# BIODIVERSITY OF RHIZOBIA IN NORTH SINAI DESERT

By

#### TAREK SAYED RAGAB

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2002.

#### **THESIS**

Submitted in Partial Fulfillment of the Requirements for the Degree of

#### **MASTER OF SCIENCE**

In

Agricultural Sciences (Microbiology)

Department of Microbiology Faculty of Agriculture Cairo University EGYPT

2008

#### APPROVAL SHEET

#### BIODIVERSITY OF RHIZOBIA IN NORTH SINAI DESERT

# M. Sc. Thesis By

#### TAREK SAYED RAGAB

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2002

# Approved by: Prof. Dr. Diaa El-Dean Soilem Research Professor of Microbiology, Institute of Soil, Water and Environmental Sciences, A.R.C. Porf. Dr. Aziz Mohamed Higazy Professor of Microbiology, Fac. Agric., Cairo University. Prof. Dr. Mohamed Fayez Fouad Professor of Microbiology, Fac. Agric., Cairo University. Dr. Mohamed Abd El-Alim Ali Assistant Professor of Microbiology, Fac. Agric., Cairo University. Date: / /

#### **SUPERVISION SHEET**

# BIODIVERSITY OF RHIZOBIA IN NORTH SINAI DESERT

M. Sc. Thesis
By

#### TAREK SAYED RAGAB

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2002

#### SUPERVISION COMMITTEE

Dr. Mohamed Fayez Fouad Professor of Microbiology, Fac. Agric., Cairo University.

Dr. Mohamed Abd El-Alim Ali Associate Professor of Microbiology, Fac. Agric., Cairo University. Name of Candidate: Tarek Sayed Ragab Degree: M.Sc.

Title of Thesis: Biodiversity of Rhizobia In North Sinai Desert

Supervisors Prof. Dr. Mohamed Fayez Fouad, Dr. Mohamed Abd El-Alim

Ali

**Department**: Microbiology

Branch: Microbiology Approval: //

#### **ABSTRACT**

Acacia saligna trees and 9 different wild herbaceous legumes grown in El-Ahrash protectorate, North Sinai, were surveyed for nodulation. Sixty five rhizobia were isolated from naturally occurring root-nodules of Acacia saligna trees and the different wild annuals. According to their cultural, morphological and physiological characteristics as well as SDS-PAGE of total cell proteins, the isolates were phenotypically categorized into two distinct groups. The first includes those isolated from Acacia saligna which were differentiated into three distinct clusters. The second group comprises isolates from wild annual legumes; analysis of their phenotypic characters building dendrograms divided them into two distinct clusters. Eight rhizobial isolates representing the various Acacia-rhizobia clusters were examined for inoculation of Acacia saligna seedlings grown in sandy soil plastic bags under sunlight or shade conditions. After 6 months, all seedlings, whether inoculated or not, formed from 4 to 87 nodules seedling<sup>-1</sup> with lower number recorded with uninoculated seedlings. In the sunny nursery, Ac22p and Ac18p were the most infective isolates inducing the formation of up to 87 and 75.6 nodules seedlings<sup>-1</sup> with maxima nodule biomass, shoot and root length and biomass. Shading severely affected nodulation of Acacia seedlings either with indigenous or introduced rhizobia. Uninoculated seedlings formed 17 nodules seedling-1 under sunlight and 4.4 nodules seedling-lunder shade conditions. Seedlings grown in the shaded nursery showed a very poor growth after 6 months. A promoting effect on growth of Acacia seedling roots was displayed by the isolates Ac-22p, Ac-23s, Ac-17s and Ac-17f where significantly taller roots were recorded with seedlings drenched with these bacterial inocula. For the first time in Egypt, sugarcane bagasse pith was used as rhizobia inocula carrier. Data recommended pre-sterilization of bagasse pith incorporated in the inocula formula. Survival of rhizobia in pith-based inocula was good or better than that recorded with peat based inocula. Instead of the imported Irish peat moss, the present data recommend sugarcane bagasse pith for rhizobial inoculant formulation.

