CHAPTER 1

INTRODUCTION

1.1 The Antarctic continent

1.1.1 Geographical position

Antarctica is a remote continent, the fourth largest continent in dimensions measuring about 13.5 million square kilometers. It is formed by two land-masses, greater or East Antarctica and lesser or West Antarctica, which both reached the South Pole position about 60 million years ago. The Antarctic Peninsula begins at 930 Km from south America from which it separated by the Drake passage, more distance from Antarctica are: the coast of Australia (2,500 Km away), the tip of Africa (4,000 Km) and New Zealand which about 2,500 Km away (Onofri *et al.,* 2007).

Antarctica is the most physically isolated continent due to its distance and patterns of ocean currents and air stream (Ellise-Evans, 1985). It contains 90% of the world's Ice and more than 70% of the world's fresh water, the Ice sheet covers about 98% of Antarctica with 2,500m thick. Oases or Ice free lands occur mainly in Antarctic Peninsula and in coastal areas. MucMurdo dry Valleys in Victoria Land represent the most extensive Ice-free zone (Onofri *et al.,* 2007).

1.1.2 The climatic conditions

The Antarctic climate is the coldest, windiest and driest known on earth making it inhospitable continent (Øvstedal and Lewis, 2001). Precipitation is rare and falls in the form of snow which mainly distributed along the coast decrease further inland and non-existent in the central region (Onofri *et al*., 2007). Antarctica can be subdivided into three climatic regions according to Holdgate (1977) called sub-Antarctic, maritime Antarctic and continental Antarctic regions corresponding to different biographical areas including the coastal, slope and ice plateau regions. Later, Øvstedal and Lewis (2001) subdivided maritime Antarctic into Northern and Southern regions.

1.1.2.1 The sub Antarctic region

The sub-Antarctic regions consist of most of the Antarctic islands north to the 60^o south: South Georgia, Marion and Prince Edward Islands, Crozet Island, Kerguelen, Heard, McDonald and Macquarie Islands. It is characterized by a cool oceanic climate with monthly temperature above freezing for at least 6 months, mean annual precipitation over 1,000 mm (Øvstedal and Lewis, 2001).

1.1.2.2 The maritime Antarctic region

The maritime Antarctic region includes the South Sandwich Islands, South Orkney Islands, South Shetland Islands, the Palmer Archipelago, and the west and north-east coast of the Antarctic Peninsula. It's characterized by a cold maritime climate, humid in the north and dry in the south areas (Lewis, 1984). Øvstedal and Lewis (2001) differentiated a Northern and a Southern regions by mean monthly temperatures are above O^0C in the warmer period (ranging from 1-3 to 3-4 months per year, moving from south to north) and rarely $<-15^oC$ in winter in the south and< -10 to -12^0C in the north. Annual precipitation ranges from less than 350 to over 500 mm (Lewis, 1984; Øvstedal and Lewis, 2001).

1.1.2.3 The continental Antarctic region

The continental Antarctica has been defined as not only the surrounding islands but also the west and north-east coasts of the Antarctic Peninsula. It's characterized by a cold arid climate on the coastal areas. Øvstedal and Lewis (2001) indicated three geobotanical zones which are Coastal, Slope and Ice Plateau. On the Coastal zone the monthly temperature are generally above 0^0C for 0-2 months in summer, in winter it range from -5 to -25^0C, and annual precipitation is between 100-150 mm water equivalents, with little rain. On the slope zone the monthly temperature are below -5^0C in summer and below -30^0C in winter, and the annual precipitation is below 100 mm water equivalent. On the Ice Plateau the monthly temperatures are below-15^0C in summer and below -50^0C in winter, precipitation is near non-existent.

The McMurdo Dry Valleys or Ross Desert is the largest ice-free area in Antarctica; Climatic conditions in this area are the harshest. In winter, air temperature ranged between -20°C and -50°C but much lower values can be reached, and during summer, the air temperature rises to -15°C, but milder temperatures may be reached according to the weather and the geographical position (Nienow and Friedmann, 1993).

1.1.3 The microbial life in Antarctica

Geographic isolation together with environmental stress makes Antarctica the first place to look for endemic organisms in order to explain evolutionary processes generating microbial speciation (Rusisi *et al.,* 2007). The dominant Antarctic conditions of low temperature, strong winds, low water availability and high ultraviolet radiation together comprise limiting factors for plant and animal life. Therefore, Antarctica is dominated by microorganisms with a high level of adaptation to these extreme conditions (Friedmann, 1993). The diversity of these microorganisms decrease along latitudinal gradients, from the maritime to the continental Antarctic zone and with increasing altitude and latitude from the coast to the Ice Slope Region (Pickard and Seppelt, 1984; Kappen, 1993; Broady, 1996).

