Isolation and Characterization of Hydrocarbon Degrading Bacteria from Petrol Contaminated Soil

Talat Yasmeen Mujahid, Abdul Wahab*, Safia Hashim Padhiar, Syed Abdus Subhan, Muneera Naz Baloch and Zaid A. Pirzada

Department of Microbiology, University of Karachi, Karachi-75270, Pakistan

Abstract: Hydrocarbon degrading bacteria were isolated from the petrol contaminated soil of Karachi to determine their biodegradation capabilities of aromatic hydrocarbons such as xylene, phenanthrene, naphthalene, biphenyl and anthracene. Twelve bacterial strains were isolated by culture enrichment technique in Bushnell Hass medium in the presence of petrol. Hydrocarbon degradation capabilities of bacterial strains were assessed by means of enumeration using spread-plate technique. Current study revealed that all of the twelve isolated bacterial strains were able to degrade aromatic hydrocarbons, particularly *Pseudomonas* sp. SA044, degraded all the tested five aromatic hydrocarbons while *Burkholderia* sp., *Ralstonia* sp., *Stenotrophomonas* sp., *Micrococcus* sp. and *Staphylococcus* sp. degraded three or more aromatic hydrocarbons. Naphthalene and phenanthrene were the most degraded aromatic hydrocarbons.

Keywords: Polyaromatic hydrocarbons (PAHs), biodegradation, bioremediation, petrol contaminated soil, enumeration, Bushnell Hass (BH) medium.

1. INTRODUCTION

Nowadays pollution is considered as one of the major problems of the world which could be either organic or inorganic in nature. Quantitatively, the organic pollutants of most concern are hydrocarbons in various forms. The most common are petroleum derivatives which includealkanes and other aliphatic and aromatic compounds and other minor constituents [1, 2].

Hydrocarbons enter into the environment through waste disposal, accidental spills, as pesticides and *via* losses during transport, storage, and use and their accumulation in the environment causes serious problems. At present, it is widely recognized that contaminated land or water systems are a potential threat to human health. Awareness of this reality has led to international efforts to remediate many of these sites, either as a response to the health risks or to control the detrimental effects on the environment caused by contamination aiming the recovery of the contaminated sites. Over the years, many cleanup methods have been developed and applied [3].

Current physical and chemical treatments of wastes are generally expensive and are not able to remove trace quantities of pollutants [4]. Biodegradation is a major mechanism that removes pollutants from the environment [5, 6] because biodegradation by microorganisms is more favorable than chemical treatment for dealing with oil pollution since the microbes modify crude oils in beneficial ways and the end products are environmentally safe to all living things. Hydrocarbon degradation by microorganism has been shown to produce non-toxic products such as water and carbon dioxide [7].

Hydrocarbons are considered to be of biological origin [8, 9] and it could be present in various forms. On the basis of structure it could be aliphatic hydrocarbons and aromatic hydrocarbons. Aromatic compounds are benzene which includes mono or polycyclic hydrocarbons (PAHs). PAHs consist of two or more than two fused benzene rings which are arranged in linear, angular or clustered form. PAHs are found naturally in the environment due to natural and anthropogenic activities [10]. PAHs are potential threat to the environment due to their toxic, mutagenic, and carcinogenic properties [11]. Moreover the ecotoxicity of 16 PAHs have been listed as of great concern by the US Environmental Protection Agency [12, 13].

It is very difficult to remove PAHs from the environment due to their high hydrophobicity which increases with increasing molecular weight and it results in higher toxicity and longer persistence in the environment 12]. But there are a variety of microorganisms (bacteria and fungi) that are capable of degrading certain PAHs therefore there is a significant interest in studying microorganisms in contaminated sites as a mean for bioremediation [14].

In present study the hydrocarbon degrading bacterial strains were isolated from petrol contaminated soil of Karachi and the isolated bacterial strains were

*Address correspondence to this author at the Department of Microbiology, University of Karachi, Karachi-75270, Pakistan;
E-mail: abwahab@uok.edu.pk

ISSN: 1814-8085 / E-ISSN: 1927-5129/15

identified and checked for their biodegradation capabilities for aromatic hydrocarbons (xylene, phenanthrene, naphthalene, biphenyl and anthracene) which are deleterious for both biotic and abiotic flora by means of enumeration using spread plate method.

2. MATERIALS AND METHODS

Sample Collection

Soil samples were used to isolate hydrocarbon degrading bacteria and the samples were collected from two different petrol pumps of Karachi in clean, sterile plastic bags and kept at 4 ^{o}C until they were proceed for further manipulations.

Chemicals and Media

The hydrocarbon substances used in this study were xylene, phenanthrene, naphthalene, biphenyl and anthracene and the organic solvents in which hydrocarbons dissolved were ether [15, 16], hexane [17]. The media used were Bushnell Hass (BH) medium (0.2 gm of $MgSO_4$, 0.02 gm of $CaCl_2$, 1.0 gm of KH_2PO_4, 1.0 gm of K_2HPO_4, 1.0 gm of NH_4NO_3, 2 drops of $FeCl_3$(0.5 M) in 1000 mL of distilled water) [11, 18] and nutrient agar for isolation and screening of hydrocarbon degrading bacteria.