Key words: Biodiversity, rhizobia, *Acacia saligna*, wild legumes, sugarcane bagasse pith, Sinai desert.

# **DEDICATION**

I dedicate this work to whom my heart felt thanks; to my wife Eman and my daughter Laila for their patience and help, as well as to my father and brothers for all the support they lovely offered along the period of my post graduation.

### ACKNOWLEDGEMENT

I wish to convey my sincere thanks, deepest gratitude and appreciation to **Dr. Mohamed Fayez** and **Dr. Mohamed Abd El-Alim.** Professors of Microbiology, Faculty of Agriculture, Cairo University for supervision, continued assistance and their guidance through the course of study and revision of the manuscript.

Sincere thanks to **Dr. Nabil A. Hegazi** Professor of Microbiology, Faculty of Agriculture, Cairo University for suggesting the problem, providing all the required facilities particularly the out-reach and field trips as well as his guidance through the course of study.

Sincere thanks to **Dr. Waffaa Ameer**, Professor of Botany, Faculty of Science, Cairo University for her help and support in the classification of plants used in the study.

Grateful appreciation is also extended to all staff members of Microbiology Department, Faculty of Agriculture, Cairo University.

## **CONTENTS**

INTRO	DUCTION
REVIE	W OF LITERATURE
1.	Nodulation of Acacia
2.	Microsymbionts of Acacia
3.	Environmental influences on Acacia symbiosis
4.	Future perspective
5.	El-Ahrash protectorate
6.	Pith as a carrier for <i>Rhizobium</i> inoculants
MATER	IALS AND METHODS
1.	Soil
2.	Carriers
3.	Collection site and host plants
4.	Isolation of rhizobia
5.	Characterization of rhizobia isolates
	a. Cultural properties, colony morphology and
	pH reaction
	b. Carbon source utilization
	c. Tolerance to NaCl concentrations
	d. Growth at different pH levels
	e. Growth at different incubation temperatures
	f. SDS-PAGE of total bacterial proteins
	Plant infection
7.	
8.	affected by rhizobial inoculation and shading Sugarcane bagasse pith-based inoculant for
0.	Acacia rhizobia
9.	Statistical analysis
	Media and nutrient solutions
	TS
	Collection site
	Characterization of rhizobia isolates
_,	a. Morphological characteristics.

b. Tolerance to NaCl concentrations	41
c. Growth at different incubation temperatures	47
d. Carbon source utilization	50
3. Numerical analysis	50
4. Plant infection test	57
5. Growth and nodulation of Acacia saligna as affected by rhizobial inocula and shading	58
6. Sugarcane bagasse pith-based inoculant for <i>Acacia</i> rhizobia	72
DISCUSSION	77
SUMMARY	88
REFERENCES	92
Arabic summary	

## LIST OF TABLES

No	Title	Page
1.	Mechanical and chemical analysis of soil samples	21
2.	Chemical analysis for Pith and Peat	22
3.	Sampling sites, altitude and season of isolation	25
4.	Water holding capacity for each carrier used in the study	33
5.	Some colony morphology characteristics of <i>Acacia</i> rhizobia	42
6.	Some colony morphology characteristics of rhizobia from some wild legumes	44
7.	Motility (M) in YMB and growth of the different <i>Acacia</i> rhizobial isolates on YMA with different pH values and Peptone Glucose Agar (P)	45
8.	Tolerance of <i>Acacia</i> rhizobia to different NaCl concentration in YMA	47
9.	Tolerance of rhizobia isolated from some wild legumes to NaCl concentration in YMA.	48
10.	Growth of the different <i>Acacia</i> Rhizobia isolates at different incubation temperatures.	49
11.	Growth of rhizobial isolates on YMA with different carbon sources	51
12.	Some traits of the rhizobia isolates used for inoculating <i>Acacia saligna</i> seedlings gown under direct sunlight or shade conditions.	58
13.	Some physical and chemical properties of the different inocula carriers	86

