CHAPTER 2 LITERATURE REVIEW

2.1 Microbial diversity in Antarctica

Antarctica is located at the southernmost continent and offers a range of extreme climatic conditions that constitutes one of the harshest environments on Earth (Cowan and Tow, 2004). Antarctica is the coldest, driest and windiest continent with the highest average elevation of all the continents. The area of Antarctica is of 14.425 million km^2 and 98% is covered with ice. Antarctica, the major cold regions of the earth, has environmental niches, which includes different soil types, sediments, rocks, melt waters, snow and ice, and each region has various nutrients, salinity and water activity (Russell, 2006).

Signy Island (60^o 42' S, 45^o 36' W) is located in South Atlantic Ocean on the southern arm of the Scotia Ridge. The total surface of Signy Island is about 20 km^2, with length and width of 8 km and 5 km respectively and a highest elevation of 280m (Figure 2.1). Signy Island experience low temperature with the mean temperature around -3.5℃ throughout the year (Gugleilmin *et al*., 2008). About 50% of the surface of Signy Island is free from ice and snow during summer. With its varied terrain, the lowland and coastal zone provides a diversity of habitats (Holdgate, 1967). The soils of Signy Island contain significant organic content which provide diverse habitats for microorganisms and soil arthropods (Holdgate, 1967).

To date, relatively few Antarctic microorganisms were described (Nichols *et al*., 2002) and has motivated microbiologist to screen the largely unexplored Antarctic continent and surrounding marine sites (Brambilla *et al*., 2001). Isolation and description of new Antarctic bacteria has continued and a number of novel findings have been reported for microbes isolated from Antarctica (Nichols *et al*., 1999). Novel

genera and species were subsequently described and founded in the Freshwater Lakes (McCammon *et al*., 1998), and sea ice (Bowman *et al*., 1997a), saline lake (Bowman *et al*., 1997d), pond sediment (Mountfort *et al*., 1998) and sandstone (Schumann *et al*., 1997) in Antarctica.

A great diversity of microorganisms were isolated from McMurdo Sound Area, Antarctica (Boyd and Boyd, 1963), Lake Fryxell in McMurdo Dry Valleys (Brambilla *et al*., 2001), maritime Antarctic Lake (Pearce *et al*., 2003) using culture dependent techniques. Bacteria ranging from subphylum *α-Proteobacteria*, *β-Proteobacteria*, *γ-Proteobacteria*, *Actinobacteria*, *Clostridium-Bacillus*, *Flavobacterium*, *Plancytomycetes* were reported (Brambilla *et al*., 2001). Microbial diversity of Antarctica were subsequently studied and specific genera found in the maritime Antarctic Lake were the *β-Proteobacteria* genera *Janthinobacterium*, *Pseudomonas* and *Herbaspirillum*, the *α-Proteobacteria* genus *Sphingomonas*, the γ-Proteobacteria genus *Aeromonas*, the *Actinobacterium* genus *Arthrobacter* and the Firmicutes genera *Paenibacillus* and *Bacillus* (Pearce *et al*., 2003) as well as methylotrophic bacteria was detected in soil and sediment samples collected from Signy Island (Moosvi *et al*., 2005). The great diversity of microorganisms thus provides significant interest in conducting research in Antarctic.

2.2 Diversity and distribution of actinobacteria in Antarctica

Actinobacteria were formerly known as actinomycetes, under the order of *Actinomycetales* based on 16S rRNA gene sequence-based phylogenetic clustering (Stackebrandt *et al.*, 1997). They are a group of high G + C, Gram-positive, aerobic, mycelia, filamentous bacteria based on 16S rRNA gene sequence-based phylogenetic clustering (Basilio *et al*., 2003; Stackebrandt *et al*., 1997; Sacramento *et al*., 2004). Actinobacteria can be found in soil and considered as one of the major communities of

microbial population present in soil (Basilio *et al*., 2003). Their presence are highly affected by the soil temperature, pH, location, soil type, moisture content, cultivation, organic matter content, aeration and vegetation (Basilio *et al*., 2003**;** Labeda, 1990). Up to date, class Actinobacteria are classified into 5 orders namely *Actinomycetales*, *Rifidobacteriales*, *Acidimicrobiales*, *Cariobacteriales*, *Sphaerobacterales* and *Rabrobacterales*, 13 suborders and 50 families based on 16S rRNA phylogenetic clustering (Zhi *et al*., 2009) (Figure 2.2).