Isolation and Screening of Bacteria

Hydrocarbon degrading bacteria were isolated from soil samples using BH medium containing petrol as a sole source of carbon. 1.0gram of soil was introduced in 250 mL Erlenmeyer flask containing 100 mL of BH medium and 100 µL petrol [11] and incubated at 37 ^{o}C for one week. After one week 1.0 mL of sample was transferred into another 250 mL Erlenmeyer flask in which 100 mL fresh BH medium and 100 µL petrol was present and further incubated for one week. At the end of second week samples were serially diluted up to 10^{-6} dilutions. Then 0.1 mL from 10^{-6} and 10^{-5} dilutions spread on BH agar. The plates were incubated at 37 ^{o}C for 5 to 7 days. Mixed colonies were obtained and each of the selected colonies was grown on BH agar plate in the presence of petrol and stored at 4 ^{o}C for further studies.

Identification of Isolates

Pure colonies were identified and characterized on the basis of their morphological characteristics and biochemical properties according to the identification scheme of Bergey's Manual of Determinative Bacteriology [19, 20].

Enumeration of Hydrocarbon Degrading Bacteria

Hydrocarbon degrading bacterial strains were enumerated by counting the CFU/mL using spread plate method on nutrient agar plate [21, 22]. Loopful of 24 hours old bacterial cultures were inoculated into 30 mL BH medium with 1 % xylene and ethereal solution of phenanthrene, 0.5 % ethereal solution of naphthalene, biphenyl and 0.5 % anthracene solution (dissolved in hexane) and left the flasks opened near the flame in hood to allow evaporation of organic solvents and were incubated 35 ^{o}C in an orbital shaker operated at 150 rpm for 8-16 days. The growth of bacteria was monitored from 0-8th day at an interval of 4 days by making serial dilutions in physiological saline up to 10^{-3} and 0.1mL from 10^{-2} and 10^{-3} were spread on nutrient agar plates. After 24 hour of incubation at 37 ^{o}C, the colonies were counted and colony forming unit per milliliter (CFU/mL) was calculated.

Growth of Hydrocarbon Degrading Strains on Nutrient Agar

In this method growth of 12 isolated bacterial strains was studied [23] on nutrient agar plates in the presence of five selected aromatic hydrocarbons (at 0.25 % w/v concentration). 100 µL of 0.25 % of each hydrocarbon solution was spread onto nutrient agar plates and the plates were kept open near the flame for 5 minutes so that organic solvent could evaporate and only hydrocarbon compounds would remain on the plates. Twelve isolated strains were streaked and incubated at 37 ^{o}C for 24-48 hours.

3. RESULTS AND DISCUSSION

ISOLATION AND IDENTIFICATION OF BACTERIAL STRAINS

Twelve bacterial strains were isolated from contaminated soil samples of two petrol pumps of Karachi by selective enrichment culture technique. For that, Bushnell Hass medium was used that contained 1% petrol. These bacterial strains were identified on the basis of morphological and biochemical characteristics by using Bergey's Manual of Determinative Bacteriology [11, 20]. The bacterial strains were identified as *Corynebacterium* sp. SA07, *Ralstonia* sp. SA09, *Burkholderia* sp. SA011, SA042, *Stenotrophomonas* sp. SA012, *Pseudomonas* sp. SA013, SA044, SA049, *Staphylococcus* sp. SA046, SA058, SA061 and *Micrococcus* sp. SA048. Table **1** shows the morphological and biochemical characteristics of the isolated bacterial strains. Results

showed the percentage presence of higher gram negative bacteria (58.3%) than gram positive bacteria (41.6%). The previous studies have also shown that the gram negative bacterial strains isolated from hydrocarbon contaminated soil was higher than gram positive bacteria [18, 24].

Enumeration of Hydrocarbon Degrading Bacteria

The enumeration is the best way to study the PAH-degrading bacterial population [25]. Therefore, hydrocarbon degrading bacterial strains were enumerated by spread plate method on nutrient agar plate [21]. It is easy to perform and very small number of organisms can be counted by this technique. Table **2** shows CFU/mL when 0.5 % concentration of naphthalene, biphenyl and anthracene was used whereas data for phenanthrene and xylene is not shown. The graphical representation of CFU/mL for naphthalene, biphenyl and anthracene are shown in Figures **1**, **2** and **3** respectively. It is clearly observed that CFU/mL increased between 4 and 8 days (Figures **1** and **2**) and 5 and 12 days which indicated that CFU/mL was increased with the passage of time, means hydrocarbon utilization by bacteria as sole source of carbon and energy. But in few strains the gradual decline in CFU/mL or no growth was observed indicating that the cells entered in stationary phase as it was also observed by Nikhil *et al*. (2013) [26]. Only *Pseudomonas* sp. SA044 was an excellent hydrocarbon degrader as it had the potential to degrade phenanthrene, naphthalene, xylene, anthracene and biphenyl. The plasmids may encode some enzymes which have potential to degrade several hydrocarbons [27]. During this study it was observed that anthracene was degraded by *Stenotrophomonas* sp. SA012 and *Pseudomonas* sp. SA044 while rest of the strains were not able to degrade anthracene due to its very low solubility in aqueous medium and slight solubility in organic solvents [28]. *Corynebacterium* sp. SA07 was able to degrade only phenanthrene while *Ralstonia* sp. SA09,

