

**CHARACTERIZATION OF ANTARCTIC
BACTERIA AND THEIR ANTIMICROBIAL
ACTIVITIES**



TAM HENG KEAT

PERPUSTAKAAN | MALAYSIA SABAH
UNIVERSITI MALAYSIA SABAH

**BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2009**

**CHARACTERIZATION OF ANTARCTIC
BACTERIA AND THEIR ANTIMICROBIAL
ACTIVITIES**

TAM HENG KEAT



PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

UMS
UNIVERSITI MALAYSIA SABAH

**THESIS SUBMITTED IN FULLFILLMENT FOR
THE DEGREE OF MASTER OF SCIENCE**

**BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2009**

tohom

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

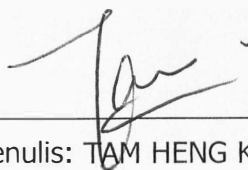
JUDUL: CHARACTERIZATION OF ANTARCTIC BACTERIA AND THEIR ANTIMICROBIAL ACTIVITIES

IZAJAH: SARJANA SAINS (MOLECULAR MIKROBIOLOGI)

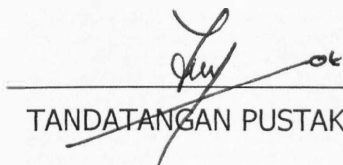
SESI PENGAJIAN: SESSI PENGAJIAN 2007-2009

Saya, TAM HENG KEAT mengaku membenarkan tesis sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan berikut:

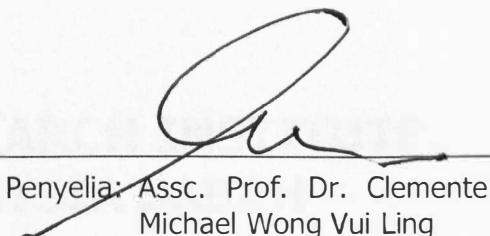
1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran Institusi Pengajian Tinggi.
4. TIDAK TERHAD



Penulis: TAM HENG KEAT



TANDATANGAN PUSTAKAWAN



Penyelia: Assc. Prof. Dr. Clemente
Michael Wong Vui Ling

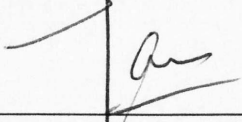
Tarikh: 20 Ogos 2009

Catatan: ® Tesis dimaksudkan SEBAGAI TESIS Ijazah Doktor Falsafah dan Sarjana secara penyelidikan atau Disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau laporan Projek Sarjana Muda (LPSM)

DECLARATION

I hereby declare that the material in this thesis is of my own effort, except for quotations, excerpts, equations, references and summaries which have been duly acknowledged and cited clearly it sources.

20 AUGUST 2009


TAM HENG KEAT
PB2007-8160



UMS
UNIVERSITI MALAYSIA SABAH

CERTIFICATION

NAME : **TAM HENG KEAT**
MATRIC NO. : **PB2007-8160**
TITLE : **CHARACTERIZATION OF ANTARCTIC BACTERIA AND
THEIR ANTIMICROBIAL ACTIVITIES**
DEGREE : **MASTER OF SCIENCE**
VIVA DATE : **10 AUGUST 2009**

DECLARED BY


1. SUPERVISOR

(Associate Professor Dr. Clemente Michael Wong Vui Ling)



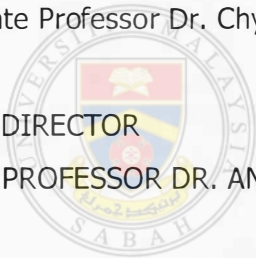
2. INTERNAL EXAMINER

(Associate Professor Dr. Chye Fook Yee)



3. DEAN / DIRECTOR

(DATIN PROFESSOR DR. ANN ANTON)



UNIVERSITI MALAYSIA SABAH

ACKNOWLEDGEMENT

Without the encouragement and participation of many people, this project would never have been accomplished. First of all, I would like to thank the Director of Biotechnology Research Institute for giving me an opportunity to further my study in their laboratories. I would like to thank the Malaysian Antarctic Research Program under the auspices of the Academic Science of Malaysia for funding for this research (Research Grant No. 95500-66). My personal and my deepest appreciation go to my supervisor, Dr. Clemente Michael Wong Vui Ling for his expertise, guidance and support throughout the development of the project and help me being my vision to most satisfying conclusion and providing the samples used in this study. The appreciation will also go to the Instituto Antartico Chileno (INACH), Chile during the expedition to King George Island (2006) and the National Centre of Antarctic and Ocean Research of India during Indian expedition to Maitri Station, Schirmacher Range (2005) for all their wonderful arrangement and logistic support for the samples collection. Prof. Son Radu is gratefully acknowledged for the supply of the pathogens for the study of antimicrobial assay.