Recent studies stated that the search for novel actinobacteria now focuses on bioprospecting in environments such as Antarctica. *Streptomyces* spp. was found to be the most abundant genus readily isolated from most aerobic soil and some aquatic environments in Antarctica (Nichols *et al*., 2002). Actinobacteria were isolated from Ice free soils of Vestfold Hills, Macquarie Island (Nichols *et al*., 2002), freshwater lakes on Signy Island, South Orkney Islands (Pearce *et al*., 2003) and McMurdo Dry Valley soils (Cameron *et al*., 1972).

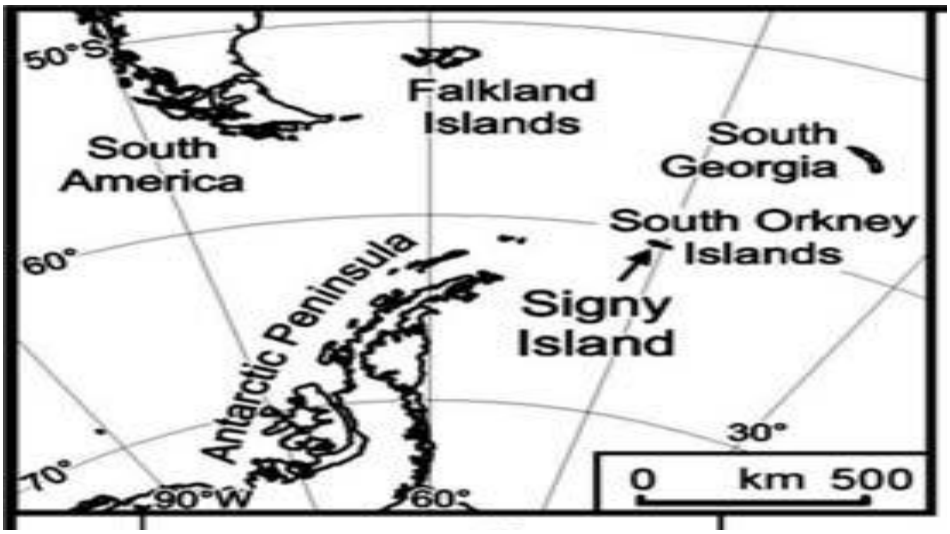


Figure 2.1 Location of Signy Island (Chong *et al*., 2009).