In Antarctica, the microbial life is mainly psychrotrophic, however thermophiles were also isolated (Alias *et al*., 2005). Sinclair and Stokes (1963) considered that psychrophiles are micro-organisms which grow rapidly at 0^oC to form macroscopically visible colonies in one or two weeks, and subdivided them into strict or obligate and facultative, depending on whether they grow rapidly below or above 20^oC. Morita (1975) redefined psychrophiles as organisms having an optimum temperature for growth at 15^oC or lower, a maximum temperature for growth at 20^oC or below, and used the term psychrotrophic for cold tolerant organisms. Finally, psychrophiles or sychrotrophs were defined by (Wynn-Williams, 1990; Gounot, 1991; Broady, 1993 and Vishniac, 1993), as cold-adapted micro-organisms which not only survive in cold but able to grow at low temperature, near 0^oC.

Although the few mycological studies and the low number of habitats available for microbial life in the Antarctic continent, a significant number of new taxa of fungi, bacteria, micro-algae and protozoa have been described (Rusisi *et al*., 2007). Some filamentous fungi and yeasts originally isolated from Antarctic continent, which have been later isolated elsewhere such as *Penicillium antarcticum* Hocking and McRae, also recorded in Denmark and Atlantic Ocean (Ruisi *et al*., 2007). Many investigations were done on the Antarctic regions for the psychrophilic bacteria and archea, occasionally for algae and rarely for fungi (Gunde-Cimerman *et al*., 2003). The study of these microorganisms gave clear picture about their biological mechanisms of adaptation (Vincent, 2000). About twenty of the new taxa described from continental Antarctica representing 8% of the whole micro flora of this area which gives an idea about the microbial diversity and occurrence when compared to higher plants and animals (Foissner, 2006). The use of molecular techniques will improve the understanding of Antarctic microbial phylogeny, biodiversity and evolution (Rusisi *et al*., 2007).

1.2 Biodiversity of Antarctic fungi

The biodiversity of Antarctic fungi has been investigated and this include floristic studies (Del Frate and Caretta, 1990; Montemartini Corte, 1991; Onofri *et al*., 1991, 1994; Onofri and Tosi, 1992; Mercantini *et al*., 1993; Montemartini Corte *et al*., 1994) ecophysiological studies (Caretta, Del Frate and Margiarotti, 1994; Zucconi *et al*., 1996; Fenice *et al*., 1997; Onofri *et al*., 2000; Tosi *et al*., 2002) and molecular studies (Vishniac and Onofri, 2002).

More recently, Selbmann *et al*., (2005) studied the phylogenetic points of view by isolating and identifying fungal strains from samples collected from different parts of Antarctic continent. The distribution of Antarctic fungi is related to the distribution of different substrates such as soils, rocks, bird feathers, dung and lichens, and to distribution of scientific research stations, mainly scattered along the coast of the continent (Leotta *et al*., 2002; Tosi *et al*., 2002; Jumpponen, Newsham and Neises *et al*., 2003; Bridge and Worland, 2004).

Fungi that have been occur in polar and sub-polar regions are reported by Tubaki, 1961; Tubaki and Asano, 1965; Wicklow, 1968; Cameron, 1971; Barker, 1977; Rounsevell, 1981; Fletcher *et al*., 1985; Kerry, 1990; Vishniac, 1993. *Sclerotium antarcticum* Bommer and Rousseau with teleomorph *Sclerotina Antarctica* are the first new fungal species isolated from Antarctica, from Damco coast, maritime Antarctic Peninsula (Gamundi and Spinedi, 1987).

Antarctic fungi can be divided into truly “indigenous fungi”, which are able to tolerate Antarctic condition and actively grow and reproduce, and “non indigenous fungi” which are received from outside the continent like South America by vectors such as animals and winds and it is difficult to distinguish between indigenous and non indigenous fungi (Rusisi *et al.,* 2007).

