DIVERSITY OF CULTURABLE PSYCHROTROPHIC BACTERIA FROM ARCTIC SOIL SAMPLES

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DEDICATION

……………This thesis is especially dedicated to my beloved parents, my supervisor: Associate professor Dr. Zaharah Binti Ibrahim, friends and well-wishers.

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ABSTRACT

The study of psychrotrophic microorganisms has gained interests in recent times because cold region serves as sources of unique microorganisms for biotechnological applications. The present study was aimed at investigating the diversity of culturable psychrotrophic bacteria associated with four frozen arctic soil samples collected from different points in Svalbard Hornsund. A total of 40 psychrophilic and psychrotolerant bacteria were isolated under aerobic conditions at 20°C. The mean viable bacterial count ranged from 1.2×10^4 to 5.4×10^5 cfu/mL. The isolates were analysed on the basis of morphological, physiological and molecular characteristics. The results of Gram stain reactions showed that Gram negative bacteria (n = 24) were predominant in the samples. Based on the 16S rRNA gene sequence analysis, the isolates (n = 40) could be categorised into five genera, comprising of *Pseudomonas* as the most abundant followed by *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Sphingomonas* belonging four phyla (Proteobacteria, Actinobacteria, Firmicutes and Flavobacteria) and one unclassified Bacterium.

ABSTRAK

Kajian terhadap mikroorganisma psikrotropik telah mendapat perhatian baru-baru ini kerana kawasan sejuk merupakan kawasan sumber bagi mikroorganisma unik untuk aplikasi bioteknologi. Kajian ini bertujuan untuk menyiasat kepelbagaian pengkulturan bakteria psikrotropik yang melibatkan empat sampel tanah beku artik yang diambil dari beberapa tempat di Svalbard Hornsund. SejumLah 40 psikropilic dan psikrotolerant bakteria telah diasingkan dalam keadan aerobik pada suhu 20°C. Nilai min bagi kiraan bakteria yang tumbuh adalah dalam anggaran antara 1.2 x 10^4 hingga 5.4 x 10^5 cfu/mL. Bakteria yang terasing telah dianalisis berdasarkan sifat morfologi, fisiologi dan sifat molekular. Keputusan kewarnaan Gram menunjukkan Gram negatif (n = 24) adalah yang paling utama dalam sampel. Berdasarkan kepada analisis urutan gen 16S rRNA, bakteria terasing (n = 40) boleh dikategorikan kepada lima genera, iaitu *Pseudomonas* yang paling banyak diikuti oleh *Arthrobacter, Bacillus, Flavobacterium* dan *Sphingomonas* serta empat phyla (Proteobacteria, Actinobacteria, Firmicutes dan Flavobacteria) dan satu tidak dapat dikelaskan Bacterium.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

%	-	Percentage
v/v	-	Volume per volume
w/v	-	Weight per volume
ºC	-	Degree Celsius
µL	-	Microliter
nm	-	Nanometer
mm	-	Millimeter
km	-	Kilometer
km^2	-	Kilometer per square
g	-	Gram
l	-	Liter
M	-	Molar
µ	-	Micro
N_2O	-	Dinitrogen monoxide
mL	-	Milliliter
mg	-	Milligram
TAE	-	Tris-acetate-EDTA
EDTA	-	Ethylene dimethyl tetra acetic acid
BLAST	-	Basic Local Alignment Search Tool
bp	-	Base pair
NCBI	-	National Centre of Biotechnology Information
O_2	-	Oxygen
CO_2	-	Carbon dioxide
cfu	-	Colony forming unit

ID	-	Identity
ABM	-	Antarctic Bacterial Medium
rpm	-	Rotation per minute
GHGs	-	Greenhouse Gases
V	-	Volt
UV	-	Ultraviolet
PCR	-	Polymerase chain reaction
DNTPs	-	Deoxynucleotide triphosphates
$MgCl_2$	-	Magnesium chloride
h	-	Hour
min	-	Minutes
sec	-	Seconds
et al.,	-	and others
sp.	-	Specie
ATP	-	Adenosine triphosphate
CH_4	-	Methane
Na_2CO_3	-	Sodium trioxocarbonate
NaOH	-	Sodium Hydroxide
NaCl	-	Sodium chloride
CCl_4	-	Carbon tetrachloride
CFCs	-	Chlorofluorocarbons
SF_6	-	Sulphur hexafluoride
HFCs	-	Hydro fluorocarbons
CH_3CCl_3	-	Methyl chloroform
H_2O_2	-	Hydrogen peroxide
OD	-	Optical density
KOH	-	Potassium hydroxide