A very special thanks is extended to lab assistant and personnel of Biotechnology Research Institute, UMS: Ms. Vidarita, Mr. Richard, Shanty, Zulkrifi Mohammed, Junainah Ismail and Johan Alexander who have been of immeasurable value to me during the progress of my project and graduation. My personal sincerest appreciation and thanks to Mr. Adrian Ng Wei Ming, Mr. Kenneth Rodriguez, Ms. Chelven Lim, Mr. Gordon Thomas, Ms. Sharon Lau, Ms. Elaine Remi, and Mr. Alex Foong, who unselfishly shared with me their valuable knowledge and experiences and was always available to answer my questions. I would like to express any appreciation to all my colleagues and friends for sharing with me a joyful life here.

Finally, I would like to express my deepest appreciation to my family and my lovely fiancée for their uncounted love, warmth and support throughout my life to all my sincerest thank you.

ABSTRACT

CHARACTERIZATION OF ANTARCTIC BACTERIA AND THEIR ANTIMICROBIAL ACTIVITIES

A total of 2582 bacterial strains were isolated from 16 soil and water samples from the King George Island and Schirmacher Range, Antarctica. Twenty three Antarctic bacterial strains inhibited the growth of one or more Gram-negative and Gram-positive food pathogens such as *Escherichia coli* 0157:H7, *Salmonella* spp., *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Vibrio* spp. and a Gram-positive food pathogen *Bacillus cereus* K15. Seven out of the 23 strains, BG5, CG21, HKAM1, MTC3, MA2, WEA1 and WEK1 were identified based on their 16S rDNA sequences and biochemical analyses. They were *Pseudomonas* sp. MTC3, *Pseudomonas* sp. CG21, *Pseudomonas* sp. MA2, *P. corrugata* WEK1, *P. migulae* WEA1, *Janthinobacterium lividum* HKAM1 and *Pedobacter cryoconits* BG5. Although most of them were affiliated to the same genus or closely related species, their biochemical, phenotypic characteristics and antibiotics resistance profiles varied. Inhibitors produced by strains MTC3, CG21 and BG5 were sensitive to protease suggesting that they have proteinaceous structures while strains WEA1, WEK1, HKAM1 and MA2 were not sensitive to catalase, lipase, α -amylase, and protease indicating four of these inhibitors have complex structures. Three out of seven Antarctic bacterial strains WEA1, WEK1 and MA2 were found to encode polyketide synthase gene, indicating the antimicrobial agent was probably produced by polyketide synthase. Antimicrobial resistance profiles of 45 Antarctic bacterial isolates were obtained. Most of the bacteria were resistance to at least of three or more types of antimicrobial agents tested while one of the bacterial isolate was susceptible to all the antimicrobial agents. These data revealed that the existence of many antimicrobial resistant strains among the Antarctic bacterial population. The plasmid sequence of pHK1 of *Pseudomonas* sp. CG21 revealed that there was no gene encoding the antimicrobial production and antimicrobial resistance on the plasmid. Basically the pHK1 plasmid carried genes encoding for plasmid replication, stability and maintenance, mobilization and genes for unknown function.

ABSTRAK

Sebanyak 2582 bakteria telah diasingkan dari 16 sampel tanah dan air yang diperolehi dari Antartika. Dua puluh tiga bakteria dari Antartika yang dapat merencatkan tumbesaran bakteria seperti *Escherichia coli* O157:H7, *Salmonella* spp., *Klebsiella pneumoniae* and *Vibrio* spp. dan patogen Gram-positif *Bacillus cereus* K15. Tujuh daripada dua puluh tiga bakteria tersebut telah dikenalpasti melalui jujukan DNA 16S rDNA dan analisis biokimia yang terdiri daripada *Pseudomonas* sp. MTC3, *Pseudomonas* sp. CG21, *Pseudomonas* sp. MA2, *P. corrugata* WEK1, *P. migulae* WEA1, *Janthinobacterium lividum* HKAM1 dan *Pedobacter cryoconits* BG5. Walaupun spesies ini tergolong dalam kumpulan genus yang sama, namun terdapat perbezaan dari segi biokimia. Bahan antibiotik yang dihasilkan oleh bakteria MTC3, CG21 dan BG5 adalah sensitif kepada protease manakala bahan antibiotik yang dihasilkan oleh bakteria WEA1, WEK1, HKAM1, MA2 tidak sensitif kepada katalase, lipase, α -amilase dan protease yang menunjukkan bahan ini mempunyai struktur yang kompleks. Tiga bakteria WEA1, WEK1 dan MA2 didapati membawa gen polyketide synthase, menunjukkan bahan antibiotik dihasilkan oleh polyketide synthase. Profil kebolehan pertahanan antibiotik daripada 45 bakteria dari Antartika menunjukkan kebanyakan bakteria dari Antartika mempunyai kerintangan peka kepada sekurang-kurangnya tiga atau lebih jenis antibiotik yang diuji. Walau bagaimanapun, dua bakteria tidak peka kepada semua antibiotik yang diuji. Data ini menunjukkan bakteria dari Antartika mempunyai kerintangan kepada antibiotik. Jujukan DNA plasmid pHK1 yang dibawa oleh *Pseudomonas* sp. CG21, menunjukkan tiada gen berkaitan dengan penghasilan antibiotik dan pertahanan antibiotik yang dibawa oleh plasmid. Plasmid ini membawa gen yang berkaitan dengan replikasi plasmid, kestabilan serta penyelenggaraan plasmid, kemobilitan dan gen yang membawa protein yang tidak dikenali.

