# FLUORESCENT PSEUDOMONADS AS PLANT GROWTH PROMOTERS AND BIOCONTROL OF ROOT-INFECTING PATHOGENS ON MAIZE PLANT

By

### **OSAMA ELSAYED MOHAMED**

B.Sc.Agric.Sc. (Biotechnology), Ain-Shams University, 2012

A Thesis Submitted in Partial Fulfillment
Of
The Requirement for the Degree of

MASTER OF SCIENCE
in
Agricultural Sciences
(Agricultural Microbiology)

Department of Agricultural Microbiology
Faculty of Agriculture
Ain-Shams University

## **Approval Sheet**

# FLUORESCENT PSEUDOMONADS AS PLANT GROWTH PROMOTERS AND BIOCONTROL OF ROOT-INFECTING PATHOGENS ON MAIZE PLANT

By

## **OSAMA ELSAYED MOHAMED**

B.Sc.Agric.Sc. (Biotechnology), Ain-Shams University, 2012

This thesis for M. Sc. degree has been approved by:

Dr. Mohammed ZakariaSedik  Prof. Emeritus of Agric. Microbiol, Fac. of Agric., CairoUniversity				
Dr. Shawky Mahmoud Selim				
Prof. of Agric. Microbiol, Fac. of Agric., Ain-Shams University				
Dr. EnasAbd El-Tawab Hassan  Prof. of Agric. Microbiol, Fac. of Agric., Ain-Shams University				
Dr.Elshahat Mohamed Ramadan				
Prof. Emeritus of Agric. Microbiol, Fac. of Agric., Ain-Shams				
University				

**Date of Examination**: 16/12/2018

# FLUORESCENT PSEUDOMONADS AS PLANT GROWTH PROMOTERS AND BIOCONTROL OF ROOT-INFECTING PATHOGENS ON MAIZE PLANT

By

### OSAMA ELSAYED MOHAMED

B.Sc.Agric.Sc. (Biotechnology), Ain-Shams University, 2012

### Under the supervision of:

#### Dr. Elshahat Mohamed Ramadan

Prof., Emeritus of Agricultural Microbiology, Department of Agric. Microbiology, Faculty of Agriculture, Ain-Shams University (Principal Supervision).

#### Dr. Enas Abd El-Tawab Hassan

Prof. of Agricultural Microbiology, Department of Agric. Microbiology, Faculty of Agriculture, Ain-Shams University.

### Dr. Elhamy Mohamed Mostafa El-Assiuty

Head Research Emeritus of Plant Pathology, Plant Pathology Research Institute, Agricultural Research Centre, Giza

#### ABSTRACT

Osama Elsayed Mohamed:Fluorescent Pseudomonads as Plant Growth Promoters and Biocontrol of Root-Infecting Pathogens on Maize Plant. Unpublished M. Sc. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2019.

Possibility of manipulating some of the efficient strains of rhizospheric fluorescent pseudomonads to manage the root-infecting pathogens of maize was studied throughout this study. Out of 110 isolates, 24 of *Pseudomonas* species, recovered from the rhizosphere of maize and sugar beetshowed high antagonistic effect against two major root-infecting pathogens of maize, namely Cephalosporium maydis and Fusarium verticillioides in vitro. Pot experiment revealed that just 4 isolates could reduce infection with both pathogens and enhance the plant growth as well. Based on the genotypic identifications, they could be identified as: Pseudomonas putida Pau9, P.putidaPau11, P.putida Psf3 enzymes revealed P.aeruginosaPsf9.Assay ofextracellular cellulase was actively produced, only by P. aeruginosa Psf9. Chitinase, however was detected in growing media of three strains, but not by P. putida Paul1. Assay of antibiotics produced by the bacterial strains showed that phenazine could, only be produced by P. aeruginosa Psf9. HCN was found to be excreted by P. putida Psf3 and P. aeruginosa Psf9. Except P. putida Pau9, the IAA could be produced by the other three strains. All strains were able to produce siderophores, and caused availability of P and K. GC-MS analyses revealed that different compounds were detected within the metabolites produced by each of Pseudomonas strains under study. Coating maize seed with the mixture of the four strains and seeding in potted-soil infested singly with one of the two target pathogens, or in combination revealed that fresh and dry weights of resulting plants were significantly increased compared with the control. Treatment caused significant increase in root lengths, insignificant increase in shoot lengths. This is correct in soil infested with C. maydis. Whereas,in soil infested with F. verticillioides, treatment caused significant results in all of the growth parameters of plants. Combining the two pathogens in soil showed that insignificant effect of coating seed with the mix of bacteria on any of shoot or root lengths. Whereas, significant results were found in shoot and root weights compared to the non-treated control.