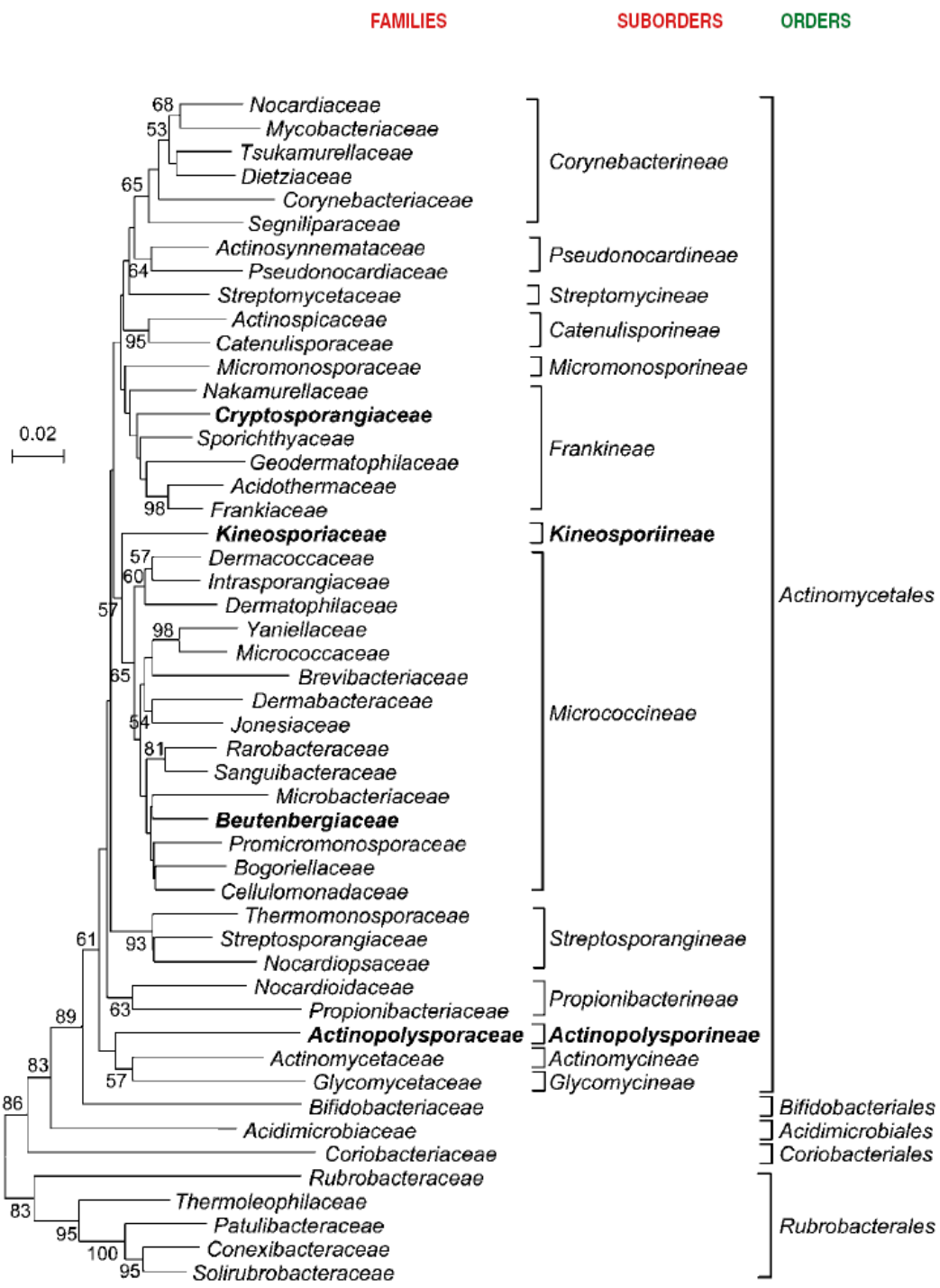


Figure 2.2 Classification of actinobacteria based on 16S rRNA sequence. The scale bar represents 5 nucleotide substitutions per 100 nucleotides (Zhi *et al*., 2009).

Novel species of actinobacteria such as *Friedmanniella antarctica* gen. nov., sp. nov. (Schumann *et al*., 1997), *Modestobacter multiseptatus* gen. nov., sp. nov (Mevs *et al*., 2000) and *Pseudonocardia antarctica* sp. nov. (Prabahar *et al*., 2004) were discovered in Antarctica. It was followed by detection of *Friedmanniella* and

Arthrobacter by culturing or amplicon library clones in Victoria Land, Antarctica (Aislabie *et al*., 2006). *Streptomyces griseus* strain NTK97 which produced the new antibiotic, Frigocyclinone, was isolated from Edmunson Point, Antarctica. The antibiotic showed antibacterial activities against Gram-positive bacteria. This shows that actinobacteria from Antarctica are capable to produce new metabolites (Bruntner *et al*., 2005). Aislabie *et al*. (2006) indicated that studies of bacterial identification in Antarctica led to the conclusion that bacteria from Antarctic soils maybe cosmopolitan, similar to those found in soils worldwide.

2.3 Isolation of actinobacteria from environmental samples

Soil is the main source for the isolation of actinobacteria and are best collected from the top 4 cm of the soil profile, as this is where most of the microbial activity takes place, and thus where most of the bacterial population is concentrated (Labeda, 1990). Soils which are not processed immediately are best stored in polyethylene bags at -20 ℃ or lower (Labeda, 1990). Actinobacteria are usually isolated from adding 100 µl of soil suspension by spread plate technique on the isolation media. Actinobacteria are generally found at numbers of 10^5 to 10^6 per gram of soil in agricultural soils (Labeda, 1990).

