



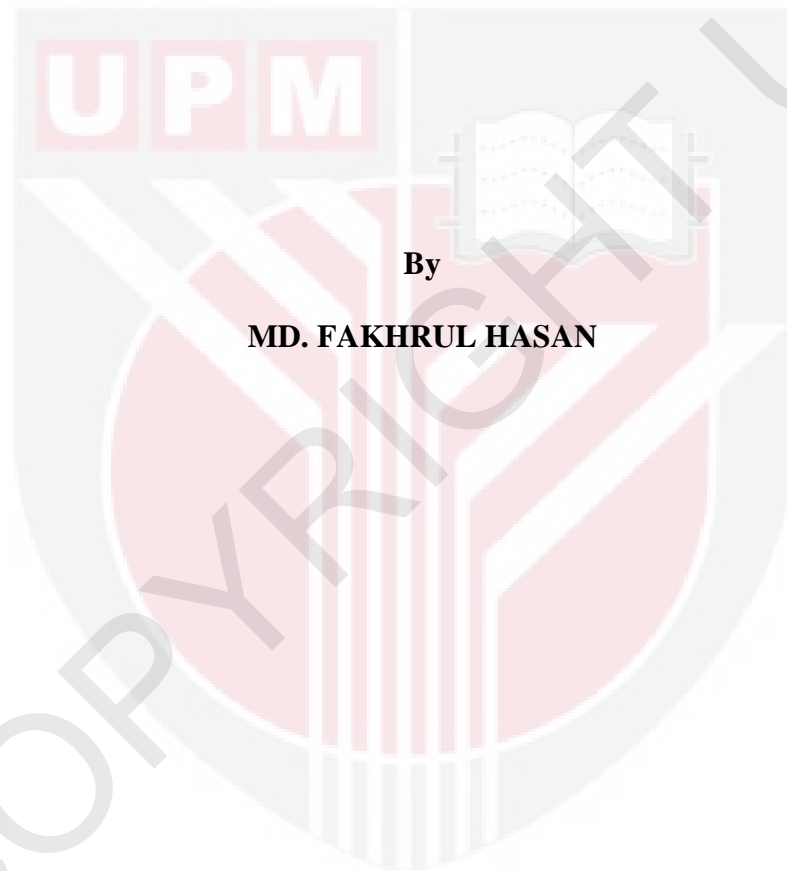
**UNIVERSITI PUTRA MALAYSIA**

**CONTROLLING ANTHRACNOSE AND POSTHARVEST  
QUALITY OF PAPAYA (*CARICA PAPAYA* L. CV. FRANGI)  
USING *BACILLUS SUBTILIS* STRAIN B34**

**MD. FAKHRUL HASAN**

**FP 2012 57**

**CONTROLLING ANTHRACNOSE AND POSTHARVEST  
QUALITY OF PAPAYA (*CARICA PAPAYA* L. CV. FRANGI)  
USING *BACILLUS SUBTILIS* STRAIN B34**



**By**

**MD. FAKHRUL HASAN**

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

**December 2012**

## *DEDICATION*

*To my beloved homeland BANGLADESH, a place of peace and love.*

*To my affectionate parents and beloved wife for encouraging me to the higher ideals of life.*

*To my daughters Fatima and Aisha, for giving me happiness and joy.*

*Finally to my Zannati son Mohammad, who have helped me to understand the reality of life.*

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

**CONTROLLING ANTHRACNOSE AND POSTHARVEST QUALITY OF  
PAPAYA (*Carica papaya* cv. FRANGI) USING *Bacillus subtilis* STRAIN B34**