Table 1: Morphological and Biochemical Characteristics of Bacterial Isolates

Characteristics	Bacterial isolates											
	SA07	**SA09**	**SA011**	**SA012**	**SA013**	**SA042**	**SA044**	**SA046**	**SA048**	**SA058**	**SA049**	**SA061**
Gram reaction	+	-	-	-	-	-	-	+	+	+	-	+
Morphology	Rods	Rods	Diplobacilli	Rods	Short rods	Rods	Rods	Cocci	Cocci	Cocci	Rods	Cocci
Motility	-	+	-	-	+	-	+	+	+	-	+	-
Oxidase	-	+	-	-	+	+	-	-	-	-	+	-
Catalase	+	+	+	+	-	+	+	+	+	+	-	+
Citrate	+/-	+	+/-	+	+	-	-	+	+/-	-	-	-
Hemolysis	γ	γ	γ	β	γ	β	β	α	γ	γ	γ	β
Glucose utilization	-	+	+	-	-	-	-	+	+	-	+	+
Sucrose utilization	-	+	-	-	-	+	-	+	+	+	+	+
Lactose utilization	-	-	+	-	-	-	-	+	+	+	-	+
Maltose utilization	-	+	-	-	-	+	+	+	+	+	-	+
Mannitolutilization	-	-	+	-	-	+	-	-	-	+	-	+
Tipple sugar iron	N/C	K/K	N/C	K/K	K/K	A/K	K/K	A/K	A/K	A/K	A/K	A/A
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+
Urea hydrolysis	+	+	+	+	+	+	-	-	-	+	+	-
Indole production	-	-	-	-	-	-	-	-	-	-	-	-
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-

Key: + = positive, **- =** negative, +/- = weakly positive, N/C = no change, K = alkaline, A = acidic.

Table 2: Colony Count of Isolated Bacterial Strains

Strains	Naphthalene (0.5 %)			Biphenyl (0.5 %)			Anthracene(0.5 %)			
	Zero day	After 4th day	After 8th day	Zero day	After 4th day	After 8th day	Zero day	After 5th day	After12th day	After 16th day
Corynebacterium sp. SA07	NG	$1x10^4$	NG	NG	$1.33x10^5$	NG	$1.5x10^5$	$1.6x10^5$	NG	NG
Ralstonia sp. SA09	$7x10^4$	$7x10^5$	$7.25x10^5$	NG	NG	NG	NG	NG	$9x10^3$	NG
Burkholderia sp. SA011	NG	NG	$6.5x10^4$	NG	$5x10^3$	$5.15x10^5$	NG	NG	NG	$1.05x10^4$
*Stenotrophomonas*sp. SA012	TNTC	NG	$2.4x10^6$	TNTC	$9.8x10^4$	NG	NG	$1.35x10^4$	$1x10^4$	$1.3x10^6$
Pseudomonas sp. SA013	NG	$2x10^5$	NG	NG	NG	$6x10^5$	$1.8 x10^5$	$6x10^5$	NG	NG
Burkholderia sp. SA042	$2.4x10^3$	$6x10^3$	$3x10^4$	NG	NG	$2.4x10^5$	$6 x10^4$	$8.5x10^5$	TNTC	NG
Pseudomonas sp. SA044	$6x10^4$	$1 x 10^5$	$8x10^5$	NG	NG	$5x10^4$	$1x10^5$	$1.3x10^5$	$1.2x10^6$	$1.5x10^6$
Staphylococcus sp. SA046	$8x10^4$	$1 x 10^4$	$1x10^3$	$4x10^3$	NG	$1.1x10^4$	NG	NG	$1.1x10^5$	$3.3x10^4$
*Micrococcus*sp. SA048	$4x10^4$	$2 x 10^4$	$3.7x10^4$	NG	NG	$6x10^4$	NG	NG	$1.5x10^5$	$2x10^4$
Pseudomonas sp. SA049	$2.5x10^4$	$1.6 x 10^5$	$5.x10^5$	NG	NG	NG	NG	NG	NG	NG
Staphylococcus sp. SA058	NG	$3.5 x 10^4$	$5x10^4$	NG	$5.3x10^5$	$1.95x10^5$	NG	$4 x10^3$	$5.8x10^4$	NG
Staphylococcus sp. SA061	TNTC	NG	$4.4x10^5$	$2x10^5$	$1x10^6$	$1.2x10^5$	NG	NG	$3 x10^3$	NG

Key: NG= no growth, TNTC = too numerous to count.