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of the Research

A large portion of the earth is characterised by low mean temperature represented by the cold environment that include; the polar region (arctic and antarctic), ocean, freshwater, sediments, soils, permafrost, ice, glaciers, cold deserts, alpine soils, lakes and snow (Fendrihan and Negoiţă, 2012). The arctic is one of the coldest environment found at the north pole of the earth and covers countries like Canada, Russia, Norway, United States, Greenland and Scandinavia with land area of around 7.2 x 10^6 km^2. Glaciers which mostly occur in Greenland, cover approximately 26% of the land area (Tarnocai, 2009). The terrestrial part of arctic is a frozen soil referred to as permafrost comprising of sediment, rock, ice and large amount of organic materials. Permafrost covers about 25% of the land surface and remains frozen for two consecutive years. It is characterised by low mean annual precipitation and poor vegetation (Wagner, 2008). But harbours unique psychrotrophic microorganisms for biotechnological processes.

Microorganisms are the smallest and early forms of life capable of carrying out various essential chemical reactions for higher organisms. Humans, plants, and animals would never have flourished without the past microbial activities that brought about the recycling of key nutrients and degradation of organic matter.

While some microorganisms are pathogenic, others are highly beneficial as they produce antimicrobial agents and enzymes of industrial applications such as lipases, amylases and cellulases. Microbial activities usually occur at low temperature and neutral pH values. However, some microorganisms can adapt and grow under either acidic or alkaline condition and are referred to as acidophiles and alkaliphiles respectively (Torsvik and Øvreås, 2008).

Microorganisms can be grouped into three based on their temperature requirements for growth; those found at ambient temperature between 20°C to 45°C are referred to as mesophiles, the heat loving microorganisms (thermophiles) survive at a temperature between of 50°C and 70°C. While the cold-adapted microorganisms found at a temperature range between -16°C and 40°C are referred to as psychrophiles or psychrotrophs (Torsvik and Øvreås, 2008). Psychrotrophic organisms found in nutrient poor environment like the permafrost soils of the polar region with little organic and inorganic nutrients are called oligotrophs (Stan-Lotter, 2012). Microbial populations from all three domains of life like prokaryotes, eukaryotes and the archaea possess unique molecular and physiological adaptations for survival in these hostile environment of low temperature, low nutrients availability and continuous exposure towards solar radiation during summer (Kim *et al*., 2010; Tehei *et al*., 2004).

Psychrotrophic microorganisms has gained interests in recent times because they are sources of novel microbial products utilised in various industrial, agricultural and biotechnological processes. Psychrotrophs are involved in a number of geochemical cycles like nitrogen fixation, sulphur and carbon cycles, degradation of natural organic compounds such as cellulose, hemi-cellulose, lignin, and chitin. Others produce natural gas (methane) and renewable forms of energy via anaerobic degradation of organic matter (Madigan *et al*., 2010). Psychrotrophs are utilized in bioremediation of toxic wastes like polyaromatic hydrocarbons, polychlorinated biphenyls and other xenobiotic compounds derived from anthropogenic sources.The optimal activities at low temperature make them useful as biocatalysts for biotransformation of heat-labile compounds at cold temperatures and for bioremediation of polluted soils and wastewaters at low temperatures (Yu *et al*., 2009). Industrial processes at low temperature prevent energy consumption and also reduces the effect of warming on the environment.

Psychrotrophs produces cold-active lipases, proteases, lactases and cellulases that are useful in food processing like bakery, meat tenderization, ripening of cheese detergents and cleaning agents. The high catalytic activities of psychrotrophic enzymes are utilized in the food industries to minimise problems related to spoilage and reduce energy consumption. Psychrotrophic microorganisms also synthesizes carotenoids (antioxidants), unsaturated fatty acids and biosurfactants that are biodegradable, non-toxic or less toxic than chemical surfactants. Biosurfactants are used in cosmetics, soap formulations and trans-dermal drug delivery systems. Other forms of biosurfactants like surfactin from psychrotrophic bacteria have been used in pharmacological applications like inhibition of hemolysis (Pruthi and Cameotra, 1997). Different types of these psychrotrophic microorganisms have been isolated from various permanently cold environment including the arctic regions.

