



UNIVERSITI PUTRA MALAYSIA

**GROWTH RESPONSE OF SWEET POTATO (*Ipomoea batatas* L.) TO
APPLICATION OF SELECTED INDIGENOUS PLANT GROWTH-
PROMOTING RHIZOBACTERIA AND NITROGEN FERTILIZATION**

FARZANA YASMIN

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By

FARZANA YASMIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

September 2007



**DEDICATED
TO
MY BELOVED HUSBAND
FOR YEARS OF LOVE AND SACRIFICE**



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

GROWTH RESPONSE OF SWEET POTATO (*Ipomoea batatas* L.) TO APPLICATION OF SELECTED INDIGENOUS PLANT GROWTH-PROMOTING RHIZOBACTERIA AND NITROGEN FERTILIZATION

By

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September 2007

Chairman: Associate Professor Radziah Othman, PhD

Faculty : Agriculture

Sweetpotato requires high amount of fertilizer for commercial cultivation which can lead to increase production cost and environmental pollution. Biofertilizer is globally accepted as an alternative source of chemical fertilizer which improves plant growth through increased uptake of water and mineral nutrients. Laboratory and field experiments were conducted to characterize the beneficial properties of plant growth-promoting rhizobacterial (PGPR) strains isolated from sweetpotato rhizosphere and to determine the effects of rhizobacterial inoculation on the growth and yield of sweetpotato. Performance of the PGPR with different levels of nitrogen fertilizer on plant growth was evaluated under field condition. *In vitro* scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies were conducted to examine the colonization of PGPR strains on roots of sweetpotato plantlets.



Results of the laboratory study showed that 15 rhizobacterial isolates were able to produce indole acetic acid (IAA). The concentration of IAA produced ranged from 3.84 to 13.33 mg L⁻¹. Addition of L-tryptophan (L-TRP) to the bacterial isolates increased the production of IAA ranging from 4.94 to 46.66 mg L⁻¹. Six isolates (40%) were able to solubilize insoluble phosphate as evident by production of clear zone on calcium phosphate medium. All isolates were able to grow in N-free media indicating their abilities to produce nitrogen which ranged from 0.74 to 1.32 ppm. Three of the isolates produced fluorescent pigment on agar plate indicated their abilities to produce siderophores. Four isolates were able to inhibit the fungal pathogens *Rhizoctonia sp.* and *Pythium sp.* The intrinsic antibiotic test showed that all isolates were resistant against Chloramphenicol (10 and 30 µg mL⁻¹), Streptomycin (10 µg mL⁻¹), Kanamycin (5 and 30 µg mL⁻¹), Penicillin (10 µg mL⁻¹) and Tetracyclin (30 µg mL⁻¹). The Biolog identification system identified the rhizobacterial isolates UPMSP2, UPMSP3, UPMSP9, UPMSP10, UPMSP12, UPMSP13, UPMSP18 and UPMSP20 as *Pseudomonas corrugate*, *Serratia ficaria*, *Klebsiella terrigena*, *Erwinia cyripedii*, *Acinetobacter radioresistens*, *Pseudomonas maculicola*, *Paenibacillus pabuli* and *Pseudomonas fuscovaginae*, respectively.

Inoculation of Sepang Oren sweetpotato cultivar with twelve rhizobacteria isolates under glasshouse condition positively affected plant growth. The highest growth was observed on plants inoculated with *Klebsiella sp.* which increased shoot dry weight by 23% compared to control. Five of the isolates

were able to produce sweetpotato storage roots. *Klebsiella* sp and *Erwinia* sp. produced higher storage root yields of 35.15 and 8.22 g plant⁻¹, respectively, compared to the other treatments. PGPR inoculation significantly increased the concentrations and uptake of N, P, K, Ca and Mg in plant tissue, total bacterial populations, soil pH, IAA and nutrients (N, P, K, Ca and Mg) concentrations. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) studies showed that *Klebsiella* sp, *Erwinia* sp, *Azospirillum brasilense*, *Bacillus sphaericus* UPMB10 could effectively colonize the sweetpotato root surface and internal region of 7 day old plantlets.