By

**MD. FAKHRUL HASAN**

**December 2012**

**Chairman : Professor Mahmud Tengku Muda Mohamed, PhD**

**Faculty : Agriculture**

Papaya (*Carica papaya* L.), a climacteric fruit, is highly vulnerable to several postharvest diseases. Among them, anthracnose caused by *Colletotrichum gloeosporioides* is the major postharvest disease which demands an effective control. This study was carried out aiming at developing an eco-friendly biocontrol agent to control the disease. Three antagonistic bacteria identified as B34, B60 and B71 were screened from 81 epiphytic bacteria isolated from leaf and fruit surfaces of papaya. These three isolates were identified as *Bacillus subtilis* based on Biolog Omniliog Gen III identification system and its 16S rDNA sequence which was compared with the related bacteria in the GenBank. All the three strains were proved to be efficacious for the control of anthracnose of papaya in *in vitro* biocontrol assays. Bacterial strain B34, B71 and B60 produced antibiotic substances which restricted the fungal growth up to 84.6, 80.6 and 79.3%, respectively compared with the control. Moreover, these strains not only able to completely inhibit the fungal growth by secreting diffusible antibiotics but also restricted 40.6, 33.4, and 34.6% of fungal growth through the antibiotic volatiles produced by strains B34, B60 and B71, respectively. In spore germination test, germination percentage decreased gradually with increased concentrations of strains'

suspension. No spores of the test fungus germinated at the concentration of  $10^8$  CFU mL<sup>-1</sup> but the performance of strain B34 was quite better than B71 and B60 at lower concentration. Mycelial growth of *C. gloeosporioides* was completely absent in  $10^8$  CFU mL<sup>-1</sup> cell suspensions of all strains. On the other hand, strain B34 showed statistically highest inhibition of growth of mycelial plug in sterilized culture filtrate treated plates compared to strain B60 and B71. However, *in vitro* performances of *B. subtilis* strain B34 against *C. gloeosporioides* showed its better efficacy compared to strains B60 and B71. The growth of strain B34 was faster in nutrient broth medium and the biomass production reached the highest level in temperatures between 30-36°C three days after incubation. The strain B34 showed its different mechanisms in controlling *C. gloeosporioides* by reducing nutrients for test fungus, producing proteolytic enzymes and secreting antibiotics to quash the cell wall as well as dissolve the fungus. Moreover, strain B34 was able to attack and degrade the hyphae of pathogen when observed under scanning electron microscope. This bacterium was found to be highly compatible with 20% *Aloe vera* gel (aloe gel) and 2% sodium bicarbonate (SBC) or mixture of both. Both of these have suppressive activity against *C. gloeosporioides* of papaya and could be used as enhancer for biocontrol efficacy of strain B34 during fruits storage. The survival and proliferation of strain B34 in papaya wounds and on fruit surfaces was not affected by aloe gel and SBC throughout the storage period. In addition, as observed from scanning electron microscopy micrograms, strain B34 was able to colonize and multiply on the surface of papaya fruits. The combination of strain B34 with aloe gel and SBC was more effective in controlling the anthracnose disease than strain B34 alone or other treatments, even superior to that obtained with fungicide Benocide® (benomyl 50% WP) both in inoculated or naturally infected fruits stored at  $12\pm 1^\circ\text{C}$  and  $90\pm 5\%$  RH for 18 and 14 days, respectively. However, this combination offered a greater control by reducing 98.2% of the disease over control in naturally infected fruits at the

end of 14 days storage at  $12\pm 1^{\circ}\text{C}$  and  $90\pm 5\%$  RH and six days post ripening at  $28 \pm 2^{\circ}\text{C}$ , which was superior to that found with Benocide<sup>®</sup> or other treatments tested. Furthermore, fruits treated with the combination of *B. subtilis*-aloe gel-SBC showed delayed climacteric respiration and ethylene evolution more than seven days compared to control with reduced rate of  $\text{CO}_2$  and  $\text{C}_2\text{H}_4$  productions. This combined treatment reduced weight loss by more than 25% compared to the control. It also markedly slowed down the ripening of fruits as shown by their retention of firmness after storage. Moreover, it also delayed changes in external colour, titratable acidity and pH without compromising fruit quality was observed in fruits that were subjected to the combined treatment. The storage life was thus extended by 15 days when compared with control. Inhibitory activity of strain B34 preserved in glycerol was also observed in this study. strain B34 preserved in 2% glycerol based liquid formulations showed economically better performance in terms of controlling *C. gloeosporioides* and number of viable propagules (CFUs) over 120 days of test period. Finally, it is clear that strain B34 not only able to store at 2% glycerol for longer time but also effective when incorporated with SBC and *Aloe vera* gel to control *C. gloeosporioides* as well as improve the postharvest quality during cold storage compared to Benocide<sup>®</sup>. This suggested that this composite coating is promising and eco-friendly to be used in commercial postharvest applications for prolonging the storage life of Frangi papaya.