1.2 Problem Statement

One of the major environmental problems the world is facing today is global warming and increasing atmospheric levels of greenhouse gases (GHGs) (Saini *et al*., 2011) from human activities (Ajam *et al*., 2012; Damien *et al*., 2013).

The arctic region with long term repositories of unique psychrotrophic microorganisms of biotechnological are affected by the current increase in atmospheric temperature. Heat emission from industrial processes and anthropogenic burning of fossil fuel causes warming of the arctic region thereby accelerating the release of more greenhouse gasses to the atmosphere from microbial degradation of soil organic carbon.

Rapid climate change in the Arctic has begun to affect the abundance, diversity and ecology of microbial species throughout the region (Ajam *et al*., 2012; Damien *et al*., 2013). These necessitate the need for studying psychrotrophic bacteria and the utilisation of their cold-active compounds in industrial processes in order to save energy and reduce global warming.

1.3 Objectives of Study

- To isolate culturable psychrotrophic bacteria from arctic soil samples
- To identify the isolates according to 16S rRNA gene sequencing analysis
- To study the diversity of the identified bacteria

1.4 Scope of the Research

Psychrotrophic bacteria associated with arctic soil samples were isolated using antarctic bacterial growth medium at a temperature of 20°C and then sub-cultured until pure bacterial cultures were obtained. The 16S rRNA gene sequence analysis was carried out to identify the isolates. The bacterial abundance and bacterial diversity in the soil samples was also analysed.

1.5 Significance of the Research

Studies of microbial community in arctic soils will provide significant contribution to the existing knowledge on the diversity and potential of microorganisms in cold environment. They may serve as model systems for exploring microbial abundance, functions and potentials that may be of great value for applications in environmental clean-up, pollution prevention, or energy saving processes. These microorganisms may also provide enzymes with special properties for biotechnological applications in the temperate zone (Chu *et al.*, 2010). For example, lactase enzyme from psychrotrophic bacteria catalyses the conversion of lactose in milk to glucose and galactose at low temperature without the need for heating that denatures protein and changes the flavour of milk.

The knowledge of biodiversity is important towards preserving the integrity, function and long-term sustainability of natural and managed terrestrial ecosystems.

REFERENCES

Ajam Yakub, S., Kannan, K., Sandeep, N. M., Raju, R. Y., Abhay, B. F., Sivanesan Saravana, D. and Tapan, C. (2012). Recent Advancements in Carbonic Anhydrase–Driven Processes for CO_2 Sequestration: Minireview. *Critical Reviews in Environmental Science and Technology, 42*. doi: 10.1080/10643389.2011.556884.

Akila, G. and Chandra, T. (2003). A novel cold-tolerant Clostridium strain PXYL1 isolated from a psychrophilic cattle manure digester that secretes thermolabile xylanase and cellulase. *FEMS Microbiology Letters, 219*(1), 63-67.

Anisimov, O. A., Vaughan, D. G., Callaghan, T., Furgal, C., Marchant, H., Prowse, Terry D. Prowse, Hjaimar Vilhmsson and John E. Walsh, J. E. (2007). Polar regions (arctic and antarctic). *Climate change, 15*, 653-685.

Assessment, A. C. I. (2004). Impacts of a Warming Arctic-Arctic Climate Impact Assessment by Arctic Climate Impact Assessment, pp. 144. ISBN 0521617782. Cambridge, UK*: Cambridge University Press, December 2004., 1*.

Bej, A. K., Aislabie, J., and Atlas, R. M. (2009).*Polar Microbiology:* The Ecology, Biodiversity and Bioremediation Potential of Microorganisms in Extremely Cold Environments, 117-158.

Blaire Steven, Richard Léveillé, Wayne H., Pollard Lyle G. and Whyte (2006). Microbial ecology and biodiversity in permafrost. Extremophiles 10:259–267.

