Alexandria University
Faculty of Agric. (Saba-Bacha)

GENETICAL STUDIES ON SOME BACTERIAL GENERA AND ITS APPLICATION IN BIODEGRADATION FOR WASTEWATER

Presented by

SUZAN MOHAMMED HASSAN MOHAMMED HASSAN

For the Degree of

MASTER OF AGRICULTURAL SCIENCE (GENETICS)

Examiner's Committee	Approved
Prof. Dr. Houssam El-Din M.F. El-Wakil Emeritus Prof. of Genetics Faculty of Agric., Saba-Bacha, Alexandria Univ.	.....
Prof. Dr. Saied Abdel Salam Dora Prof. of Genetics and Vice Dean for Post Graduate studies Faculty of Agric., Kafr El-Sheikh Univ.	.....
Prof. Dr. Ahmed El-Sayed Mohamed Khaled Prof. of Genetics Faculty of Agric., Saba-Bacha, Alexandria Univ.	.....
Prof. Dr. Mohammed Said M. Abdel Megeed Prof. of Genetics Faculty of Agric., Kafr El-Sheikh Univ.	.....

2008

جامعة الإسكندرية
كلية الزراعة . سابا باشا

دراسات وراثية على بعض الاجناس البكتيرية وتطبيقاتها فى الهدم الحيوى لمخلفات الصرف الصحى

مقدمة من

سوزان محمد حسن محمد حسن

للحصول على درجة
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BY

SUZAN MOHAMMED HASSAN MOHAMMED HASSAN

A Thesis Submitted in Partial Fulfillment of the Requirements Governing the Award of the Degree of

MASTER OF AGRICULTURAL SCIENCES

(GENETICS)

Department of Agricultural Botany

From

Alexandria University

2008

لجنة الإشراف :

الأستاذ الدكتور/ أحمد السيد محمد خالد .....

أستاذ الوراثة

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الأستاذ الدكتور/ محمد سيد محمد عبد المجيد .....

أستاذ الوراثة

كلية الزراعة – جامعة كفر الشيخ

SUPERVISION'S COMMITTEE

Prof. Dr. Ahmed El Saied Mohammed Khaled .....

Prof. of Genetics, Faculty of Agriculture, Saba Bacha, Alexandria University

Prof. Dr. Mohammed Said M. Abd Elmagid .....

Prof. of Genetics, Faculty of Agriculture, Kafr El-Sheikh University

ACKNOWLEDGMENT

The author wishes to express his sincere gratitude to **Prof. Dr. Amal abou Alnasr,** Head of Genetics department, Faculty of Agriculture, Saba Bash, University of Alexandria, Egypt, for her valuable instructions and encouragement during the investigation ALSO Prof. of Genetics Faculty of Agriculture, University of Alexandria, for suggesting and helping in the success of this work.

Deep appreciation and gratitude to **Prof. Dr. Hossam El Dein M. Fathy El-Wakil** Prof. of Genetics Faculty of Agriculture, Saba Bacha, University of Alexandria, for valuable advice throughout the course of the investigation and the preparation of the manuscript.

I would like to thank **Prof. Dr Saied Abdel Salam Dora,** Prof. of Genetics, Vice-Dean for Graduate Studies and Research, Faculty of Agriculture, Kafr El-Sheikh University for valuable suggestion and help through the investigation and preparation of manuscript.

My deep appreciation to **Prof**. **Dr. Ahmed El Saied Mohammed Khaled** Prof. of Genetics, Faculty of Agriculture, Saba Bacha, Alexandria University , for supervision, guidance, and help throughout the investigation and preparation of manuscript.

I would like to express my deep appreciation to **Prof Dr. Mohamed Said Abd elmageed**, Prof of Genetics, Faculty of Agriculture, Kafr El -Shikh University, for suggesting the problems, guidance, supervision,encouragement, valuable advice, continuous help throughout the investigation, preparation of the manuscript, and putting the thesis in its final form.

