Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Detecting genetics of several isolated bacterial species from soils by hydrocarbons

Rana AH AL-Lami[1]*, Ithar Kamil Al-Mayaly[2]

1. Biology Department, College of Science for Women, University of Baghdad, Baghdad, Iraq
2. Biology Department, College of Science, University of Baghdad, Baghdad, Iraq

** Corresponding author's E-mail: rana.a@csw.uobaghdad.edu.iq*

ABSTRACT

The presence of hydrocarbons in the soil is considered one of the main problems of pollution. In our current study, eight samples isolated from soil saturated with hydrocarbons were taken from different areas of Baghdad, Iraq. In this study, 5 isolates belonging to *Pseudomonas aeruginosa* by 99%, 4 isolates to *Klebsiella pneumoniae* by 98%, and 3 isolates to *Enterobacter hormaechei* by 97% were diagnosed in different ways. A molecular examination was also conducted by 16sRNA. We recorded *P. aeruginosa*, *K. Pneumoniae* and *E. hormaechei* as new local isolates in NCBI. In addition, a comparison was made between our isolates and the global isolates to determine the degree of convergence in the evolutionary line. The genes *alkB* and *nahAc7* were diagnosed in *P. aeruginosa* capable of degradation hydrocarbons. The aim of this study was to identify the bacterial species that resist the presence of hydrocarbons in the soil and also to diagnose some genes in the bacteria responsible for degradation of hydrocarbons in order to find the biological treatment methods.

Keywords: hydrocarbon, *Pseudomonas aeruginosa*, 16sRNA, alkB gene, nahAc7 gene.
Article type: Research Article.

INTRODUCTION

Crude oil is a complex composition of oil extracted from various reservoirs and from hydrocarbons. Hydrocarbons are the most common fuel in the world and represent the basis for encouraging the growth of microorganisms (Litvinenko 2020) Hydrocarbons are divided into four categories: aliphatic, asphalts, aromatics, and resins. In the past, they were divided into low molecular weight alkanes that volatilize easily (Leahy & Colwell 1990). It is clear that the widespread use of petroleum products causes pollution throughout the environment and that many microorganisms have the ability to hydrolyse hydrocarbons in oil-polluted areas. So, we found many studies that diagnose microorganisms and the possibility of analysing them for pollutants (Chaillan *et al.* 2004; Allamin *et al.* 2020; Obaid *et al.* 2022; Sahi *et al.* 2022). Many types of bacteria can decompose the polluted oil in the soil through its high and accurate enzymatic activity. Removing organic pollution using microorganisms is one of the safe economic ways to treat oil pollution. Bacterial diversity plays a major role in the analysis of hydrocarbons in the ecosystem (Das & Chandran 2011; Chandra *et al.* 2013). Bacterial species that degrade hydrocarbons are identified by genetic methods based on 16 sRNA genes

MATERIALS AND METHODS

Isolation of bacteria from the soil

Eight samples of soil contaminated with hydrocarbons were collected from sites beside the oil refinery in Baghdad in sterile containers and transferred to the laboratory for isolation.

Isolation methodology

1- Modified Mineral Salt (MMS) medium was prepared according to (Duvnjak *et al.* 1982).

Caspian Journal of Environmental Sciences, Vol. 20 No. 4 pp. 813-819 Received: Feb. 02, 2022 Revised: April 26, 2022 Accepted: June 03, 2022
DOI: 10.22124/CJES.2022.5771

Publisher: University of Guilan, Iran

2- Five grams of soil was added to 50 mL MMS and placed in an incubator at 37 ºC by continuous shaking for 2 days
3- A series of dilutions was prepared as 1 mL of the suspension is transferred to 9 mL of the dilution solution including 10^{-1} to 10^{-6}.
4- Spreading 0.1 mL of each dilution on agar plate by a glass L-shape rod (Kannan *et al.* 2018)

Isolation of bacterial strains
Pure colonies were isolated and cultured on selective media, then morphological tests were performed as well as Gram stain and Vitek-2 compacted.