Field inoculation of five bacterial strains in combination with three levels of nitrogen fertilizer (0, 33, and 100 kg N ha⁻¹) in the form of urea significantly (P<0.05) influenced growth and storage root yield of Sepang Oren sweetpotato. The highest storage root yield (12.59 kg plot⁻¹) was observed in plants inoculated with *Klebsiella* sp. applied with 33 kg N ha⁻¹. Significant interaction between PGPR inoculation and N fertilization was observed on the uptake of N, K and Ca in shoots. Bacterial Inoculation and N fertilization significantly stimulated the soil bacterial population at various stages of plant growth. The highest population of 2.63X10⁷ CFU g (dry wt.)⁻¹ soil was observed in soil inoculated with *Klebsiella* sp. applied with 33 kg N ha⁻¹. However, the population of bacteria in soil declined after the 2nd and 3rd month of inoculation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PERTUMBUHAN KELEDEK (*Ipomoea batatas* L.) KESAN DARI
PENGUNAAN RIZOBAKTERIA PENGALAK TUMBESARAN
TANAMAN ASLI TERPILIH DAN PEMBAJAAN NITROGEN**

Oleh

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Pengerusi: Profesor Madya Radziah Othman, PhD

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Tanaman keledek memerlukan penggunaan baja yang amat tinggi untuk ditanam secara komersial dan ini mengakibatkan peningkatan kos pengeluaran dan pencemaran alam sekitar. Bajabio kini diterima di peringkat global sebagai sumber alternatif kepada baja kimia yang boleh membantu pertumbuhan pokok dengan meningkatkan pengambilan air dan nutrien. Kajian di makmal dan di lapangan telah dilakukan untuk menentukan ciri-ciri kebaikan penggunaan strain rizobakteria penggalak tumbesaran tanaman (PGPR) yang telah diasingkan dari rizosfera keledek, dan untuk mengkaji kesan inokulasi rizobakteria keatas pertumbuhan dan hasil pengeluaran keledek. Keupayaan PGPR bersama pembajaan nitrogen pada pasas berbeza terhadap pertumbuhan pokok telah dinilai pada keadaan lapangan. Kajian in vitro mikroskop elektron pengimbas (SEM) dan mikroskopi elektron pancaran (TEM) telah dilakukan untuk mengkaji pengkolonian strain PGPR keatas akar anak pokok keledek.



Keputusan kajian menunjukkan 15 isolat rizobakteria boleh menghasilkan asid indol asetik (IAA). Kepekatan IAA yang dihasilkan adalah diantara 3.84 hingga 13.33 mg L⁻¹. Penambahan L-tryptophan (L-TRP) kepada isolat bakteria meningkatkan pengeluaran IAA pada julat kepekatan dari 4.94 hingga 46.66 mg L⁻¹. Enam isolat (40%) berupaya melarutkan fosfat berdasarkan bukti penghasilan zon jernih pada medium kalsium fosfat. Semua isolat boleh tumbuh dalam media bebas N menunjukkan keupayaan menghasilkan nitrogen yang ber julat diantara 0.74 hingga 1.32 ppm. Tiga daripada isolat mengeluarkan pigmen berpendarfluor pada agar yang menunjukkan keupayaan menghasilkan siderofor. Empat isolat berupaya merencat kulat patogen, *Rhizoctonia* sp. dan *Pythium* sp. Kajian antibiotik intrinsik menunjukkan kesemua isolat resistan terhadap Chloramphenicol (10 dan 30 µg mL⁻¹), Streptomisin (10 µg mL⁻¹), Kanamisin (5 dan 30 µg mL⁻¹), Penisilin (10 µg mL⁻¹) dan Tetrasiklin (30 µg mL⁻¹). Sistem identifikasi Biolog telah mengenalpasti isolat rizobakteria UPMSP2, UPMSP3, UPMSP9, UPMSP10, UPMSP12, UPMSP13, UPMSP18 dan UPMSP20, sebagai *Pseudomonas corrugate*, *Serratia ficaria*, *Klebsiella terrigena*, *Erwinia cyripedii*, *Acinetobacter radiomonas*, *Pseudomonas maculicola*, *Paenibacillus pabuli* dan *Pseudomonas fuscovaginae*.

