ISOLATION AND CHARACTERIZATION OF BACTERIA FROM SOILS COLLECTED FROM THE SCHIRMACHER OASIS, ANTARCTICA

CHEE JO ANNE

THIS DISSERTATION IS PRESENTED TO FULFILL THE PARTIAL REQUIREMENT TO OBTAIN A BACHELOR DEGREE OF SCIENCE WITH HONOURS

BIOTECHNOLOGY PROGRAM
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

April 2007

UMS UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: ISOLATION AND CHARACTERIZATION OF BACTERIA FROM SOILS COLLECTED FROM THE SCHIRMACHER OASIS

Ijazah: SARJANA MUDA DENGAN KEPUJIAN (KURSUS BIOTEKNOLOGI)

SESI PENGAJIAN: 2004/2005

Saya CHEE JO ANNE HS2004-2457

(HURUF BESAR)

mengaku membenarkan tesis (LPS/Sarjana/Doktor Falsafah)* ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik 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 antara institusi pengajian tinggi.
4. **Sila tandakan (/)

[ ]	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
[✓]	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
[ ]	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: B21, JLN KENARI BIRU 2, TMN KENARI JAYA, 08000 SG PETANI, KEDAH

DR MICHAEL CLEMENTE W.V.L

Nama Penyelia

Tarikh: 27/4/07

Tarikh: 27/4/07

CATATAN: * Potong yang tidak berkenaan.

** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

CONFESSION

I hereby confess that, this is my own work and all the sources I have used or quoted have been indicated and acknowledged by the means of full references.

12 March 2007

CHEE JO ANNE

HS2004-2457

VERIFIED BY

Signature

1 SUPERVISOR
(DR. CLEMENTE MICHAEL WONG VUI LING)

2. EXAMINER 1
(DR. ROZIAH KAMBOL)

3. DEAN
(SUPT/KS PROF. MADYA DR. SHARIFF A. KADIR S. OMANG)

ACKOWLEDGEMENT

First of all, I would like to extend my deepest gratitude to my supervisor, Dr. Michael Wong for his guidance, support and encouragement throughout the duration of my final year project. His optimism upon my ability and upon life itself has gave me new insights from which I can see the world through a different point of view. I appreciate all the opportunities he gave me throughout my final year project.

I would also like to thank the following people for their assistance, advice and guidance without which this project would not have been successful. They are Chelven, Xn Yii, Gordon, Kenneth, Adrian, Melvin, Awang, Sharon and Elaine. My sincerest appreciation goes to them for sharing their valuable knowledge and experience with me, and mentoring me throughout my final year project duration. I also thank the lab assistants, Vidarita, Richard and Azian for assisting me throughout my project. Finally, I would like to thank my fellow friends who had been working in the lab with me. Their support and encouragement throughout the year has kept me going without gives up. A special thanks to my best friends, Kumuda and Nur Khairunnisa, for sharing ideas and always being there for me.

I am blessed to have a loving family who guided and supported me all the way throughout all the decisions I make in life. Their unconditional love for me has become the wings in my life from which I can soar and become what I am today. I am truly grateful for what they have imparted to me throughout my life. Finally, I would like to thank God for his grace and love, shining throughout the walks of my life and lighting the path before me.

ABSTRACT

Antarctica's drift towards South Pole 38 million years ago had allowed microorganisms to adapt to extreme conditions and thus, offer a unique diversity. The objective of this study is to isolate bacteria from soil samples obtained from Schirmacher Oasis, identify selected microorganisms by sequencing their 16S rDNA, and determine the relationship between these microbes by carrying out phylogenetic analysis. Bacteria were isolated by incubation of samples L43, L45, U5, West 2 and West 5 in MRS, Marine, Luria-Bertani, *Streptomycetes* and Antarctica Bacteria media. 77 six pure isolates were obtained and observed for their colony and cell characteristics. 45 of the isolates were Gram positive and 33 were Gram negative. Among 24 selected isolates, nine bacterial strains were psychrotolerant, 14 bacterial strains were mesophilic and one was undistinguished. RAPD analysis revealed that 23 out of the 24 strains were different. Ten isolates (C2, C4, H1, H3, H4, J1, J2, L8, L9 and N3) were identified based on their 16S rDNA sequences. The 16S rDNA genes were partially sequenced and the resulting sequence was analyzed using BLAST. Results revealed that C2 was from *Micrococcus gen.*; C4 from *Arthrobacter gen.;* H1 and J1 from *Rhodococcu ge.;* H3 and H4 from *Ralstonia gen.;* J2 linked to *Cohnella gen.;* L8 from *Streptomyces gen.;*, L9 from *Microbacterium gen;*, and N3 from *Bradyrhizobium gen.* Phylogenetic analysis using ClustalW software indicated that these isolates can be classified into three groups: the first group consists of isolates C2, L8 and L9; the second group consists of isolates C4, J2, N3, H3 and H4; and the third group consists of isolates H1 and J1.