Molecular identification of isolates
DNA was extracted from 8 isolates using isolation tools according to the company's instructions (Promega, USA)

Primers

Primer name	Seq.	Annealing Temp (°C)	Product size (bp)	No.
27F	5`-AGAGTTTGATCCTGGCTCAG-3`	60	1500	9
1492R	5`-TACGGTTACCTTGTTACGACTT-3`			10
alkB-F	5`-TCGAGCACATCCGCGGCCACCA-3`	60	330	11
alkB-R	5`-CCGTAGTGCTCGACGTAGTT-3`			
nahAc7-F	5`-ACTTGGTTCCGGAGTTGATG-3`	57	136	12
nahAc7-R	5`-CAGGTCAGCATGCTGTTGTT-3`			

Primer preparation

Concentration (pmol µL^{-1})	Vol. of nuclease free water (µL)	Primer Name
27F	300	100
1492R	300	100
alkB-F	300	100
alkB-R	300	100
nahAc7-F	300	100
nahAc7-R	300	100

PCR component calculation

No. of Reaction	4	rxn	Annealing temperature of primers	57, 60
Reaction Volume /run	25	µL	No. of primer	3
		%	No. of PCR Cycles	30
Aliquot per single rxn	22 µL of master mix per tube and adding 3 µL of template			

PCR program

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	57, 60	00:30	
Extension	72	01:00	
Final extension	72	07:00	1
Hold	10	10:00	

Standard Sequencing

PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation, Korea. The results were received by email then analysed using geneious software.

RESULTS AND DISCUSSION

Results showed that the soil saturated with hydrocarbons contains different types of bacteria as 12 bacterial isolates were isolated by culturing on selective media. Colonies appeared on the MacConkey medium were between 3 lactose-fermenting isolates and 9 non-fermenting ones. All isolates exhibited negative in gram stain. The results showed that 5 isolates belong to the genus of bacteria.

Molecular identification of isolates

All isolates were genetically characterized by 16sRNA and their sequences were made. The results revealed that five of the isolates belonged to *Pseudomonas aeruginosa*, 4 isolates to *Klebsiella pneumoniae* and 3 to *Enterobacter hormaechei* (Fig. 1)

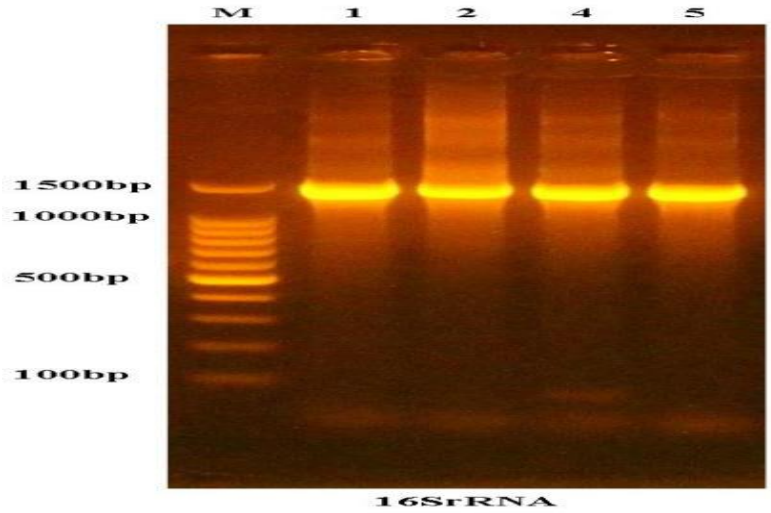

Fig. 1. Results of the amplification of *16s RNA gene* of unknown bacterial species were fractionated on 1.5% agarose gel electrophoresis stained with Ethidium Bromide; M: 100bp ladder marker. Lanes 1-5 resemble 1500 bp PCR products.