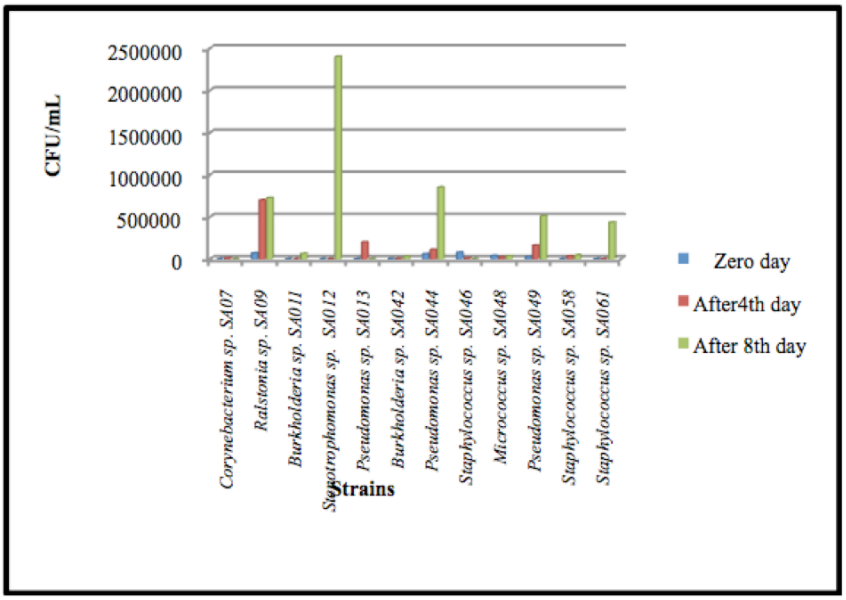


Figure 1: CFU/mL of isolated strains degrading naphthalene (0.5%).

Pseudomonas sp. SA049 and *Staphylococcus* sp. SA061 were able to degrade phenanthrene, naphthalene and xylene. *Burkholderia* sp. SA011 and *Micrococcus* sp. SA048 degraded all studied hydrocarbons except anthracene. *Stenotrophomonas* sp. SA012 degraded phenanthrene, naphthalene, and anthracene. *Pseudomonas* sp. SA013 degraded phenanthrene, biphenyl and xylene. *Burkholderia* sp. SA042 degraded naphthalene and biphenyl while *Staphylococcus* sp. SA046 degraded biphenyl and *Staphylococcus* sp. SA058 degraded only naphthalene. On the basis of CFU/mL, the results of aromatic

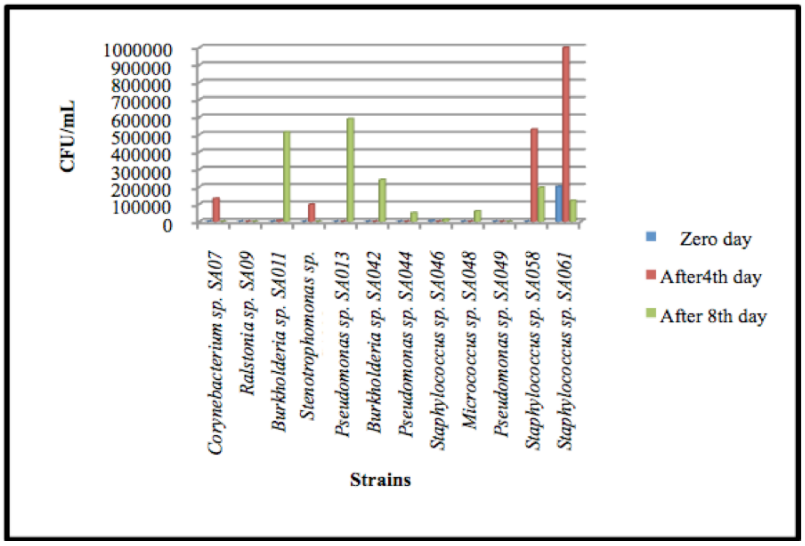


Figure 2: CFU/mL of bacterial strains degrading biphenyl (0.5%).

