CONTENTS

			PAGE
ΑB	STRAG	CT	ii
		VLEDGEMENTS	iv
	NTEN'		V
		ΓABLES	viii
		FIGURES	ix
		SYMBOLS	xi
		ABBREVIATIONS	xii
CH	IAPTI	ERS	
1.	INTE	RODUCTION	1
_,	1.1	Rationale	1
	1.2	Objective	2
	1.3	Hypotheses	3 3
	1.4	Scopes	3
	1.5	Expected Benefits	3
2.	LIST	TERATURE REVIEWS	4
	2.1	The Actinomyces, Genus Streptomyces	4
	2.1.1	Definition of Streptomyces	4
	2.1.2	Identification of Streptomyces	6
	2.1.3	The Cultivation of <i>Streptomyces</i>	9
	2.1.4	The metabolism of <i>Streptomyces</i>	11
	2.1.5	The Application of <i>Streptomyces</i>	12
	2.2	Chitin	12
	2.2.1		13
		Biosynthesis Properties of Chitins	14
	2.2.3	1	15
	2.2.4	Applications of chitin and its derivatives	16
	2.2.5	Chitin-degrading enzymes	17
	2.3	Damping off Disease in Greenhouse	18
	2.3.1 2.3.2	Symptoms of Common Diseases Damping-off Pathogens	19 21
	2.3.2	Biological Control of Damping off Disease in Greenhouse	21
	2.4.1	The Interactions Types of Biological Control	22
	2.4.2	Biological Control Product	24
3.	CHI	FINOLYTIC STREPTOMYCETE ISOLATED FROM	26
		MITE MOUND AT KANCHANABURI, THAILAND	
	3.1	Abstract	26
	3.2		26
	3.3		28
	3.3.1	Isolation of Plant Pathogenic Fungi causing Seedling Damping Of Diseases in Economic Plant Nursery	ff 28

			PAGI
	3.3.2	Isolation and Screening for Antagonistic Actinomycetes against	28
		Fungal Seedling Damping Off Diseases	
	3.3.3.	Antagonistic Test on Dual Culture Agar Plate	29
	3.3.4	Identification of the Selected Actinomycetes	29
	3.3.5	Comparison Test between Selected Actinomycetes,	33
	2.4	S. fradiae and S. rubrolavendulae	22
	3.4.	Results and Discussions	33
	3.4.1	Plant Pathogenic Fungi causing Seedling Damping Off Diseases	33
	3.4.2	Isolation of Soil Actinomycetes	37
	3.4.3	Antagonistic Activity on Dual Culture Agar Plate	39
	3.4.4 3.4.5	Identification of the Selected Antagonistic Actinomycetes	39 43
	3.4.3	Characteristic comparison of <i>Streptomyces</i> sp. strain S4, <i>Streptomyces fradiae</i> , and <i>Streptomyces rubrolavendulae</i>	43
	3.5	Conclusion	47
	3.3	Conclusion	4/
4.	ANT	AGONISTIC MECHANISMS OF STREPTOMYCES SP.	48
••		AIN S4 AGAINST SEEDLING DAMPING OFF FUNGI	
	4.1	Abstract	48
	4.2	Introduction	48
	4.3	Materials and Methods	50
	4.3.1		50
	4.3.2	Antagonistic Test of <i>Streptomyces</i> sp. strain S4 against	51
		Phytophthora infestans and Pythium aphanidermatum	
	4.3.3	Evaluation of Biological Control Mechanisms of <i>Streptomyces</i> sp. strain S4	52
	4.3.4	Biological Activity of Antagonistic <i>Streptomyces</i> sp. strain S4	53
	4.4.	to Phytophthora infestans and Pythium aphanidermatum Results and Discussions	54
	4.4. 4.4.1	Production of Extracellular Hydrolytic Enzymes	54 54
	4.4.1	Antagonistic Activity of <i>Streptomyces</i> sp. strain S4 against	56
	4.4.2	Phytophthora infestans and Pythium aphanidermatum	50
	4.4.3	Biological Control Activity of Antagonistic <i>Streptomyces</i> sp.	61
	7.7.3	strain S4 against <i>P. infestans</i> and <i>P. aphanidermatum</i>	01
	4.5.	Conclusions	63
			32
5.	BIOI	OGICAL CONTROL PRODUCT OF STREPTOMYCES S	SP.64
	STR	AIN S4 FOR CONTROLLING PLANT SEEDLING	
	DAM	IPING OFF DISEASE	
	5.1	Abstract	64
	5.2	Introduction	65
	5.3	Materials and Methods	66
	5.3.1	1	66
		for Solid State Fermentation	
	5.3.2	Experimental Design for Cell Mass Production	66
		in Solid State Fermentation Medium	

