ISOLATION, IDENTIFICATION AND CHARACTERISATION OF BACTERIA FROM DECEPTION ISLAND, ANTARCTIC

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

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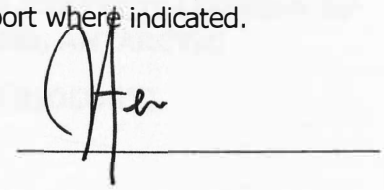

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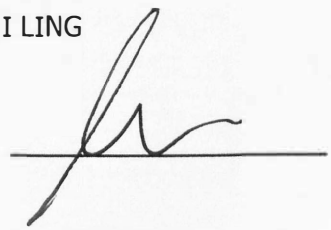

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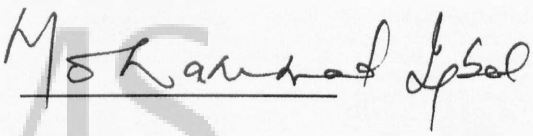

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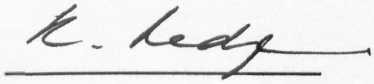

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ABSTRACT

ISOLATION AND CHARACTERISATION OF THERMOPHILES FROM DECEPTION ISLAND, ANTARCTIC

Deception Island, an active stratovolcano is one of the South Shetland Islands in the Antarctic. It provides excellent conditions for thermophilic bacteria to grow because of high ground temperatures in specific areas, where the temperatures are above the mesophilic range. The aims of this study were to isolate some thermophiles from two locations in the Deception Island, and to determine their enzyme profiles. Eight strains of bacteria B1, B2, B3, B4, B5, B6, B7 and B8 were isolated at a growth temperature of 40°C. The eight strains were Gram-positive bacilli and harboured endospores. These bacteria were identified based on their 16S rDNA sequences. Three of them were duplicates of the other strains and therefore only five were used for the subsequent analyses. The eight bacterial strains B1, B2 (and B8), B3, B4 (and B5), and B6 (and B7) had the highest percentage similarities to *Paenibacillus barengoltzii* (96%), *Aneurinibacillus danicus* (92%), *Aneurinibacillus migulanus* (91%) *Paenibacillus timonensis* (96%) and *Bacillus weihenstephanensis* (99%) respectively. Those with less than 97% similarity to known species are possibly novel species. The growth rates of the five bacterial strains were highest at 37°C when compared to 40°C, 50°C and 60°C. Strain B1 was found to produce amylase, xylanase, and cellulase. Strain B2 was able to produce amylase and cellulase. Strain B3 was able to produce amylase, xylanase and cellulase while strain B4 was found to produce amylase, xylanase and cellulase. The enzyme produced by these bacteria may be useful for the industries.

ABSTRAK

Pulau Deception adalah sebuah stratovolcano aktif di Kepulauan Shetland Selatan, Antartika. Ia menyediakan syarat yang terbaik untuk bakteria termofilik untuk berkembang kerana suhu tanah tinggi di kawasan tertentu, di mana suhu adalah melebihi julat mesofilik. Tujuan kajian ini adalah untuk mengasingkan beberapa termofili dari dua lokasi di Pulau Deeption dan untuk menentukan profil enzim mereka. Lapan jenis bakteria B1, B2, B3, B4, B5, B6, B7 dan B8 telah dikultur pada suhu 40°C. Kelapan-lapan jenis bacteria adalah Gram positif dan mempunyai endospora. Bakteria ini telah dikenal pasti berdasarkan jujukan rDNA 16S masing-masing. Tiga bakteria ditemui sama dengan bakteria lain. Disebankan itu, hanya lima bakteria digunakan untuk analisis seterusnya. Lapan jenis bakteria B1, B2 (dan B8), B3, B4 (dan B5), dan B6 (dan B7) mempunyai peratusan persamaan tertinggi dengan Paenibacillus barengoltzii (96%), Aneurinibacillus danicus (92%), Aneurinibacillus migulanus (91 %) Paenibacillus timonensis (96%) dan Bacillus weihenstephanensis (99%). Bakteria yang mempunyai kurang daripada 97% persamaan, adalah kemungkinan spesies baru. Kadar pertumbuhan lima strain bakteria adalah tertinggi pada 37ºC berbanding 40°C, 50°C dan 60°C. B1 didapati menghasilkan amilase, xylanase, dan selulase. B2 mampu untuk menghasilkan amilase dan selulase. B3 dapat menghasilkan amilase, xylanase, dan selulase manakala B4 didapati menghasilkan amilase, xylanase, dan selulase. Enzim yang dihasilkan oleh bakteria ini mungkin berguna untuk industri.