Blaire Steven, Geoffrey Briggs, McKay, Chris P., Pollard, Wayne H., Greer, Charles W. and Whyte, Lyle G. (2007). Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture-dependent and culture-independent methods. FEMS Microbiol Ecol. 513 – 523.

Brenchley, J. (1996). Psychrophilic microorganisms and their cold-active enzymes. *Journal of industrial microbiology, 17*(5-6), 432-437.

Brenner, D. J., Staley, J. T. and Krieg, N. R. (2005). Classification of procaryotic organisms and the concept of bacterial speciation. *Bergey's Manual® of Systematic Bacteriology* (pp. 27-32): Springer.

Change, C. (2007). Synthesis Report: Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change Core Writing Team: IPCC, Geneva, Switzerland.

Chapin III, F. S., Jefferies, R. L., Reynolds, J. F., Shaver, G. R., Svoboda, J. and Chu, E. W. (1991). Arctic ecosystems in a changing climate*:* an ecophysiological perspective: *Academic Press*.

Chu, H., Fierer, N., Lauber, C. L., Caporaso, J. G., Knight, R. and Grogan, P. (2010). Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. *Environmental Microbiology*, *12*(11), 2998-3006.

Damien, J. F., Colm, D. E., Colette, C. F. and Kevin, P. M. (2013). Carbon sequestration and the role of biological carbon mitigation: A review. *Renewable and Sustainable Energy Reviews, 21*. doi: 10.1016/j.rser.2012.12.038.

Derry, A., Staddon, W., Kevan, P. and Trevors, J. (1999). Functional diversity and community structure of micro-organisms in three arctic soils as determined by sole-carbon-source-utilization. *Biodiversity and Conservation, 8*(2), 205-221.

Dhillon, A., Teske, A., Dillon, J., Stahl, D. A., and Sogin, M. L. (2003). Molecular characterization of sulphate-reducing bacteria in the Guaymas Basin. *Applied and environmental microbiology, 69*(5), 2765-2772.

Dutta, K., Schuur, E., Neff, J. and Zimov, S. (2006). Potential carbon release from permafrost soils of Northeastern Siberia. *Global Change Biology, 12*(12), 2336-2351.

Feller, G., Narinx, E., Arpigny, J., Zekhnini, Z., Swings, J. and Gerday, C. (1994). Temperature dependence of growth, enzyme secretion and activity of psychrophilic Antarctic bacteria. *Applied microbiology and biotechnology, 41*(4), 477-479.

Fendrihan, S. and Negoiţă, T. G. (2012). Psychrophilic microorganisms as important source for biotechnological processes: *Springer*.

Ganzert, L., Bajerski, F. and Wagner, D. (2014). Bacterial community composition and diversity of five different permafrost-affected soils of North East Greenland. *FEMS microbiology ecology*.

Gjertz, I. and Persen, E. (1987). Confrontations between humans and polar bears in Svalbard. *Polar Research, 5*(2), 253-256.

Goldfarb, K. C., Karaoz, U., Hanson, C. A., Santee, C. A., Bradford, M. A., Treseder, K. K., Mathew D. Wallenstein and Brodie, E. L. (2011). Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. *Frontiers in microbiology, 2*.

Hall, B. G. (2004). Phylogenetic trees made easy: a how-to manual (Vol. 547): Sinauer Associates Sunderland.

Hassol, S. J. (2004). Impacts of a warming Arctic-Arctic climate impact assessment: Cambridge University Press.

Hébraud, M. and Potier, P. (1999). Cold shock response and low temperature adaptation in psychrotrophic bacteria. *Journal of molecular microbiology and biotechnology, 1*(2), 211-219.

Henttonen, H., Fuglei, E., Gower, C. N., Haukisalmi, V., Niemimaa. R.A.IMS J. and N.G.Yoccoz (2001). Echinococcus multilocularis on Svalbard: introduction of an intermediate host has enabled the local life-cycle. The Polar Environmental Centre, N-9296 Troms˘, Norway.

Hoover, R. B. and Pikuta, E. V. (2009). Psychrophilic and psychrotolerant microbial extremophiles in polar environments.

Illeris, L., Michelsen, A. and Jonasson, S. (2003). Soil plus root respiration and microbial biomass following water, nitrogen, and phosphorus application at a high arctic semi desert. *Biogeochemistry, 65*(1), 15-29.