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

**PENGAWALAN ANTRAKNOS DAN KUALITI LEPAS TUAI BETIK (*Carica papaya* cv. FRANGI) MENGGUNAKAN *Bacillus subtilis* STRAIN B34**

Oleh

**MD. FAKHRUL HASAN**

**Disember 2012**

**Pengerusi : Profesor Mahmud Tengku Muda Mohamed, PhD**

**Fakulti : Pertanian**

Betik (*Carica papaya* L.), satu strain buah berklimakterik sangat mudah diserang beberapa penyakit lepas tuai. Di antaranya, antraknos yang disebabkan oleh *Colletotrichum gloeosporioides* merupakan penyakit lepas tuai utama yang memerlukan pengawalan yang berkesan. Oleh itu, kajian ini dilakukan bertujuan untuk membangunkan agen kawalan biologi mesra alam bagi mengawal *Colletotrichum gloeosporioides*. Tiga bakteria antagonis dikenali sebagai B34, B60 dan B71 telah disaring daripada 81 bakteria epifit yang diasing daripada permukaan daun dan buah betik. Ketiga-tiga bakteria tersebut telah dikenalpasti sebagai *Bacillus subtilis* berdasarkan sistem pengenalan Biolog Omniliog Gen III dan urutan rDNA 16S yang telah dibandingkan dengan GenBank bakteria berkaitan. Ketiga-tiga strain bakteria ini telah terbukti berkesan untuk mengawal antraknos betik secara biologi hasil ujian makmal. Bakteria strain B34, B71 dan B60 menghasilkan bahan antibiotik yang telah menghadkan pertumbuhan kulat masing-masing sehingga 84.6, 80.6 dan 79.3% berbanding kawalan. Selain itu, ia bukan sahaja dapat menghalang pertumbuhan kulat sepenuhnya dengan merembeskan antibiotik yang boleh tersebar,

tetapi juga menyekat 40.61, 33.36 dan 34.6% pertumbuhan kulat melalui penghasilan antibiotik meruap oleh bakteria masing-masing strain B34, B60 dan B71. Dalam ujian percambahan spora, peratusan percambahan menurun secara beransur-ansur dengan peningkatan kepekatan larutan bakteria. Tiada sebarang spora kulat yang diuji telah bercambah pada kepekatan sebanyak  $10^8$  CFU mL<sup>-1</sup> tetapi prestasi strain B34 adalah lebih baik berbanding B71 dan B60 pada kepekatan yang lebih rendah. Pertumbuhan miselium *C. gloeosporioides* adalah tiada sepenuhnya dalam piring petri yang dirawat dengan larutan sel  $10^8$  CFU mL<sup>-1</sup> bagi semua strain. Sebaliknya, strain B34 secara statistik menunjukkan perencatan pertumbuhan tertinggi bagi palam miselium dirawat dengan turasan kultur steril dalam piring petri berbanding strain B60 dan B71. Walau bagaimanapun, keputusan ujian dalam makmal bagi *B. subtilis* strain B34 terhadap *C. gloeosporioides* menunjukkan keberkesanan yang lebih baik berbanding strain B60 dan B71. Pertumbuhan strain B34 adalah lebih cepat dalam larutan media nutrien dan pengeluaran biojisim mencapai tahap tertinggi di antara suhu 30-36°C tiga hari selepas pengeraman. Strain B34 menunjukkan mekanisme yang berbeza dalam mengawal *C. gloeosporioides* dengan mengurangkan nutrien bagi kulat yang diuji, menghasilkan enzim proteolitik dan merembeskan antibiotik untuk menghancurkan dinding sel serta melarutkan kulat. Selain itu, strain B34 mampu menyerang dan merosakkan hifa patogen apabila diperhatikan di bawah mikroskop imbasan elektron. Bakteria inididapati sangat serasi dengan 20% *Aloe vera* gel (gel lidah buaya) dan 2% natrium bikarbonat (SBC) atau campuran kedua-duanya. Kedua-duanya mempunyai aktiviti bersifat menindas terhadap *C. gloeosporioides* betik dan boleh digunakan sebagai penambah keberkesanan kawalan biologi strain B34 semasa penyimpanan buah. Kelangsungan hidup dan perkembangbiakan strain B34 dalam

