

Being the coldest area on Earth, Antarctica continent rates amongst the least hospitable of habitats for living organisms. It is without question one of the most extreme environments with harsh and challenging climate conditions and landscape far off the tolerated range from a human point of view. However, not surprisingly there are living organisms harbouring literally any part of the continent, from the tip of the icebergs to the most arid soils in Antarctica Dry Valleys (Tindall, 2004), with many pieces of the puzzle still missing.

Microorganisms are well known for their ability to adapt to almost any environment and the extremely harsh Antarctic climate is of no exception. A climate characterized with blistering cold, low water availability, constant freeze-thaw and wet-dry cycles, high incident radiation (UV) and insufficient levels of nutrients (Baskin, 2005). Under these circumstances when plant diversity is at minimum, the biological activities of the ecosystem depend almost entirely on microorganisms (Simmons, 2009). These remarkably flexible creatures become the main players of the crucial environmental processes in this most unwelcoming face of Mother Nature.

It is known that soil ecosystems in general contain a diverse range of microbial life. Even though the extremely unfavourable conditions of Antarctica may imply very low cell densities, this value has been shown to be around 10^6 - 10^8 prokaryotic cells per gram soil (Smith *et al.*, 2006). This might potentially indicate high level of species diversity in soil samples of Antarctica, amongst which may rest entirely novel species or even new genera and families of microorganisms.

It has been merely more than two decades since the attempt to educate the public as to the enormous part microorganisms play in human life (Hunter-Cevera, 1998). Extremophiles (microorganisms generally thriving in extreme environments) compose their own rightful category considering their unique abilities. Their discovery has

revolutionized many industries including pharmaceuticals, food and chemical industries as well as environmental biotechnology (Demirjian *et al.*, 2001). Not to mention their leading role of telling the history of life on Earth as the oldest inhabitants of the planet, and even the possibility of life existence on other planets (Hunter-Cevera, 1998).

There are two main approaches to investigate microbial diversity in any given environment; culture-dependent and culture-independent methods (Bull, 2004). The classical culture-dependent methods employed in assessing biodiversity of bacterial communities face many shortcomings due to the hardships in mimicking the original habitat conditions. Therefore, microbiologists turned to alternative techniques that were cultivation-independent, molecular-based methods to study phylogenetic relations of microorganisms (Bull, 2004).

The approaches for uncovering the vast majority of the residing microorganisms in the samples must allow a thorough examination of the microbial diversity. The recent advantages of the culture-independent molecular techniques benefit the researchers in this area where the results of culturing studies are considerably limited (Smith *et al.*, 2006). Genomic sequences such as 16S rDNA fragments allow the assessment of a more extensive range of biodiversity than that acquired by cultivation studies. Consequently, a very rich diversity can be observed when the two are compared (Smith *et al.*, 2006).

1.1 Objectives

The purpose of this study is to estimate bacterial diversity of soil samples from three locations in Antarctica using culture-independent approach by:

- PCR amplification of bacterial 16S rRNA gene from total genomic DNA extracted from the soil samples of study sites
- Separation of the resultant amplicons by construction of clone libraries
- Selection of unique phlotypes using restriction fragment length polymorphism (RFLP) analysis
- Identification of phlotypes using DNA sequencing of isolated 16S rRNA genes
- Phylogenetic analysis of the identified bacteria
- Assessing bacterial diversity in the clone libraries corresponding to each study site