

CONTENTS

	Page
ABSTRACT IN THAI.....	I
ABSTRACT IN ENGLISH.....	III
ACKNOWLEDGEMENTS.....	V
CONTENTS.....	VI
LIST OF TABLES.....	IX
LIST OF FIGURES.....	X
LIST OF ABBREVIATIONS.....	XII
 CHAPTER	
I INTRODUCTION.....	1
1.1 Background / Problem.....	1
1.2 Research objectives.....	2
1.3 Research hypothesis.....	3
1.4 Scope and limitation of the study.....	3
1.5 Expected results.....	3
II LITERATURE REVIEW.....	4
2.1 Melioidosis	4
2.1.1 Epidemiology of Melioidosis.....	4
2.1.2 Clinical presentation.....	5
2.2 <i>Burkholderia pseudomallei</i>	6
2.2.1 Virulence factors of <i>B. pseudomallei</i>	7
2.2.2 Type III Secretion Systems (T3SS) of <i>B. pseudomallei</i>	9
2.3 Method for diagnosis melioidosis.....	10
2.4 CRISPR/CAS: CRISPR (Clustered regularly interspaced short palindromic repeats)	12
2.4.1 CRISPR/Cas12a.....	13

CONTENTS (Continued)

	Page
III MATERIALS AND METHODS.....	16
3.1 Materials.....	16
3.1.1 Bacterial strains.....	16
3.1.2 Culture media.....	16
3.1.3 Chemicals and Reagents.....	17
3.2 Methods.....	18
3.2.1 Sample preparation.....	18
3.2.2 DNA extraction of <i>B. pseudomallei</i> by boiling method.....	18
3.2.3 Selection of specific <i>B. pseudomallei</i> genes and gRNA design.....	18
3.2.4 Detection of <i>orf2</i> and <i>orf11</i> of T3SS-1 of <i>B. pseudomallei</i> by polymerase chain reaction (PCR) amplification.....	19
3.2.5 <i>In vitro</i> digestion activity of Lba Cas12a (Cpf1) on <i>B. pseudomallei</i> DNA target.....	20
3.2.6 Measurement the signal of single stranded DNA fluorophore-quencher (ssDNA-FQ reporter) of FAM-BHQ1 in CRISPR/Cas12a system.....	21
3.2.7 Determination the specificity of selected target site <i>orf2</i> and <i>orf11</i> from T3SS-1 of <i>B. pseudomallei</i> with another Gram-negative bacilli (<i>B. thailandensis</i>).....	21
IV RESULTS AND DISCUSSION.....	23
4.1 Detection of <i>orf2</i> and <i>orf11</i> of T3SS-1 of <i>B. pseudomallei</i> by polymerase chain reaction (PCR) amplification.....	23
4.2 Determination of the specificity of selected target site <i>orf2</i> and <i>orf11</i> from T3SS-1 <i>B. pseudomallei</i> with another Gram-negative bacilli.....	26
4.3 <i>In vitro</i> digestion activity of Lba Cas12a (Cpf1) on <i>B. pseudomallei</i> DNA target.....	29
4.4 Measurement the signal of single stranded DNA fluorophore-quencher (ssDNA-FQ reporter) of FAM-BHQ1 in CRISPR/Cas12a system.....	34

CONTENTS (Continued)

	Page
V CONCLUSION.....	37
REFERENCES.....	39
APPENDICES.....	43
APPENDIX A SEQUENCE OF OPEN READING FRAMES 2 (<i>ORF2</i>) AND OPEN READING FRAMES 11 (<i>ORF11</i>) FROM T3SS-1 OF <i>B. PSEUDOMALLEI</i> K96243.....	44
APPENDIX B BLASTN RESULTS OF <i>B. PSEUDOMALLEI</i> K96243 AND PRIMERS BLAST OF <i>ORF2</i> AND <i>ORF11</i>	48
APPENDIX C EQUIPMENTS AND INSTRUMENTS.....	50
CURRICULUMVITAE.....	50

LIST OF TABLES

Table	Page
3.1 The sequence of specific gRNA for <i>orf2</i> and <i>orf11</i> gene of <i>B. pseudomallei</i>	19
3.2 Forward and reverse primers used for detection of <i>orf2</i> and <i>orf11</i> genes of <i>B. pseudomallei</i> and PCR product size.....	20
3.3 The sequence of ssDNA-FQ reporter used in CRISPR/Cas12a system.....	21

LIST OF FIGURES

Figure	Page
2.1 Clinical presentations of melioidosis.....	5
2.2 Gram stain of <i>B. pseudomallei</i>	7
2.3 Colony morphology of <i>B. pseudomallei</i>	7
2.4 A schematic representation of the <i>B. pseudomallei</i> intra- and intercellular life cycles.....	9
2.5 Cas12a (Cpf1) with a bound crRNA targeting a genomic site recognizes the PAM sequence 5'-TTTV-3'.....	14
4.1 The PCR products of <i>orf2</i> and <i>orf11</i> from T3SS-1 of the wild type <i>B. pseudomallei</i>	24
4.2 The PCR products of <i>orf2</i> from T3SS-1 of clinical isolate <i>B. pseudomallei</i> exhibited 250 bp using 2% agarose gel electrophoresis.....	25
4.3 The PCR products of <i>orf11</i> from T3SS-1 of clinical isolate <i>B. pseudomallei</i> exhibited 335 bp using 2% agarose gel Electrophoresis.....	26
4.4 The PCR result of amplifying <i>orf2</i> from <i>B. thailandensis</i>	28
4.5 The PCR result of amplifying <i>orf11</i> from <i>B. thailandensis</i>	29
4.6 The gRNA binding to target sequences of <i>orf2</i> from T3SS-1 of <i>B. pseudomallei</i>	31
4.7 The gRNA binding to target sequences of <i>orf11</i> from T3SS-1 of <i>B. pseudomallei</i>	31
4.8 <i>In vitro</i> digestion activity of RNA-guided enzyme Cas12a or CRISPR/Cas12a on target sites of <i>orf2</i> and <i>orf11</i> from T3SS-1 of the wild type <i>B. pseudomallei</i>	32
4.9 <i>In vitro</i> digestion activity of RNA-guided enzyme Cas12a or CRISPR/Cas12a on target sites of <i>orf2</i> from T3SS-1 of clinical isolate <i>B. pseudomallei</i>	33

LIST OF FIGURES (Continued)

Figure	Page
4.10 <i>In vitro</i> digestion activity of RNA-guided enzyme Cas12a or CRISPR/Cas12a on target sites of <i>orf11</i> from T3SS-1 of clinical isolate <i>B. pseudomallei</i>	34
4.11 The signal of FAM fluorophore of cleavage products of <i>orf2</i> from T3SS-1 of <i>B. pseudomallei</i> comparing with the fluorescence signals of control groups.....	36
4.12 The signal of of FAM fluorophore of cleavage products of <i>orf11</i> from T3SS-1 of <i>B. pseudomallei</i> comparing with the fluorescence signals of control groups.....	36

LIST OF ABBREVIATIONS

°C	Degree Celsius
g	Gram
h	Hour
min	Minute
mL	Milliliter
nm	Nanometer
OD	Optical density
µg/ml	Microgram per milliliter
µg	Microgram
µl	Microliter
µ	Micrometer
RT	Room temperature
RPM	Revolutions per minute
MW	Molecular Weight
pmole	Picomole
nmol	Nanomole
bp	Base pair
PCR	Polymerase chain reaction
NC	Negative Control
Temp	Temperature
ng/µl	Nanogram per milliliter
nt	Nucleotide
V	Volt