ABSTRAK

Antartika yang telah mengalami hanyutan benua ke arah kutub Selatan 38 juta tahun lalu telah menyebabkan sesetengah mikroorganisma menyesuaikan diri dalam keadaan lampau ini dan melahirkan suatu diversiti mikroorganisma yang unik. Matlamat kajian ini adalah untuk mengasingkan bakteria dari sampel tanah yang dikumpul dari Oasis Schirmacher, Antartika; mengenalpasti mikroorganisma tertentu dengan menjujukkan 16S rDNAnya, dan mengenalpasti hubungan antara mikroorganisma tertentu melalui analisis filogeni. Bakteria telah diasingkan melalui pengeraman sampel-sampel L43, L45, U5, West 2 dan West 5 atas media Luria-Bertani, *Streptomycetes*, Marin, MRS dan Bakteria Antartika. 77 asingan bakteria telah berjaya diperolehi dan sifat-sifat koloni dan sel dikaji. 45 asingan adalah Gram positif dan 33 adalah Gram negatif. Dalam 24 bakteria, sembilan bakteria adalah psychrotolerant, 14 adalah mesofilik dan satu tidak dapat diketahui. Analysis RAPD telah menunjukkan bahawa 23 daripada 24 bakteria ini adalah berlainan. Sepuluh asingan (C2, C4, H1, H3, H4, J1, J2, L8, L9 dan N3) dikenalpasti melalui jujukan gen 16S rDNAnya. Sebahagian gen ini telah dijujukkan dan analysis dijalankan dengan menggunakan 'basic local alignment search tool' (BLAST). Keputusan menunjukkan bahawa C2 berasal dari genus *Micrococcus*; C4 berasal dari genus *Arthrobacter*; H1 dan J1 berasal dari genus *Rhodococcus*; H3 dan H4 berasal dari genus *Ralstonia*; J2 dikaitkan dengan genus *Cohnella*; L8 berasal dari genus *Streptomyces*; L9 berasal dari genus *Microbacterium* dan N3 berasal dari genus *Bradyrhizobium*. Analisis fologeni menggunakan program ClustalW memnunjukkan bahawa asingan into boleh diklasifikasikan kepada tiga kumpulan: kumpulan pertama terdiri daripada asingan C2, L8 and L9; kumpulan kedua terdiri daripada asingan C4, J2, N3, H3 and H4; dan kumpulan ketiga terdiri daripada asingan H1 and J1.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

No. Table **Page**

LIST OF PHOTOS

ABBREVIATIONS

bp	base pair
g	gram
kb	kilo base
M	molar
mg	milligram
mM	millimolar
μl	microlitre
mins	minutes
pmole	picomole
rpm	revolution per minute
sec	seconds
%	percent
°C	degree Celcius
BLAST	Basic Local Alignment Search Tool
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoside triphosphate
EDTA	Ethylenediamine tetraacetic acid
LB	Luria Bertani
$MgCl_2$	Magnesium chloride
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA Analysis

RE	Restriction enzyme
rDNA	ribosomal Deoxyribonucleic acid
SDS	Sodium dodecyl sulphate
Taq	*Thermus aquaticus*
TBE	Tris Borate EDTA
TE	Tris-HCl EDTA
UV	ultraviolet
V	volt
w/v	weight over volume
X	times
X-gal	5-bromo-4-chloro-3-indolyl b-D-galactopyramoside

CHAPTER 1

INTRODUCTION

Microorganisms (or microbes) are organisms that are microscopic and too small to be seen by the naked eye. These organisms can exist as single cells or cell clusters. Generally, microorganisms are able to carry out life processes of growth, energy generation, and independent reproduction.

Life on Earth depends much on microorganisms. In the absence of microorganisms, higher life forms would never have arisen and sustained. In fact, the lives of humans, plants and animals have now been intimately tied to microbial activities (Madigan *et al.*, 2003). The microbes ensures an adequate supple of nutrients to higher forms of life by playing a major role in recycling of nutrients. Thus, the microbes are responsible for degrading organic matters.

Understanding of microbes is important because they could act as disease agents, neutral agents, or even useful agents to man. As a disease agent, they could cause disease to humans and other animals (Madigan *et al.*, 2003). On the other hand, understanding of useful microbes could lead to the discovery of useful compounds such as antimicrobial agents and enzymes. In fact, it was even concluded that

Antarctic soils represent an untapped reservoir of novel, cold-active antimicrobial producers (O'Brien *et al*, 2004).

The Antarctica land covers an area of 14 million km^2. This continent's temperatures are usually below 0°C throughout the year; with the lowest temperature recorded at -89.6°C (Phillpot, 1985). At such temperatures, water has already formed ice and thus, very little free water is available in the Antarctica. Thus, the temperature conditions and the unavailability of free water results in an extreme condition for survival.

