

بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ



HOSSAM MAGHRABY



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



HOSSAM MAGHRABY

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم
قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغييرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HOSSAM MAGHRABY



بعض الوثائق

الأصلية تالفة



HOSSAM MAGHRABY



بالرسالة صفحات

لم ترد بالأصل



HOSSAM MAGHRABY

**STUDIES ON BIOLOGICAL CONTROL OF
POTATO BROWN ROT DISEASE**

B 12771

By
Nevein Anwar Shehata
(B. Sc. In Botany, 1992)
Faculty of Science
Cairo University (Beni Suef branch)



Thesis

Submitted in Partial Fulfillment
of the Requirements
For the Degree of

MASTER OF SCIENCE

in

BOTANY (PLANT PATHOLOGY)

Supervisory Committee

Prof. Dr Nabil S. Farag
Deputy Director of
Plant Pathology Research
Institute, Giza
Agricultural Research Center

Prof. Dr Mohamed A. Saleh
Chief Researcher in
Central Lab. of Pesticides,
Dokki-Giza
Agricultural Research Center

Dr. Suzan A. Abdallah
Associate Prof. of
Plant Pathology, Botany Dep.
Faculty of Science, Zagazig Univ. (Benha branch)

Botany Departement
Faculty of Science (Benha branch)
Zagazig University

December 2001

ACKNOWLEDGMENT

Thanks to God the most high and merciful for giving me the strength from the beginning until the end to carry out this work.

I am deeply grateful to Prof. Dr. Nabil Sobhy Farag, deputy director of Plant Pathology Institute Research Institute, Giza, for suggesting the problem for his valuable advices, enormous help and full support on supervision of this work.

I am thankful to Prof. Dr. Abdel Allah Salleh, Chief researcher, Central Lab of Pesticides, Agricultural Research Center, Dokki for his supervision.

I am indebted to Dr Suzan Abd-Elhaleam Abdallah, associate Prof. Of plant pathology, Botany department, Faculty of Science, for her encouragement, helpful supervision and directive comments.

I am also thankful to Prof Dr Hussein Yossery Olama the head of Botany Dept, Faculty of Science, Zagazig Univ. (Benha branch) for offering the facilities needed to conduct part of this work.

I am thankful also to Prof Dr Youssef H. El-Daoudi Head of Central Administration for the Plant the Quarantine and Director of Potato Brown Rot Project for offering every possible facility.

Thanks also due to Prof. Dr. Faiza Gabriel Fawzy, Head of Research of bacterial disease in Potato Brown Rot Project, my colleagues in the Potato Brown Rot Project and in Plant Pathology Institute who helped to make this study possible by all means.

I am thankful also to Prof. Dr. Jaap Janse (Head of Bacteriology Dept. Wageningen, The Netherlands) and Prof. Dr. David Stead (Head of Bacteriology Central Science Lab York UK.) for their valuable guidance, training and cooperation while I was conducting Fatty acid analysis, protein electrophoresis, PCR and lyophilization of the strains used in this work in their labs.

CONTENTS

Acknowledgment	i
Contents	ii-v
List of tables	vi-vii
List of figures	viii-ix
List of abbreviations	x-xii
INTRODUCTION	1-2
REVIEW OF LITERATURE	3-27
1. Epidemiological and Ecological Studies	3-6
1.1. Epidemiological studies	3-5
1.2. Ecological studies	5-6
2. Detection and race identification of <i>Ralstonia solanacearum</i>	6-11
2.1. Detection	7-8
2.1.1. Using traditional techniques	7
2.1.2. Using serological methods	7-8
2.2. Identification	8-11
2.2.1. Fatty acid profiling	8
2.2.2. SDS-PAGE protein electrophoresis	8
2.2.3. Genetic fingerprints	9-10
2.2.4. Integrated Identification methods	10-11
3. Pathogenicity and Pathogenesis	12-15
4. Susceptibility and Hypersensitivity	15-17
4.1. Susceptibility of different potato cultivars	15-16
4.2. Hypersensitive response	16-17
5. Control of <i>Ralstonia solanacearum</i>	17-27
5.1. Using resistant varieties	17
5.2. Chemical control	18-19
5.3. Biological control	19-27