There are leaks of millions of litres oil entering the soil and resulting in one of the biggest problem in the world, i.e., pollution by hydrocarbons. Hydrocarbons are also affected by many factors, including environmental factors, pollution sources and the presence of microorganisms (Head *et al.* 2006; Stroud *et al.* 2007). Microorganisms, especially bacteria, play a major role in dismantling hydrocarbons and detoxifying the places where they are found, since they converted into water and carbon dioxide with no effect on the environment and are harmless to humans. Therefore, studies focus on the types and quantities of microbial groups involved in the degradation of hydrocarbons, as well as environmental factors that affect rates of microbial metabolism (Rodrigues *et al.* 2009; Chikere *et al.* 2011). Some strains of *P. aeruginosa* are capable of breaking down hydrocarbons by employing a number of metabolic procedures (Silva *et al.* 2006). It was also found that the strains isolated from the environment are highly efficient in degrading hydrocarbons compared to clinical strains, indicating their high efficiency in adaptation and evolution to deal with adverse conditions (Grady *et al.* 2017). Although *Enterobacter* spp. and *Klebsiella* spp. were described that few of them as hydrocarbons decomposer, some authors mentioned that it has the ability to biologically decompose some diverse pollutants such as organic phosphorous and halogens as well as hydrocarbons, and its efficiency elevates once the medium has low salinity (Rodrigues *et al.* 2009)

Registration of 16sRNA gene isolates in NCBI

The differences were found in the gene sequences of the isolates *P. aeruginosa*, *K. Pneumoniae* and *E. hormaechei* which are local Iraqi isolates from the soils saturated with hydrocarbons, compared to the global isolates. These differences led to the testing and documentation of these isolates in the NCBI as new local Iraqi isolates, and they were accepted according to the accession numbers of *P. aeruginosa* MZ950650.1, *K. Pneumoniae* MZ950643.1

and *E. hormaechei* MZ950647.1 for each isolate. Phylogenetic trees showed the comparison between local and global isolates recorded by a number of countries and the extent of the differences between them (Figs. 2, 3 and 4).

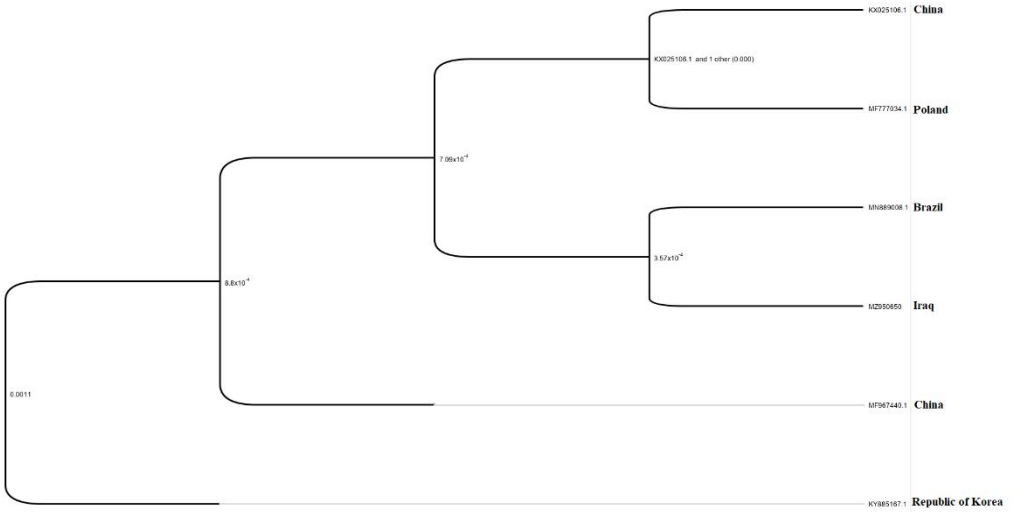

Fig. 2. Phylogenetic tree of 16sRNAgene for locally *P. aeruginosa* isolates with global strain by accession no. (MZ950650.1).

Fig. 3. Phylogenetic tree of 16sRNA gene for locally *K. Pneumoniae* isolates with global strain by accession no. (MZ950643.1).