			PAGE
	5.3.3	Production of the Antagonistic Cell Powder for making Biological Control Product	68
	5.3.4	Shelf Life and Efficacy of the <i>Streptomyces</i> sp. strain S4 Biological Control Product	69
	5.4	Results and Discussions	69
	5.4.1	Chemical Composition of Shrimp Head and Rice Bran	69
	5.4.2	The Growth of <i>Streptomyces</i> sp. strain S4 in Solid State Fermentation Media	70
	5.4.3	The Cell Mass Production of Streptomyces sp. strain S4	74
	5.4.4	Shelf Life of Biological Control Product of <i>Streptomyces</i> sp. strain S4	75
	5.4.5	Efficacy of Biological Control Product of <i>Streptomyces</i> sp. strain S4 in Controlling of Seedling Damping Off Disease	76
	5.4.6	Shelf Life of <i>Streptomyces</i> sp. strain S4 Formulation	72
	5.4.7	Efficacy of <i>Streptomyces</i> sp. strain S4 formulated in controlling of seedling damping off	76
	5.5	Conclusion	77
6.	CONCLUSION AND RECOMMENDATIONS		78
	6.1	Conclusion	78
	6.2	Recommendations	80
RE	FERE	NCES	81
AP	PEND	IX	100
	A	Proximate Analysis	100
	В	Media Composition	104
	C	Reagents and Buffer Solution	109
	D	Experiment Analysis	112
	E	Molecular Analysis	114
	F	Nucleotide Database	119
CU	RRIC	ULUM VITAE	131

LIST OF TABLES

TAH	3LE	PAGE
2.1	The observed properties for bacterial classification and identification	5
2.2	Contents of Chitin	14
2.3	Types of interspecies antagonisms leading to biological control of plant pathogens.	25
3.1	List of pathogenic microorganism	31
3.2	The colony appearance of actinomycetes isolated from organic-rich soil samples	38
3.3	Physiological and biochemical properties of the isolated S4	38
3.4	Antagonistic activity of actinomycetes isolates against pathogenic	39
	fungi of seedling damping off disease.	
3.5	Gene bank accession numbers along with the alignments of sequences obtained with reported 16S rRNA gene sequences in the gene bank and	43
2.6	highest similarity with different Streptomyces species.	4.5
3.6	The cultural characters of <i>Streptomyces</i> sp. strain S4, <i>S. fradiae</i> , and <i>S. rubrolavendulae</i>	45
3.7	Physiology and biochemical properties of <i>Streptomyces</i> sp. strain S4, <i>S. fradiae</i> , and <i>S. rubrolavendulae</i>	46
3.8	Morphology of <i>Streptomyces</i> sp. strain S4, <i>S. fradiae</i> , <i>S. rubrolavendulae</i>	47
4.1	Ability of <i>Streptomyces</i> sp. strain S4 to inhibit mycelial growth of fungal pathogens in liquid medium	1 57
4.2	Biological and chemical control of tomato and chili damping off disease caused by <i>P. infestans</i>	62
4.3	Biological and chemical control of Chinese spinach damping off disease caused by <i>P. aphanidermatum</i>	63
5.1	Range of the independent variables for response surface methodology	67
5.2	The experimental design of central composite design of response surface methodology (2 ³ CCD)	68
5.3	Chemical Composition of Shrimp head powder and Rice bran	69
5.4	The experimental responses of dependent variable growth	71
5.5	Analysis of variance (ANOVA) for the fitted quadratic polynomial mode for cell mass production.	el 71