## LIST OF FIGURES

No	Title	Pag
1	Satellite over view of El-Ahrash Protectorate, North Sinai	24
2	The naturally occurring <i>Acacia saligna</i> trees in El-Ahrash Protectorate	26
3.	Acacia saligna tree	26
4.	Some wild annual legumes	27
5.	Nodule morphology of <i>Acacia saligna</i> nodules	39
6.	Colony morphology of Some Rhizobial isolates from <i>Acacia</i> saligna A, Ac11F; B, Ac 9; C, Ac 1; D, Ac15s	46
7.	UPGMA dendrogram of the phenotypic traits of <i>Acacia</i> and wild legume rhizobia isolates	54
8.	SDS-PAGE of the total protein profile of rhizobia isolates	55
9.	SDS-PAGE dendrogram for rhizobia isolates from <i>Acacia</i> trees and wild legumes	56
10.	Growth chamber in which plant infection technique was conducted.	57
11.	The experimental nursery in Rafah, North Sinai	59
12.	Total nodule number (nodules. Seedling <sup>-1</sup> ) formed by the 6-month-old seedlings grown with or without rhizobial inocula under sunny or shaded nursery.	60
13.	Total and tap-root nodule numbers (nodules. Seedling <sup>-1</sup> ) formed by the 6-month-old seedlings grown with or without rhizobial inocula under sunny or shaded nursery	62
14.	Dry weight (g. Seedling <sup>-1</sup> ) of nodules formed by the 6-month- old seedlings grown with or without rhizobial inocula under sunny or shaded nursery.	62
15.	Plant heights of the 6-month-old Acacia grown with or without rhizobial inoculation under shade or direct sunlight conditions	63

16.	Shoot heights of the 6-month-old <i>Acacia</i> grown with or without
17.	Shoot dry biomass (g.seedling <sup>-1</sup> ) of the 6-month old <i>Acacia</i> seedlings grown with or without rhizobial inocula under sunny or shaded nursery.
18.	Root length (cm.seedlings <sup>-1</sup> ) of the 6-month-old seedlings grown with or without rhizobia inoculation under direct sunlight or shade.
19.	Root dry biomass (g.seedlings <sup>-1</sup> ) of the 6-month-old seedlings grown with or without rhizobia inoculation under direct sunlight or shade.
20.	Numbers (nod.seedling <sup>-1</sup> ) of nodules formed by the 1-year old <i>Acacia</i> seedlings inoculated or not with different rhizobial Isolates.
21.	Nodule biomass of the one-year old <i>Acacia</i> seedlings grown with or without rhizobia under sunlight.
22.	Plant heights (cm.seedling <sup>-1</sup> ) of the one-year old <i>Acacia</i> inoculated or not with different rhizobial isolates
23.	Shoot heights (cm.seedling <sup>-1</sup> ) of the one-year old <i>Acacia</i> inoculated or not with different rhizobial isolates.
24.	Shoot dry weight of the one-year old <i>Acacia</i> seedlings grown with or without rhizobia under sunlight.
25.	Root dry weight of the one-year old <i>Acacia</i> seedlings grown with or without rhizobia under sunlight
26.	Root heights (cm.seedling <sup>-1</sup> ) of the one-year old <i>Acacia</i> inoculated or not with different rhizobial isolates
27.	Different survival patterns of the isolate Ac15s in pith-based inocula stored at different temperatures.
28.	Rhizobial colonies recovered from sterilized –pith based Inoculum with low number of contamination
29.	Survival of <i>Acacia</i> rhizobial isolate Ac-15s in different inocula formulations at 30 °C
<ul><li>30.</li><li>31.</li></ul>	inocula formulations at 4°C room temp
	5 · · · · · · · · · · · · · · · · · · ·