Isolation of actinobacteria is based on its selection of nutrients which aid the growth of actinobacteria and adding of antibiotics to prevent growth of fungi (Hirsch, 1983; Labeda, 1990). Various isolation media have been design to aid the isolation of actinobacteria while reducing growth of fungi and other bacteria. Formulations such as Conn's agar, Krainsky's medium and Kuster-Williams agar which is poor in organic nitrogen and growth factors effectively controlled other bacterial populations and aid in isolating soil actinomycetes. These media were used for the isolation of actinomycetes, particularly *Streptomyces* during the discovery of a large variety of saprophytic aerobic

actinomycetes (Srinivasan *et al*., 1991). Several useful isolation media has been recommended to isolate different genera of actinobacteria, namely Gauze mineral medium, Starch-casein agar, arginine-glycerol-salts agar, arginine-vitamin (AV) agar, colloidal chitin agar, M3 agar and Humic acid-salts-vitamin agar (Labeda, 1990).

Various enrichments can be employed for the isolation of various types of actinobacteria (Labeda, 1990) where soil actinobacteria has a slower growth rate compared to soil bacteria. Approaches of selective nutrients, using high carbon sources such as glucose, glycerol, starch and high nitrogen sources such as nitrate or casein promotes the growth of actinobacteria and greatly reduces the number of bacteria on isolation plates. On the other hand, certain minerals, namely NaCL, K_2HPO_4, $MgSO_4.7H_2O$, $CaCO_3$ and $FeSO_4.7H_2O$ were found to favour the growth of actinobacteria while addition of cholesterol and sodium azide enhances the growth of *Norcardia* (Waksman, 1967; Labeda, 1990). Addition of chitin supplements into isolation media also aids the isolation of actinomycetes which have the ability to hydrolyse this carbohydrate, while relatively few bacteria and fungi among the soil population can utilize it. Many microorganisms i.e actinobacteria utilize sole carbon and nitrogen source in chitin (El-Nakeeb and Lechevalier, 1963). Previous reports also showed that chitin agar aids the development of actinomycetes while suppressing the growth of bacteria and fungus (Hsu and Lockwood, 1975). Addition of rose Bengal (Ottow, 1972) or sodium propionate (Crook *et al*., 1950) into isolation media also helps to reduce the spreading growth of fungi (Ottow, 1972). Actinobacteria in media containing rose Bengal are easily recognized as small, intensively pink coloured colonies that develop inside or on a slightly pink medium (Alef and Nannipieri, 1995). Actinobacteria were known to be comparatively salt tolerant (Waksman, 1961). Mackay (1977) stated that addition of sodium chloride are useful in isolating actinobacteria

while Starch casein medium with the addition of up to 4.6% NaCl showed growth of *Streptomyces* while suppressing the growth of bacteria.

A range of culture media were described for the cultivation in the International Streptomyces Project (ISP). Media such as Yeast malt extract agar (ISP2), oatmeal agar (ISP3), Inorganic salts starch agar (ISP4), glycerol asparagine agar (ISP5), Peptone-yeast extract iron agar (ISP6) and Tyrosine agar (ISP7) are recommended for the characterization of *Streptomyces* sp. The ISP2 media is the standard media for morphological studies of actinomycetes. ISP characterization publications remain the best set of descriptions for a group of actinobacteria (Shirling and Gottlieb, 1966).

Addition of antibiotics was employed to improve isolation. The main antibiotics, cyloheximide and nystatin can be routinely incorporated into actinobacteria isolation agar at approximately 50 µg/ml each, to inhibit fungus growth (Hirsch, 1983; Labeda, 1990; Imada *et al*., 2007). Different concentrations of antibiotics were known to be used to isolate specific genera of actinobacteria (Srinivasan *et al*., 1991).

Reports show that Antarctic bacterial soil isolates or psychrophiles may have both their optimum and upper limit between 15 ℃-20 ℃ (Russell, 2006). Psychrophiles can also grow at 0 ℃ or below, with their optimum temperature above 15 ℃ while the maximum temperature for psychrotolerants can be until 30 ℃-40 ℃ (Russell, 2006). Reports showed that actinobacteria were cultured and isolated at 4 ℃ (Moncheva *et al*., 2002) and 28 ℃ (Nedialkova and Naidenova, 2005).