However, Antarctica has a few small lakes which are free of snow and ice during summer. At this period, microorganisms would abort from hibernation and resume their life cycle by growing, mating and reproducing. Life in Antarctic is restricted to a small range of animals such as penguins, seals, blue whales and other small sized creatures such as microscopic mites, lice, and nematodes. Although only a small number of animals live in Antarctic as compared to other continents, these animals are able to contribute a significant amount of organic matters to the soil. As the soil collects depositions from organisms and receives inputs from animals and ocean life, these organic materials supports the lives of microorganisms (Walker, 2005). Summer stays for about six months and after this period, microorganisms would start hibernating again when winter comes and the lake freezes.

Microbial populations live in locations in an environment called habitat. In different habitats, the microbial diversity varies. On Earth, there are vast numbers of habitats which shows a great variety of conditions. These differences include temperature, humidity, salinity, acidity and light intensity.

The environment in Antarctica is extremely cold with high moisture, rough winds, high ultraviolet radiation, occasional blizzards and continuous dark and light periods (Margesin & Schinner, 1999). Here, the microbial community have to adapt to these environments and thus, could be different from those found at tropical countries. Only microbes which can withstand these extreme conditions are able to survive. Therefore, it is possible to discover microbes with special properties to survive in such extreme environment, in which these microbes might be rare in the tropics.

The Antarctic microbial diversity is special because the continent has been separated from other continents for about 38 million years. Throughout these years, evolution of microbes occurred in other parts of the world but much slower in the Antarctic due to its cold weather. Hence, this may provide us with microbes that are different from other parts of the world. Additionally, these organisms are important because they act as an important tool for us to understand the history of earth, and their adaptation to the cold environment may provide us with useful genes in biotechnology applications.

The aims of this study are: (1) To isolate bacteria and fungi from samples obtained from the Schirmacher Oasis, Antarctica. (2) To identify microorganisms of interest by sequencing their 16S rDNA. (3) To determine the relationship between microorganisms of interest by carrying out phylogenetic analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Antarctica

The 14 million km^2 landmass of Antarctica is an extreme condition for survival. The continental temperatures are usually below 0°C throughout the year; with the lowest temperature recorded at -89.6°C (Phillpot, 1985). With such temperatures, the permanent ice sheets are formed and this leaves very little free water for survival. Antarctica has a few small, ice-covered lakes which are free of snow and ice during summer. Soil from these lakes may contain depositions from organisms that may have existed in the lakes during summer. The marine soils, too, are free of ice during summer receive inputs from animals and ocean life (Walker, 2005).

Life in Antarctic is restricted to a small range of animals. Marine life in Antarctic includes penguins, seals and blue whales (Cox, 1974). The land fauna consists of small sized creatures such as microscopic mites, lice, and nematodes. These lives contribute a significant quantity of organic material to soils (Walker, 2005). Such content of nutrient in soils allows microbial growth although it is limited only to those who can withstand the extreme environment. Microorganisms not only face an extreme cold environment, but also osmotic stress. Salinity controls the density of cold seawater (Neshyba, 1987). When ice forms, most of the salt is excluded from the crystal lattice. As a result, the salinity and density of the remaining waters. Seawater near Antarctic has the highest density in the world and thus, microorganisms have to be able to withstand this condition too (Kennett, 1982).

Antarctic has a long geologic history. For 552 million years, Antarctic has been part of Gondwana land. The isolation and cooling of Antarctica began about 38 million years ago (Hawkesworth *et al.*, 1999). According to the plate tectonics theory, continental drift and seafloor spreading has caused the isolation of continents. Initially Antarctic waters were from 15°C to 20°C. As the Antarctic continent drift towards the South Pole, the water temperatures fell from 15°C to 0°C. As a result, there is an extensive and thick ice regime within Antarctica between 37 and five million years ago (Webb, 1990).

Before Antarctica developed its permanent ice caps some 14 million years ago, the Antarctic waters were from 15°C to 20°C (Webb & Harwood, 1987). During this period, microbes may have existed in this continental crust. As the Antarctic continent drifts towards the South Pole, the microbes face a reduction in temperature throughout the years. At the same time, the Antarctica environment which is extremely cold with high moisture, occasional blizzards, high ultraviolet radiation and continuous dark and light periods offers a bigger challenge for survival (Margesin & Schinner, 1999). As the microbes adapt to the cold climate, the descendants of the microbes may have live in subglacial rock crevices, lakes, and sediments and perhaps even in the glacial ice itself (Price, 2000). By the time the ice sheets were permanent, the microbial life may have been limited to only the summer period where free water is available. When winter arrives, the microbes may hibernate or produce antifreeze compounds to obtain free water. The antifreeze is mainly secreted outside the cell to protect the cell against possible damages caused by freezing of environmental water (Sun *et al*., 1995).