TABLE OF CONTENTS

| | PAGE |
|---|------|
| TITLE | i |
| DECLARATION | ii |
| DECLARED BY | iii |
| ACKNOWLEDGEMENT | iv |
| ABSTRACT | v |
| ABSTRACT | vi |
| TABLE OF CONTENTS | vii |
| LIST OF FIGURES | x |
| LIST OF TABLES | xii |
| LIST OF ABBREVIATIONS | xiii |
| LIST OF SYMBOLS | xv |
| LIST OF APPENDIXES | xvi |
| CHAPTER 1: INTRODUCTION | 1 |
| 1.1 Background | 1 |
| 1.2 Objectives | 3 |
| CHAPTER 2: LITERATURE REVIEW | 4 |
| 2.1 Overview of Antarctica | 4 |
| 2.1.1 Introduction | 4 |
| 2.1.2 Temperature Classes of Bacteria | 6 |
| 2.1.3 Antarctic Bacteria | 7 |
| 2.1.4 Biotechnological Aspects of Antarctic Bacteria | 9 |
| 2.1.5 Antimicrobial Activity | 10 |
| 2.2 Plasmid | 14 |
| 2.2.1 Introduction | 14 |
| 2.2.2 Antimicrobial Agent's Production | 15 |
| 2.2.3 Antibiotic Resistance Plasmid | 16 |
| 2.3 Food-Borne Pathogenic Bacteria | 17 |
| 2.3.1 <i>Escherichia</i> spp. | 17 |
| 2.3.2 <i>Salmonella</i> spp. | 18 |
| 2.3.3 <i>Klebsiella</i> spp. | 19 |
| 2.3.4 <i>Enterobacter</i> spp. | 19 |
| 2.3.5 <i>Vibrio parahaemolyticus</i> | 20 |
| 2.3.6 <i>Bacillus cereus</i> | 20 |
| 2.4 Antimicrobial Agents | 22 |
| 2.4.1 Antimicrobial Agents and Sources | 22 |
| 2.4.2 Antibiotics and Growth Factor Analogs | 27 |
| 2.4.3 Bacteriocins | 29 |
| 2.4.4 Organic Acid and Oxygen Metabolites | 30 |
| 2.5 Antibiotic Resistance | 31 |
| 2.5.1 Introduction | 31 |
| 2.5.2 Mechanism of Antibiotic Resistance | 32 |
| 2.5.3 Antibiotic Resistance of Environmental Bacteria | 32 |

| | |
|--|-----|
| CHAPTER 3: METHODOLOGY | 35 |
| 3.1 Isolation of Antarctic Bacteria | 35 |
| 3.2 Pathogens and Their Cultivation | 35 |
| 3.3 Detection of Antimicrobial Production | 36 |
| 3.4 Sensitivity of Inhibitors to Enzymes | 36 |
| 3.5 Morphology and Physiological of Bacteria | 38 |
| 3.5.1 Morphology of Colonies and Gram Staining | 38 |
| 3.5.2 Non-staining Test of Gram Reaction | 38 |
| 3.5.3 Motility Test | 39 |
| 3.6 Growth Studies of Bacteria | 39 |
| 3.7 Biochemical Characterization | 39 |
| 3.8 DNA Extraction using Phenol: Chloroform | 40 |
| 3.9 16S rDNA Amplification | 41 |
| 3.10 Purification of the PCR Products | 41 |
| 3.11 Preparation of Competent Cells | 42 |
| 3.12 Cloning | 42 |
| 3.13 Small Scale Preparation of Plasmid | 43 |
| 3.14 Restriction Digestion of the Clones | 43 |
| 3.15 Sequencing and Phylogenetic Analysis of 16S rDNA | 43 |
| 3.16 Nucleotide Sequence Accession Numbers for 16S rDNA | 44 |
| 3.17 Identification of Polyketide Synthase Gene | 44 |
| 3.18 Nucleotide Sequence Accession Numbers for PKS Genes | 44 |
| 3.19 Antimicrobial Susceptibility Tests | 45 |
| 3.20 Analysis of Plasmid Contents | 45 |
| 3.21 Restriction Digestion of the Plasmid | 46 |
| 3.22 Cloning of the Digested Fragments from Plasmid | 46 |
| 3.23 Sequencing and Analysis of Plasmid Sequence | 47 |
| 3.24 Nucleotide Sequence Accession Numbers for Plasmid | 48 |
| CHAPTER 4: RESULTS | 49 |
| 4.1 Isolation of Antarctic Microbes | 49 |
| 4.2 Screening for Antimicrobial Activities | 50 |
| 4.3 Sensitivity of Inhibitors to Enzymes | 57 |
| 4.4 Morphology and Physiology of the Bacteria | 58 |
| 4.5 Growth Studies of the Bacteria | 60 |
| 4.6 Biochemical Properties of the Bacteria | 62 |
| 4.7 16S rDNA Sequencing and Phylogenetic Analysis | 68 |
| 4.8 Identification of PKS | 72 |
| 4.9 Antibiotics Resistance Profiles | 76 |
| 4.10 Plasmid Content | 84 |
| 4.11 Cloning of the Plasmid | 85 |
| 4.12 Sequence Characterization and Data Analysis of Plasmid Sequence | 87 |
| 4.13 Toxin-antitoxin System | 93 |
| 4.14 Rep Protein | 106 |
| 4.15 Mobilization Protein | 114 |
| 4.16 ORF3 as a Putative Protein | 121 |