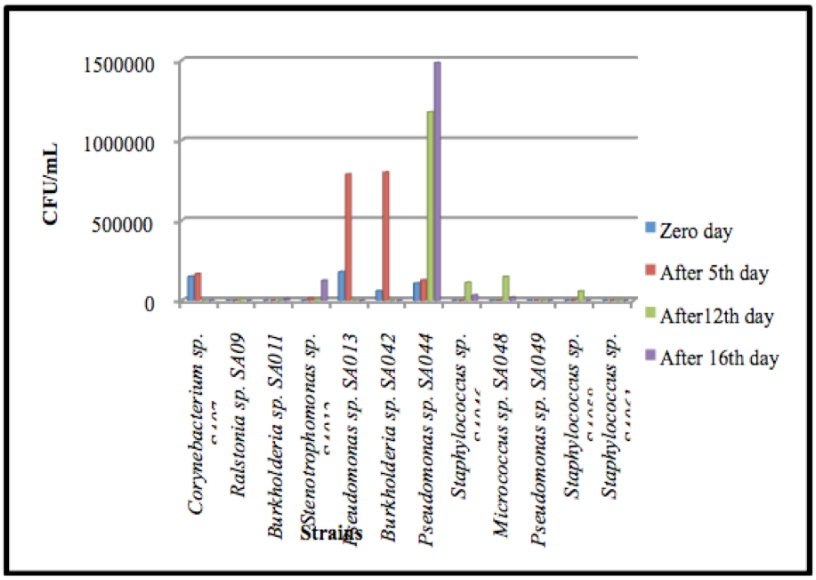


Figure 3: CFU/mL of isolated strains degrading anthracene (0.5%).

hydrocarbon degradation by selected strains that were isolated from petrol contaminated soil are summarized in Table **3** and the percentage of bacterial strains that degraded aromatic hydrocarbons (naphthalene, phenanthrene, biphenyl, anthracene and xylene) in Figure **4**. It was observed that the most degraded aromatic hydrocarbons were naphthalene and phenanthrene i.e. out of 12 , 9 (75 %) of the isolated bacterial strains had the potential to degrade naphthalene and phenanthrene as both of these hydrocarbons are widely distributed in the environment and can be easily degraded by microorganisms [29]. The results also show that the majority of the isolated strains that degraded phenanthrene also degraded naphthalene except *Corynebacterium* sp. SA07 that only degraded phenanthrene. Phenanthrene is degraded by some soil bacteria through one of two different routes. In one route, 1-hydroxy-2-naphthoic acid is oxidized to 1, 2-dihydroxynaphthalene, which is further degraded *via* the naphthalene pathway to salicylate which can be further metabolized. In the other pathway, the ring of 1-hydroxy-2-naphthoic acid is cleaved and further metabolized *via* the phthalate pathway. This suggests that phenanthrene and naphthalene share common metabolic pathway [30].

Growth of Isolated Bacterial Strains on Hydrocarbon Containing Nutrient Agar Plates

The growth pattern of hydrocarbon degrading strains was also studied [23] on nutrient agar plate by using 0.25 % of five hydrocarbons i.e. naphthalene, phenanthrene, biphenyl, anthracene and xylene.

Table 3: Hydrocarbon Degradation by Isolated Bacterial Strains

Strains	Hydrocarbons				
	Phenanthrene	Naphthalene	Biphenyl	Anthracene	Xylene
Corynebacterium sp. SA07	+	-	-	-	-
Ralstonia sp. SA09	+	+	-	-	+
Burkholderia sp. SA011	+	+	+	-	+
Stenotrophomonas sp. SA012	+	+	-	+	-
Pseudomonas sp. SA013	+	-	+	-	+
Burkholderia sp. SA042	-	+	+	-	-
Pseudomonas sp. SA044	+	+	+	+	+
Staphylococcus sp. SA046	-	-	+	-	-
Micrococcus sp. SA048	+	+	+	-	+
Pseudomonas sp. SA049	+	+	-	-	+
Staphylococcus sp. SA058	-	+	-	-	-
Staphylococcus sp. SA061	+	+	-	-	+

Key: + = degradation of hydrocarbon, - = no degradation of hydrocarbon.

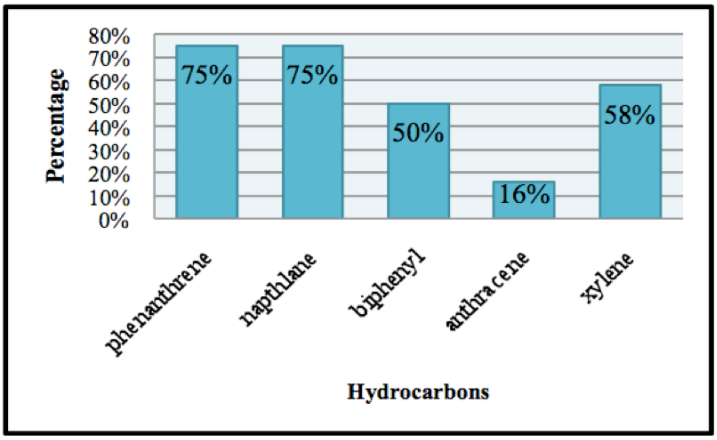
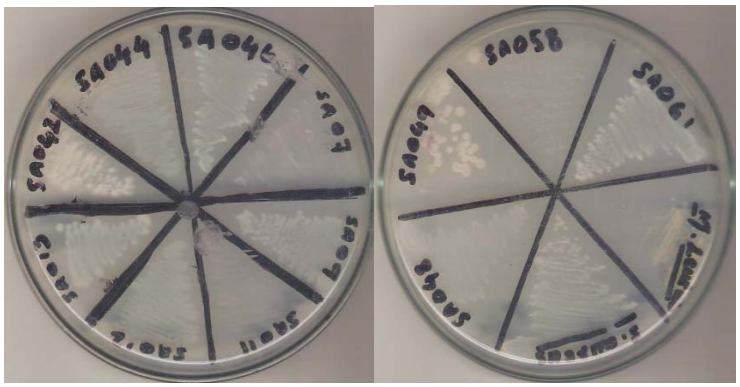


Figure 4: Percentage of hydrocarbon degrading bacterial strains.