I am grateful to **Mr. Ramdan Mohamed El -Sayed**, Operation Manager of Estern wastewater filtaration station traeatment -Alexandria for supplying the materials and for his help during this work.

Finally, I wish to express my grateful thanks to **my hasband and my dughter Mnnaht allah Ali Hassan**, for their kind help and complete this thesis .

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. **Table (7): DNA fragments of RAPD for treated and untreated *Bacillus* bacteria using four random primers.**

Primers	AATCCGCAAT					CGCGGAATGC					AATCGGTACT					CTCGAATGCA				
DNA fragments	B	B1	B2	B3	H	B	B1	B2	B3	H	B	B1	B2	B3	H	B	B1	B2	B3	H
1600																				
1550													+		+					
1500																				
1450																				
1350																			+	
1250					+															
1200					+	+						+								
1180												+								
1150	+	+			+								+	+	+		+		+	+
950																+			+	
900		+					+													
850		+	+			+	+	+	+											
800	+		+						+	+										
750	+		+														+	+		
700		+	+								+	+	+	+	+					
680	+		+	+	+		+	+	+	+										
600	+					+	+													
550																+		+	+	
500			+				+													
450		+			+						+							+		
350													+							
300												+		+						
280																		+		

Where: B: *Bacillus* sp. without addition of $ZnsO_4$ in their growth medium, B1: *Bacillus* sp. under the treatment of 0.5 mg/L of $ZnSO_4$, B2: *Bacillus* sp. under the treatment of 1.0 mg/L of $ZnSO_4$, B3: *Bacillus* sp. under the treatment of 1.5 mg/L of $ZnSO_4$, and H: Hybrid between *Bacillus* sp. and *Pseudomonas* sp.

Table (6): DNA fragments of RAPD for treated and untreated *Pseudomonas* bacteria using four random primers.

Primers	AATCCGCAAT					CGCGGAATGC					AATCGGTACT					CTCGAATGCA				
DNA fragments	P	P1	P2	P3	H	P	P1	P2	P3	H	P	P1	P2	P3	H	P	P1	P2	P3	H
1600													+							
1550														+	+					
1500																				
1450												+								
1350												+								
1250					+			+												
1200					+			+	+											
1180																				
1150	+	+			+	+			+		+	+			+					+
950																				
900	+	+																		
850		+	+	+		+		+	+							+			+	
800		+	+	+						+										
750			+					+	+											
700	+			+		+					+	+		+	+			+		
680	+	+	+		+			+	+	+										
600						+	+													
550																				
500																+				
450			+		+													+		
420																				
350	+															+				
280																	+			
220																+				

Where:

P *Pseudomonas* sp. without addition of $PbNO_3$ in their growth medium.

P1: *Pseudomonas* sp. under the treatment of 0.5 mg/L of $PbNO_3$.

P2: *Pseudomonas* sp. under the treatment of 1.0 mg/L of $PbNO_3$.

P3: *Pseudomonas* sp. under the treatment of 1.5 mg/L of $PbNO_3$.

H: Hybrid between *Bacillus sp*. and *Pseudomonas sp*

CHAPTER 1

INTRODUCTION

Wastewater treatment was the most important problem in recent years. Thus, advances in molecular biology have extended our understanding of the metabolic processes related to microbial transformation of heavy metals and petroleum hydrocarbons (**Puntambekar** and **Ranjeker**, 1989 and **Mezzanotte**, 2002). By establishing conditions which maximize rates and extents of microbial growth, hydrocarbon access, and transformation, highly accelerated and bioreactor-based petroleum waste degradation processes have been implemented. Microbes may be exploited to break stable oilfield emulsions to produce pipeline quality oil. Molecular approaches are being used to broaden the substrate specificity and increase the rates and extents of desulfurization. Bacterial processes are being commercialized for removal of H_2S and sulfoxides from petrochemical waste streams. Microbes also have potential for use in removal of nitrogen from crude oil leading to reduced nitric oxide emissions provided that technical problems similar to those experienced in biodesulfurization can be solved. Thus, new strains of *Bacillus* were presented that produce lipase and other enzymes for the degradation of oleaginous materials such as fats, greases and cooking oils, and protease enzymes to degrade proteins. This novel strain and the enzymes produced thereby have a number of applications, including wastewater treatments, agricultural uses, laundry and dish detergents, drain cleaners and spot removers, among others **(Kozo *et al.,* 1979; Raquel *et al.*, 2007 and Reshma *et al.,* 2007).**