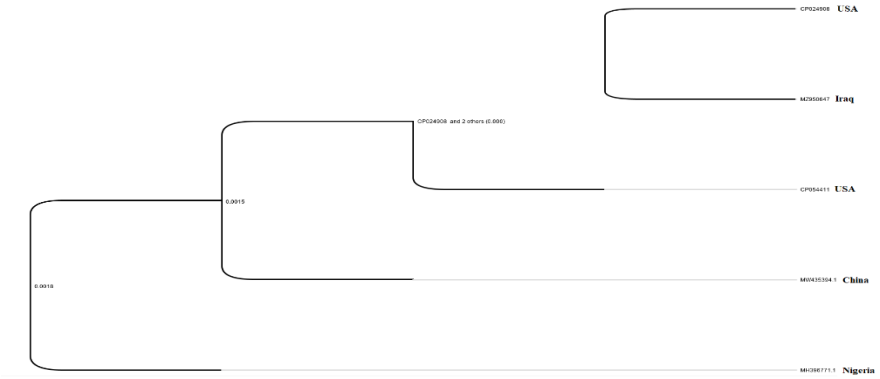

Fig. 4. Phylogenetic tree of 16sRNA gene for locally *E. hormaechei* isolates with global strain by accession no. (MZ950647.1).

The differences between the local isolates registered in NCBI and the global isolates registered are caused by mutations that improve the efficiency level of bacteria to resist difficult environmental conditions through the secretion of enzymes that help biodegrade hydrocarbons in the environment in which they live. These mutations and adaptations helped focus on biological treatment that reduces economic damage and be environmentally friendly.

Identification of ***alkB gene*** and ***nahAc7 gene*** in ***P. aeruginosa***

P. aeruginosa is one of the best known microorganisms has a high ability to break down hydrocarbons, so it is one of the most important tools for bioremediation of soil contaminated with hydrocarbons (Aparna *et al.* 2012). It has the ability to produce reductants surfactants, which increases the rate of hydrocarbons decomposition (Satpute *et al.* 2010). The abundances in alkane monooxygenase (alkB) and naphthalene dioxygenase genes (***nahAc7*)** in genome of ***P. aeruginosa*** was observed. Their abundance greatly helps in the decomposition of alkanes and naphthalene and plays a great role in the decomposition of hydrocarbons in general. Also gene expression is greatly affected by the amount of oil and salt present (Wasmund *et al.* 2009). The results of our study showed the presence of 100% of *alkB* gene and *nahAc7*gene of 50% in Fig. 5 which is attributed to the reason for the abundance of *alkB* gene because this gene has the ability to break down the short and medium chain alkanes (C_6-C_{15}; Kleinsteuber *et al.* 2006, Ulrich *et al.* 2009).

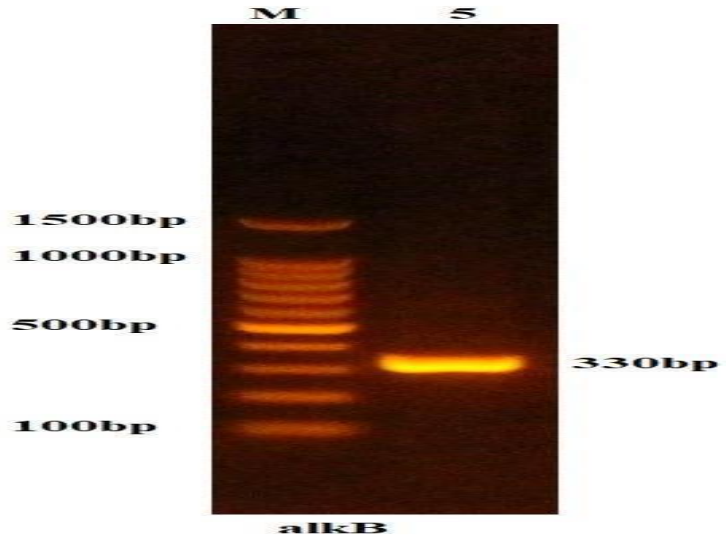

Fig. 5. Results of the amplification of *alkB gene* of *P. aeruginosa* were fractionated on 1.5% agarose gel electrophoresis stained with Ethidium Bromide; M: 100bp ladder marker. Lanes 5 resemble 330bp PCR products.