luka betik dan pada permukaan buah-buahan tidak terjejas oleh gel lidah buaya dan SBC sepanjang tempoh penyimpanan. Di samping itu, seperti yang diperhatikan melalui mikrogram mikroskop imbasan elektron, strain B34 dapat menjajah dan mengganda pada permukaan buah betik. Gabungan strain B34 dengan gel lidah buaya dan SBC adalah lebih berkesan dalam mengawal penyakit antraknos daripada strain B34 sahaja atau rawatan lain, malahan dilihat lebih baik daripada racun kulat Benocide<sup>®</sup> (benomyl WP 50%) samada bagi buah yang disuntik atau dijangkiti semulajadi yang masing-masing disimpan pada  $12\pm 1^{\circ}\text{C}$  dan  $90\pm 5\%$  RH selama 18 dan 14 hari. Walau bagaimanapun, gabungan ini menawarkan satu kawalan yang lebih baik dengan mengurangkan 98.2% pengurangan penyakit berbanding kawalan bagi buah yang dijangkiti semulajadi pada akhir 14 hari penyimpanan pada  $12\pm 1^{\circ}\text{C}$  dan  $90\pm 5\%$  RH dan enam hari selepas kemasakan pada  $28\pm 2^{\circ}\text{C}$ , yang mana adalah lebih baik berbanding Benocide<sup>®</sup> atau rawatan lain yang diuji. Tambahan pula, buah yang dirawat dengan gabungan *B. subtilis*-gel lidah buaya-SBC menunjukkan penangguhan respirasi klimakterik dan evolusi etilena lebih daripada tujuh hari berbanding kawalan dengan pengurangan kadar penghasilan  $\text{CO}_2$  dan  $\text{C}_2\text{H}_4$ . Rawatan gabungan ini mengurangkan kehilangan berat lebih daripada 25% berbanding dengan kawalan. Ia juga melambatkan kemasakan buah secara ketara seperti yang ditunjukkan oleh pengekal kepejalan selepas penyimpanan. Selain itu, ia melambatkan perubahan warna luaran, asid tertitrat dan pH tanpa mengkompromi kualiti buah pada buah yang dirawat dengan rawatan kombinasi. Jangkahayat simpanan dapat dipanjangkan selama 15 hari apabila dibandingkan dengan kawalan. Pengekalan aktiviti perencatan strain B34 yang disimpan dalam gliserol juga diperhatikan dalam kajian ini. strain B34 yang disimpan dalam rumusan 2% gliserol berasaskan cecair dari segi ekonomi menunjukkan prestasi

tertinggi mengawal *C. gloeosporioides* dan bilangan bahan biak berdaya saing (CFUs) selama 120 hari tempoh penyimpanan. Akhirnya, adalah jelas bahawa strain B34 bukan sahaja dapat disimpan pada gliserol 2% untuk masa yang lama tetapi juga berkesan dalam penggabungan dengan SBC dan gel lidah buaya untuk mengawal *C. gloeosporioides* serta mengekalkan kualiti lepas tuai semasa penyimpanan sejuk berbanding untuk Benocide®. Ini mencadangkan bahawa salutan komposit ini menjanjikan sebagai sesuatu yang mesra alam untuk digunakan dalam aplikasi lepas tuai komersial bagi memanjangkan hayat penyimpanan betik Frangi.

## ACKNOWLEDGEMENTS

All praise is to Almighty Allah for His endless blessings, kindness, guidance, strength, and will to successfully complete my doctoral study. May His name be glorified and praised.

