IDENTIFICATION AND CHARACTERIZATION OF SOME GENES RELATED TO HYDROCARBON COMPOUNDS DEGRADATION

BY

HEBA ABDEL-HALIM RADWAN ABDEL-HALIM
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2007
M.Sc. Agric. Sci. (Genetics), Fac. Agric., Cairo Univ., 2013

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APPROVAL SHEET

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APPROVAL COMMITTEE

Dr. MOHAMED MUSTAFA IBRAHIM ……...…...…
Professor of Genetics, Fac. Biotech., Misr University for Science and Technology

Dr. NAGLAA ABDELMONEM ABDALLAH…….
Professor of Genetics, Fac. Agric., Cairo University

Dr. ABDELHADI ABDALLAH ABDELHADI ……...……
Professor of Genetics, Fac. Agric., Cairo University

Date: 17/6/2019

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. HAGGAG SALAH ZAIN MOSTAFA
Professor of Genetics, Fac. Agric., Cairo University

Dr. ABDELHADI ABDALLAH ABDELHADI
Professor of Genetics, Fac. Agric., Cairo University

Dr. AHMED ZEIN EL-ABDEEN ABDEL-AZIZ
Professor of Biochemistry, Fac. Biotech., Misr University of Science and Technology

Dr. AHMED NAGIB EL-SAYED SHARAF
Late Professor of Genetics, Fac. Agric., Cairo University

Name of Candidate: Heba Abdel Halim Radwan **Degree:** Ph.D.
Title of Thesis: Isolation and Characterization Of Some Genes Related To Polyaromatics Hydrocarbon Degrading Enzyme
Supervisors: Dr. Haggag Salah Zein
Dr. Abdelhadi Abdallah Abdelhadi
Dr. Ahmed Zein Elabdeen Abdel-Aziz
Dr. Ahmed Najib Elsayed Sharaf
Department: Genetics **Approval: 17/6 /2019**

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are recalcitrant hazardous environmental contaminants. Various strategies, including chemical and physical like oxidation, fixation, leaching, and electrokinetic or biological-based techniques were used for remediation of polluted sites. Bioremediation of PAHs, via PAH-degrading endophytic and rhizospheric microbes, represented as a time-/cost-effective way for ecorestoration. Four bacterial strains were isolated from contaminated soil on MSM supplemented with anthracene, alpha-naphthalene or catechol as sole carbon sources. These isolates were identified by *16S rRNA* gene as *Bacillus anthracis, B. cereus, B. mojavensis and B. subtilis*. The degradation efficiency on the selected aromatic compounds was tested by HPLC analysis. *B. subtilis* showed the highest degradation efficiency of anthracene (99%) after five days of incubation. *B. subtilis* showed the highest catechol 1, 2 dioxygenase activity in MSM supplemented with anthracene. The enzyme was purified by gel filtration chromatography and characterized (70 kD, K_m 2.7 µg and V_{max} 178 U/mg protein). The *catechol 1,2 dioxygenase* gene from the identified four bacterial strains were isolated and submitted to GenBank (accession numbers MG255165-MG255168). The gene expression level of *catechol 1,2 dioxygenase* gene was upregulated ≃ 2.5fold during the 24 hr of incubation period in the presence of Fe^{3+}. Furthermore, *B. subtilis* is a promising strain to be used in bioremediation of aromatic compounds-contaminated environments.

Keywords: *Bacillus subtilis*, catechol 1,2 dioxygenase, bioremediation, PAHs, qPCR.

DEDICATION

I dedicate this work to whom my heartfelt thanks to Prof. Dr. Ahmed Nagib El-sayed Sharaf, and all my family. A special feeling to my Dad Abdelhalim and my mom Katifa, their words of encouragement ring in my ears and their endless support along the period of my post-graduation. My sister Noha and my little brother Mohammed have never left my side.

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LIST OF ABBREVIATIONS

Abbrev.	Full term
AH	Aromatic hydrocarbon
B(a)P	Benzo[a]pyrene
bp	Base pair
HMW	High molecular weight
HPLC	High performance liquid chromatography
KDa	Kilo Dalton
LB	Luria broth
LMW	Low molecular weight
MSM	Mineral salt media
PAH	Poly aromatics hydrocarbon
PCR	Polymerase Chain Reaction
qPCR	Quantative Polymerase Chain Reaction
SDS	Sodium dodecyl sulfate
TEMED	Tetramethylethylenediamine

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