ABSTRAK

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS / ABBREVIATION

A	Adenine
ATP	Adenosine triphosphate
&	And
α	Alpha
bp	Base pair
β	Beta
cmc	Carboxymethyl cellulose
k	Carrying capacity
C	Cytosine
Da	Dalton
°C	Degree Celsius
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
dNTP	Deoxyribonucleotide triphosphate
E. coli	*Escherichia coli*
g	Gram
G	Guanine
HCl	Hydrochloric acid
kb	Kilo base pair
km	Kilometre
km^2	Kilometre square
<	Less than
L	Litre
LB	Luria broth
m	Metre

µg	Microgram
µl	Microlitre
µm	Micrometre
mg	Milligram
ml	Millilitre
mm	Millimetre
mM	Millimolar
M	Molar
>	More than
-	Negative / To
X	No
BLASTn	Nucleotide-nucleotide BLAST
/	Per
OD	Optical density
th	Ordinal number suffix
%	Percentage
pmol	Picomole
pcr	Polymerase chain reaction
+	Positive
pH	Potential Hydrogen
rpm	Revolution per minute
RNA	Ribonucleic acid
rDNA	Ribosomal ribonucleic acid
NaCl	Sodium chloride
SDS	Sodium dodecyl sulfate
sp.	Species

SD	Standard deviation
Taq	*Thermus aquaticus*
X	Times
™	Trade mark
T	Thyine
U	Unit
U	Uracil
V	Volt
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Background

Prokaryotes are found all over the earth, which includes soil, sediment, marine and terrestrial subsurface, animals and plant tissues and play an important role in the biogeochemical cycles of the biosphere and represent vast novel valuable bioactive compounds for health or industry. There are many enzymes and antimicrobial compound have been successfully isolated from laboratory cultured microorganisms as reported by Patrick *et al.*, 2003. According to Curtis *et al.*, 2002, soil is the most suitable environment for microbial reservoir and contains wide microbial diversity.

The Antarctica is situated at the South Pole, which is at the south point on the earth which sits in the Antarctic region of the Southern Hemisphere and surrounded by the Southern Ocean. Since it is mostly covered by ice sheet, it is the driest and coldest place on the earth. The Antarctica was formed 25 million years ago when the 'supercontinent' Gondwanaland broke apart. Gondwanaland was known to be covered by forests and was inhabited by various ancient life-forms and experienced a tropical climate.

Since Antarctica is so dry and cold, there are no complex organism is living there except for bacteria. Bacteria dominate most of the Antarctica ecosystems and are essentially involved in food chains (Franzmann *et al.*, 1997). Due to the fact that Antarctica is less intruded, thus the microbial community is preserved without introduction of new bacteria into the area. The microbial diversity remains unchanged since the formation of Antarctica. Therefore, according to Fransmann *et al.*, 1997, the microorganisms that inhabit Antarctica ecosystems have the potential for economic benefits in biotechnology. Moreover, microbial diversity especially the

prokaryotes are being studied extensively to provide more understanding regarding the origin of life and its evolution because they were the first inhabitants of the earth at the origin of life (Brock, 1978).

Extremophiles are mircroorganisms that are able to live in extreme environment. The reason being that they are able to produce enzymes and proteins that can withstand extreme temperature, salinity, pH and pressure in order to sustain their life in the extreme environment. Adaptation of these bacteria to hot environments (Stetter, 1999), production of heat-stable enzymes (Niehaus, 1999), structural and functional relationships of these enzymes (Zeikus, 1998) and their biotechnological and industrial applications (Eichler, 2001; Haki, 2003) are among the major areas of research on these microorganisms provide a valuable resource for exploitation in novel biotechnological processes (Nicolaus *et al.*, 1999).

In spite of the ice-bound Antarctica continent, it depends on solar heat in the summer to support a few terrestrial life, and water supply from some volcanic activity that warms the soil and steam coming out from fumaroles condensation (Logan, 2000). Deception Island is formed from a collapse caldera, which as a result of subsidence of magma chamber (Marti *et al.*, 1996). Although Deception Island is known to be an active volcanic island, but, only almost half of the island is free from ice. In these ice free regions, it could have contained the microorganisms which have originated from Gondwaland that was once an ice free land. Deception Island is a volcanic island; the soils are hot and the microorganisms that are in the soil could have adapted to high temperature.