First and foremost, I would like to express my heartfelt appreciation and utmost gratitude to my supervisor Professor Dr. Professor Dr. Mahmud Tengku Muda Mohamed for his continuous support and invaluable guidance for my Ph. D study, for his patience, motivation and enthusiasm. During my doctoral study, he provided sound advice, good teaching and friendly company, and shared a lot of his expertise, research insight and best ideas. I simply could not imagine having a better advisor and friendlier mentor for Ph. D study. I believe that one of the main gains of my doctoral study was working with Prof. Mahmud.

With a great deal of luck, I got an excellent Supervisory Committee. I owe an immense debt to the rest of my supervisory committee, Associate Professor Dr. Jugah B Kadir, Dr. Phebe Ding for their encouragement, insightful comments and critical review. This thesis could not have been done without their strong supervision.

It is my greatest pleasure to acknowledge Universiti Putra Malaysia for providing Graduate Research Assistance Scholarship for the study. I am also grateful to my employer organization Patuakhali Science and Technology University for granting my leave to pursue my study.

Thanks are also extended to all the staffs of Postharvest, Plant Pathology, Agro Technology and Microbiology Laboratories for their willing assistance and help during my entire period of research. I want to extend my sincere thanks and gratitude to Azhar, Mohammad, Parviz, Humam, Rubel, Amin, Kausar, Hamizah, Aysha, Munirah, Bungah and Alisah for their abundant help in different ways.

I respectfully acknowledge the blessings and good wishes of my parents, teachers, brothers, sister and relatives. Special gratitude go to my wife, daughters and my beloved zannati son for their endless love, great sacrifice, patience and support during the study period.

I certify that a Thesis Examination Committee has met on -/-/- to conduct the final examination of Md. Fakhrul Hasan on his PhD thesis entitled “**Controlling anthracnose and postharvest quality of papaya (*carica papaya* cv. Frangi) using *Bacillus subtilis* strain B34**” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Siti Hajar Ahmad, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Zainal Abidin b Mior Ahmad, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Puteri Edaroyati bt Megat Wahab, PhD**

Lecturer  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

**Elhadi Yahia, PhD**

Professor  
Food and Agriculture Organization (FAO) of the United Nation  
Egypt  
(External Examiner)

---

**SEOW HENG FONG, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:.....

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Mahmud Tengku Muda Mohamed, PhD**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Jugah Bin Kadir, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Phebe Ding, PhD**

Lecturer  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

---

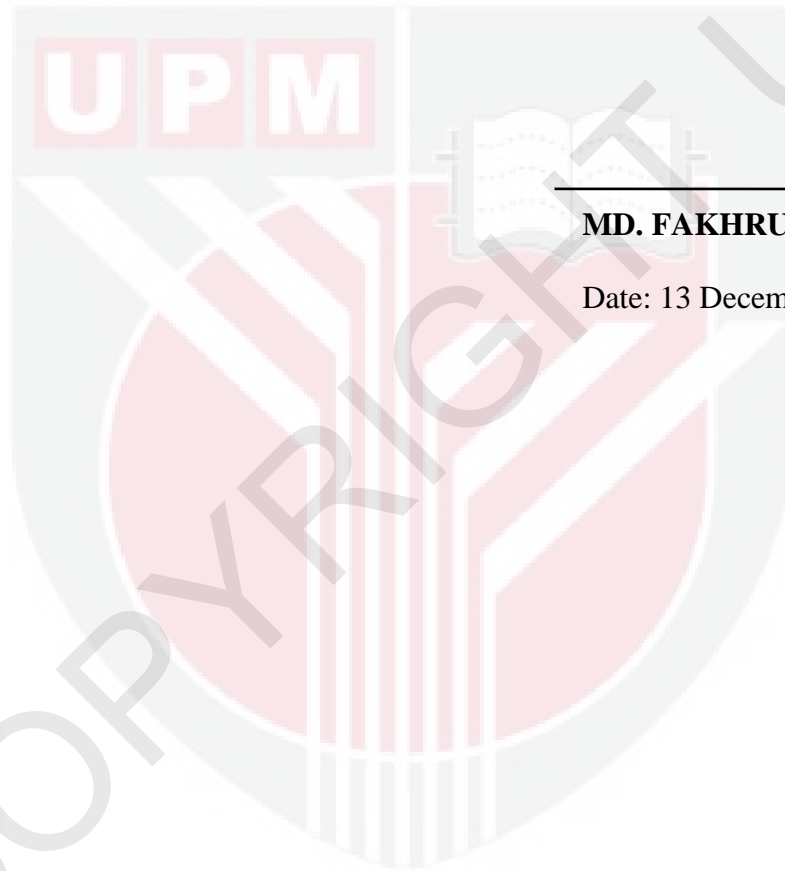
**BUJANG BIN KIM HUAT, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.