Microbes only reproduce in the summer when the temperature is slightly higher and free water is available (Ratkowsky *et al.*, 1983). Therefore, the growth period of these microbes are very short as compared to those in temperate regions where microbes grow throughout the year. As a result, evolution which occurs in the Antarctic is far less than those in temperate regions. Therefore, the Antarctic microbes are important because they can show us the microbes which have lived also in temperate regions millions of years ago before they undergo much evolution to form the microbes we have today in temperate regions. As a result, the Antarctic microbes act as an important tool for us to understand the history of earth (Miller *et al.*, 2004).

Up to this day, very little is known about psychrophilic bacteria as they have very unique adaptations to the polar regions (Deming, 2002). In fact, it is not even certain yet whether life on earth originate in a hot or cold environment. Antarctic could provide us with answers to these questions. Scientists have found that this land could provide us with much information on the paleoclimate and also the environmental history of earth. In fact, we could now study atmospheric science, depletion of ozone, and adaptation of organisms to extreme environment by studying the remote and extreme conditions of Antarctic. Thus, Antarctica is now an important key in understanding of global and environmental concerns (Miller *et al.*, 2004).

Schirmacher Oasis (70°46′04″–70°44′21″S; 11°49′54″– 11°26′03″E) is located approximately 70 km south of Princes Astrid coast (Singh *et al.*, 2006). It consists of a number of rocky hills and valleys, where its elevation varies from zero to 236 meters above sea level. The oasis is three km wide and 20 km long. The area has three types of lakes: proglacial Lake, land-locked lake and epi-shelf lake (Singh *et al.*, 2006). The average annual temperature is around –10°C with a mean wind velocity of about 10 ms^{-1}. According to India Meteorological Department (IMD), the highest temperature of the year was 9.2°C during the polar summer on 30–31 December 2003, and the lowest temperature of the year was –33°C during polar winter on 24 July 2003. When summer arrives, the polar ice melts and water often flows into the lakes. Earlier studies had suggested that life at Schirmacher Oasis is dominated by lichens, mosses and algae (Pandey *et al.*, 1995). Schirmacher Oasis is an interesting place for many scientists especially in the study of bacterial populations, yeast and fungi (Shivaji *et al.*, 1987).

There are two stations located at the Schirmacher Oasis – the Maitri Station, built by the Indians in 1988 (Photo 2.1) and Novolazarevskaya Research Station, managed by the Russians. Samples for this project have been obtained near the Maitri Station. The infrastructure available at the Maitri station has allowed scientists to conduct research in

REFERENCES

Abdul Hamid, N. S., Zen, H. B., Ong, B. T., Halifah, Y. M., Saari, N. and Abu Bakar, F. 2003. Screening and identification of extracellular lipase-producing thermophilic bacteria from a Malaysian hot spring. *World Journal of Microbiology and Biotechnology* **19** (9), 1573-0972.

Aboderin, F. I. & Oyetayo, V. O. 2005. Haematological studies of rats fed different doses of probiotic, *Lactobacillus plantarum*, isolated from fermenting corn slurry. *Pakistan Journal of Nutrition* **5,** 102-105.

Antoun, H., Beauchamp, C. J., Goussard, N., Chabot, R. & Lalande, R. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). *Plant Soil* **204**, 57–67.

Aragno, M. 1978. Enrichment, isolation and preliminary characterization of a thermophilic, endospore-forming hydrogen bacterium. *FEMS Microbiology Letters* **3**, 13-15.

Beeson, K. E., Erdner, D. L., Bagwell, C. E., Lovell, C. R. and Sobecky, P. A., 2002. Differentiation of plasmids in marine diazotroph assemblages determined by randomly amplified polymorphic DNA analysis. *Microbiology* **148**, 179-189.

Bindschaldler, R.A. (ed.). 1990. *Searise: A multidisciplinary research initiative to predict rapid changes in global sea level caused by collapse of marine ice sheets.* NASA Conference Publications 3075. National Aeronautics and Space Administration, Washington.

Borsodi, A. K., Micsinai, A., Kovacs, G., Toth, E., Schumann, P., Kovacs, A. L., Boddi, B. and Marialigeti, K. 2003. *Pannonibacter phragmitetus* gen. nov., sp. nov., a novel alkalitolerant bacterium isolated from decomposing reed rhizomes in a Hungarian soda lake. *International Journal Systematic and Evolutionary Microbiology* **53**, 555-561.

Boutros, N., Gonullu, N., Casetta, A., Guibert, M., Ingrand, D. and Lebrum, L. 2002. *Ralstonia pickettii* Traced in Blood Culture Bottles. *Journal of Clinical Microbiology* **40** (7), 2666-2667.

Breukers, A., Lansink, A. O., Van der Werf, W. and Huirne, R. 2003. Bio-economic modeling of potato brown rot in the Netherlands. EPPO Bulletin **33** (3), 525-527.