| | | |
|-------------------|--|-----|
| 4.17 | Other ORFs | 122 |
| CHAPTER 5: | DISCUSSION | 125 |
| 5.1 | Isolation of Bacteria | 125 |
| 5.2 | Antimicrobial Activities | 126 |
| 5.3 | Sensitivity to Enzymes | 128 |
| 5.4 | Polyketide Synthase Gene | 129 |
| 5.5 | Characterization of Inhibitor Producing Bacteria | 130 |
| 5.6 | Inhibitor of Producing Bacteria | 133 |
| 5.7 | Antibiotics Resistance and Plasmid Contents | 134 |
| 5.8 | Genes on The Plasmid | 138 |
| CHAPTER 6: | CONCLUSION | 147 |
| REFERENCES | | 149 |



UMS
UNIVERSITI MALAYSIA SABAH

LIST OF FIGURES

| | PAGE |
|--|------|
| Figure 2.1 : Schimarczer Oasis | 4 |
| Figure 2.2 : Growth Rate Versus Temperature for Five Environmental Classes of Prokaryotes. | 6 |
| Figure 2.3 : Mode of Action of Antimicrobial Agents | 28 |
| Figure 3.1 : "Half-moon" Appearance. | 37 |
| Figure 4.1 : Pigmentation by Antarctic Bacteria. | 50 |
| Figure 4.2 : Zone of Inhibition Produced by HKAM1. | 53 |
| Figure 4.3 : Inhibition Produced by BG5, BG6 and WEK1. | 54 |
| Figure 4.4 : Zone of Inhibition Produced by CG20 and CG21. | 54 |
| Figure 4.5 : "Half-moon" Appearance After Protease Treatment. | 57 |
| Figure 4.6 : Growth Rate of the Seven Bacterial Strains. | 61 |
| Figure 4.7 : Formation of Pink Colonies by CG22 and WEA1 on MacConkey Agar. | 62 |
| Figure 4.8 : 16S rDNA Amplification of Microbes HKAM1 and BG5. | 69 |
| Figure 4.9 : PCR Amplification of the 16S rDNA. | 69 |
| Figure 4.10 : Phylogenetic Trees Constructed of 16S rDNA. | 71 |
| Figure 4.11 : PCR Amplification of Type II PKS Primers with Annealing Temperature at 58°C. | 72 |
| Figure 4.12 : Agarose Gel Showing Single Amplicon Amplified Using Type II PKS Primers. | 72 |
| Figure 4.13 : NJ Tree Constructed of Three PKS Proteins. | 75 |
| Figure 4.14 : Antibiotics Resistance by BG5 and WEK1. | 76 |
| Figure 4.15 : Antibiotics Resistance by DI5 and Dr4. | 78 |
| Figure 4.16 : Bart Chart of Total Bacteria Versus Antibiotics Resistance. | 83 |
| Figure 4.17 : Plasmid Extraction of Bacteria. | 84 |
| Figure 4.18 : Restriction Digestion Profiles of the Plasmid. | 86 |
| Figure 4.19 : Genetic Map of Plasmid pHK1. | 88 |
| Figure 4.20 : Genetic Map of Putative Open Reading Frames (ORF). | 89 |
| Figure 4.21 : Restriction map of pHK1 showing the cut site of the restriction enzymes. | 91 |
| Figure 4.22 : Restriction map of pHK1 showing the cut sites of the restriction enzymes. | 92 |
| Figure 4.23 : Genetic map indicates the localization of <i>orf6</i> gene. | 93 |
| Figure 4.24 : Nucleotide Sequence and Amino Acid Sequence the Orf5 and Orf6. | 94 |
| Figure 4.25 : NJ Tree of PemK-like Proteins with Orf6 based on PSI-BLAST. | 97 |
| Figure 4.26 : Conserved Domain of Orf6 with PemK Proteins. | 97 |
| Figure 4.27 : Alignment of Amino Acid Sequence of PemK Proteins. | 98 |
| Figure 4.28 : NJ Tree of PemK-like Proteins with Orf6 Based on CDD. | 98 |
| Figure 4.29 : Alignment of Amino Acid Sequence of Toxin Proteins. | 99 |