(Liu *et al.* 2015) results were in support of our findings that the abundance of the gene expression of *alkB* gene and the gene of *nahAc7* played a major role in the decomposition of hydrocarbons. In addition, this is what (Shahreza *et al.* 2019) supported that *alkB* gene is abundantly available in *P. aeruginosa* genome and that it plays a major role in the destruction of pollutants especially hydrocarbons. It is clear from the study that there is a large group of microorganisms that have the ability to biodegrade hydrocarbons by various mechanisms and enzymes all of which are under genetic control. The availability of a set of genes in the genome of some bacterial strains makes this bacteria an important organism used in biological treatments, which are considered safe economic and environmentally friendly methods as the decomposition products are not harmful to humans or the environment.

REFERENCES

Allamin, IA, Halmi, MIE, Yasid, NA, Ahmad, SA, Abdullah, SRS & Shukor, Y 2020, Rhizodegradation of petroleum oily sludge-contaminated soil using *Cajanus cajan* increases the diversity of soil microbial community. Scientific Reports, 10: 1-11. DOI: 10.1038/s41598-020-60668-1.

Aparna, A, Srinikethan, G & Smitha, H 2012, Production and characterization of biosurfactant produced by a novel Pseudomonas sp. 2B. *Colloids and Surfaces B: Biointerfaces*, 95: 23-29, DOI: 10.1016/j.colsurfb .2012.01.043.

Chaillan, F, Le Flèche, A, Bury, E, Phantavong, YH, Grimont, P, Saliot, A & Oudot, J 2004, Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. *Research in Microbiology*, 155: 587-595. DOI: 10.1016/j.resmic.2004.04.006.

Chandra S, Sharma R, Singh K & Sharma, A 2013, Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. *Annals of Microbiology*, 63: 417-431, DOI: 10.1007/s13213-012-0543-3.

Chikere, CB, Okpokwasili, GC & Chikere, BO 2011, Monitoring of microbial hydrocarbon remediation in the soil. 3 *Biotech*, 1: 117-138, DOI: 10.1007/s13205-011-0014-8.

Das, N & Chandran, P 2011, Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology Research International*, 2011: 941810, DOI: 10.4061/2011/941810.

Duvnjak, Z, Cooper, DG & Kosaric, N 1982, Production of surfactant by *Arthrobacter paraffineus* ATCC 19558. *Biotechnology and bioengineering*, 24.1: 165-175.

Grady, SL, Malfatti, SA, Gunasekera, TS, Dalley, BK, Lyman, MG, Striebich, RC, Mayhew, MB, Zhou, CL, Ruiz, ON & Dugan, LC 2017, A comprehensive multi-omics approach uncovers adaptations for growth and survival of Pseudomonas aeruginosa on n-alkanes. *BMC genomics*, 18: 1-19.

Head, IM, Jones, DM & Roling, WF 2006, Marine microorganisms make a meal of oil. *Nature Reviews Microbiology*, 4: 173-182.

Kannan, MN, Sethi, S, Badoni, A, Chamoli, V & Bahuguna, NC 2018, Isolation and characterization of bacterial isolates from agriculture field soil of Roorkee region. Journal of Pharmacognosy and Phytochemistry, 5: 108-110.

Kleinsteuber, S, Riis, V, Fetzer, I, Harms, H & Muller, S 2006, Population dynamics within a microbial consortium during growth on diesel fuel in saline environments. *Applied and Environmental Microbiology*, 72: 3531–3542, DOI: https://doi.org/10.1128/AEM.72.5.3531-3542.2006.

Lane, DJ 1991, 16S/23S rRNA sequencing in: Nucleic acid techniques in bacterial systematic. E, Stackebrandt & M, Goodfellow (Eds.), John Wiley & Sons Ltd, Chichester England.