Novel mutant strain *Pseudomonas fluorescens* 3P has been found to be capable of degrading and removing anionic and/or non-ionic surface active agents, detergents and like materials. Thus, this mutant could be cultured in wastewater from any type of industrial plant containing anionic and/or non-ionic surface active agents. The mutant strain of this invention can be employed in ion exchange resin treatment systems, in tricking filter systems, in carbon adsorption systems, in activated sludge treatment systems, in outdoor lagoons or pools, etc. (**James**, **2006).** Molecular biology technique presented a huge helpful for wastewater treatment, **Wikstrom (200٢)** demonstrated that microbial community changes over time in a nitroaromatic-contaminated groundwater upon amendment with hydrocarbons previously unknown to the microbial community (extrinsic) and hydrocarbons previously known to the microbial community (intrinsic).

Microbial growth, biodegradation, and community structure changes measured by random amplified polymorphic DNA (RAPD) and quantitative PCR (qPCR) targeting catechol-2,3-dioxygenase (C23O) genes were monitored over time. All amended substrates were biodegraded after both substrate amendments except for 2,4-DNT, which was only partially degraded after the second amendment. Unique microbial communities were developed in flasks amended with phenol, benzoic acid, and naphthalene. In addition protoplast fusion technique was applied for enhancement heredity desirable genotype between different bacterial genera. **Kanevski *et al.* (1983)** carried out hybridization by fusion between protoplasts of *A. tumefaciens* and different *Pseudomonas* sp. All the eight strains which selected on screening media were true chromosomal hybrid and heredity tumor formation as a character of *A. tumefaciens*, but nine strains which were selected by

mechanical isolation were resistant for two different pesticides as a remark of expressing, the *Pseudomonas* plasmids. **Gleba *et al.* (1986)** fused protoplasts of *Seratia sp.* with protoplasts of *Agrobacterium tumefaciens*. All the obtained 57-hybrid strains contained lysopine dehydrogenase, which is encoded by T-DNA, in both callus tissue and shoots. They found that, in six of 12 strains, chromosomes from both parents were present and found the nearly diploid chromosome numbers of both species, while in the other six cell lines, one of the parents was predominate. Esterase and amylase isoenzymes patterns and chloroplast DNA restriction analysis were detected. Furthermore, **Muller and Schieder (1987**) showed that protoplasts of a *Nicotiana paniculata* cell line could be fused with protoplasts of nitrate reductase deficient cnx68 cell line of *Pseudomonas*. From about 1.1 X 10^7 cultured protoplasts, 18 cell lines were obtained. Four of them showed two dominant and two recessive markers, while three cell lines lacked one or more markers.

The main objective of this study could be summarized as follow:

1- Isolation, purification and identification of bacterial genera characterized with their ability for wastewater biodegradation through different microbial tests.
2- Assay the ability of bacterial isolates from different genera for wastewater biodegradation.
3- Induction of a bacterial mutant which is superior in wastewater biodegradation comparing with its parents.
4- Improvement of wastewater biodegrading bacterial isolates under study through applied genetical technique (protoplast fusion technique).
5- Application of different molecular biological techniques (protein fingerprinting and Random Amplified Polymorphic DNA (RAPD) to study and compare parental and hybrid bacteria which are produced by protoplast fusion.