Janda, J. M. and Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of Clinical Microbiology, 45*(9), 2761-2764.

Jill Harrison, C. and Langdale, J. A. (2006). A step by step guide to phylogeny reconstruction. *The Plant Journal, 45*(4), 561-572.

Karnachuk, O. V., Pimenov, N. V., Yusupov, S. K., Frank, Y. A., Frank, Y. A., Kaksonen, A. H., Puhakka, J A., Ivanov, M.V., Lindström, B.E. and Tuovinen, O. H. (2005). Sulfate reduction potential in sediments in the Norilsk mining area, northern Siberia. *Geomicrobiology Journal, 22*(1-2), 11-25.

Kaštovská, K., Elster, J., Stibal, M. and Šantrůčková, H. (2005). Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (High Arctic). *Microbial ecology, 50*(3), 396-407.

Khawar, S. S., Timothy, J. W., David, W., Sheree, Y., Michelle, A. A., Mark, V. B., Federico M. and Ricardo, C. (2013). Psychrophiles. *Annual Review of Earth and Planetary Sciences, 41*. doi: 10.1146/annurev-earth-040610-133514.

Kim, E. H., Cho, K. H., Lee, Y. M., Yim, J. H., Lee, H. K., Cho, J.-C. and Hong, S. G. (2010). Diversity of cold-active protease-producing bacteria from arctic terrestrial and marine environments revealed by enrichment culture. *The Journal of Microbiology, 48*(4), 426-432.

Klemsdal, T. (2010). Svalbard and Jan Mayen. In *Encyclopaedia of the World's Coastal Landforms* (pp. 581-588). Springer Netherlands.

Konstantinidis, K. T. and Stackebrandt, E. (2013). Defining taxonomic ranks *The prokaryotes* (pp. 229-254): Springer.

Lee, Y. M., Kim, E. H., Lee, H. K. and Hong, S. G. (2014). Biodiversity and physiological Characteristics of Antarctic and Arctic lichens-associated bacteria. *World Journal of Microbiology and Biotechnology, 30*(10), 2711-2721.

Lewin, A., Wentzel, A. and Valla, S. (2013). Metagenomics of microbial life in extreme Temperature environments. *Current Opinion in Biotechnology, 24*(3), 516-525. Doi: 10.1016/j.copbio.2012.10.012.

Madigan, M. T., Clark, D. P., Stahl, D. and Martinko, J. M. (2010). *Brock Biology of Microorganisms 13th edition*: Benjamin Cummings.

Maharana, A. K. and Ray, P. (2014). Application of Plackett-Burman Design for improved cold temperature production of lipase by psychrotolerant Pseudomonas sp. AKM-L5. *Int. J. Curr. Microbiol. App. Sci, 3*(4), 269-282.

Morgan-Kiss, R. M., Priscu, J. C., Pocock, T., Gudynaite-Savitch, L. and Huner, N.

P. (2006). Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiology and Molecular Biology Reviews, 70*(1), 222-252.

Morozkina, E., Slutskaya, E., Fedorova, T., Tugay, T., Golubeva, L. and Koroleva, O. (2010). Extremophilic microorganisms: Biochemical adaptation and biotechnological application (review). *Applied biochemistry and microbiology, 46*(1), 1-14.

Mostofa, K. M., Liu, C.-q., Gao, K., Li, S., Vione, D. and Mottaleb, M. A. (2013). Impacts of global warming on biogeochemical cycles in natural waters *Photobiogeochemistry of Organic Matter* (pp. 851-914): Springer.

Moyer, C. L. and Morita, R. Y. (2007). Psychrophiles and psychrotrophs. *eLS*. doi: 10.1002/9780470015902.a0000402.pub2.

Nilsson, A. E. (2009). A changing Arctic climate*:* Science and policy in the Arctic Climate Impact Assessment: *Springer.*

Oechel, W. C., Hastings, S. J., Vourlrtis, G., Jenkins, M., Riechers, G. and Grulke, N. (1993). Recent change of Arctic tundra ecosystems from a net carbon dioxide sink to a source. *Nature, 361*(6412), 520-523.

Overmann, J. (2013). Principles of enrichment, isolation, cultivation, and preservation of prokaryotes *The Prokaryotes* (pp. 149-207): Springer.