---

**MD. FAKHRUL HASAN**

Date: 13 December 2012

## TABLE OF CONTENTS

	<b>Page</b>
<b>DEDICATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	vi
<b>ACKNOWLEDGEMENTS</b>	x
<b>APPROVAL</b>	xii
<b>DECLARATION</b>	xiv
<b>LIST OF TABLES</b>	xx
<b>LIST OF FIGURES</b>	xxi
<b>LIST OF ABBREVIATIONS</b>	xxiv
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
<b>2 LITERATURE REVIEW</b>	<b>8</b>
2.1 Papaya	8
2.1.1 Postharvest losses	9
2.1.2 Postharvest losses of papaya	10
2.1.3 Papaya diseases	11
2.1.3.1 Field diseases of papaya	11
2.1.3.2 Postharvest diseases of papaya	12
2.1.4 Postharvest disease control of papaya	14
2.2 Biological control of postharvest diseases of fruits	15
2.3 Mechanisms of biocontrol of postharvest diseases	17
2.3.1 Competition for space, and nutrients and space	18
2.3.2 Attachment and direct parasitism	19
2.3.3 Production of antibiotics	20
2.3.4 Induced resistance	20
2.4 Isolation and screening of antagonist	21
2.5 Characterization of selected biocontrol agents	23
2.6 Biocontrol activity of <i>Bacillus subtilis</i>	23
2.6.1 Biocontrol of <i>B. subtilis</i> on postharvest diseases	24
2.6.2 A variety of biocontrol-related activities of <i>B. subtilis</i>	25
2.6.2.1 Involvement in direct antagonism through antibiosis	25
2.6.2.2 Involvement in plant systemic resistance elicitation	26
2.6.2.3 Involvement of enzyme to control pathogen	27
2.7 Enhancement of the efficacy of biocontrol agents	28
2.7.1 Addition of salt additives in the microbial culture	30
2.7.1.1 Addition of sodium bicarbonate	30
2.7.1.2 Sodium bicarbonate in postharvest diseases	32
2.7.1.3 Mechanism of sodium bicarbonate to control fungi	33
2.7.2 Addition of <i>Aloe vera</i> gel	33
2.7.2.1 <i>Aloe vera</i> gel to control postharvest diseases and	

	quality of fruit	35
	2.7.2.2 Effect of <i>Aloe</i> gel on the quality and storage life of fruits	36
2.8	Formulation of bacterial biopesticides	38
<b>3</b>	<b>SCREENING AND <i>IN VITRO</i> BIOCONTROL ACTIVITY OF ANTAGONISTIC BACTERIA AGAINST <i>COLLETOTRICHUM GLOESPORIOIDES</i> IN PAPAYA</b>	
3.1	Introduction	39
3.2	Materials and methods	40
3.2.1	Source of epiphytic bacteria	40
3.2.2	Isolation of the epiphytic bacteria	41
3.2.3	Preparation of conidial suspension of <i>C. gloeosporioides</i>	41
3.2.4	<i>In vitro</i> screening of antagonistic bacteria	42
3.2.5	Dual culture assay	42
3.2.6	Preparation of aqueous antagonist suspension	43
3.2.7	Preparation of filter sterilized culture filtrate	43
3.2.8	Identification and confirmation of the identity of bacterial antagonists	44
3.2.8.1	BIOLOG identification	44
3.2.8.2	Molecular identification	44
3.2.9	Antagonism	45
3.2.9.1	Production of diffusible antifungal substances	45
3.2.9.2	Production of volatile antifungal substances	46
3.2.9.3	Determination of antifungal activity of <i>B. subtilis</i> strain against the mycelial growth of <i>C. gloeosporioides</i>	47
3.2.9.4	Spore germination test	47
3.2.9.5	Study on hyphal morphology	48
3.2.10	Experimental design and statistical analysis	48
3.3	Results	49
3.3.1	Isolation of antagonistic bacteria from leaf and fruit surface of papaya	49
3.3.2	Screening and selection of antagonistic bacteria	49
3.3.3	Identification of antagonistic bacterial strains	51
3.3.4	Production of diffusible and volatile antifungal substances	54
3.3.5	Effect of cell suspensions and filter sterilized culture filtrate of three strains of <i>B. subtilis</i> on mycelial growth of <i>C. gloeosporioides</i>	56
3.3.6	Inhibition of germination of <i>C. gloeosporioides</i> spores	57
3.3.7	Effect on hyphal morphology of <i>C. gloeosporioides</i>	59
3.4	Discussion	60