**Keywords:** Fluorescent Pseudomonads, *Cephalosporiummaydis*. *Fusarium verticillioides*, Maize plant, GC-MS analyses bioagent.

#### **ACKNOWLEDGEMENT**

Praise and thanks be to ALLAH, the most merciful for assisting and directing me to the right way.

There are few opportunities in the most people's lives to officially prove their gratitude to people who were mentors and supporters at different stages of our lives. However, we do not forget to seal those feelings on paper.

I would like to express my deep gratitude to **Dr. Elshahat Mohamed Ramadan** Prof. of Agricultural Microbiology, Faculty of
Agriculture, Ain Shams University, for his supervision, helpful
encouragement, critical reading and revising the manuscript.

I would like to express my sincere thanks, and deep gratitude to **Dr. Enas Abd El-Tawab Hassan** Prof. of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, for her fruitful discussion, criticism and valuable help throughout of this study.

Deep thanks and appreciation are extended to **Dr. Elhamy Mohamed Mostafa El-Assiuty,** Chief of Research, Plant Pathology
Research Institute, Agricultural Research Centre, for his encouragement,
motivation and providing all needs and facilities during the preparation of
this thesis.

Cordially, it is my pleasure to express my deep gratitude and sincere appreciation to **Dr. Zeinab Mohamed Fahmy** Chief of Research, Plant Pathology Research Institute, A.R.C, for her support and valuable encouragement throughout executing this study.

I would, also like to thank all members of Maize and Sugar Crops Research Department, Plant Pathology Research Institute, A. R. C. for their cordial assistance.

Finally, I would like to thank my parents, my wife, brothers and sister for their love, patience, interest, support and continuous encouragement.

## **CONTENTS**

	Page
LIST OF TABLE	
LIST OF FIGURES	
INTRODUCTION	1
REVIEW OF LITERATURE	3
1. Maize plant as an economical crop	3
2. Pathogens infected maize plant	3
3. Fluorescent pseudomonads involved in suppression of plant	
root pathogens and improving plant growth	4
A. Antagonism by production of lytic enzymes	5
B. Phytohormones production	6
C. Production of siderophores	7
D. Antibiotic production	7
E. Production of Hydrogen cyanide (HCN)	8
F. Phenolic compound	9
G. Phosphate solubilization	10
H. Potassium solubilization	10
4. Performance of plant affected by microbial inoculants	11
MATERIAL AND METHODS	13
1. Isolation and identification of maize infected pathogens	13
A. Cephalosporium maydis (Harpophora maydis)	13
B. Fusarium verticillioides (F.moniliforme)	13
2. Isolation of rhizospheric fluorescent pseudomonads	14
3. Assessment the antagonistic activity of fluorescent	
pseudomonads	14
A. In vitro antagonistic activity	14
B. In vivo evaluation of antagonist bacterial isolates	15
4. Identification of the most efficient <i>Pseudomonas</i> isolate	15
4.1. Biochemical characterization of <i>Pseudomonas</i> spp	15
4.2. Molecular identification of <i>Pseudomonas</i> spp	15