Leahy, JG & Colwell, R 1990, Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews*, 54: 305-315. PMid:2215423

Litvinenko, V 2020, The role of hydrocarbons in the global energy agenda: The focus on liquefied natural gas. *Resources*, 9: 59, DOI: 10.3390/resources9050059.

Liu, Q, Tang, J, Bai, Z, Hecker, M & John, PG 2015, Distribution of petroleum degrading genes and factor analysis of petroleum contaminated soil from the Dagang Oilfield, China. *Published online*, Jun 18. DOI: 10.1038/srep11068.

Obaid, RF, Kadhim Hindi, NK, Kadhum, SA, Jafaar Alwaeli, LA & Jalil, AT 2022, Antibacterial activity, anti-adherence and anti-biofilm activities of plants extracts against *Aggregatibacter actinomycetemcomitans*: An in vitro study in Hilla City, Iraq. *Caspian Journal of Environmental Sciences*, 20: 367-372.

Park, JW & Crowley, E 2006, Dynamic changes in nahAc gene copy numbers during degradation of naphthalene in PAH-contaminated soils. *Applied Microbiology and Biotechnology*, 72: 1322-1329, DOI: 10.1007/s00253-006-0423-5.

Rodrigues, DF, Sakata, SK, Comasseto, JV, Bı´cego, MC & Pellizari, VH 2009, Diversity of hydrocarbon-degrading Klebsiella strains isolated from hydrocarbon-contaminated estuaries. *Journal of Applied Microbiology*, 106: 1304-1314.

Sahi, MK, Haran, MS & Hanoon, MB 2022, Effect of inoculation with *Bacillus* spp., *A. chroococcum*, *P. fluorescens* and phosphorous levels on the amount of major nutrients in maize (*Zea mays* L.) irrigated with saline water. *Caspian Journal of Environmental Sciences*, 20: 533-537.

Satpute, SK, Banat, IM, Dhakephalkar, PK, Banpurkar, AG & Chopade, BA 2010, Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. *Biotechnology Advances*, 28: 436-450.

Shahreza, H, Akhavan Sepahy, A, Hosseini, F & Khavari Nejad, R 2019, Molecular Identification of Pseudomonas Strains with Polyethylene Degradation Ability from Soil and Cloning of alkB Gene. *Archives of Pharmacy Practice*, 10: 43-48.

Silva, P, Rosa, M, Arelis, A.R, Oca, JMD, José, M & Domingo, CM 2006, Biodegradation of crude oil by pseudomonas aeruginosa at18 strain. *Chemical Technology*, 26: 70-77.

Smits, TH, Ro¨thlisberger, M, Witholt, B & van Beilen, JB 1999, Molecular screening for alkane hydroxylase genes in Gram-negative and Gram-positive strains. *Environmental Microbiology*, 1: 307-317.

Stroud, JL, Paton, GI & Semple KT 2007, Microbe-aliphatic hydrocarbon interactions in soil: implications for biodegradation and bioremediation. *Journal of Applied Microbiology*, 102: 1239-1253.

Turner, S, Pryer, KM, Miao, VPW & Palmer, JD 1999, Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *Journal of Eukaryotic Microbiology*, 46: 327-338, DOI: 10.1111/j.1550-7408. 1999.tb04612. x.

Ulrich, AC, Guigard, SE, Foght, JM, Semple, KM, Pooley, K, Armstrong, JE & Biggar, KW 2009, Effect of salt on aerobic biodegradation of petroleum hydrocarbons in contaminated groundwater. *Biodegradation*, 20: 27-38.

Wasmund, K, Burns, KA, Kurtboke, DI & Bourne, DG 2009, Novel alkane hydroxylase gene (alkb) diversity in sediments associated with hydrocarbon seeps in the timor sea, Australia. *Applied and Environmental Microbiology*, 75: 7391-7398.

Bibliographic information of this paper for citing:

AL-Lami, R,A, Al-Mayaly, I,K 2022, Detecting genetics of several isolated bacterial species from soils by hydrocarbons. Caspian Journal of Environmental Sciences, 20: 813-819.