Buchanan, R.E., and Gibbons N.E. 1974. *Bergey's* Manual of Determinative Bacteriology, 8th edition (ed.) The Williams and Wilkins Company (Publisher), Baltimore.

Coenye, T., Goris, J., Vos, P. D., Vandamme, P. and LiPuma, J. 2003. Classification of *Ralstonia pickettii*-like isolates from the environment and clinical samples as *Ralstonia insidiosa* sp. nov. *International Journal of Systematic and Evolution Microbiology* **53**, 1075-1080.

Cox, C. B. 1974. Vertebrate palaeodistributional patterns and continental drift. *Journal of Biogeography,* **1** (2), 75-94.

Damiani, G., Amedeo, P., Bandi, C., Fani, R., Bellizza, D. and Sgaramella, V., 1996. *Bacteria identification by PCR-based techniques*. In: Adolph, K. W. (eds), Microbial Genome Methods. CPC Press Inc. USA.

Delamare, A. P. L, Artico, L. O., Grazziotin, F. G., Echeverrigaray, S., Costa, S. O. P. 2002. Total protein electrophoresis and RAPD fingerprinting analysis for the identification of *Aeromonas* at the species level. *Brazilian Journal of Microbiology* **33**, 358-362.

Deming, J. W., 2002. Psychrophiles and Polar Regions. *Current Opinions on Microbiology* **5**, 301-309.

Drewry, D. J. 1983, *Antarctica: Glaciological and geophysical folio*. Scott Polar Research Institute, Cambridge, England.

Efroymson, R. A. and Alexander, M. 1991. Biodegradation by an *Arthrobacter* Species of Hydrocarbons Partitioned into an Organic Solvent. *Journal of Applied and Environmental Microbiology* **57** (5), 1441–1447.

Ehling-Schulz, M., Svensson, B., Guinebretiere, M., Lindback, T., Andersson, M., Schulz, A., ricker, M., Christiansson, A., Granum, P. E., Martlbeuer, E., Nguyen-The, C., Salkinoja-Salonen, M. and Scherer, S., 2005. Emetic toxin formation of *Bacillus cereus* is restricted to a single evolutionary lineage of closely related strains. *Microbiology* **151**, 183-197.

Fegan, M. and Prior, R. 2005. Diverse members of the Ralstonia solanacearum species complex cause bacterial wilts of banana. *Australasian Plant Pathology* **35** (2), 93-101.

Funke, G., von Graevenitz, A., and Weiss, N. 1994. Primary identification of *Aureobacterium spp*. isolated from clinical specimens as *"Corynebacterium aquaticum"*. *Journal of Clinical Microbiology* **32**, 2686-2691.

Gorkiewicz, G., Feierl, G., Schober, C., Dieber, F., Kofer, J., Zechner, R., Zechner, E. L. 2003. Species-specific identification of campylobacters by partial 16S rRNA gene sequencing. *Journal of Clinical Microbiology* **41** (6), 2537-2546.

Hawkesworth, C.J., Kelly, S., Turner, S., LeRoex, A and Storey, B.C. 1999. Mantle processes during Gondwana break up and dispersal. *Journal of African Earth Science* **28**, 239-261.

Hayatsu, M., Hirano, M., Nagata, T. 1999. Involvement of Two Plasmids in Degradation of Carbaryl by *Arthrobacter* sp. Strain RC100. *Journal of Applied and Evolutionary Microbiology* **65** (3), 1015-1019.

Helmke, E. and Weyland, H. 2004. Psychrophilic versus psychrotolerant bacteria-occurrence and significance in polar and temperate marine habitats. *Cell Molecular Biology* **50** (5), 553-561.

Holm-Hansen, O., S.Z. El-Sayed, G. A. Franceschini & R.L. Cuhel. 1977. *Primary production and the factors controlling phytoplankton growth in the southern ocean.* in "Adaptations within Antarctic ecosystems" (G.A. Llano, ed), Smithsonian Institution, Washington, 11-50.

Homer F. Swift M.D, 1921. Preservation of stock cultures of bacteria by freezing and drying. *The Journal of Experimental Medicine* **33**, 69-75.

Junge, K., Gosink, J. J., Hoppe, H. G. & Staley, J. T. 1998. *Arthrobacter, Brachybacterium,* and *Planococcus* isolates identified from antarctic sea ice brine. Description of *Planococcus mcmeekinii*, sp. nov. *Journal of Systematic and Applied Microbiology* **21,** 306-314.

Karigar, C., Mahesh, A., Nagenahalli, M. & Dae, J. Y. 2006. Phenol Degradation by Immobilized Cells of *Arthrobacter citreus. Biodegradation* **17,** 47-55.

Kennett J.P. 1982. *Marine geology*. Prentice-Hall, Englewood Cliffs, New Jersey.