5.	Molecular identification of Fusarium
5.1.	DNA extraction.
5.2.	PCR amplification
6.	Molecular identification of Cephalosporium
6.1.	DNA extraction
6.2.	DNA extraction
7.	Phylogenetic analysis
8.	Characterization of antagonistic bacteria
8.1.	Production of bacterial lytic enzymes
A.	Production of cellulase
B.	Pectinase activity
C.	Chitinase activity
8.2.	Detection of antibiotic production genetically
8.3.	Phosphate solubilization
8.3.	1. Qualitative assay
8.3.	2. Quantitative assay
8.4.	Potassium solubilization
8.5.	Production of indole 3- acetic acid (IAA)
8.6.	Production of HCN
8.7.	Quantitative assessment of siderophore production
1-	Preparation of standard curve of deferoxamine mesylate
2-	Preparation of culture for examination
3-	Quantitative siderophores
9.	Characterization of antifungal metabolites by Gas
	Chromatography Mass Spectrometry (GC-MS)
10.	Effect of bioagent on fungal pathogen infected maize plant,
	under pot experiment
10.1	. Parameters measured
A.	Vegetative parameters
B.	Chemical analysis
1-	Nitrogen, phosphorus and potassium content
2-	Chlorophyll and beta carotenoid contents

a. Enzyme activity assay
11. Statistical analysis
RESULTS
1. Identification of pathogenic fungus of maize plant
1.1. Phenotypic identification of <i>Cephalosporium maydis</i>
1.2. Genotypic identification of <i>Cephalosporim maydis</i>
1.3. Phenotypic identification of <i>Fusaruim</i>
1.4. Phylogenatic analysis of <i>Fusarium</i>
2. Isolation of the fluorescent pseudomonads
3. Antifungal activity
4. Evaluation of selected cultures as biocontrol agent <i>in vivo</i>
5. Biochemical characterization of fluorescent pseudomonads
6. Genotypic identification for the most potent antagonist
bacterial isolates.
7. Biologcal activities of the efficient bacterial strains
7.1. Production of extracellular enzymes
7.2. Antibiosis of <i>Pseudomonas</i> strains
7.3. Production of hydrogen cyanide (HCN)
7.4. Production of siderophores
7.5. Production of phytohormones by <i>Pseudomonas</i> strains
7.6. Phosphate solubilization.
7.7. Potassium availability
8. Analyses of secondary metabolites produced by the four
efficient Pseudomonas strains
9. Performance of maize plant in soil infested with root-infecting
pathogen and biocontrol agent
9.1. Vegetative parameters
9.2. Accumulation of pigment and enzyme activity of mize plant
DISCUSSION
SUMMARY
REFERENCES
ARABIC SUMMARY

# LIST OF TABLES

No.		P
1	Sequence of specific primer used for Pseudomonas spp	
	identification	
2	Primers for PCR analysis of tested antibiotics	
3	Number of fluorescent pseudomonads isolates recovered	
	from different sources	
4	Efficiency of coating maize seed with fluorescent	
	pseudomonad isolates in managing root-infecting	
	pathogens of maize under greenhouse conditions	
5	Biochemical characterization for identification of the	
	most potent Pseudomonas isolates	
6	Assessment of enzymatic activity of selected	
	Pseudomonas strains	
7	Potency of Pseudomonas strains for producing	
	antimicrobial compounds	
8	Production of IAA and gibberellins by tested strains	
9	GC-MS analyses for the metabolites produced by	
	Pseudomonas putida psf 3in liqid culture	
10	GC-MS analyses for the metabolites produced by	
	Pseudomonas aeruginosa psf 9 in liqid culture	
11	GC-MS analyses for the metabolites produced by	
	Pseudomonas putida Pau 9 in liquid culture	
12	GC-MS analyses for the metabolites produced by	
	Pseudomonas putida Paul 1 in liquid culture	
13	Effect of the combination of the four bacterial mixture	
	and C.maydis on parameters of maize plant growth under	
	greenhouse conditions	
14	Effect of the combination of the four bacterial mixture	
	and F. verticillioides on growth parameters of maize	
	plants under greenhouse conditions	

No.		Page
15	Effect of the combination of the four bacterial mixture	
	and C.maydis + F. verticillioides on growth parameters	
	of maize plants under greenhouse conditions	48
16	Effect of the combination of four bacterial mix and	
	fungal pathogens on chlorophyll and carotenoides	
	contents of mize plant under greenhouse condition	51
17	Effect of combination of bacterial mixture and fungal	
	pathogens on peroxidase activity of maize plant under	
	greenhouse conditions	52