Penginokulatan Kultivar Sepang Oren dengan 12 isolat rizobakteria di dalam rumah kaca mempengaruhi secara positif pertumbuhan pokok. Pertumbuhan pokok yang tertinggi dapat dilihat pada pokok yang diinokulasi dengan *Klebsiella* sp. dengan peningkatan berat kering pucuk sebanyak 23%



berbanding kawalan. Lima daripada isolat berupaya menghasilkan ubi. *Klebsiella* sp. dan *Erwinia* sp. menghasilkan ubi keledak yang tinggi masing-masing, 35.15 dan 8.22 g pokok⁻¹, berbanding rawatan lain. Inokulasi PGPR meningkatkan dengan ketara kepekatan dan pengambilan nutrien N, P, K, Ca dan Mg tisu pokok, jumlah populasi bakteria, pH tanah, kandungan IAA dan nutrien tanah (N, P, K, Ca dan Mg). Mikroskopi elektron pengimbas (SEM) dan mikroskopi elektron pancaran (TEM) menunjukkan bahawa *Klebsiella* sp. *Azospirillum brasilense*, *Bacillus sphaericus* UPMB10 boleh mengkolonisasi secara berkesan permukaan dan kawasan dalaman akar anak pokok berumur 7 hari.

Penginkulatan di lapangan lima strain rizobakteria bersama 3 paras baja nitrogen (0, 33, dan 100 kg N ha⁻¹) dalam bentuk urea telah mempengaruhi dengan signifikan ($P < 0.05$) pertumbuhan pokok dan penghasilan ubi keledak Sepang Oren. Penghasilan ubi keledak tertinggi (12.59 kg plot⁻¹) dapat dilihat pada pokok yang telah diinokulasi dengan *Klebsiella* sp. yang ditambah 33 kg N ha⁻¹. Interaksi signifikan diantara inokulasi PGPR dengan pembajaan N telah diperolehi keatas penyerapan nutrien N, K dan Ca dalam pucuk. Inokulasi bakteria dan pembajaan N merangsang dengan ketara populasi bakteria pada pelbagai peringkat pertumbuhan. Populasi bakteria tertinggi sebanyak 2.63×10^7 CFU g (berat kering)⁻¹ tanah dapat dilihat pada tanah diinokulasi dengan *Klebsiella* sp. yang ditambah 33 kg N ha⁻¹. Walau bagaimanapun populasi bakteria dalam tanah merosot selepas 2 dan bulan 3 diinokulasikan.

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I certify that an Examination Committee met on 21st of September 2007 to contract the final examination of Farzana Yasmin on his Doctor of Philosophy thesis entitled “Growth Response of Sweet potato (*Ipomoea batatas* L.) to Application Selected Indigenous Plant Growth-Promoting Rhizobacteria and Nitrogen Fertilization” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommended that the candidate be awarded the relevant degree. The committee members for the candidate are as follows:

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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	xi
DECLARATION	xiii
LIST OF TABLES	xviii
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS	xxii
CHAPTER	
I INTRODUCTION	1
II REVIEW OF LITERATURE	6
2.1 Sweetpotato	6
2.2 Sweetpotato plants	7
2.3 Storage root	10
2.4 Factors affecting sweetpotato production	12
2.4.1 Climate	12
2.4.2 Soil type	12
2.4.3 Soil moisture	13
2.4.4 Cultural practices	14
2.4.4.1 Seed bed preparation and plant spacing	14
2.4.4.2 Weed control	15
2.5 Nutritional requirement	15
2.6 Plant growth promoting rhizobacteria (PGPR)	16
2.7 Mechanisms of plant growth promotion	18
2.7.1 Phytohormone production	18
2.7.1.1 Synthesis of phytohormones	18
2.7.1.2 Factors affecting IAA biosynthesis	20
2.7.2 Nitrogen fixation	21
2.7.3 Uptake of nutrients	21
2.7.4 Phosphate solubilization	23
2.7.4.1 Mechanisms of phosphate solubilization	23
2.7.5 Biological control	24
2.7.5.1 Competition	24
2.7.5.2 Antibiosis	26
2.7.5.3 Parasitism	26
2.7.6 Factors affecting growth and colonization of PGPR	27