Keswani, J. and W. B. Whitman. 2001. Relationship of 16S rRNA sequence similarity to DNA hybridization in prokaryotes. *Int. J. Syst. Evol. Microbiol.* **51,** 667-678.

Kolaczkowski, B. and Thornton, J. W. 2004. Performance of maximum parsimony andlikelihood phylogenetics when evolution is heterogenous. *Nature* **431**, 980-984.

Konopka, A., Zakharova, T., Bischoff, M., Oliver, L., Nakatsu, C. and Turco, R. F. 1999. Microbial Biomass and Activity in Lead-Contaminated Soil. *Applied and Environmental Microbiology* **65** (5), 2256-2259.

Kosono, S., Maeda, M., Fuji, F., Arai, H. and Kudo, T. 1997. Three of the seven bphC genes of *Rhodococcus erythropolis* TA421, isolated form a termite ecosystem, are located on an indigenous plasmid associated with biphenyl degradation. *Applied and Environmental Microbiology* **63** (8), 3282-3285.

Lee, J. S., Lee, K. C. and Park, Y. H. 2006. *Microbacterium koreense* sp. nov., from sea water in the South Sea of Korea. *International Journal of Systematic and Evolutionary Microbiology* **56,** 423-427.

Lee, M., Kim, M.K., Singleton, I., Goodfellow, M., Lee, S.-T. 2006. Enhanced biodegradation of diesel oil by a newly identified *Rhodococcus baikonurensis* EN3 in the presence of mycolic acid. *Journal of Applied Microbiology* **100 (2)**, 325–333.

Li, W.J., Chen, H. H., Kim, C. J., Park, D. J., Tang, S. K., Lee, J. C., Xu, L. H. and Jiang, C. L. 2005. *Microbacterium halotolerans* sp. nov., isolated from a saline soil in the west of China. *International Journal of Systematics and Evolutionary Microbiology* **55**, 67-70.

Liu, H., Xu, Y., Ma, Y., Zhou, P. 2000. Characterization of *Micrococcus antarcticus* sp. nov., a psychrophilic bacterium from Antarctica. *International Journal of Systematic and Evolutionary Microbiology* ***50*** (2), 715-719.

Lodwig, E. M., A. H. F. Hosie, A. Bourdes, K. Findlay, D. Allaway, R. Karunakaran, J. A. Downie, and P. S. Poole. 2003. Amino-acid cycling drives nitrogen fixation in the legume-*Rhizobium* symbiosis. *Nature* **422,** 422-426.

Loveland-Curtze, J., P. P. Sheridan, K. R. Gutshall, and J. E. Brenchley. 1999. Biochemical and phylogenetic analyses of psychrophilic isolates belonging to the *Arthrobacter* subgroup and description of *Arthrobacter psychrolactophilus* sp. nov. *Archieve of Microbiology* **171**, 355-363

Madigan, M. T., Martinko, J. M. and Parker, J., 2003. *Brock Biology of Microorganisms.* 10th Edition Ed. Pearson Education Inc. USA.

Margesin, R. and Schinner, F. 1999. *Cold-Adapted Organisms*. Springer, Heidelberg, Germany. ISBM 3-540-64973-5.

Martinez, R. J., Wang, Y., Raimondo, M. A., Coombs, J. M., Barkay, T., & Sobacky, P. A. 2006. Horizontal Gene Transfer of P_{IB}-Type ATPases among Bacteria Isolated from Radionuclide- and Metal-Contaminated Subsurface Soils. *American Society for Microbiology* **72** (5), 3111-3118.

Master, E. R. and Mohn, W. M. 1998. Psychrotolerant Bacteria Isolated from Arctic Soil That Degrade Poychlorinated Biphenyls at Low Temperatures. *Journal od Applied and Environmental Microbiology* **64** (12), 4823-4829.

Medigue, C., Krin, E., Pascal, G., Babe., V, Bernsel, A., Bertin, P. N., Cheung, F., Cruveiller, S., D'Amico, S., Duilio, A., Fang, G., Feller, G., Ho, C., Mengenot, S., Marino, G., Nilsson, J., Parrilli, E., Rocha, E. P. C., Rouy, A., Sekowska, A., Tutino, M. L., Vallenet, D., Heijne, G. V. and Danchin, A. 2005. Coping with cold: The genome of the versatile marine Antarctica bacterium Pseudoalteromonas haloplanktis TAC125. *Genome Research* **15**, 1325-1335.

Miller, G.H., Wolfe, A., Forman, S. L. and Olsen, C. 2004. *Geochronology of high-latitude paleolimnology in (Smol, J. and Last, W. eds) long-term environmental change in Arctic and Antarctic lakes*, R. Pienitz, M.S.V. Douglas and J.P. Smol (eds), 2004. Kluwer Academic Publishers, Dordrecht, The Netherlands, 167-182.

