رِسْمِ اللهِ الرَّدُمنِ الرَّحِيمِ

"وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ

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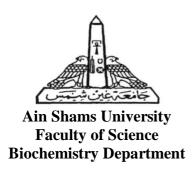
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وَالشَّمَاحَةِ فَيُنَبِّنُكُمْ رِمَا كُنْتُمْ تَعْمَلُونَ

وَالشَّمَاحَةِ فَيُنَبِّنُكُمْ رِمَا كُنْتُمْ تَعْمَلُونَ

حدَقَ الله العَظِيم

(التوقة 10)



## Molecular Characterization of Petroleum Compounds-Degrading Bacteria Isolated from Petroleum Contaminated Soil

A Thesis submitted to Faculty of Science

Ain Shams University In Partial Fulfillment Of

Master Degree in Biochemistry

Submitted by

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# Declaration

I declare that this thesis has been composed by myself and the work therein has not been submitted for a degree at this or any other university.

I would like to thank my family, in particular my mother and sisters. I have no doubt that without their patience and faith in me; I would have gone down that long difficult path which I began. I dedicate my thesis to them as well as my friends.

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**Degrading Bacteria Isolated from Petroleum Contaminated Soil** 

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Title: Molecular Characterization of Petroleum Compounds-Degrading Bacteria Isolated from Petroleum Contaminated Soil

### ABSTRACT

There is a growing public concern over the soil and groundwater contamination by petroleum hydrocarbons, owing to rapid industrial development and its broad environmental distribution, which can reach soil, groundwater and air. Pollution by petroleum hydrocarbons is widespread as a result of accidental oil spills, leaking underground storage tanks, oil extraction, and processing operations, producing a significant environmental burden. Biodegradation is a natural process carried out by soil and aquatic microorganisms whereby organic wastes are biologically degraded under controlled conditions to a harmless state, or to levels below concentration limits. The main objective of this study was to isolate bacterial strains capable of the degradation of petroleum hydrocarbons in contaminated soil and to characterize these bacterial strains using biochemical and molecular techniques. A total of fifty-four bacterial cultures were isolated from a long term hydrocarbon contaminated soil. Five isolates designed RAM03, RAM06, RAM13, RAM17 and MS30 were selected based on their relatively higher growth on broth basal salt medium amended with high concentrations of crude oil or BTEX (as a sole source of carbon), emulsion index, surface tension, and degradation percentage. The bacterial isolates (RAM03, RAM06, RAM13, RAM17 and MS30) were identified as Ochrobactrum cytisi, Ochrobactrum

anthropi, Sinorhizobium meliloti 3 Ochrobactrum anthropi, and Ochrobactrum lupini respectively, according to the analysis of 16S rRNA gene sequence. The capability of these bacterial strains to degrade crude oil or BTEX was assessed under in vitro conditions, in culture medium and soil. The tested bacterial strains revealed a promising potential for bioremediation of petroleum oil contamination as they could degrade more than 84% of total petroleum hydrocarbons (TPH) in modified basal salt medium supplemented with 4 % crude oil after 30 day. Moreover four of these strains could remove 43.5 - 54 % of TPH from contaminated soil after 30 day. These bacteria could effectively remove both aliphatic and aromatic petroleum hydrocarbons, and they are able to produce bio-surfactant. These data indicate that these isolates may have the potential for use in bioremediation of petroleum hydrocarbon contaminated soil.

### LIST OF ABBREVIATIONS

**A** Anthrathene

**B** Benzene

**bp** Base Pair

**BTEX** Benzene, Toluene, Ethyl benzene and Xylenes

C Chatecol

**CFU** Colony Forming Unit

CTAB N-cetyl-N,N,N- Trymethyl Ammonium Bromide

**E** Ethylbenzene

**EDTA** Ethylenediaminetetraacetic acid

**EI** Emulsion index

**EPA** Environmental Protection Agency

**ETBE** Ethyl tert-butyl ether

**FID** Flame ionization detectore

**GA** Gentisic acid

**GC–MS** Gas chromatography mass spectrscopy

**HMN** 2,2,4,4,6,8,8-Heptamethylnonane

**LB** Luria Bertani

**LUSTs** Leaking underground storage tanks

MBSM Modified Basal Salt Medium

MCL Maximum Contaminant Level

**mM** Mili mole

### LIST OF ABBREVIATIONS

mol Mole

MTBE Methyl tert-butyl ether

mV Milli volt.
nm Nanometer

NRC National Research Council

**OH'** Hydroxyl radicals

**PAHs** Polycyclic aromatic hydrocarbons

**PCA** Protochatechuic acid

**PCR** Polymerase Chain Reaction

**Ph** Phenantherene

PHA Polyhydroxyalkanoates

**ppb** Parts per billion

**Py** Pyrene

SA Salicylate

SDS Sodium Deodosyl Sulphate

T Toluene

**TBA** Tert-butyl alcohol

**TBE buffer** Tris Borate EDTA

**TE buffer** Tris EDTA

**TPHs** Total Petroleum Hydrocarbons

**USTs** Underground storage tanks

VOCs Volatile organic carbons

X Xylene

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