Soil actinomycetes favour in growing in neutral or slight alkaline conditions, but they also grow in acidic and even alkali conditions. Alkaliphilic actinomycetes are capable in growing within the pH 5.0 to 9.5-10.0 (Selyanin *et al*., 2005) while acidophilic actinomycetes grow within pH 3.5-6.5. Acidophilic actinomycetes have optimum growth at pH 5.0 while the upper limit of pH of their growth may be close to pH7.0 (Zakalyukina *et al*., 2002). However, the population of actinomycetes isolated

from neutral conditions are higher compared to acidic and alkali conditions (Basilio *et al*., 2003).

Actinobacteria grow more slowly compared to most bacteria and fungi, and hence are likely to be more masked in culture plates of ordinary media. Actinobacteria are capable of growing in media containing low nitrogen; this property is used to prevent the development of the more rapidly spreading colonies of bacteria. The list of novel Actinobacteria and products found in microbiologically poorly explored areas such as China and Australia suggests that careful exploration of new habitats might continue to be useful (Okazaki and Naito, 1986; Nolan and Cross, 1988). However, some actinomycetes grow slowly or do not produce spores. Hence, it is essential to reduce the competition from *Streptomyces* or other bacteria and provide suitable growth factors for the growth of novel actinomycetes (Srinivasan *et al*., 1991). Previous reports showed that most isolates isolated were identified as genus *Streptomyces* when conventional isolation techniques were applied (Nolan and Cross, 1988). *Streptomyces* spp. were known as the dominant bacteria in soils and their isolation is extremely high compared to other bacterial taxa (Midayoh *et al*., 1997). Due to their ease of isolation and the intensive screening programs carried out over several decades on them, there is growing interest in non-streptomycete Actinobacteria as sources of novel compounds (Nichols *et al*., 2002). The availability of novel Antarctic species, generally isolated from extreme environment, opens the door for possible biotechnological exploration (Nichols *et al*., 1999).

Many factors must be considered for isolation purposes. In this study, the isolation mediums used were in one-tenth strength as high nutrient levels may inhibit the growth of actinobacteria strains. Hence, isolation of actinobacteria from extreme environments, which includes Antarctica, requires a selective method to aid the growth of Actinobacteria.

2.4 Characterization and dereplication of actinobacteria

Characterization and dereplication methods are adopted for the classification and description of actinobacteria. Actinobacteria species can be characterized based on morphological, cultural physiological, biochemical and chemotaxonomic properties (Moncheva *et al*., 2002; Antonova-Nikolova, 2005). 16S rRNA has also been considered as a useful tool to identify the strains (Jiang *et al*., 2008). Advances in microscopic and chemotaxonomic methods have greatly enhanced the abilities of scientists to differentiate genera of actinomycetes (Dietz and Currie, 1996).

2.4.1 Morphological characterization of actinobacteria

Actinobacteria can be distinguished easily from other Gram-positive bacteria by their morphological appearance. Actinobacteria exhibit filamentous characteristics in which the filamentous element are known as hyphae. Development of actinobacteria starts with the germination of spores, hyphae or inoculum. On solid agar media, the inoculum grows into the medium and develops into substrate mycelium, followed by vertically growing hyphae will then penetrate the substrate and form aerial mycelium, which grows on the surface of the agar (Midayoh *et al*., 1997). However, actinomycetes only germinate and extend their hyphae when the environmental conditions are favourable such as favourable humidity and temperature. (Midayoh *et al*., 1997). Nevertheless, aerial mycelium may be absent in *Micromonospora* or *Actinoplanes* (Midayoh *et al*., 1997) or *Nocardia* strains, which form true mycelium in the early stages and subsequently fragmentized into rods and cocci (Srinivasan *et al*., 1991).

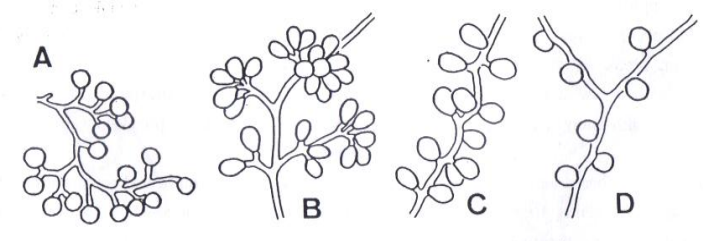
Actinobacteria normally exhibit dry, powdery aerial mycelium morphologically giving them a distinct appearance (Midayoh *et al*, 1997). Actinobacteria colonies may be raised, flat and sometimes covered with a leathery layer. Actinobacteria produces various pigments, ranging from white, yellow, orange, rose, red, purple, blue, green,

brown and black. The colonies may be completely compact or may demonstrate different zones of growth. However, the size of the colony also depends on its age, species and growth conditions (Midayoh *et al*., 1997). They exhibit particular characteristics and can be distinguished morphologically from other bacteria.