Ministry of Earth Sciences, India. 2006. *Draft Comprehensive Environmental Evaluation of New Indian Research Base at Larsemann Hills, Antarctica.* http://www.ncaor.gov.in/Draftcee/file4of14_chapter1.pdf

Mira, A., Pushker, R., Legault, B. A., Moreira, D. and Rodiguez-Valera, Francisco. 2004. Evolutionary relstoinships of *Fusobacterium nucleatum* based on phylogenetic analysis and comparative genomics. *BMC Evolutionary Biology* **4**, 50.

Morey, R. E., Galloway, R. L., Bragg, S. L., Steigerwalt, A. G., Mayer, L. W., Levett, P. N. 2006. Species-Secific Identification of *Leptospiraceae* by 16S rRNA Gene Sequencing. *Journal of Clinical Microbiology* **44** (10), 3510-3516.

Morgan, G. J. 1998. Emile Zuckerkandl, Linus Pauling, and the Molecular Evolutionary Clock, 1959-1965. *Journal of the History of Biology* **31**, 155-178.

Morita, R. Y. 1975. Psyhrophilic bacteria. *Bacteriological Reviews* **39**, 144-167.

Nei, M. and Kumar, S., 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press. USA.

Neshyba, S. 1987. *Oceanography: Perspectives on a Fluid Earth.* John Wiley & Sons, New York.

O'Brien, A., Sharp, R., Russel, N. J. and Roller, S., 2004. Antarctic bacteria inhibit growth of food-borne microorganisms at low temperature. *FEMS Microbiology Ecology* **48**, 157-167.

Okatani, A. T., Hayashidani, H., TAkahhashi, T., Taniguchi, T., Ogawa, M., Kaneko, K. 2000. Randomly amplified polymorphic DNA analysis of *Erysipelothrix* spp. *Journal of Clinical Microbiology* **38**, 4332-4336.

Oliveria, I. A, Vasconcellos, M. J., Seldin, L, Paiva, E, Vargas, M. A., Horta de Sa, N. M. 2000. Random amplified polymorphic DNA analysis of effective *Rhizobium* sp. associated with beans cultivated in Brazilian cerrado soils. *Brazilian Journal of Microbiology* **31** (1), 39-44.

Pandey, K. D. and Kashyap, A. K. 1995. *Diversity of algal flora in six freshwater streams of Schirmacher Oasis, Antarctica.* Tenth Indian expedition to Antarctica, Scientific Report, DOD Technical Publication, **3**, 87–96.

Phillpot, H.R. 1985. *Physical geography – climate, In "Key environments: Antarctica* (W.N.Bonner & D.W.H Walton, eds.), Pergamon Press, Oxford, 23-38.

Pipke, R., Amrhein, N. 1988. Degradation of the Phosphonate Herbicide Glyphosate by *Arthrobacter atrocyaneus* ATCC 13752. *Journal of Applied and Environmental Microbiology* **54** (5), 1293-1296.

Price, P. B., 2000. A habitat for psychrophiles in deep Antarctic ice. *Proceedings National Academic of Science 97* **3**, 1247-1251.

Ratkowsky, A., Lowry, R. K., Mcmeekin, T. A., Stokes, A. N., Chandler, R. E. 1983. Model for bacterial growth throughout the entire biokinetic range. *Journal of Bacteriology* **154**, 1222-1226.

Romanenko L. A., Zhukova, N. V., Rohde, M., Lysenko, A. M., Mikhailov, V. V, Stackerbrandt, E. 2003. *Glaciecola mesophila* s. nov., a novel marine agar-digesting bacterium. *International Journal of Systematic and Evolutionary Microbiology* **53**, 647-651.

Reddy, G. S. N., Prakash, J. S. S., Matsumoto, G. I., Stackebrandt, E. & Shivaji, S., 2002. *Arthrobacter roseus* sp. nov., a psychrophilic bacterium isolated from an Antarctic cyanobacterial mat sample. *International Journal of Systematic Evolution Microbiol* **52**, 1017–1021.

Rivas, R., Trujillo, M. E., Sanchez, M., Mateos, P. F., Martinez-Molina, E. and Velaquez, E. 2004. *Microbacterium ulmi* sp. nov., a xylanolytic, phosphate-solubilizing bacterium isolated from sawdust of *Ulmus nigra. International Journal of Systematic and Evolutionary Microbiology* **54**, 514-517.

Schenk, A., Aragno, M. 1979. *Bacillus schlegelii*, a new species of thermophilic, facultatively chemolithoautotrophic bacterium oxidizing molecular hydrogen. *Journal of General Microbiology* **115**, 333-341.

Shivaji, S. 1987. *A preliminary note on bacteria and yeasts of Antarctica.* Fourth Indian scientific expedition to Antarctica, Department of Ocean Development, Technical Publication **4**, 55–157.

Shivaji, S., Reddy, G. S. N., Aduri, R. P., Kutty, R., Ravenschlag, K. 2004. Bacterial diversity of a soil sample from Schirmacher Oasis, Antarctica. *Cellular and Molecular Biology* **50**, 525-536.