#### **4 COLONIZATION, SURVIVAL AND MECHANISMS OF ACTION OF *BACILLUS SUBTILIS* B34 ON PAPAYA FRUITS AGAINST *COLLETOTRICHUM GLOEOSPORIOIDES***

4.1	Introduction	64
4.2	Materials and methods	66
4.2.1	Preparation of the fruits	66
4.2.2	Culture of <i>C. gloeosporioides</i> and conidial suspension preparation	66
4.2.3	Antagonistic bacterial strain	66
4.2.4	Antagonist attachment, colonization and population dynamics on surface of papaya	67
4.2.5	Incubation time on biomass production of strain B34	68
4.2.6	Selection of suitable temperature	68
4.2.7	Selection of liquid culture media	69
4.2.8	Mechanisms of actions	69
4.2.8.1	<i>In vivo</i> competition for nutrients	69
4.2.8.2	Bioassay test for antibiotic production by the bacterial antagonist	70
4.2.8.3	Interactions between <i>C. gloeosporioides</i> and <i>B. subtilis</i> on papaya fruits	71
4.2.8.4	Proteolytic enzyme activity	71
4.2.9	Experimental design and statistical analysis	72
4.3	Results	72
4.3.1	Attachment and colonization of <i>B. subtilis</i> B34 on papaya fruit surface	72
4.3.2	Effect of cultural condition on biomass production of <i>B. subtilis</i> B34	75
4.3.3	Mechanisms of actions of <i>B. subtilis</i> B34 against <i>C. gloeosporioides</i>	77
4.3.3.1	<i>In vivo</i> competition for nutrients	77
4.3.3.2	Antibiotic activity of <i>B. subtilis</i> B34 against <i>C. gloeosporioides</i>	78
4.3.3.3	SEM observation of the bacteria-pathogen <i>in vivo</i> direct interaction	79
4.3.3.4	Production of lytic enzymes by <i>B. subtilis</i> B34	81
4.4	Discussion	82

#### **5 IMPROVEMENT OF BIOCONTROL EFFICACY OF *BACILLUS SUBTILIS* B34 WITH SODIUM BICARBONATE AND *ALOE VERA* GEL TO CONTROL ANTHRACNOSE OF PAPAYA DURING STORAGE**

5.1	Introduction	88
5.2	Materials and methods	91
5.2.1	Fruits	91
5.2.2	Culture of <i>C. gloeosporioides</i> and conidial suspension preparation	91
5.2.3	Experimental treatments	91
5.2.4	Preparation of aqueous suspension of <i>B. subtilis</i>	92
5.2.5	Preparation of sodium bicarbonate solutions	92