32.	Correlation coefficient between the total nodule numbers and	
	seedling shoot heights	83
33.	Survival of rhizobial <i>Acacia</i> isolate Ac-15s in different	
	inoculant formula at 4 or 30°C after 6 months	85

#### INTRODUCTION

The leguminase constitutes one of the largest and most widely distributed families of flowering plants occupying habitats ranging from rain forests to arid zones (Raven and Polhill, 1981). Many members of the group are of considerable ecological and economical significance, due in part to their ability to form nitrogen-fixing symbioses with bacteria from the Rhizobiaceae. Acacia species are members of the Leguminosae comprising over 1500 of shrubs and trees with a wide distribution in Africa, Australia, South America and Asia. Their potential as multi-purpose plants in providing fire wood, pulp, timber, forage for cattle and fruit and gum has been well documented (Barnet and Catt, 1991). Most of these woody plants produce nodules and fix nitrogen in an endosymbiotic association with either rhizobia or bradyrhizobia (Njiti and Gliana, 1996). Despite the importance of such tree, there is little information on the nodulation status of the majority of Egyptian Acacia trees. The necessity of characterizing indigenous rhizobia cannot be overemphasized. It is crucial to establish which rhizobia nodulate host (s) and how efficiently in terms of N<sub>2</sub>-fixation, in order to develop broad host-range inocula for the various Egyptian sites. It has been already shown the wide spread occurrence of indigenous rhizobia and nodulation status of various tree host species. The need arises to isolate and characterize *Acacia* spp. root-nodulating bacteria to select the proper effective and competitive strains tolerant to different environmental stresses prevailing in Egyptian deserts.

Inoculation of legumes with rhizobia is a common international practice, which ensures and maximizes biological nitrogen fixation and enhances the plant yield potentials without using exogenous N fertilizers. Generally, inoculants are prepared by the addition of rhizobial cells to a powdered carrier followed by a period of incubation (Roughley and Plusford, 1982) to allow the development of maximum rhizobia cell numbers. Inoculants are commercially sold in powder, liquid and/or granular form. The powder form (rhizobial and peat powder) is the most popular method for industrial legume inocula production. However, liquid and granular products are available in limited quantities in North America and Europe (Stephens and Rask, 2000). Our objective was to study the feasibility of using pith as a carrier for bacterial inoculants following the common production and application procedures for most peat-based inoculant, peat was used as a reference carrier.

#### **REVIEW OF LITERATURE**

Leguminosae, with close to 20,000 species, is the third largest family in the plant kingdom (Doyle and Luckow, 2003). Legumes are important to many agronomic systems due to their symbiotic capacity for biological nitrogen fixation (Vance and Graham, 1995). Symbiosis is an interaction between the host legume root system and specialized soil bacteria, collectively referred to as rhizobia, resulting in the morphogenesis of nodules that harbor these nitrogen-fixing bacteria. There are six main genera of rhizobia, including Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium, Sinorhizobium. Recent reports have expanded the list of nodulating symbiotic bacteria to include strains of Blastobacter, Burkholderia, Devosia, Ensifer, Methylobacterium, Ochrobactrum, and Ralstonia (Ngom et al., 2004 and Sawada et al., 2003). Much of our current understanding of legume-rhizobia symbiosis has resulted from coordinated research efforts on model systems using the legumes Glycine max, Medicago truncatulata, and Lotus japonicus, along with their rhizobial symbionts (Choi et al., 2004; Stacey et al., 2004; Young et al., 2003). These legumes have been selected either because of their agronomic importance or due to the practicalities associated with a relatively simple genome that is suitable for genetic manipulation. There are no model systems for woody legumes; however, a substantial amount of scientific information exists describing symbiotic N<sub>2</sub> fixation in woody legumes. One of the most studied genera of woody legumes is Acacia.