	2.7.6.1 Soil organic matter	27
	2.7.6.2 Soil pH	28
	2.7.6.3 Soil moisture	29
	2.7.6.4 Bacterial factors	29
III	CHARACTERIZATION OF BENEFICIAL PROPERTIES OF PLANT GROWTH PROMOTING RHIZOBACTERIA ISOLATED FROM SWEETPOTATO RHIZOSPHERE	34
	3.1 Introduction	34
	3.2 Materials and methods	36
	3.2.1 Screening for IAA producing activity	36
	3.2.2 Screening for phosphate solubilizing activity	36
	3.2.3 Screening for nitrogen producing activity	37
	3.2.4 Screening for siderophore producing activity	37
	3.2.5 Screening for antagonistic activity	38
	3.2.6 Intrinsic antibiotic resistance (IAR) test	38
	3.2.7 Identification of the rhizobacteria isolates using biolog system	39
	3.2.8 Statistical analysis	40
	3.3 Results	40
	3.3.1 IAA production	40
	3.3.2 Phosphate solubilizing activity	41
	3.3.3 Total nitrogen production	43
	3.3.4 Siderophore production	43
	3.3.5 Test for antagonistic activity of bacteria against pathogens	44
	3.3.6 Identification of the rhizobacteria isolates using biolog system	46
	3.3.7 Intrinsic antibiotic resistance (IAR) test	46
	3.4 Discussion	48
	3.5 Conclusion	52
IV	SELECTION OF RHIZOBACTERIAL ISOLATES FOR IMPROVED GROWTH AND YIELD OF SWEETPOTATO UNDER GLASS HOUSE CONDITION	53
	4.1 Introduction	53
	4.2 Materials and methods	54
	4.2.1 Preparation of soil and planting materials	54
	4.2.2 Preparation of inoculum and planting	55
	4.2.3 Shoot and root weight	56
	4.2.4 Plant tissue analysis	56
	4.2.5 Soil microbial population	57

4.2.6 Concentrations of IAA in soil	57
4.2.7 Soil pH and nutrient analysis	58
4.2.8 Statistical analysis	58
4.3 Results	59
4.3.1 Plant growth and storage root formation	59
4.3.2 Nutrient concentrations in shoots	60
4.3.3 Nutrient uptake in shoots	61
4.3.4 Population of bacteria and concentrations of IAA in soil	63
4.3.5 Soil pH and nutrient concentration analysis	64
4.3.6 Correlation analysis	65
4.4 Discussion	66
4.5 Conclusion	68
V COLONIZATION OF SWEETPOTATO ROOTS BY RHIZOBACTERIAL ISOLATES	69
5.1 Introduction	69
5.2 Materials and methods	71
5.2.1 Plantlet growth in vitro	71
5.2.2 Sample preparation for scanning electron microscope (SEM)	72
5.2.3 Sample preparation for transmission electron microscope (TEM)	73
5.2.4 Rhizoplane bacterial population	74
5.2.5 Endophytic (Root internal region) bacterial population	74
5.2.6 Statistical analysis	75
5.3 Results	75
5.3.1 SEM micrograph	75
5.3.2 TEM micrograph	76
5.3.3 Population of rhizoplane and endophytic bacteria	83
5.4 Discussion	83
5.5 Conclusion	86
VI EFFECT OF PGPR INOCULATION AND N FERTILIZATION ON YIELD OF SWEETPOTATO	88
6.1 Introduction	88
6.2 Materials and methods	89
6.2.1 Experimental location	89
6.2.2 Land preparation and field lay out	90
6.2.3 Preparation of Inoculum	91
6.2.4 Planting and crop management	91
6.2.5 Fertilizer application	92
6.2.6 Harvesting and shoot and storage root yield	92



6.2.7 Nutrient concentration in shoots	93
6.2.8 Nutrient concentration in storage root	93
6.2.9 Storage root starch and crude protein content	93
6.2.10 Soil analysis	94
6.2.11 Leaf chlorophyll content	94
6.2.12 Statistical analysis	95
6.3 Results	95
6.3.1 Shoot growth	95
6.3.2 Storage root yield	96
6.3.3 Marketable yield	99
6.3.4 Nutrient concentration and uptake in shoots	99
6.3.5 Nutrient, starch and crude protein concentrations of storage root	102
6.3.6 Nutrient content of storage root	102
6.3.7 Total bacterial population in soil	105
6.3.8 Concentrations of IAA in soil	107
6.3.9 Soil pH and nutrient concentration	110
6.4 Discussion	110
6.5 Conclusion	114
VII GENERAL DISCUSSION AND CONCLUSION	115
REFERENCES	120
APPENDICES	139
BIODATA OF THE AUTHOR	161
LIST OF PUBLICATIONS	163