Overpeck, J., Hughen, K., Hardy, D., Bradley, R., Case, R., Douglas, M. Bruce Finney, Zielinski, G. and Jennings, A. (1997). Arctic environmental change of the last four centuries. *Science, 278*(5341), 1251-1256.

Pabis, K., Kędra, M. and Gromisz, S. (2014). Distinct or similar? Soft bottom polychaete Diversity in Arctic and Antarctic glacial fjords. *Hydrobiologia, 742*(1), 279-294.

Panikov, N. S. (2009). Microbial activity in frozen soils *Permafrost soils* (pp. 119-147): Springer.

Panikov, N. S., and Sizova, M. V. (2007). Growth kinetics of microorganisms isolated from Alaskan soil and permafrost in solid media frozen down to− 35 C. *FEMS microbiology ecology, 59*(2), 500-512.

Peeters, K., and Willems, A. (2011). The gyrB gene is a useful phylogenetic marker for exploring the diversity of Flavobacterium strains isolated from terrestrial and aquatic habitats in Antarctica. *FEMS Microbiology Letters, 321*(2), 130-140.

Powers, E. M. (1995). Efficacy of the Ryu nonstaining KOH technique for rapidly determining gram reactions of food-borne and waterborne bacteria and yeasts. *Applied and environmental microbiology, 61*(10), 3756-3758.

Pruthi, V., and Cameotra, S. (1997). Production and properties of a biosurfactant synthesized byArthrobacter protophormiae an antarctic strain. *World Journal of Microbiology and Biotechnology, 13*(1), 137-139.

Robinson, C. H., Michelsen, A., Lee, J., Whitehead, S., Callaghan, T. V., Press, M. and Jonasson, S. (1997). Elevated atmospheric CO_2 affects decomposition of Festuca vivipara (L.) Sm. litter and roots in experiments simulating environmental change in two contrasting arctic ecosystems. *Global Change Biology, 3*(1), 37-49.

Russell, N. J. (2003). Psychrophily and resistance to low temperature. *Extremophiles (Life under extreme environmental Condition), Encyclopedia of Life Support Systems (EOLSS) Developed under the Auspices of the UNESCO. Eolss Publishers, Oxford, UK. http://www. eolss. net*.

Sabri, A., Jacques, P., Weekers, F., Baré, G., Hiligsmann, S., Moussaïf, M. and Thonart, P. (2000). *Effect of temperature on growth of psychrophilic and* *psychrotrophic members of Rhodotorula aurantiaca.* Paper presented at the Twenty-First Symposium on Biotechnology for Fuels and Chemicals.

Saini, R., Kapoor, R., Kumar, R., Siddiqi, T. and Kumar, A. (2011). CO_2 utilizing microbes. A comprehensive review. *Biotechnology Advances, 29*(6), 949-960.

Shen, L., Yao, T., Xu, B., Wang, H., Jiao, N., Kang, S., Liu, X. and Liu, Y. (2012). Variation of culturable bacteria along depth in the East Rongbuk ice core, Mt. Everest. *Geoscience Frontiers, 3*(3), 327-334.

Shivaji, S., Pratibha, M., Sailaja, B., Kishore, K. H., Singh, A. K., Begum, Z., Anarasi U., Parabagaran. S.R., Reddy, G.N.S. and Srinivas, T. (2011). Bacterial diversity of soil in the vicinity of Pindari glacier, Himalayan mountain ranges, India, using culturable bacteria and soil 16S rRNA gene clones. *Extremophiles, 15*(1), 1-22.

Siddiqui, K. S., Williams, T. J., Wilkins, D., Yau, S., Allen, M. A., Brown, M. V., and Cavicchioli, R. (2013). Psychrophiles. *Annual Review of Earth and Planetary Sciences*(0).

Sokołowski, A., Szczepańska, A., Richard, P., Kędra, M., Wołowicz, M. and Węsławski, J. M. (2014). Trophic structure of the macrobenthic community of Hornsund, Spitsbergen, based on the determination of stable carbon and nitrogen isotopic signatures. *Polar Biology*, 1-14.