Therefore, screening of microbial bioactive compound from Antarctic microorganisms is important route that lead to the discovery of novel bioactive and therapeutic chemicals. It is the lesser-known and new microbial taxa that offer biotechnological potential and interest. Due to the extreme environment in Antarctic, it may have unique population of microorganism that produces novel bioactive compounds. Scientists are now actively involved in search of novel

microorganism biodiversity that could provide these potential microorganisms in unusual or extreme environments, such as the Antarctica. However, despite the numerous discoveries of bacteria from Antarctica, little is known about the diversity of thermophiles in Deception Island. Hence, the present study is set out to determine the biodiversity of extremophiles in Deception Island.

1.2 Objectives

Objectives of this study are:

(i) To isolate, identify and characterize bacteria from the Deception Island.

(ii) To screen the bacteria for amylase, cellulase, xylanase, protease and mannanase enzymatic activity.

CHAPTER 2

Literature Review

2.1 Antarctic in general

The Antarctica is situated at the South Pole, which is at the south point on the earth which sits in the Antarctic region of the Southern Hemisphere and surrounded by the Southern Ocean as shown in figure 2.1.

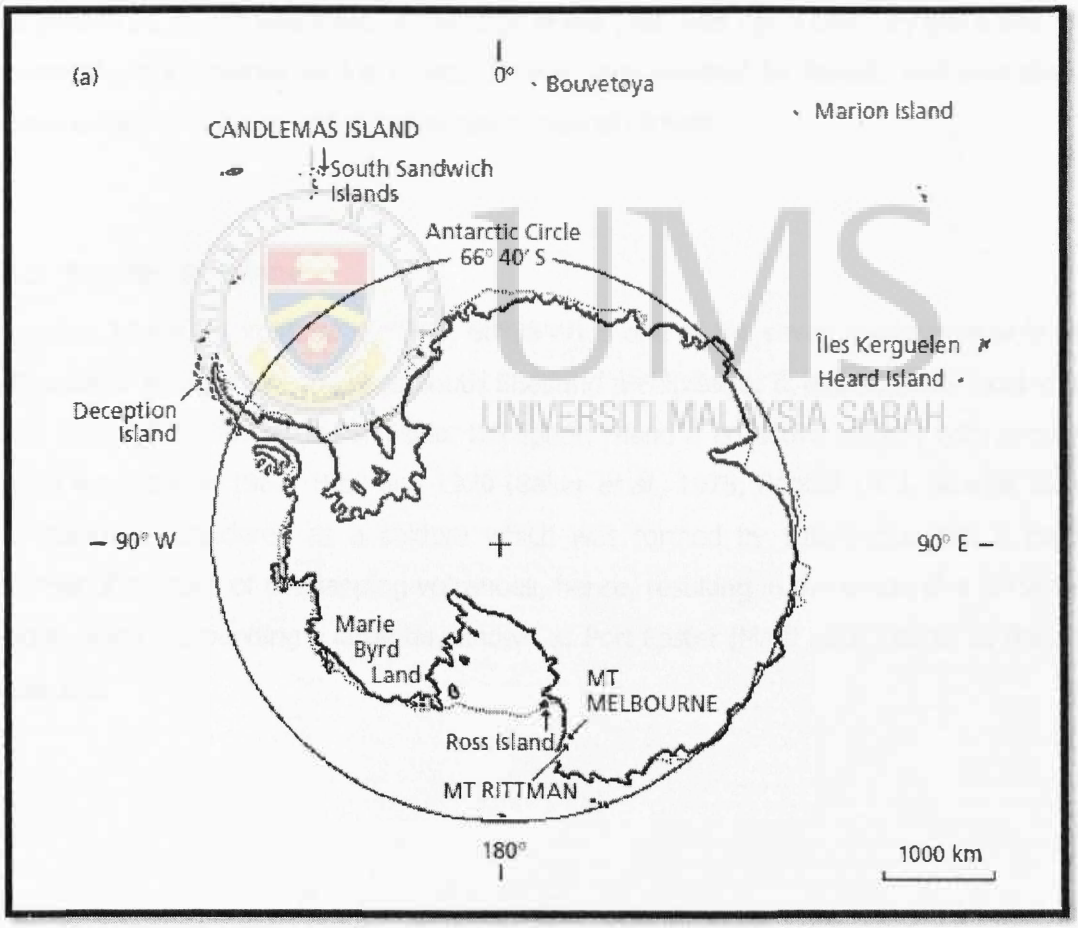

Figure 2.1: Labelled map of Antarctica
Source: Logan *et al.*, 2000

The Antarctica expands approximately 14 million making it the fifth-largest continent after Asia, Africa, North America and South America. About 98% of the land is covered by