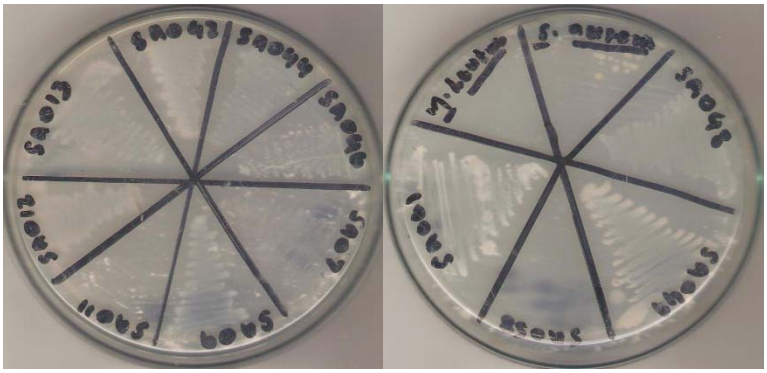
Table 4: Growth of Bacterial Strains on Nutrient Agar Plates in the Presence of 0.25 % Aromatic Hydrocarbons

Strains	Hydrocarbon substrates				
	Phenanthrene	Naphthalene	Biphenyl	Anthracene	Xylene
Corynebacterium sp. SA07	+	+++	+	+++	++
Ralstonia sp. SA09	+++	+++	+++	+	++
Burkholderia sp. SA011	+	+++	++	+++	++
Stenotrophomonas sp. SA012	+++	+++	+	+++	+++
Pseudomonas sp. SA013	+++	+++	+	+++	+++
Burkholderia sp. SA042	+++	+++	+++	+++	+++
Pseudomonas sp. SA044	+++	++	+++	+++	+++
Staphylococcus sp. SA046	+++	+++	+++	+++	+++
Micrococcus sp. SA048	+++	+	++	+++	++
Pseudomonas sp. SA049	+++	+	+	+++	++
Staphylococcus sp. SA058	-	+	++	+++	+
Staphylococcus sp. SA061	+++	+	+++	+++	+++

Key: + = less growth, ++ = moderate growth, +++ = heavy growth, - = no growth.



a

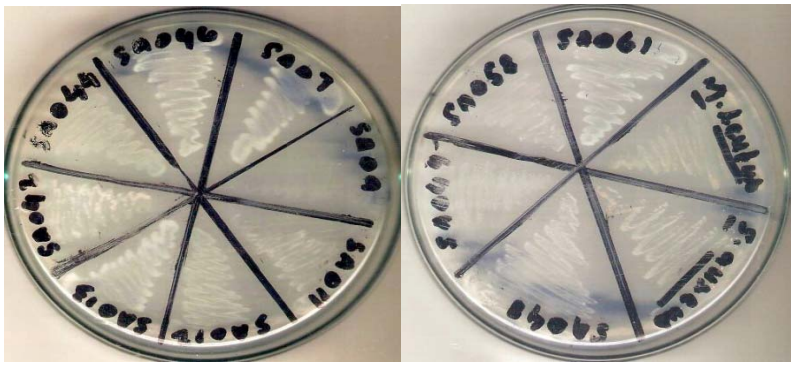


b

c

d

(Figure 5). Continued.



e

Figure 5: a: Growth of isolated bacterial strains on nutrient agar plates containing xylene (0.25 %). **b:** Growth of isolated bacterial strains on nutrient agar plates containing phenanthrene (0.25 %). **c:** Growth of isolated bacterial strains on nutrient agar plates containing naphthalene (0.25 %). **d:** Growth of isolated bacterial strains on nutrient agar plates containing biphenyl (0.25 %). **e:** Growth of isolated bacterial strains on nutrient agar plates containing anthracene (0.25 %).

M. luteus and *S. aureus* were used as control as they have been reported as hydrocarbon degraders [31, 32]. It is shown in Table **4** that all twelve selected strains were able to grow on nutrient agar plate in the presence of all five selected hydrocarbons except *Staphylococcus* sp. SA058 which could not grow in the presence of phenanthrene. Their growth on nutrient agar is shown in Figures **5a-e**. All the strains showed their growth after 24 - 48 hours in the presence of naphthalene, phenanthrene, anthracene and xylene. Biphenyl containing plates showed growth after three days of incubation. By using this method it was concluded that microorganisms prefer to grow best when the concentration of hydrocarbon decreased although it was also reported that growth of organisms increased as the concentration of phenanthrene increased [33], but every organism has its own level of tolerance because hydrocarbons on one hand are source of carbon and energy and on the other hand they are toxic too [31], therefore microorganisms' degradation capability decreases when high concentration of hydrocarbons is used [34].