Onofri *et al*., (2005) reported that (0.6%) of known fungi species is represented by water moulds (kingdom Chromista), while (99.4%) is composed of true fungi, including yeasts and filamentous fungi, which together include species belonging to phyla of Chytridiomycota, Zygomycota, Ascomycota and Baisidiomycota. Among these some species are endemic and the other is indigenous being able to show active growth and reproduce in Antarctica. The indigenicity of some species can be indicated by the high number of isolation over the years as in *Geomyces pannorum* (Link) Sigler & J.W. Charmich., *Cryptococcus vishniacii* Vishniac & Hempfling, and *Thelebolus microspores* (Berk and Broome) Kimbr, commonly recorded in Antarctica (Rusisi *et al*., 2007).

Microfungal strains having meristematic growth also have been recorded found associated with rocks and some of them isolated from the ice free areas of Victoria Land

and described as endemic genera and species (Onofri, 1999; Selbmann *et al*., 2005). The meristematic fungi are a group that show very little and slow expansion growth, cauliflower-like colonies and reproduced by isodiametric enlargement with subdividing cells. From a phylogenetic point of view they represent a quite heterogeneous group of fungi (Sterflinger *et al*., 1999).

Most of the fungi recorded from Antarctic continent are anamorphic forms, which show a sexual reproduction in a simple way as they conclude their life cycles in a shorter time without high metabolic costs (Rusisi *et al*., 2007). Few exceptions have been reported such as *Thelebous* spp. (Ascomycota) frequently collected in Antarctic continent. More simplification of life cycles can be indicated in Cryptoendolithic black fungi which living in the extreme conditions of the ice-free zones in Victoria Land as most of them are unable to produce anamorphic reproductive structures (Rusisi *et al.,* 2007).

Rock inhabiting fungi can be divided into two ecological groups, one is hyphomycetes of soil and hyphomycetes of epiphytic origin and the second one is black melanized microscopic fungi (Gorbushina, Beck and Schulte, 2005), which show meristematic growth and form microcolonies into rocks (Gorbushina *et al*., 1993; Sterflinger *et al*., 1999; Ruibal *et al*., 2005), and they have been named microclonial fungi (Sterflinger, 2005).

Keratinophilic fungi is present in the areas influenced by plants and animals (Marshal, 1998), and has been reported in Antarctic Peninsula (Caretta and Piontelli, 1977) and Signy Island (Pugh and Allsop, 1982). *Geomyces pannorum* is an example of keratinophilic fungi which has been reported by Finotti *et al*., (1996).

Xerophilic or xerotolerant fungi is another group of fungi which can live in dry area where little water is available, *Urantiogriseum, Wellemia sebi* and *Aspergillus versicolor* are examples for xerophilic fungi (Samson *et al*., 1995; PetroviVc *et al*., 2000).

1.2.1 Thermal Classes of Antarctic Fungi

By following the definition of Morita (1975) for bacteria, both psychrophilic and psychrotrophic organisms are able to grow at 0^0C. Psychrophiles having the optimum temperature for growth at about 15^0C or lower, a maximum growth temperature of 20^oC or below, whereas psychrotrophic fungi have a maximum growth temperature above 20^0C. The Antarctic mycoflora is mainly composed of psychrotolerants (Kerry, 1990; Zucconi *et al.,* 1996; Azmi and Seppelt, 1997). Fungi in Antarctica are present as psychrophiles, psychrotrophs or mesophilic psychrotolerants, thermotolerants and thermophilics, these two last ecological groups were found mainly in heated sites on Antarctic active Volcanoes (Onofri *et al*., 2007).

1.2.2 Physiological adaptations of fungi to continental Antarctic conditions

Antarctica is characterized by extreme environmental conditions which limiting the life only to microorganisms with high level of adaptation. These microorganisms usually exhibit different physiological and morphological adaptive strategies at the same times when single strategies are not specific for single stress factor (Rusisi *et al*., 2007).

1.2.2.1 Low temperature

Low temperature is usually identified in biology with subzero temperature with a lower limit of -20^{0}C, below which no life processes persist (Rivkina *et al.*, 2000). Terrestrial organisms in Polar Regions must survive long period with subzero and daily freeze-thaw cycles (Montiel, 2000). Low temperature will limit the microbial enzyme activity, membrane integrity (Russell, 1990; Crowe *et al.*, 1992), and restrict the availability of liquid water for the hydration of biomolecules and as a medium for biochemical process (Wynn-Williams and Edwards, 2001). Several physiological mechanisms of cold-tolerance have been reported in fungi and usually a combination of these strategies is employed by Antarctic microorganisms which include: antifreezes production, high supercooling activity, dehydration ability, freeze tolerance, selection of micro and nano-habitats, life in habitats with snow cover, anoxia tolerance (being encased in ice) (Robinson, 2001).