LIST OF FIGURES

FIGU	JRE	PAGI
2.1	The life cycle of <i>Streptomyces</i> species	4
2.2	The molecular toolbox to characterize the structural	6
	and functional diversity of microorganisms in the environment	
2.3	Seven sections of spore chain in the genus <i>Streptomyces</i>	8
2.4	The spore surface types	9
2.5	The polyketide compounds	12
2.6	Structure of chitin, chitosan and cellulose.	13
2.7	The cuticle of the arthropod <i>Homarus americanus</i>	15
	displays a variety of different architectures at multiple length scales.	
2.8	The three polymorphic configurations of chitin.	16
2.9	Disease Cycle of Damping-off and Seed Decay	19
2.10	This is a simplified disease cycle for late blight of potato and tomato	20
2.11	Pythium Root Rot Life Cycles	21
3.1	The plant pathogenic fungi	34
3.2	P. infestans	35
3.3	The mycelium and the asexual reproduction of <i>P. aphanidermatum</i>	36
3.4	The colony of actinomycetes on nutrient agar	37
3.5	Micrographs of actinomycetes strain S4 mycelium	40
3.6	Micrographs of actinomycetes strain S4 spores	40
3.7	The colony of <i>Streptomyces</i> sp. strain S4 on nutrient agar	41
3.8	Nucleotide sequence of the 2.013-kb 16s rRNA gene	42
5.0	of Streptomyces sp. strain S4.	.2
3.9	Phylogenetic tree of 16S rRNA gene sequences between	44
	Streptomyces sp. strain S4 and members of genus Streptomyces	
4.1	The growth and chitinase activity of <i>Streptomyces</i> sp. strain S4	54
	cultured in colloidal chitin medium strain S4 in colloidal chitin medium.	
4.2	The growth and cellulase activity of <i>Streptomyces</i> sp. strain S4 cultured in CMC medium	55
4.3	The growth, chitinase and cellulase production of <i>Streptomyces</i> sp.	56
	strain S4 in solid-state fermentation medium	
4.4	Photographs of <i>in vitro</i> interactions between <i>Streptomyces</i> sp.	56
	strain S4 and <i>P. infestans</i> in a dual culture on PDA.	
4.5	Photographs of <i>in vitro</i> interactions between <i>Streptomyces</i> sp.	57
	strain S4 and <i>P. aphanidermatum</i> in a dual culture on PDA	
4.6	The heat and non-heat inactivated culture supernatant	58
	of Streptomyces sp. strain S4 in CMC medium showed inhibitory	
	effect on <i>P. aphanidermatum</i> growth.	
4.7	The heat and non-heat inactivated culture supernatant	59
	of <i>Streptomyces</i> sp. strain S4 in colloidal chitin medium	
	showed inhibitory effect on <i>P. aphanidermatum</i> growth	
4.8	Scanning electron micrographs of the antagonist and <i>P. infestans</i>	60
	at 5 days co-cultured in PDA on cover glass	

FIGURE		PAGE
4.9	Scanning electron micrographs of the antagonist and <i>P. aphanidermatum</i> at 5 days co-cultured in PDA on cover glass	60
4.10	Scanning electron micrographs of the deformation of the fungal mycelium by the antagonist at 5 days after co-cultured	61
5.1	Contour plot to study the effect of substrate as ratio of shrimp head and rice bran and initial moisture (%) on the growth production (logCFU/g) at inoculum size coded level of zero	72
5.2	Contour plot to study the effect of substrate as ratio of shrimp head and rice bran and inoculum size on the growth production (logCFU/g) at initial moisture (%) coded level of zero	73
5.3	Contour plot to study the effect of initial moisture (%) and inoculum site on the growth production (logCFU/g) at substrate as ratio of shrimp head and rice bran coded level of zero	73
5.4	Selected solid state fermentation of Streptomyces sp. strain S4	74
5.5	Streptomyces sp. strain S4 powder stored at 4°C and room temperature (RT)	75
5.6	Efficacy of Biological Control Product of <i>Streptomyces</i> sp. strain S4 in controlling of <i>P. aphanidermatum</i> .	76
D.1	Standard curve of reducing sugar by DMAB method using NAG as standard sugar	112
D2	Standard curve of reducing sugar by DNS method using glucose as standard sugar	112

LIST OF SYMBOLS

 $\begin{array}{cccc} ^{\circ}C & = & & Degree \ Celcius \\ \beta & = & Beta \end{array}$

LIST OF ABBREVIATIONS

g	=	Gram(s)
mg	=	Milligram(s)
rpm	=	Revolution(s) per minute
1	=	Liter(s)
ml	=	Milliliter(s)
W/V	=	Weight/volume
M	=	Molarity
h	=	Hour(s)
ISP	=	International Streptomyces Project
min	=	minute(s)
N	=	Normality
mm	=	Millimeter(s)
mM	=	Millimolar
μl	=	Microliter(s)
V	=	Volt(s)
LB	=	Luria broth
PCR	=	Polymerase chain reaction
DNA	=	Deoxyribonucleic acid
bp	=	Base pair
f	=	Forward
r	=	Reverse
sec	=	Second(s)
μg	=	Microgram(s)
μm	=	Micrometer(s)
kb	=	Kilo base pair
ng	=	Nanogram(s)
U	=	Unit
CFU	=	Colony forming unit
BCAs	=	Biological control agent(s)
OD	=	Optical density
pН	=	Hydrogen ion
UV	=	Ultraviolet