| | |
|--|-----|
| Figure 4.30 : NJ Tree of Toxins with Orf6 Based on CDD. | 100 |
| Figure 4.31 : Genetic map indicates the localization of <i>orf5</i> gene. | 102 |
| Figure 4.32 : Conserved Domain of Orf5 with PemI-like Proteins. | 103 |
| Figure 4.33 : NJ Tree of PemI-like Proteins with Orf5 Based on PSI-BLAST. | 103 |
| Figure 4.34 : NJ Tree of PemI-like Proteins with ORF5 Based on CDD. | 104 |
| Figure 4.35 : Alignment of Amino Acid Sequence of Orf5 Proteins. | 105 |
| Figure 4.36 : Genetic map indicates the localization of <i>orf4</i> gene. | 106 |
| Figure 4.37 : NJ Tree of Rep Proteins with Orf4 Based on PSI-BLAST. | 108 |
| Figure 4.38 : Nucleotide Sequence and Amino Acid Sequence of <i>orf4</i> and <i>oriV</i> . | 109 |
| Figure 4.39 : Conserved Domain of Orf4 with Rep3 Proteins.. | 110 |
| Figure 4.40 : Alignment of Amino Acid Sequence of Rep Proteins | 111 |
| Figure 4.41 : NJ Tree of Rep Proteins with Orf4 Based on CDD. | 113 |
| Figure 4.42 : Genetic map indicates the localization of <i>orf2</i> gene. | 114 |
| Figure 4.43 : Conserved Domain of Orf2 with Relaxase Superfamily. | 116 |
| Figure 4.44 : Alignment of Amino Acid Sequence of Mobilization Proteins. | 117 |
| Figure 4.45 : NJ Tree of Mobilization Proteins with Orf2 Based on PSI-BLAST. | 119 |
| Figure 4.46 : NJ Tree of Rep Proteins with Orf2 Based on CDD. | 119 |
| Figure 4.47 : Nucleotide Sequence and Amino Acid Sequence of <i>orf2</i> . | 120 |
| Figure 4.48 : Genetic map indicates the localization of <i>orf3</i> gene. | 121 |
| Figure 4.49 : NJ Tree of Entry Exclusion Proteins with Orf3 Based on PSI-BLAST. | 122 |
| Figure 4.50 : Nucleotide Sequence and Amino Acid Sequence of <i>orf1</i> , <i>orf7</i> and <i>orf8</i> . | 123 |
| Figure 4.51 : Conserved Domain of Orf7 with DUF262 Superfamily. | 124 |
| Figure 4.52 : Alignment of Amino Acid Sequence of DUF262 Proteins. | 124 |
| Figure 5.1 : Schematic Diagram of the Toxin-antitoxin Systems | 140 |
| Figure 5.2 : NJ Tree of Rep Proteins with Orf4 Based on CDD. | 142 |
| Figure 5.3 : Sequence Alignment of the Rep Proteins of the Theta-type Iteron-containing Class. | 143 |
| Figure 5.4 : Schematic HTH motif in Rep proteins. | 144 |

LIST OF TABLES

| | PAGE |
|--|-------|
| Table 2.1 : Antimicrobial Agents and Source Organisms | 11 |
| Table 2.2 : Antimicrobial Classes and Properties. | 25-26 |
| Table 4.1 : Identification of Inhibitor-producers from Antarctica Against Thirteen Food Pathogens. | 51 |
| Table 4.2 : Inhibition Profiles of the Bacteria Against Thirteen Food and Human Pathogens. | 56 |
| Table 4.3 : Sensitivity of Inhibitors to Enzymes. | 58 |
| Table 4.4 : Morphology of Bacterial Colonies. | 59 |
| Table 4.5 : Growth Studies of Inhibitor Producing Bacteria. | 60 |
| Table 4.6 : Biochemical Tests using API20NE and API20E | 64 |
| Table 4.7 : Enzyme Assays. | 65 |
| Table 4.8 : Carbon Utilization Profiles. | 67 |
| Table 4.9 : Similarity Analysis of 16S rDNA sequence of Antarctic Bacteria with GenBank. | 68 |
| Table 4.10 : Similarity Analysis of Three Ketoacyl Synthase Proteins. | 74 |
| Table 4.11 : Antibiotics Resistance Profile of Seven Antarctic Bacteria | 77 |
| Table 4.12 : Antibiotics Resistance Profile of Antarctic Bacteria Isolated from Deception Island. | 81 |
| Table 4.13 : Antibiotics Resistance Profile of Antarctic Bacteria Isolated from Estrellas Lake. | 82 |
| Table 4.14 : Putative ORFs and Homology. | 90 |
| Table 4.15 : Similarity Analysis of Orf6 with GenBank. | 95 |
| Table 4.16 : Similarity Analysis of Orf5 with GenBank. | 102 |
| Table 4.17 : Similarity Analysis of Orf4 with GenBank. | 107 |
| Table 4.18 : Similarity Analysis of Orf2 with GenBank. | 115 |
| Table 4.29 : Similarity Analysis of Orf3 with GenBank. | 122 |
| Table 5.1 : A summary of putative genes found on the plasmid pHK1. | 139 |