5.3.1 Bacteriocinogenic strains	20-22
5.3.2. Antagonistic sporeforming bacteria	22-23
5.3.3. Antagonistic non-sporeforming bacteria	23-26
5.3.4. Actinomycetes	26-27
MATERIALS AND METHODS	28-55
1. Isolation of the pathogen:	28-30
1.1. From tubers showing typical symptoms	28-29
1.2. From tubers with latent infection	29
1.3. Isolation of the pathogen from different habitats	29-31
1.3.1. From water streams	29-30
1.3.2. From soil	30-31
2. Isolation of antagonistic bacteria	31-32
2.1. From rhizosphere and soil of potatoes	31-32
2.2. From rhizosphere and soil of eggplant	32
2.3. From naturally infected potato tubers	32
3. Identification of bacteria	33-41
3.1. Biochemical tests	33
3.2. Immunofluorescence antibody stain (IFAS)	33-35
3.2.1. IFAS testing of bacterial pathogen-containing tubers	34-35
3.2.2. IFAS testing of bacterial pathogen in different habitats	35
3.3. Identification by fatty acid analysis	35-36
3.4. Protein Electrophoresis	36-38
3.5. Polymerase Chain Reaction (PCR)-based methods	38-41
4. Antagonistic bacteria and biological control	42-44
4.1. <i>In vitro</i> trials	42-43
4.1.1. <i>In vitro</i> testing of antagonism	42
4.1.2. Effect of trace elements on the antagonistic potential of the tested bacteria	42-43

4.2. <i>In vivo</i> trials	43-44
5. Media used	45-49
5.1. Buffers used for IFAS test	49-50
5.2. Reagents for FAA	50-51
5.3. Electrophoresis reagents for PE	51-52
5.4. PCR reagents and programmes	53-55
RESULTS AND DISCUSSIONS	56-118
1. Isolation and Identification of the Pathogen	56-74
1.1. Isolation and Pathogenecity	56-59
1.2. Identification of the pathogen	60-74
1.2.1. Physiological and Biochemical characteristics	63-64
1.2.2. Immunofluorescens antibody staining test	65-68
1.2.3. Fatty acid analysis of <i>R. solanacearum</i>	69-70
1.2.4. Protein electrophoresis of <i>R. solanacearum</i>	71-72
1.2.5. Polymerase chain reaction (PCR)-based methods	73-74
2. Isolation of the pathogen from different Habitats	75-79
2.1. Isolation from watercourses	75-76
2.2. Isolation from soil	77-79
3. Isolation, identification and testing of the antagonistic bacteria	80-103
3.1. Isolation of antagonistic bacteria from Potato, Eggplant and Sweet pepper rhizosphere.	80-86
3.2. Isolation of antagonists from infected potato tubers	87-88
3.3. Identification of the antagonistic bacteria	89-103
3.3.1. Biochemical and physiological characteristics	89-92
3.3.2. Fatty acid profiling	93-101
3.3.3. Identification by protein profiling	102-103
4. Biological control under greenhouse conditions:	104-118
4.1. Effect of different application methods of antagonistic strains on wilt development on potato cultivars.	104-108
4.2. Effect of application of two selected bacterial biocontrol agents	

on wilt development of potato in sterilized and non sterilized-soils	109-111
4.3. Effect of application of one biocontrol agent on wilt development of tomato seedlings in sterilized and non sterilized-soils	112-113
5. Effect of trace elements on the antagonistic potential of the tested bacteria	114-118
6. GENERAL DISCUSSION	119-123
7. SUMMARY	124-127
REFERENCES	128-144
ARABIC SUMMARY	

List of tables

Table	Title	Page
(1)	Isolation of <i>R. solanacearum</i> from diseased tubers and latently tubers and pathogenicity on tomato and potato plants	58
(2)	Physiological and biochemical characteristics of <i>R. solanacearum</i>	64
(3)	Immunofluorescence antibody stain test (IFAS) of latently infected potato samples	67
(4)	Fatty acids (FA) determined in extracts of <i>Ralstonia solanacearum</i> .	70
(5)	Isolation of <i>R. solanacearum</i> from watercourses in different governorates in Egypt.	76
(6)	Isolation of <i>R. solanacearum</i> from soil in different governorates in Egypt.	79
(7)	Densities of total bacterial flora in potato rhizosphere of three late maturing potato cultivars	81
(8)	Bacterial densities and antagonists in rhizosphere of different potato cultivars, Eggplant and sweet pepper at different growth stages.	85
(9)	Characteristics of antagonists isolated from infected potato tubers from different governorates in Egypt during 1996-1997.	88