Srinivas, T. N. R., Nageswara Rao, S. S. S., Vishnu Vardhan Reddy P., Pratibha, M. S. Sailaja, B., Kavya, B. and Shivaji, S. (2009). Bacterial Diversity and Bioprospecting for Cold-Active Lipases, Amylases and Proteases, from Culturable Bacteria of Kongsfjorden and Ny- Ålesund, Svalbard, Arctic *Current microbiology* 59(5), 537–547.

Stan-Lotter, H. (2012). Physico-chemical boundaries of life: *Springer.*

Stan-Lotter, H. and Fendrihan, S. (2012). Adaption of microbial life to environmental extremes: *Springer.*

Tarnocai, C. (2009). Arctic permafrost soils *Permafrost Soils* (pp. 3-16): Springer.

Tehei, M., Franzetti, B., Madern, D., Ginzburg, M., Ginzburg, B. Z., Giudici-Orticoni, M. T., Bruschi, M. and Zaccai, G. (2004). Adaptation to extreme environments: macromolecular dynamics in bacteria compared in vivo by neutron scattering. *EMBO reports, 5*(1), 66-70.

Torsvik, V. and Øvreås, L. (2008). Microbial diversity, life strategies, and adaptation to life in extreme soils *Microbiology of Extreme Soils* (pp. 15-43): Springer.

Trevors, J. T. (1998). Bacterial biodiversity in soil with an emphasis on chemically-contaminated soils. *Water, Air, and Soil Pollution, 101*(1-4), 45-67.

Van Trappen, S., Vandecandelaere, I., Mergaert, J. and Swings, J. (2005). *Flavobacterium fryxellicola* sp. nov. and *Flavobacterium psychrolimnae* sp. nov., novel psychrophilic bacteria isolated from microbial mats in Antarctic lakes. *International journal of systematic and evolutionary microbiology, 55*(2), 769-772.

Vardhan Reddy, P. V., Shiva Nageswara Rao, S. S., Pratibha, M. S., Sailaja, B., Kavya, B., Manorama, R. R., Singh, S.M., Naga, T.,Srinivas, R. and Shivaji, S. (2009). Bacterial diversity and bioprospecting for cold-active enzymes from culturable bacteria associated with sediment from a melt water stream of Midtre Lo enbreen glacier, an Arctic glacier. *Research in Microbiology, 160*(8), 538-546.

Verde, C., di Prisco, G., Giordano, D., Russo, R., Anderson, D. and Cowan, D. (2012). Antarctic psychrophiles: models for understanding the molecular basis of survival at low temperature and responses to climate change. *Biodiversity, 13*(3-4), 249-256.

Vincent, W. F. (2000). Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. *Antarctic Science, 12*(03), 374-385.

Vincent, W. F. (2010). Microbial ecosystem responses to rapid climate change in the Arctic. *The ISME journal, 4*(9), 1087.

Vincent, W. F., Callaghan, T. V., Dahl-Jensen, D., Johansson, M., Kovacs, K. M., Michel, C., Prowse T., Reist, J. D and Sharp, M. (2011). Ecological implications of changes in the Arctic cryosphere. *Ambio, 40*(1), 87-99.

Wagner, D. (2008). Microbial communities and processes in Arctic permafrost environments *Microbiology of extreme soils* (pp. 133-154): Springer.

Whyte, L. G., Bourbonniere, L., and Greer, C. W. (1997). Biodegradation of petroleum hydrocarbons by psychrotrophic Pseudomonas strains possessing both alkane (alk) and naphthalene (nah) catabolic pathways. *Applied and environmental microbiology, 63*(9), 3719-3723.

Włodarska-Kowalczuk, M., Kukliński, P., Ronowicz, M., Legeżyńska, J. and Gromisz, S. (2009). Assessing species richness of macrofauna associated with macroalgae in Arctic kelp forests (Hornsund, Svalbard). *Polar biology, 32*(6), 897-905.

Yu, Y., Li, H., Zeng, Y., and Chen, B. (2009). Extracellular enzymes of cold-adapted bacteria from Arctic sea ice, Canada Basin. *Polar biology, 32*(10), 1539-1547.

Zhang, W., Parker, K., Luo, Y., Wan, S., Wallace, L. and Hu, S. (2005). Soil microbial responses to experimental warming and clipping in a tallgrass prairie. *Global Change Biology, 11*(2), 266-277.