (Daniel *et al*., 2017).But the implementation of these methods is whether costly or does not allow the complete removal of hydrocarbon pollutants. Bioremediation is the most promising approach of environmental biotechnology that uses microorganisms to detoxify hydrocarbons contaminants into eco-friendly products (Vidali, 2001)**.** Bioremediation works fundamentally on biodegradation that transform complex natural contaminants in to less complex and soluble compounds such as carbon dioxide, water and inorganic by products with the help of microbes i.e. bacteria, fungi, yeasts, molds, algae, etc. Microorganisms utilize hydrocarbons as a source of carbon and energy and turn them in to water and gases that return back in to ecosystem (Das and Chandran, 2011).

Additionally, the metabolic pathways that hydrocarbon degrading bacteria carry out can be either aerobic or anaerobic. Aerobic neutralization of hydrocarbons requires oxygen as essential electron acceptor (Van Hamme *et al*., 2003). While anaerobic degradation needs an alternative electron acceptor for example nitrate and sulfate (Widdel and Rabus, 2001).However, for maximum and effective assimilation of hydrocarbon by microorganisms, various physiochemical factors are important (Roberts, 1992). They contain oxygen, temperature, pH, salinity, light, carbon, nitrogen and phosphorus sources. These parameters have direct influence on hydrocarbon degradation ability of microbes (Palmisano *et al*., 2000; Kerr and Capone, 1998; Munoz *et al*., 2003).Hence optimization of these factors provokes degradation of hydrocarbons at faster rates.

A widespread hydrocarbon degrading microflora are present in nature, therefore hydrocarbon utilizing bacteria (HUB) are the most abundant and preferred for bioremediation applications as compared to other microorganisms, because of their easy cultivation strategies. The most dominant hydrocarbon utilizing bacterial species *are*

Pseudomonas, Rhodococcus, Mycobacterium, Bacillus, Acinobacter, Providencia, Flavobacter, Corynebacterium, Streptococcus and *Serretia* (Bhattacharya *et al*., 2002).

The main objective of bioremediation plan should be the generation of optimum conditions for bacterial growth and activities that would further induce the biodegradation of hydrocarbons (Balba *et al*., 1998).So, the goal of this study was to screen hydrocarbon degrading bacteria from hydrocarbon contaminated soil and to explore their potential to accumulate different hydrocarbon containing compounds.

MATERIALS AND METHODS

Sample collection

Soil samples were aseptically collected from hydrocarbon polluted sites of mechanic workshops, oil changer shops, car washing area and soil contaminated with generator oil, Karachi, Pakistan in sterile bags and stored at 4°C till use.

Isolation of hydrocarbon utilizing bacteria

Soil samples were inoculated in Nutrient broth containing diesel and generator oil as substrate in separate flasks. Inoculated flasks were incubated at 37°C for 3 days. Serial dilutions of the inoculated broth were prepared for up to 10^{-6} in normal saline. 100 µL of 10^{-6} dilution was placed on nutrient agar plates containing 1 % diesel and oil separately using spread plate method. The plates were then incubated at 37°C for 24 hours.

Identification of hydrocarbon utilizing bacteria

Isolated colonies of different morphology were picked and further purified on Nutrient agar plates containing 1 % diesel and oil. Colonies that showed zone of hydrolysis on diesel and oil were selected. Colony morphology, Gram's staining and other biochemical tests of the pure culture were performed. The selected cultures were named as HRA-1, HRA-2, HRA-3, HRA-4 and HRA-5.

Biomass determination of isolated cultures on various hydrocarbons

The entire selected five *Bacillus* species were inoculated separately in Nutrient broth containing 1% (v/v) diesel and generator oil as carbon source. After inoculation flasks were incubated at 37°C for 24, 48 and 72 hours. Biomass of the selected cultures was determined by taking Absorbance at 600 nm against the un-inoculated Nutrient broth containing hydrocarbons.

Estimation of hydrocarbon utilization on minimal media

Bushnell-Hass mineral salt media was prepared to determine the utilization of hydrocarbon by the selected bacterial isolates. For each culture, two sets of 50 ml of BH mineral salt media were prepared. 100 µL of benzene and xylene were added separately in BH minimal salt media. Each culture was inoculated in BH medium containing benzene and xylene and incubated at 37°C for a week. The growth was estimated by taking absorbance at 600 nm.

RESULTS AND DISCUSSION

Characterization of hydrocarbon utilizing bacteria (HUB)

Among all isolated bacterial strains, five best hydrocarbon degrading bacteria namely, HRA1, HRA2, HRA3, HRA and HRA5 were selected on the basis of hydrocarbon assimilation via preliminary qualitative screening. For taxonomic identification of HUB, studies on cultural, cellular and biochemical features of each bacterial isolates were carried out. According to it, all hydrocarbon utilizing bacteria were identified as *Bacillus* species.