5.2.6	Preparation of <i>Aloe vera</i> gel (aloe gel)	92
5.2.7	Compatibility test of strain B34 with sodium bicarbonate and aloe gel	93
5.2.8	Effect of sodium bicarbonate and aloe gel on <i>in vitro</i> growth and spore germination of <i>C. gloeosporioides</i>	93
5.2.9	Effect of sodium bicarbonate and aloe gel on fruit colonization by <i>B. subtilis</i> B34	94
5.2.10	Biocontrol activity of <i>B. subtilis</i> B34 enhanced with sodium bicarbonate and aloe gel on papaya fruits pre inoculated with <i>C. gloeosporioides</i>	95
5.2.10.1	Fruit inoculation and lesion measurement	95
5.2.10.2	Biocontrol activity on naturally infected fruits	96
5.2.10	Experimental design and statistical analysis	97
5.4	Results	98
5.4.1	Compatibility of strain B34 with sodium bicarbonate and aloe gel	98
5.4.2	Effect of SBC on mycelia growth and spore germination of <i>C. gloeosporioides</i>	99
5.4.3	Effect of aloe gel on mycelial growth and spore germination of <i>C. gloeosporioides</i>	103
5.4.4	Effect of SBC and aloe gel on fruit colonization by <i>B. subtilis</i> B34	105
5.4.5	Biocontrol activity of <i>B. subtilis</i> B34 enhanced with SBC and aloe gel on papaya fruits pre-inoculated with <i>C. gloeosporioides</i>	107
5.4.6	Biocontrol activity on naturally infected fruits	111
5.5	Discussion	117
<b>6</b>	<b>POSTHARVEST APPLICATION OF <i>BACILLUS SUBTILIS</i> B34 WITH SODIUM BICARBONATE AND <i>ALOE VERA</i> GEL COATING ON THE STORABILITY AND QUALITY OF PAPAYA</b>	
6.1	Introduction	125
6.2	Materials and methods	127
6.2.1	Fruits and treatments	127
6.2.2	Determination of respiration rate and ethylene production in papaya during storage	128
6.2.3	Determination of physical characteristics	129
6.2.3.1	Weight loss	129
6.2.3.	Determination of pulp firmness	129
6.2.3.	Determination of external peel colour	130
6.2.4	Determination of chemical characteristics	130
6.2.4.1	Determination of titratable acidity	131
6.2.4.2	Determination of pH	131
6.2.4.3	Determination of soluble solids concentration	132
6.2.4.4	Determination of ascorbic acid	133
6.2.5	Sensory evaluation of ripe fruit	133
6.2.6	Experimental design and statistical analysis	134
6.3	Results	134
6.3.1	Respiration rate and ethylene production	134

6.3.2	Changes in physical characteristics	137
6.3.2.1	Weight loss	137
6.3.2.2	Flesh firmness	138
6.3.2.3	External peel colour	140
6.3.3	Changes in chemical characteristics	142
6.3.3.1	Titrateable acidity	142
6.3.3.2	pH	143
6.3.3.3	Soluble solids concentration	144
6.3.3.4	Ascorbic acid content	145
6.3.2	Sensory analysis	147
6.4	Discussion	148
<b>7</b>	<b>EFFICACY OF <i>Bacillus subtilis</i> B34 STORED IN GLYCEROL AGAINST ANTHRACNOSE OF PAPAYA</b>	
7.1	Introduction	159
7.2	Material and methods	161
7.2.1	Preparation of liquid based formulation	161
7.2.2	<i>In vitro</i> viability assay of <i>B. subtilis</i> in liquid formulations	161
7.2.3	<i>In vitro</i> antagonistic assay of <i>B. subtilis</i> against <i>C. gloeosporioides</i> of different formulations	162
7.2.4	<i>In vivo</i> biocontrol assay of <i>B. subtilis</i> against <i>C. gloeosporioides</i> of different formulations	162
7.2.5	Experimental design and statistical analysis	162
7.3	Results	163
7.3.1	Effect of glycerol concentrations on the viability and population of <i>B. subtilis</i>	163
7.3.2	<i>In vitro</i> effect of different formulation of <i>B. subtilis</i> against <i>C. gloeosporioides</i>	165
7.3.3	<i>In vivo</i> effect of different formulation of <i>B. subtilis</i> against <i>C. gloeosporioides</i>	166
7.6	Discussion	168
<b>8</b>	<b>SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH</b>	
8.1	Summary	171
8.2	Conclusion	172
8.3	Recommendation	173
	<b>REFERENCES</b>	174
	<b>APPENDICES</b>	206
	<b>BIODATA OF STUDENT</b>	216
	<b>LIST OF PUBLICATIONS</b>	217