LIST OF ABBREVIATIONS

| | | |
|-----------|---|---|
| rRNA | - | Ribosomal ribonucleic acid |
| rDNA | - | Ribosomal deoxyribonucleic acid |
| WHO | - | World Health Organization |
| STEC | - | Shiga toxin-producing <i>Escherichia coli</i> |
| EHEC | - | Enterohemorrhagic <i>Escherichia coli</i> |
| ETEC | - | Enterotoxigenic <i>Escherichia coli</i> |
| EPEC | - | Enteropathogenic <i>Escherichia coli</i> |
| EIEC | - | Eenteroinvasive <i>Escherichia coli</i> |
| HUS | - | Hemolytic–uremic syndrome |
| THF | - | Tetrahydrofolate |
| DHF | - | Dihydrofolate |
| PABA | - | Paraminobenzoic acid |
| mRNA | - | Messenger RNA |
| tRNA | - | Transfer RNA |
| ATP | - | Adenosine triphosphate |
| LAB | - | Lactic acid bacteria |
| Gly | - | Glycine |
| Tyr | - | Tyrosine |
| Asn | - | Asparagine |
| Val | - | Valine |
| Xaa | - | Histidine |
| Cys | - | Cysteine |
| FAO | - | Food and Agriculture Organization |
| Ala | - | Alanine |
| ABA | - | Aminobutyric acid |
| Ala-S-Ala | - | Lanthionine |
| ABA-S-Ala | - | ρ -methyllanthionine |
| TSA | - | Tryptic Soy Agar |
| LBA | - | Luria-Bertani agar |
| ABM | - | Antarctic Bacterial Medium |
| NB | - | Nutrient broth |
| NA | - | Nutrient agar |

| | |
|-------------------|--|
| KOH | - Potassium hydroxide |
| dNTP | - 2'-dexoyribonucleoside-5'-triphosphates |
| EDTA | - Ethylenediaminetetraacetic acid |
| PCR | - Polymerase chain reaction |
| TAE | - Tris-acetate-EDTA |
| SDS | - Sodium lauryl sulfate |
| NaCl | - Sodium chloride |
| MgCl ₂ | - Magnesium chloride |
| BLAST | - Basic local alignment search tool |
| MEGA | - Molecular Evolutionary Genetics Analysis |
| MCS | - Multiple cloning sites |
| CDS | - Coding sequence |
| ORF | - Open reading frame |
| RBS | - Ribosome binding site |
| CDD | - Conserved Domain Database |



UMS
UNIVERSITI MALAYSIA SABAH

LIST OF SYMBOLS & UNITS

| | | |
|--------------------|---|----------------------|
| β | - | Beta |
| $^{\circ}\text{C}$ | - | Degree Celsius |
| < | - | Less than |
| > | - | More than |
| γ | - | Gamma |
| α | - | Alpha |
| % | - | Percent |
| cfu | - | Colony forming units |
| g | - | Gram |
| <i>g</i> | - | Graviti |
| S | - | Subunit |
| ml | - | Milliliter |
| mM | - | Millimolar |
| M | - | Molar |
| mg | - | Milligram |
| w/v | - | Weight per volume |
| s | - | Second |
| cm | - | Centimeter |
| X | - | Times |
| μl | - | Microliter |
| min | - | Minute |
| - | - | Minus |
| rpm | - | Rotation per minute |
| pmol | - | Pico mol |
| U | - | Unit |
| μM | - | Micromolar |
| V | - | Volt |
| Kb | - | Kilo base |
| <i>g</i> | - | Gravity |

LIST OF APPENDIXES

| | PAGE |
|--|------|
| Appendix A: 16S rDNA Sequence Fact Files and Alignments | 177 |
| Appendix B: PKS Genes Sequence Fact Files and Alignments | 198 |
| Appendix C: pUC19 | 206 |
| Appendix D: Plasmid pMWHK01 Sequence Fact Files and Alignments | 207 |



UMS
UNIVERSITI MALAYSIA SABAH

CHAPTER 1

INTRODUCTION

1.1 Background

In the late 1960 and early 1970s, notable achievements in developing antibiotics to combat pathogenic diseases had led to the misconception that the war between infectious diseases and human was over (Spellberg *et al.*, 2004). However, currently, medical centers are experiencing a rise in antimicrobial resistance of pathogenic bacteria and the susceptibility of pathogens against antibiotics is steadily decreasing. The emergence of multiple drug resistance in clinical *Escherichia coli* O157:H7, *Vibrio cholerae* and *Salmonella enterica* from the tropics such as Malaysia is one of the manifestations of this phenomenon (Radu *et al.*, 2001; Radu *et al.*, 2002; Tunung *et al.*, 2007). Many isolates were found to be resistant not only to the β -lactam family of antibiotics but also to the new aminoglycosides such as tobramycin and gentamicin and this poses a serious therapeutic problem for the clinicians and public health agencies (Hogan and Kolter, 2002; Haryani *et al.*, 2007). The exhibition of resistance indicates to us that there is a need to look for new antibiotics to keep pace with emergence of resistance caused by microbiological agents and genetic mutation of pathogenic bacteria.