Alteration of membrane lipid composition is one of adaptive strategy used by microorganisms in Antarctica (Russell, 1990). At low temperature the membrane lipids were alter from the liquid crystalline to the gel phase which will cause Freeze and/or dehydration damage to cells (Crowe *et al.*, 1987). An increasing of the degree of fatty acids un-saturation will lowered the temperature at which the transition occurs which helping cells to function at low temperatures (Rusisi *et al.*, 2007). An increase in the linoleic and arachidonic acids production were observed in A Antarctic strains of *Mortierella alapina* peyronel, *Mortierella Antarctica* Linnem, and *Cadophora fastigiata* Lagerb & Melin when cultivated at low temperatures (Maggi *et al.*, 1991), and changes in fatty acid composition in response to low temperatures were observed in Antarctic strains of *Geomyces vinsceus* Dal Vesco and *Geomyces pannorum (Link)* Sigler & J.W. Charmich (Finotti *et al*., 1993).

Dehydration and osmotic stress are induced by low temperature in fungi and these can result in synthesis of different compatible solutes with enzyme activity protection roles. Glycerol consider one of these compatible solutes and the most efficient ones with cryoprotective ability during desiccation or freezing are sugars such as trehalose (Weinstein *et al*., 2000) and mannitol (Feofilova *et al*., 1994), they can stabilize membranes maintaining their integrity and function. Trehalose is the most distributed disaccharide in fungi (Thevelein, 1984), together with sugar alcohols and glycogen appears to be a general stress protecting in the cytosol (Cooke andWhipps, 1993), and known to stabilize membrane during dehydration (Goodrich *et al*., 1988). This trehalose accumulates in fungal hyphae and its concentration increased to double in excised alpine mycorrhizal roots when they exposed to low temperatures (Niedere *et al*., 1992). Also presence of polyols which act as physiological buffering agents and show evidence of potential cryoprotectant role (Jennings, 1984).

Production of exopolysaccharides represents another response to stress conditions used by fungi (Rusisi *et al*., 2007), they action has still not been fully clarified but it has been suggested they could protect cells by changing the permeability to Na^+ and K^+ ions during freezing and thawing and act on the viscosity of extracellular solution avoiding excessive stresses. They also could alter the structure of water within and around the cells forming a glass structure during freezing which controls water crystallization to a certain level (Rusisi *et al*., 2007). Production of cold-active enzymes by some Antarctic fungi also will contribute in the ability to survive at low temperature (Fenice *et al*., 1998).

1.2.2.2 Low water availability

Although 70% of the Earth's fresh water is in Antarctica, it is approximately present in the form of ice, and in some ice-free areas such as the McMurdo Dry Valleys, precipitation represented only by snow (Rusisi *et al*., 2007). Strong winds will enhance desiccations which increase the evaporation, and the main source of moisture is come by transient water melted under the solar heating of the substratum during the austral summer. Maritime Antarctica contains larger amounts of liquid water where milder conditions present (Rusisi *et al*., 2007). Low availability of liquid water is one of the most stress factors which limiting the distribution and abundance of terrestrial organisms in Antarctica and organisms mainly distributed along the coast where melted water frequently occur (Rusisi *et al*., 2007).

Generally fungi have much lower minimum values of water activity (a_w) for growth than bacteria so, many Antarctic fungal species consider as xerophilic i.e. they able to grow under condition of low water availability, or Xerotolerant they able to grow under dry conditions (Onofri *et al*., 2007). Many xerophilic fungal strains from Antarctica are recorded such as *Wallemia sebi* (a_w 0.69), *Eurotium rubrum* (a_w 0.70), *Pencillium chrysogenum* (a_w 0.78), and *Aspergillus sydowii* (a_w 0.78) (Samson *et al*., 1995; Petrovic *et al*., 2000).

High evaporation rates will lead to high salt concentration in shallow ponds and on rock and soil surface (Rusisi *et al*., 2007). Microorganisms adapted to matrix water stress resulting from drought or binding of water into ice, are also can adapted to osmotic, since high salinity causes the same effects as freezing due to osmotic imbalance (Gunde-Cimerman *et al*., 2003).

Microorganisms under desiccation and low water availability conditions react by producing osmoregulators substances or by absorbing solutes from the substratum (Onofri *et al*., 2007). The accumulation of these intracellular osmoregulators at high concentrations does not interfering with enzyme activity and metabolism and these compatible solute belong to several classes of compounds: polyols and melanin (Bell and Wheeler, 1986; Butler and Day, 1998; Kogej et al, 2004), mycosporines (Volkmann *et al*., 2003), sugars and sugars derivatives such as glycerol, arabitol, mannitol and trehalose (Grant, 2004). Production of exopolysaccharide in response to dehydration can be seen in *phoma herbarum* from Antarctica (Selbmann *et al*., 2002).