Simpson, K. L., Pettersson, B. and Priest, F. G., 2001. Characterization of lactobacilli from Scotch malt whisky distilleries and description of *Lactobacillus ferintoshensis* sp. Nov., a new species isolated from malt whisky fermentations. *Microbiology* **147**, 1007-1016.

Singh, S. M., Puja, G., Bhat, D. J. 2006. Psychrophilic fungi from Schirmacher Oasis, East Antarctica. *Current Science* **90**, 1388-1392.

Smith K, Neafie R, Yeager J, Skelton H. 1999. *Micrococcus folliculitis* in HIV-1 disease. *British Journal of Dermatology* **141** (3), 558-61.

Snel, B., Bork, P., Huynen, M. A. 1999. Genome phylogeny based on gene content. *Nature Genetics* **21**, 108-110.

Somerville, W., Thiber, L., Schwartzman, K., Behr, M. A. 2005. Extraction of *Mycobacterium tuberculosis* DNA: a question of containment. *Journal of Clinical Microbiology* **43**, 2996-2997.

Stackerbrandt, E., 2002. *Phylogeny based on 16S rRNA/DNA*. In: Atkins, D. (eds) Encyclopedia of Life Science. Macmillan Publishers, USA.

Stackebrandt, E. and B. M. Goebel. 1994. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **44**, 846-849.

Stragevitch, L., Davila, S. G. 1997. Application of a generalized maximum likelihood method in the reduction of multicomponent liquid-liquid equilibrium data. *Brazilian Journal of Chemical Engineering (online)* **14** (1), cited 2007-03-11. http://www.scielo.br/scielo.php?

Sun, X., Griffith, M., Pasternak, J. J., Glick, R., 1995. Low temperature growth, freezing survival and production of antifreeze protein by the plant growth providing *rhizobacterium, Pseudomonas putida* GR12-2, *Canada Journal of Microbiology* **41,** 776-784.

Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using neighbor-joining method. *Proceedings of the National Academy of Sciences, USA* **101**, 11030-11035.

Tavares, M. G., Ribeiro, E. H., Campos, L. A. O., Barros, E. G., Oliveira, M. T. V. A. 2001. Inheritance pattern of RAPD markers in *Melipona quadrifasciata* (Hymenoptera:Apidae, Meliponinae). *Journal of Heredity* **92**, 279-282.

Teng, J. L. L, Woo, P. C. Y., Leung, K. W., Lau, S. K. P., Wong, M. K. M. and Yuen, K., Y. 2003. Pseudobacteraemia in a patient with neutropenic fever caused by a novel paenibacillus species: *Paenibacillus hongongensis* sp. nov. *Molecular Pathology* **56**, 29-35.

Thompson, J. D., Higgins, D. G., Gibson, T. J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673-4680.

Touzel, J. P., O'Donohue, M., Debeire, P., Samain, E. and Breton, C. 2000. *Thermobacillus xylanilyticus* gen. nov., sp. nov., a new aerobic thermophilic xylan-degrading bacterium isolated from farm soil. *International Journal of Systematic and Evolutionary Microbiology* **50**, 315-320.

Trejo-Estrada, S. R., Sepulveda, I. R., Crawford, D. L. 1998. In vitro and in vivo antagonism of *Streptomyces violaceusniger* YCED9 against fungal pathogens of turfgrass. *World Journal of Microbiology and Biotechnology* **14**, 865-872.

Walker, T. R. 2005. Vertical organic inputs and bio-availability of carbon in an Antarctic coastal sediment. *Polish Polar Research* **26** (2), 91-106.

Wayne, L. G., D. J. Brenner, R. R. Colwell, et al. 1987. International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* **37,** 463-464.

Webb, P.-N. 1990. The Cenozoic history of Antarctic and its global impact. *Antarctica Science* **2**, 3-21.

Webb, P. N. & Harwood, M. 1987. Terrestrial flora of the Sirius formation: its significance for late cenozoic glacial history. *Antarctic Journal of the United State,* **21,** 7-11.

Welsh, J., McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res*earch **18,** 7213-7218.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research.* ***18***, 6531-6535.

Woese, C. R., Magrum, L. J., Fox, G. E. 1978. Archaebacteria. *Journal of Molecular Evolution* **11**, 245-252.

Woese, C.R., Kandler, O., Wheelis, M. L. 1990. Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences*, USA **87**, 4576-4579.

Yang, S., Doolittle, R. F., Bourne, P. E. 2004. Phylogeny determined by protein domain content. *Proceedings of the National Academy of Sciences of the United Stated of America* **102**, 373-378.

Yassin, A. F. 2005. *Rhodococcus triatomae* sp. nov., isolated from a blood-sucking bug. *International Journal of Systematic and Evolutionary Microbiology* **55**, 1575-1579.