The emerging of food-borne microbial pathogens has been a serious threat to human's health resulting in food poisoning (Haryani *et al.*, 2007). Furthermore, the threat of bioterrorism with multiple drug resistant pathogens such as Anthrax and Cholera highlight the need for continued intensification of antimicrobial research (Gilligan, 2002; Thomson *et al.*, 2004). Another factor is the downtrend of the antibiotic research and development even though there is an increase in pathogen resistance and the fact that no new classes of antibiotics have been developed since 1963. Although there were 2 new antibiotics with narrow spectrum drugs (linezolid and daptomycin), that have been approved, there is no new structural classes of antibiotic that have been launched into the market since 1963, when the quinolone, nalidixic acid was approved (Carpenter and Chambers, 2004; Hancock, 2007). However, a new class of antimicrobial agent, oxazolidinones was introduced in 1999

CHAPTER 1

INTRODUCTION

1.1 Background

In the late 1960 and early 1970s, notable achievements in developing antibiotics to combat pathogenic diseases had led to the misconception that the war between infectious diseases and human was over (Spellberg *et al.*, 2004). However, currently, medical centers are experiencing a rise in antimicrobial resistance of pathogenic bacteria and the susceptibility of pathogens against antibiotics is steadily decreasing. The emergence of multiple drug resistance in clinical *Escherichia coli* O157:H7, *Vibrio cholerae* and *Salmonella enterica* from the tropics such as Malaysia is one of the manifestations of this phenomenon (Radu *et al.*, 2001; Radu *et al.*, 2002; Tunung *et al.*, 2007). Many isolates were found to be resistant not only to the β -lactam family of antibiotics but also to the new aminoglycosides such as tobramycin and gentamicin and this poses a serious therapeutic problem for the clinicians and public health agencies (Hogan and Kolter, 2002; Haryani *et al.*, 2007). The exhibition of resistance indicates to us that there is a need to look for new antibiotics to keep pace with emergence of resistance caused by microbiological agents and genetic mutation of pathogenic bacteria.

The emerging of food-borne microbial pathogens has been a serious threat to human's health resulting in food poisoning (Haryani *et al.*, 2007). Furthermore, the threat of bioterrorism with multiple drug resistant pathogens such as Anthrax and Cholera highlight the need for continued intensification of antimicrobial research (Gilligan, 2002; Thomson *et al.*, 2004). Another factor is the downtrend of the antibiotic research and development even though there is an increase in pathogen resistance and the fact that no new classes of antibiotics have been developed since 1963. Although there were 2 new antibiotics with narrow spectrum drugs (linezolid and daptomycin), that have been approved, there is no new structural classes of antibiotic that have been launched into the market since 1963, when the quinolone, nalidixic acid was approved (Carpenter and Chambers, 2004; Hancock, 2007). However, a new class of antimicrobial agent, oxazolidinones was introduced in 1999

after 40 years of effort. Thus, there is a need to discover new classes of antibiotics with new mechanisms of action, directed against new antibacterial targets. These antibiotics should be free of cross-resistance to previously existing antibiotics, to slow down resistance development against antibiotics (Labischinski, 2001). Without innovative public policy and additional financial support, fewer and fewer antibiotics will be available to treat the increasing number of drug-resistant and dangerous microbes that threaten the global community.

Antibiotic resistance requires a renewed effort to screen for antimicrobial agents effective against pathogenic bacteria resistant to existing antibiotics. Antarctica offers an interesting environment to seek for new classes of antibiotics because of its extremes of climate, habitat and biogeography. This region offers a vast potential for the development of novel applications for those natural products produced by Antarctic bacteria such as application of bioactive compounds and cold-adapted enzymes in the food, cosmetic and pharmaceutical industries (Franzmann, 1996). The microbial biomass can be immense in Southern Ocean blooms and freshwater cyanobacterial mats, species richness is generally more restricted than it is in temperate regions. This microbial biomass provides a broad variety of taxa with a diverse gene pool (Wynn-Williams, 1996). However, the antagonistic properties of cold-loving organisms have not yet been explored as extensively as those of the mesophiles. These antimicrobial agents produced in cold environments need to function at low temperatures for the organisms to gain a competitive advantage during their growth cycle in these extreme environment. Such cold-active antimicrobial compounds may be amenable to exploitation in industrial applications including chilled-food preservation or as new antibiotics against increase resistance of pathogens (O'Brien *et al.* 2004).