1.2.2.3 High UV radiation

UV radiation is one of the most important and widely recognized stress factors in Antarctica. Exposure to high UV radiation will cause damages to DNA, proteins including cell membrane lipoproteins, and organelles (Karentz, 1994) and also affect ecosystems and biological evolution (Cockell and Blaustein, 2001).

Terrestrial microorganisms in habitats exposed to high UV radiation produce different pigments protecting against UV damages which they located either extracellular or inside the cell to protect metabolic critical molecules (Rusisi *et al*., 2007). Some of Antarctic microorganisms are investigated for their ability to bear the increase of UV radiation by producing photoprotector compounds as MAAS (mycosporine-like-amino acids) or usnic acid (Ferreyra *et al*., 1999; Purvis, 2000; Shick and Dunlap, 2002).

Some studies have examined the UV radiation effect on Antarctic fungal species and communities and between fungal taxa recorded in Antarctica, some melanised strains are known for their UV resistance such as *Alternaria alternate*, *Stachybotrys chartarum* and *Ulocladium consortiale* (Rusisi *et al*., 2007).

1.3 Bioactivity of Antarctic fungi

1.3.1 Definition of biological activity

Biological activity is an expression describing the beneficial or adverse effects of a drug on living matter. The main type of biological activity is substance's toxicity. Activity is generally dosage- dependent and it is uncommon to have effects ranging from beneficial to adverse for one substance when going from low to high doses. A material is considered bioactive if it has interaction with or effect on any cell tissue in the human body. Pharmacological activity is generally used to describe beneficial effects, i.e. the effect of drug candidates.

1.3.2 Definition of secondary metabolites

Secondary metabolites are compounds produced by organisms that are not required for primary metabolic processes which possessing chemical structures quite different from primary metabolites from which they were produced such as sugars, amino acids and organic acids (Demain, 1995). Secondary metabolites, also known as idiolites, are not essential for growth of microorganisms but serve diverse functions in nature. The most advantage of secondary metabolites to a producing-organism is that they may allow an organism to survive in its ecological niche (Ellen *et al*., 2008).

Classes of fungal secondary metabolites include polyketides (e.g. aflatoxin and fumonisins), non-ribosomal peptides (e.g. sirodesmin, peramine and siderophores such as ferricrocin), terpenes (e.g. T-2 toxin, deoxynivalenol (DON)), and indole terpenes (e.g. paxilline and lolitrems), and the biosynthesis of secondary metabolites are usually under the control of clustered genes (Ellen *et al*., 2008).

1.3.3 Fungal ecology as a source of bioactive natural product

1.3.3.1 Fungal natural products

Natural products have been particularly important in the development of effective therapies for cancer, malaria, bacterial and fungal infections, and CNS and cardiovascular diseases (Newman *et al*., 2000). For instance, as of 2003 over 60% of all drugs in clinical trials against cancer are either natural products or derived directly or indirectly from natural products leads (Cragg and Newman, 2005).

Among the most significant properties of fungi is their ability to produce secondary metabolites with a broad range of biological activities such as antimicrobial activity. Antibacterial agents such as penicillin and cephalosporin are the best known examples, but a variety of other compounds with pharmacological activities have also been discovered as fungal metabolite (Demain *et al*., 2005), and many important pharmaceuticals have been discovered through the studies of fungal chemistry (Masurekar, 2005; Demain *et al*., 2005).

Other medically important compounds include cyclosporine, mycophenolic acid, the ergot alkaloids and many other fungal metabolites have been discovered as potential pharmaceuticals with a wide range of pharmacologically relevant activities in mechanism-based and whole-organism assays (Caporale, 1995; Masurekar, 2005).