CONCLUSION

From the study, it is concluded that hydrocarbon degrading microorganisms are found in oil or petrol contaminated soil and can easily be isolated from these contaminated sites, although it is very difficult to work with aromatic hydrocarbons due to their volatility and toxic effects. However, in the present study twelve bacterial strains were isolated which had the potential to degrade aromatic hydrocarbons. Among the isolated hydrocarbon degrading bacteria

Pseudomonas sp. SA044, SA049 and SA013, *Burkholderia* sp. SA011, *Ralstonia* sp. SA09, *Stenotrophomonas* sp. SA012, *Micrococcus* sp. SA048 and *Staphylococcus* sp. SA061 degraded three or more aromatic hydrocarbons. Most of the isolated bacteria were found to degrade and naphthalene and phenanthrene. Bacterial strains capable of degrading complex hydrocarbons present in the environment have a potential to be used as an effective tool for removing ecotoxic compounds.

ACKNOWLEDGEMENT

This research work was funded by a grant from Dean Faculty of Science, University of Karachi (DFS grant 2012-2013) to Dr. Abdul Wahab.

REFERENCES

[1] Chikere CB, Okpokwasili GC, Chikere BO. Monitoring of microbial hydrocarbon remediation in the soil. Biotechnology 2011; 1: 117-138.

[2] Atlas RM, Philp J. Bioremediation: applied microbial solutions for real-world environmental cleanup. American Society for Microbiology (ASM) Press 2005; 78-105

[3] Pawar RM. The effect of soil pH on degradation of polycyclic aromatic hydrocarbons (PAHs) 2012. http://uhra.herts.ac.uk/bitstream/handle/2299/8965/08177224%20Pawar%20Rakesh%20-%20finalPhD%20submission.pdf?sequence=1

[4] Shukor MY, Dahalan FA, Jusoh AZ, Muse R, Shamaan NA, Syed MA. Characterization of a diesel-degrading strain isolated from a hydrocarbon-contaminated site. Journal of Environmental Biology 2009; 30(1): 145-150.

[5] Samaei MR, Mortazavi SB, Bakhshi B, Jafari AJ. Isolation and characterization of bacteria degrading n-hexadecane from soil.International Proceedings of Chemical, Biological and Environmental Engineering (IPCBEE) 2012; 40.

[6] Pepi M, Cesa`ro A, Liut G, Baldi F. An Antarctic psychrotrophic bacterium *Halomonassp*. ANT-3b, growing on n-hexadecane, produces a new emulsyfying glycolipid. FEMS Microbial Ecology 2005; 53: 157-166. http://dx.doi.org/10.1016/j.femsec.2004.09.013

[7] Toledo FL, Calvo C, Rodelas B, Lopez JG. Selection and identification of bacteria isolated from waste crude oil with

polycyclic aromatic hydrocarbons removal capacities. Systematic and Applied Microbiology 2006; 29: 244-252. http://dx.doi.org/10.1016/j.syapm.2005.09.003

[8] Panda SK, Kar RN, Panda CR. Isolation and identification of petroleum hydrocarbon degrading microorganisms from oil contaminated environment. International Journal of Environmental Sciences 2013; 3: 5.

[9] Surridge AKJ. Denaturing gradient gel electrophoresis characterisation of microbial communities in polycyclic aromatic hydrocarbon and polychlorinated biphenyl contaminated soil 2007. http://upetd.up.ac.za/thesis/available/etd-05282007-123145/unrestricted/00front.pdf

[10] Coitinho JB, Costa DMA, Guimara˜es SL, Miranda de Go´es A, Nagem RAP. Expression, purification and preliminary crystallographic studies of NahF, a salicylaldehyde dehydrogenase from *Pseudomonas putida* G7 involved in naphthalene degradation. Acta Crystallographica Section F: Structural Biology and Crystallization Communications 2012; 68: 93-97. http://dx.doi.org/10.1107/S174430911105038X

[11] Hemalatha S, Veeramanikandan P. Characterization of Aromatic Hydrocarbon Degrading Bacteria from Petroleum Contaminated Sites. Journal of Environmental Protection 2011; 2: 243-254. http://dx.doi.org/10.4236/jep.2011.23028

[12] Chauhan A, Rahman F, John G, Oakeshott, Jain RK. Bacterial metabolism of polycyclic aromatic hydrocarbons: strategies for bioremediation. Review of Indian Journal of Microbiology 2008; 48: 95-113. http://dx.doi.org/10.1007/s12088-008-0010-9

[13] Coral G, Karagoz S. Isolation and characterization of phenanthrene-degrading bacteria from a petroleum refinery soil. Annals of Microbiology 2005; 55(4): 255-259.

[14] Daane LL, Harjono I, Zylstra GJ, Haggblom MM. Isolation and characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizosphere of salt marsh plants. Applied and Environmental Microbiology 2001; 67(6): 2863-2691. http://dx.doi.org/10.1128/AEM.67.6.2683-2691.2001

[15] Kiyohara H, Nagao K, Yana K. Rapid screen for bacteria degrading water-insoluble, solid hydrocarbons on agar plates. Applied and Environmental Microbiology 1982; 43: 454-457.