(10)	Biochemical and physiological characteristics of the Fluorescent Pseudomands.	90
(11)	Biochemical and physiological characteristics of <i>Acinetobacter spp.</i> and <i>Stenotrophomonas maltophilia</i> .	92
(12)	Fatty acid analysis (FAA) of Fluorescent Pseudomonads.	94-95
(13)	Fatty acid analysis (FAA) of <i>Acinetobacter spp.</i>	97
(14)	Fatty acid analysis FAA of <i>Stenotrophomonas maltophilia</i>	98
(15)	Fatty acid analysis FAA of <i>Bacillus cereus</i>	99
(16)	Summary of FA profiles for different bacterial genera isolated from different habitats in Egypt during 1996-1998	101
(17)	Effect of application of bacterial biocontrol agents on wilt development on Diamant and Nicola potato cultivars in greenhouse	107-108
(18)	Effect of application of the selected bacterial biocontrol agents on wilt development of potato in sterilized and non sterilized-soils	110
(19)	Effect of application of one selected bacterial biocontrol agent on wilt development of tomato seedlings in sterilized and non sterilized-soils	113
(20)	Effect of trace elements on the antagonistic potential of bacterial strains, against <i>Ralstonia solanacearum</i> on KB medium.	117

List of figures

Fig.	Title	Page
(1)	Symptoms of potato brown rot disease on tuber caused by <i>Ralstonia solanacearum</i> race 3 biovar 2.	59
(2)	Disease symptoms on of 3 week-old tomato plants, 7 days after inoculation with 1×10^6 cfu of isolates of <i>Ralstonia solanacearum</i> at room temperature (28° C).	59
(3)	(a) Growth characteristics of <i>R. solanacearum</i> (race 3 biovar 2) isolates on King' s medium (KB) 2 days after incubation at 28° C. (b) Growth characteristics of <i>R. solanacearum</i> potato derived on Selective Medium South Africa (SMSA), 4 days at 28° C., note, the irregular and slimy appearance of bacterial colonies.	61
(4)	The phenotypic variation in <i>R. solanacearum</i> isolates, from latently infected tubers, grown on casamino peptone glucose Agar medium (CPG) containing 2,3,5 triphenyltetrazolium chloride (TTC) medium; a, first culturing (7 days after incubation), b and c after 3 and 6 successive subculturing respectively. The virulent colonies were irregular with red centers and luxuriant slime. The avirulent colonies were afluidal or butyrous with deep red pigmentation.	62
(5)	Fluorescence cells of <i>R. solanacearum</i> from latent infected, potato tubers.	68
(6)	Protein electrophoresis of <i>R. solanacearum</i> using Gel Compar	

	software (Applied Math, Korticht Belgium)	72
(7)	Identification of various isolates of <i>R. solanacearum</i> race 3 biovar 2, from infected potato tubers (symptomless and with typical brown rot symptoms) during 1997 using Nucleic acid-based techniques.	74
(8)	Antagonism against <i>R. solanacearum</i> : Antagonistic isolates of <i>Acinetobacter baumannii</i> , <i>Stenotrophomonas maltophilia</i> and <i>Pseudomonas putida</i> from potato.	86
(9)	Protein electrophoresis of <i>Acinetobacter baumannii</i> and <i>Pseudomonas putida</i> compared to <i>R. solanacearum</i> using Gel Compar software	103
(10)	Wilt symptoms on potato plants under green house conditions	111
(11)	Effect of trace elements on bacterial antagonism <i>in vitro</i> bioassay to <i>R. solanacearum</i> race 3 biovar 2. The antagonistic isolates were <i>A. baumannii</i> , <i>S. maltophilia</i> and <i>Pseudomonas fluorescens</i> from potato rhizosphere, eggplant rhizosphere and naturally infected potato tubers. Isolate of <i>R. solanacearum</i> , inoculated on King' s medium (KB). Trace elements added were Fe^{+++} , Zn^{++} , and Mn^{++} at a concentration of 100 μ M.	118