Almost 4,000 secondary metabolites of fungal origin are known to possess biological activities and the majority coming from the species *Penicillium, Aspergillus, Acremonium* and *Fusarium* (Dreyfuss and Chapela, 1994). However, fungi are effective in producing compounds with relevant activity to other therapeutic areas such as HIV (Singh *et al*., 2005). Fungal diseases have become increasingly common, and there are several risk groups of growing population (e.g., AIDS and chemotherapy patients) that are particularly susceptible to opportunistic fungal infections (Koltin, 1990; Wong-Beringer and Kriengkauykiat, 2003). The need for new antifungal agents in both medicine and agriculture continues to grow. Although effective antifungal agents are relatively abundant, many types of fungal infections recur after end of treatment, and others (e.g., nail infections) are particularly difficult to treat effectively (Baker *et al*., 2005). So, fungi are considered the logical sources to be exploring in search of agents that regulate fungal growth or modulate the activities of fungal enzymes (Gloer, 2007).
More importantly, very few drugs are available that are therapeutically useful in the treatment of systemic fungal infections (Richardson and Marriott, 1987; Balkovec, 1998; Wong-Beringer and Kriengkauykiat, 2003).

Moreover, fungal products show significant potential as natural agrochemicals, with important examples including nodulisporic acids (insecticides/antiparasitics), strobilurins (fungicides) and various phytotoxins (herbicides) (Gardner and McCoy, 1992; Anke and Sterner, 2002; Liu and Li, 2005).

Fungal metabolites are renewable resources, and different methods can be developed for large-scale production of important fungal metabolites using established techniques such as modification of metabolite structure and improvements in metabolite production efficiency accomplished through strain mutation, medium variation, and optimization of culture conditions (Masurekar, 2005). For instance, manipulation of metabolite-producing cultures eventually resulted in a 6,000-fold improvement in penicillin production (Demain, 1992), and a 900-fold improvement in compactin production (Chakravarti and Sahai, 2004).

Recently, it has become difficult to find new bioactive natural products from microbial sources due to expensive programmes needed (Gloer, 2007). For example, the need to dereplicate cultures considers a source of great expense (Corley and Durley, 1994), and viewed by many as negative feature of continued screening efforts. In addition, long-term dependence on screening of total numbers of *actinomycetes* and common fungi isolated mainly from soil samples as sources of bioactive metabolites, while other, less widely studied niche groups have been largely neglected (Gloer, 2007).

In attention to the specific types of organisms chosen for screening and the habitats from which they are isolated, together with disinterest in fungi that are slow-growing, more difficult to isolate, and/or difficult to adapt to standard liquid fermentation protocols, combine to intensify the problem. Therefore, many taxonomic and ecological groups of fungi have not been systematically explored for useful secondary metabolites, despite literature evidence that directly or indirectly indicates their potential in this area (Gloer, 2007).

The importance of seeking isolates for industrial screening programs from relatively unexplored niche groups or substrates has been recognized (Monaghan and Tkacz, 1990; Miller, 1991; Dreyfuss and Chapela, 1994; Bills, 1995; Caporale, 1995), and a number of programs have made significant efforts to expand their scope in order to include such isolates (Gloer, 2007).

1.3.4 Biological activity of Antarctic fungi

Antimicrobial activities of fungi from extreme environment were less investigated and only few secondary metabolites from Antarctic fungi have been reported (Montemartini *et al*., 2000). For examples, a few of the *penicillium* spp. strains isolated from the sediments of ponds in continental Antarctica showed an antibacterial activity similar to B- lactame antibiotics (Montemartini *et al*., 2000).

Nedialkova and Naidenova (2004) investigated a total of 40 *actinomycetes* strains isolated from Antarctica for antagonistic activity against test microorganisms and this initial screening resulted in 60% of the strains exhibited inhibition potential against test-microorganisms. Ten of this had a broader spectrum of antibacterial activity and could be used in the development of new substances for pharmaceutical or agricultural purposes. Some investigators indicated production of secondary antimicrobic compounds by some strains of Antarctic fungi and 29% of micro fungal species with antimicrobial activities were isolated from benthic mats of different Antarctic lakes (Marinelli *et al*., 2004).

1.4 Research objectives

It is important to continue the screening for novel bioactive compounds as the number of microorganisms resistant to the existing antibiotics is growing every year. More research is needed to be done in order to find new antibiotics from unexplored natural environments such as Antarctic continent.

In this research we try to find fungi species that may produce unique secondary metabolites with novel antibiotic properties to be exploited in pharmaceutical purposes, Therefore, the objectives of this study are:

1. To screen the antimicrobial activity of Antarctic fungi against Gram-positive bacteria, Gram-negative bacteria and yeasts using plug assay method,

2. To confirm the bioactivity of Antarctic fungi by agar disk diffusion method,

3. To quantitatively measure the minimum inhibitory concentration (MIC) of the fungi extracts,

4. To quantitatively measure the minimum bactericidal concentrations (MBC) of the fungi extract.