[16] Rasool SA, Ijaz S, Subhan SA. Hydrocarbon degradation by marine bacteria: Screening and genetic manipulation. Pakistan Journal of Botany 2003; 35(1): 37-44.

[17] Johnsen AR, Bendixen K, Karlson U. Detection of microbial growth on polycyclic aromatic hydrocarbons in microtiter plates by using the respiration indicator WST-1. Applied and Environmental Microbiology 2002; 68(6): 2683-2689. http://dx.doi.org/10.1128/AEM.68.6.2683-2689.2002

[18] Subathra MK, Immanuel G, Suresh AH. Isolation and identification of hydrocarbon degrading bacteria from Ennore creek. Bioinformation 2013; 9(3): 150-157. http://dx.doi.org/10.6026/97320630009150

[19] Chaudhary VK, Borah D. Isolation and molecular characterization of hydrocarbon degrading bacteria from tannery effluent.International Journal of Plant Animal and Environmental Sciences 2011; 1(2): 36-49

[20] Khan JA, Rizvi SHA. Isolation and characterization of micro-organism from oil contaminated sites. Advances in Applied Science Research 2011; 2(3): 455-460.

[21] Obuekwe IS, Semple KT. Impact of Al and Fe on the development of phenanthrene catabolism in soil. Journal of Soils and Sediments 2013; 13(9): 1589-1599. http://dx.doi.org/10.1007/s11368-013-0759-2

[22] Kafilzadeh F, Pour FH. Degradation of naphthalene, phenanthrene and pyrene by *Pseudomonas* sp. and *Corynebacteriumsp*. in the landfills.International Journal of Biosciences 2012; 2(9): 77-84.

[23] Ahmed F, Hasibullah M, Ferdouse J, Anwar MN. Microbial degradation of petroleum hydrocarbons. Bangladesh Journal of Microbiology 2010; 27: 10-13.

[24] Lopez MEM, Rodriguez-Casasola MT, Rios-Leal E, Esparza-Garcia F, Chavez-Gomez B, Rodriguez-Vazquez R, Barrera-Cortesa J. Fungi and bacteria isolated from two highly polluted soils for hydrocarbon degradation. Acta Chimica Slovenica 2007; 54: 201-209.

[25] Zhao B, Wang H, Mao X, Li R. A rapid screening method for bacteria degrading polycyclic aromatic hydrocarbons. Letters in Applied Microbiology 2009; 49: 408-410. http://dx.doi.org/10.1111/j.1472-765X.2009.02668.x

[26] Nikhil T, Deepa V, Rohan G, Satish B. Isolation, characterization and identification of diesel engine oil degrading bacteria from garage soil and comparison of their bioremediation potential. International Research Journal of Environment Sciences 2013; 2(2): 48-52.

[27] Obayori OS, Salam LB. Degradation of polycyclic aromatic hydrocarbons: Role of plasmids. Scientific Research and Essays 2010; 5(25): 4093-4106.

[28] Neeraja P, Madhusudhan NCH, Shanker S, Ravikumar M. Isolation and characterization of anthracene degrading bacteria from soil contaminated with petroleum wastes. International Journal of Research in Pharmaceutical Sciences 2013; 4(3): 380-385.

[29] Morzik A, Piotrowska-Seget Z, Labuzek S. Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. Polish Journal of Environmental Studies 2003; 12(1): 15-25.

[30] Kiyohara H, Torigoe S, Kaida N, Asaki T, Iida T, Hayashi H, Takizawa N. Cloning and characterization of a chromosomal gene cluster, pah, that encodes the upper pathway for phenanthrene and naphthalene utilization by *Pseudomonas putida* OUS82. Journal of Bacteriology 1994; 176(8): 2439-2443.

[31] Jyothi K, Babu KS, Clara NK, Kashyap A. Identification and isolation of hydrocarbon degrading bacteria by molecular characterization. Helix 2012; 2: 105-111.

[32] Chikere CB, Okpokwasili GC, Ichiakor BO. Characterization of hydrocarbon utilizing bacteria in tropical marine sediments. African Journal of Biotechnology 2009; 8(11): 2541-2544.

[33] Srujana K, Khan AB. Isolation and characterization of polyaromatic hydrocarbon degrading soil microbes from automobile workshop sediments. Journal of Environmental Science and Technology 2012 ; 5(1): 734-83.

[34] Dongfeng Z, Weilin W, Yunbo Z, Qiyou L, Haibin Y, Chaocheng Z. Study on isolation, identification of a petroleum hydrocarbon degrading bacterium *Bacillus fusiformissp*. and influence of environmental factors on degradation efficiency. Environment Protection 2011; 13(4): 74-82.

Received on 22-01-2015 Accepted on 27-02-2015 Published on 05-03-2015

http://dx.doi.org/10.6000/1927-5129.2015.11.32