Biodegradation potential of diesel and generator oil by hydrocarbon utilizing bacteria (HUB)

Although diesel oil constitutes an important combustible fuel for engines and vehicles, but its unintentional release in environment damages terrestrial and marine ecosystems. The requirement of nature friendly cleaning of these pollutants prompts the development of advances in bioremediation process elucidated that the active petroleum hydrocarbon degraders were microorganisms in contaminated ecological sites (Nwinyi *et al*., 2014; Leahy and Cowell, 1990). In present study, the bacterial growth was evaluated by recording changes in absorbance at 600nm in the presence of 1% (v/v) diesel. It was observed that growth of all isolates increased with the increase in time. Strain HRA-4 showed highest growth rate which indicates that strain HRA-4 poses great capability to utilize hydrocarbon **(Fig. 1).** These findings support with the previous data on hydrocarbon degradation (Kim and Picardal 2000; Nwinyi, 2011).

For the degeneration of generator oil by isolated *Bacillus* species, it was noticed that strain HRA-1 demonstrated remarkable decomposition of hydrocarbons as compared to other strains, though other *Bacillus* species were also able to use hydrocarbons as sole source of carbon **(Fig, 2).**

Numerous researches on the bioremediation of hydrocarbon pollutants by *Bacillus* species in extreme surroundings are available. Ijah and Antai (2003) elaborated that the most dominant bacterial species with crude oil utilizing capacity in highly contaminated soil environment is *Bacillus*. The presence of endospore makes *Bacillus* species highly resistant to high concentration of hydrocarbons in soil.

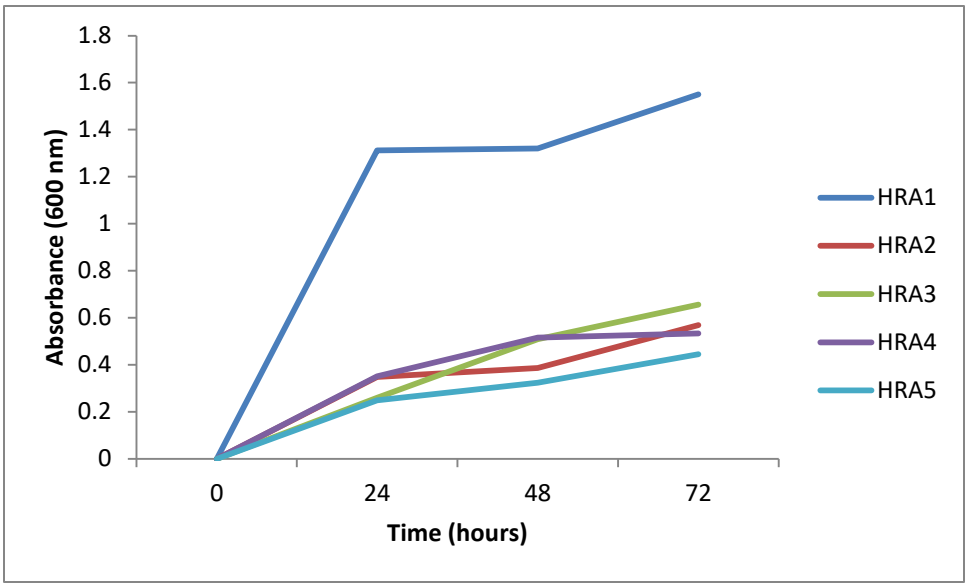

Fig. 1. Growth of isolated bacterial strains in the presence of diesel.

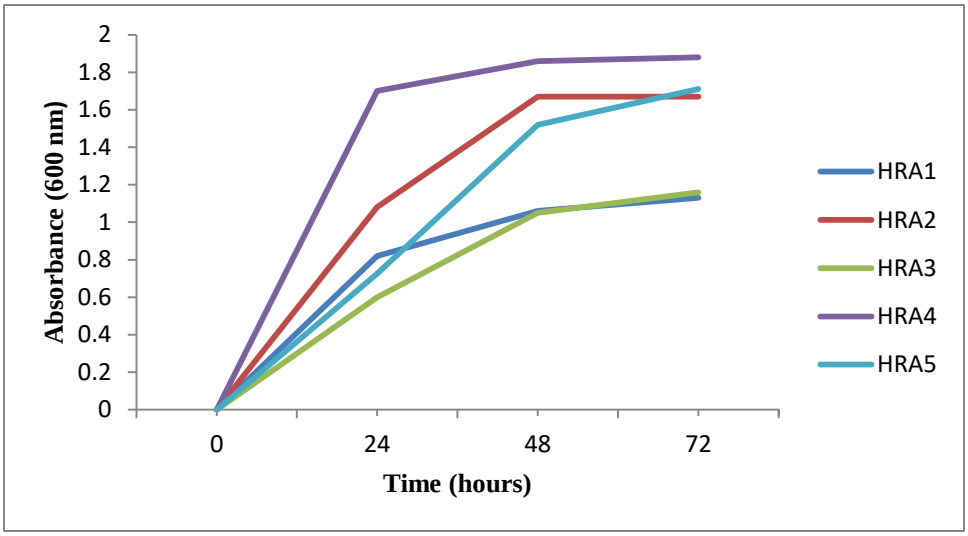

Fig 2. Growth of isolated bacterial strains in the presence of generator oil.

