بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

"وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ
وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الْغَيْبِ
وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ"

صدَقَ اللهُ العَظِيم

(التوبة 105)

Ain Shams University
Faculty of Science
Biochemistry Department

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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2015

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.

Mohamed Eraky Mohamed

Approval Sheet

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ACKNOWLEDGMENTS

الحمد لله رب العالمين

Praise is to Allah, the lord of all creatures who taught man the whole science and the names of all things.

This Master would never have been completed without the efforts of several people who really I appreciate their instructive support.

I am greatly indebted to ***Prof. Dr. Ahmed Mohamed Salem****, Professor of Biochemistry, Department of Biochemistry, Faculty of Science, Ain Shams University, for his supervision, valuable advice and his kind help, reading and criticizing the manuscript and encourangement, his accuracy and precious comments made me fell enthusiastic to finish the thesis perfectly. His valuable guidance and ultimate support are greatly appreciated.*

I would like to express my deep thanks to ***Prof. Dr. Reda A. I. Abou shanab,*** *Professor of Environmental Biotechnologyt, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research & Technology Applications, Alexandria, who suggest the topic of this thesis and who meticulously supervised the execution of the experimental studies and was always present tremendous help in the preparation and revision of the manuscript. Thanks for his scientific guidance, valuable suggestions, brilliant ideas, undeniable help, moral support, provision of facilties and criticism throughout this study. I appreciate his support and understanding, which drove me to perform to my maximum potential and complete my degree. I am very proud to perform research under his supervision.*

I am particularly indebted to ***Dr. Abdel-Rahman B. Abdel-Ghaffar****, Assistant professor of Biochemistry, Department of Biochemistry, Faculty of Science, Ain Shams University, for his valuable assistance and device, kind help and encourengement.*

ACKNOWLEDGEMENTS

I wish to thank ***Dr/Ahmed Hadad****, researcher of Environmental Biotechnology, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research & Technology Applications, Alexandria, for his support and encourengement.*

Sincere thanks to all my colleagues in the Genetic Engineering and Biotechnolgy Research institute, City of Scientific Research and Technology Applications.

I would like to thank whole heartedly ***my mother*** *and* ***family members*** *whose love and unconditional support, both on academic and personal front, enabled me to see the light of this day.*

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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 و *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

mol	Mole
MTBE	Methyl tert-butyl ether
mV	Milli volt.
nm	Nanometer
NRC	National Research Council
$OH^{\bullet}$	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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