ice sheet that averages at least 1.6 km in depth making it the coldest, driest, windiest and the highest elevated continent with annual precipitation of only 200 mm along the coast and far less inland. The maximum temperature ranging between 5°C and 15°C at the coast during summer and reaching a minimum of between -80°C and -90°C in the interior during winter time. Due to its harsh environment, it has no permanent resident, but, however, there are people coming to do scientific research on the continent and its nearby islands. Besides that, only a small amount of organisms that survived the harsh environment which includes algae, mites, nematodes, penguins, seals, bacteria, fungi, plants and protista while some vegetation only occur in the tundra area. Until recently, ancient ecosystems consisting of several types of bacteria have been living trapped deep beneath glaciers. The Antartica used to be a part of the supercontinent Gondwana 170 million years ago. The Gondwana land gradually breaks apart over time and become the Antartica as we know it today which was formed 25 million years ago. Antarctica, in the past, was not a cold, dry place and most importantly, not covered by ice sheets. It was once covered by forests and inhabited by various ancient life-forms and experienced a tropical climate.

2.1.1 Deception Island

Deception Island (coordinate: 62°57'S, 60°38'W) is one of the seven islands that is located in Bransfield Strait, that comprises South Shetland archipelago. It is specifically located 100 km to north of the Antarctic Peninsula. Deception Island is an active volcano with history of several eruptions in 1967, 1969 and 1970 (Baker *et al.*, 1975; Roobol 1973, Smellie, 2001). The island is considered as a caldera which was formed by subsidence into a magma chamber of a group of overlapping volcanoes, hence, resulting in the shape of a horseshoe-shaped island surrounding a large bay known as Port Foster (Marti *et al.*, 1996) as shown in figure 2.2.

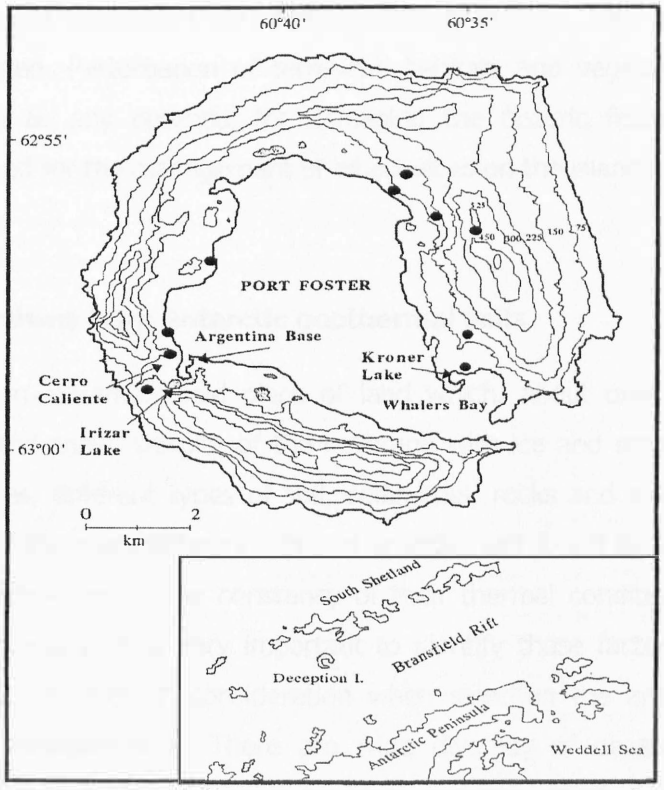

Figure 2.2: Deception Island and points of sampling; geothermal areas ⬚; lakes ●

Source: Llarch A. *et al.*, 1997

The island is approximately circular with 17 km in maximum diameter. There are two peaks on the east side of the island rising to 539 m at Mount Pond and 452 m at Mount Kirkwood, whereby, half of the island is being covered by permanent ice. Over 57% of Deception Island is covered by ice caps, glaciers, ice-cored moraines and pyroclasts. The climate of Deception Island is very similar to the northern Maritime Antarctic, which was characterized by a long cold summer with mean monthly air temperatures ranging from 0 to 2.5°C in November to March, and 0 to -10°C from April to October. Snow is the main precipitation throughout the year with reading 900mm, but during the summer, rainfall, fog and low cloud are quite frequent. There is little sunshine on the island and strong winds occur all the time (Smith, 2005).

Since its discovery in 1820's, the island has been of considerable commercial and scientific interest. Other than its scientific and historical values, Deception Island has become a major tourist attraction and being the most visited site in the Antarctic, with record of approximately 15000 visitors made at four sites. Sealing and whaling were the