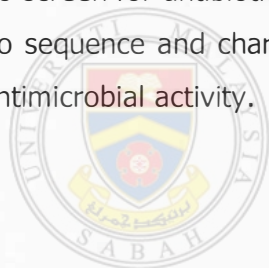
The emergence of multiple antibiotic resistant strains of pathogens is common due to constant exposure to various antibiotics. However, it is not known whether there is any multiple resistant strain of bacteria that is not constantly exposed to antibiotics such as the bacteria from Antarctica. Antarctic ecosystem is one of the pristine environments on Earth and offers researchers a unique opportunity to study microbial diversity and evolution (Franzmann, 1996; Vincent, 2000). Ecological studies have reported that antibiotic resistance is becoming a global phenomenon.

The ability to resist to antibiotics is usually due to the repetitive exposure of the bacteria to antibiotics (Baquero and Blazquez, 1997) but there are cases in antibiotics resistance have been found in bacteria populations without apparent antibiotic selection pressures as shown in Antarctic ecosystems (Kobori *et al.*, 1984; De Souza *et al.*, 2006). However, antibiotics resistance is probably intrinsic and endemic to a particular bacterial species, or selected due to antibiotics occurring naturally in the environment (Bonnedahl *et al.*, 2008). A review of available literature indicates that not many studies have been conducted on antibiotics resistance of the Antarctic bacteria.

1.2 Objectives

The objectives of the study were:

- i. To screen for strains with antimicrobial activity against food pathogens.
- ii. To identify and characterize Antarctic bacteria with antimicrobial activities.
- iii. To screen for antibiotics resistance profiles of Antarctic bacteria.
- iv. To sequence and characterize the plasmid genome of a bacteria exhibiting antimicrobial activity.



UMS
UNIVERSITI MALAYSIA SABAH

LITERATURE REVIEW

2.1 Overview of Antarctica

2.1.1 Introduction

Antarctica (Figure 2.1) is characterized by an extremely cold environment, gigantic icebergs and permanent ice shelves with the temperature rarely rises above freezing point. Microorganisms may have existed in the continental crust and their descendants may live in sub-glacial rock crevices, lakes, and sediments before the permanent ice cap formed in million years ago. The extreme conditions of Antarctica (low temperature, low humidity and high radiation) offer great opportunity for studies relating to modeling living organisms in other planets, model systems for adaptation and cell growth at low temperature, intercontinental contacts and effect on the organisms between changes of glacial and post-glacial periods (Price, 2000; Satyanarayana *et al.*, 2005).



Figure 2.1 : Schirmacher Oasis.
Source : Bruenjes, 2003.

Recent evidence has suggested that microbial communities can survive on wind-deposited sediment particles within liquid water inclusions in permanently ice-covered Antarctic lakes (Price, 2000). In coastal areas, seal and penguin rookeries may contribute significant quantities of organic material to soils; which are high in nutrients. However, soils from inshore waters that have undergone a rapid freeze-

thaw cycle can be lethal to the survival of microorganisms (Wynn-Williams, 1996). Although these unique and extreme environments limit the diversity of organism, microorganisms are dominant in Antarctica. However, they survive and grow in the Antarctic soils under condition of low temperature, high osmotic stress and the strain of the freeze-thaw phase during the transition of winter to summer which is critical for the onset of microbial activity. Microorganisms need to be efficient at rapidly switching their metabolism on and off according to prevailing conditions (Russell, 2006).

Antarctica is a novel environment and its unique biodiversity has attracted companies, bioprospectors, scientists, academicians and scholars to visit Antarctica every year to conduct research in order to find useful bioactive compounds. The lack of knowledge regarding to Antarctic biota initiates the effort to isolate novel organisms. Besides, the biochemistry of Antarctic organisms involves in adaptation in extreme environments offers an opportunity to discover novel bioactive compounds (Lohan & Johnston, 2005). Moreover, studies about psychrophilic bacteria are lesser compared to thermophiles and it is not clear whether life on earth originated from a hot or cold environment.

Antarctica can be classified into three major soil zones based on climate and moisture availability, the dry valleys and bare ground on the Trans Antarctic Mountains, the oases of coastal greater Antarctica, and the maritime Antarctic Peninsula (Claridge and Campbell, 1984). The maritime Antarctica is defined as the southern polar region where the mean air temperature in a month is above freezing point in the summer and the mean monthly temperature only occasionally falls below -10°C . However, the sub-Antarctic and maritime Antarctic regions have milder climate, higher water content and greater biodiversity compared to southernmost and continental Antarctic regions where both regions have much lower temperature and arid conditions (Holdgate, 1964). The maritime Antarctica includes South Sandwich Islands, South Orkney Islands, South Shetland Islands (King George Island, Deception Island, etc.), western coastal fringe of the Antarctic Peninsula south to Marguerite Bay, Bouvetoya and Peter I Oy (Spaul, 1973).