LIST OF TABLES

Table		Page
1	Bacterial Species Involved in Phytohormone Production	18
2	Selected Plant Growth Promoting Properties of Rhizobacterial Isolates	42
3	Intrinsic Antibiotic Resistance (IAR) Test of Rhizobacterial Isolates	47
4	Effect of Rhizobacterial Inoculation on Growth Parameters and Storage Root Formation of Sweetpotato Plants	60
5	Effect of Rhizobacterial Inoculation on Nutrient Concentration in Sweetpotato Shoots	61
6	Effect of Rhizobacterial Inoculation on Uptake of N, P, K, Ca and Mg in Sweetpotato Shoots	62
7	Effect of Rhizobacterial Inoculation on Population of Bacteria and Concentration of IAA in Soil	63
8	Effect of Rhizobacterial Inoculation on soil pH and Nutrient Concentration	64
9	Correlation between Sweetpotato yield, soil microbial population, soil IAA and nutrient uptake of Sweetpotato Plants	65
10	Effect of Rhizobacterial Inoculation on Population of Bacteria of the rhizoplane and endophytes	83
11	Effect of Rhizobacterial Inoculation and N Fertilization Rate on Dry Weights of Shoot, Storage Root and Shoot to Storage Root Ratio	96
12	Effect of Rhizobacterial Inoculation and N Fertilization Rate on Total Storage Root Yield, Sweetpotato Production and Marketable Grade Storage Root	98
13	The Effect of Rhizobacterial Inoculation and N Fertilization on Sweetpotato Shoot Nutrient Concentration	100



14	The Effect of Rhizobacterial Inoculation and N Fertilization on Sweetpotato Shoot Nutrient Uptake and Chlorophyll Content	101
15	Effect of Rhizobacterial Inoculation and N Fertilization Rate on Nutrient Concentration, Starch and Crude Protein Content of Storage Root	103
16	Effect of Rhizobacterial Inoculation and N Fertilization Rate on Nutrient Content of Sweetpotato Storage Roots	104
17	Effect of Rhizobacterial Inoculation and N Fertilization on soil pH and Nutrient Concentration	109



LIST OF FIGURES

Figure		Page
1	Different Parts of Sweetpotato Plants	9
2	Parts inside the Storage roots	11
3	Phosphate solubilizing test of rhizobacteria	41
4	Siderophore test of rhizobacteria	44
5	Antagonistic activity of bacteria against <i>Rhizoctonia</i> sp.	45
6	Antagonistic activity of bacteria against <i>Pythium</i> sp.	45
7	Inoculated sweetpotato roots at 7 days of growth	72
8a	SEM micrographs of uninoculated sweetpotato roots plantlets	77
8b	Root Surface Colonization of <i>Klebsiella</i> on sweetpotato roots plantlets	77
8c	Root Surface Colonization of <i>Erwinia</i> on sweetpotato roots plantlets	78
8d	Root Surface Colonization of <i>Azospirillum</i> on sweetpotato roots plantlets	78
8e	Root Surface Colonization of <i>Bacillus</i> on sweetpotato roots plantlets	79
9a	TEM micrographs of uninoculated sweetpotato roots plantlets	80
9b	Internal Colonization of <i>Klebsiella</i> on sweetpotato roots plantlets	80
9c	Internal Colonization of <i>Erwinia</i> on sweetpotato roots plantlets	81
9d	Internal Colonization of <i>Azospirillum</i> on sweetpotato roots plantlets	81



9e	Internal Colonization of <i>Bacillus</i> on sweetpotato roots plantlets	82
10	Storage root yield of sweetpotato in 33 Kg N fertilizer rate with PGPR	97
11	Effect of Rhizobacterial inoculation and Nitrogen on soil bacterial population at different sweetpotato growth stages	106
12	Effect of Rhizobacterial inoculation and Nitrogen on concentration of IAA in soil at different sweetpotato growth stages	108



LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CFU	Colony forming unit
Ca	Calcium
CaCl ₂	Calcium chloride
CaHPO ₄	Calcium hydrogen-phosphate
CIP	International Potato Centre
CM Value	Chlorophyll Meter value (measurement on leaf greenness by SPAD meter-502, MINOLTA™)
CRD	Completely Randomized design
DOA	Department of Agriculture
FeCl ₃	Ferric chloride
IAA	Indole-3-acetic acid
K	Potassium
K ₂ HPO ₄	Dipotassium hydrogen-phosphate
Mg	Magnesium
MOP	Muriate of potash
N	Nitrogen
OD	Optical density
P	Phosphorous
PDA	Potato dextrose agar
PDYA	Potato dextrose yeast-extract agar
PGPR	Plant Growth-Promoting Rhizobacteria
PSB	Phosphate solubilizing bacteria



R/S	Root to Shoot ratio
RCBD	Randomized complete block design
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope
TSP	Triple Super Phosphate
TRP	Tryptophen