Utilization of benzene and xylene on BH minimal salt media by isolated *Bacillus* species

HR-1 was found to be potent degrader for benzene as compared to other isolated strains **(Fig. 3)** while HRA-5 was capable to degrade xylene **(Fig. 4)** although other strains also degraded xylene and benzene in considerable amount. These both strains could be an ideal tool for efficient cleaning of benzene and xylene in different ecological niches. Singh *et al* (2009) described the benzene consumption by *Bacillus* species under aerobic and anaerobic conditions. *Pseudomonas* species have also been reported for bioremediation of BTX (Benzene, Toluene, Xylene) (Otenio *et al*., 2005)

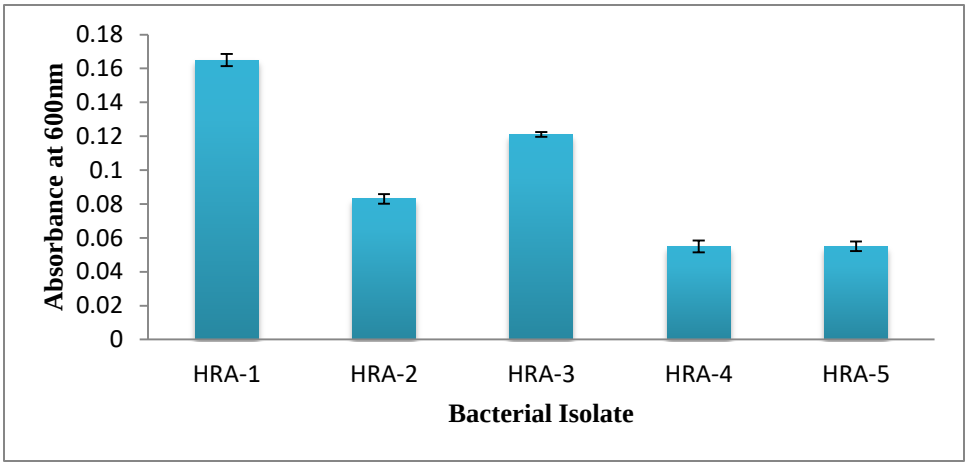

Fig 3. Utilization of Benzene by bacterial isolates.

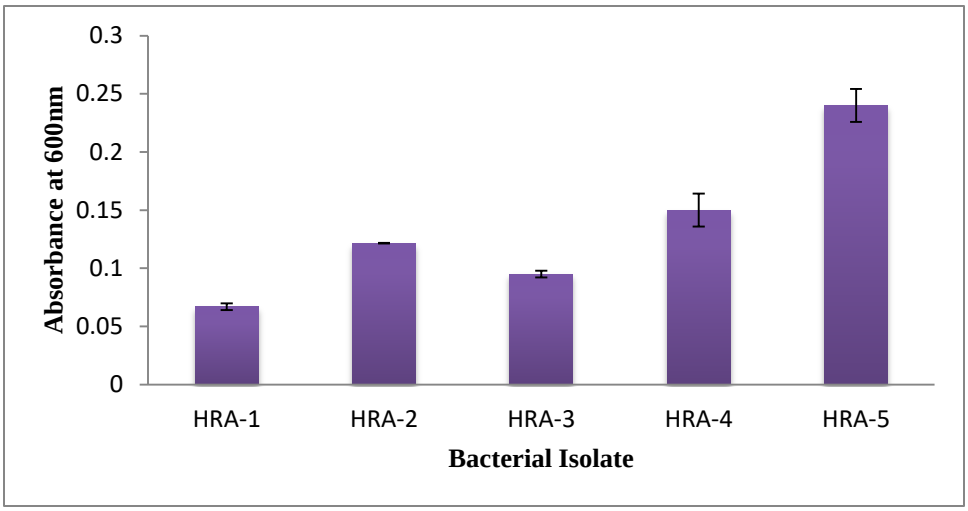

Fig 4. Utilization of Xylene by bacterial isolates.

Conclusion

Bioremediation of hydrocarbon pollutants is a useful strategy to clean up natural environment in eco-friendly manner. Current work is based on hydrocarbon removal from contaminated soil by isolated *Bacillus* species. Strains HRA-1, HRA-4 and HRA-5 showed effective potential for hydrocarbon decomposition.

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