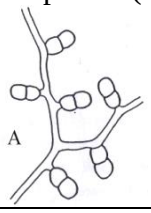
Microscopic observation is important in distinguishing the actinobacterial species. The naked eye and the dissecting microscope gives us information about colonial features and mycelial characteristics while the optical microscope reveals the morphological structures of hyphae and spores of actinobacteria (Midayoh *et al*., 1997).

Spore formation is the most important morphological criterion to identify actinobacteria. Spore formation of actinomycetes can be classified in three configurations: individually (monosporous), in chains (disporous, oligosporous, polysporous), or enclosed in sporangia. The shape of spores may range from globose, ovoid, doliform, rod-shaped, allantoid and reniform The sporulating aerial hyphae of *Streptomyces* can be differentiated into the following main types namely, rectiflexibiles, retinaculiaperti, spira and verticillati (Midayoh *et al*., 1997). The spore chain production and mycelium can be easily distinguished by using the cover slip method and view under a light microscope.

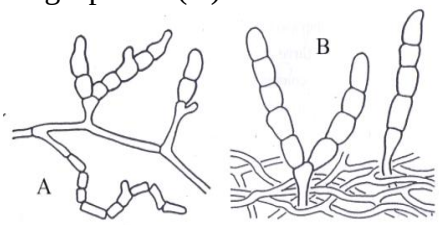
i. Monosporous: (A) Micromonospora, (B) Thermomonospora (C) Saccharomonospora (D) Thermoactinomyces



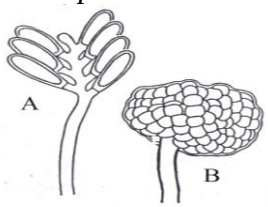
ii. Disporus: (A) Microbispora



iii. Oligosporus: (A) *Nocarida revicalena* (B) *Catelatospora*



iv. Sporangioiophores: (A) Sporangiophore of *Planomonospora venezuelensis* with short branches in alternation forming together with the monosporous sporangia in a palm leaf pattern; (B) Simple unbranched sporangiophore of *Actinoplanes*.



v. Polysporus: *Streptomyces* sp. (A) Rectiflexibles type (B) Retinaculiaperli type (C) Spira type (D) Verticillati type

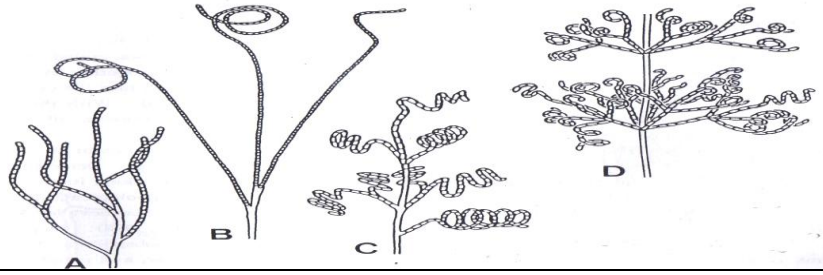


Figure 2.3 Spore formations in actinobacteria (Midayoh *et al*., 1997).

2.4.2 Chemotaxonomic characterization of actinobacteria

DAP analysis by paper chromatography of whole-cell hydrolysates was used to differentiate between *Streptomyces* and *Norcardia* because of its similar morphology (Becker *et al*., 1964). Subsequently, various actinomycete genera were classified into four cell wall types based on their cell wall components (Becker *et al*., 1965). Subsequently, a simplified method of thin layer chromatography was proposed to identify the diaminopimelic acid in the cell walls of actinobacteria (Staneck and Roberts, 1974), which is a more simplified method with increased sensitivity and rapid development. The generic status of actinobacteria can be confirmed by its chemotaxonomic properties (Dietz and Currie, 1996). Actinobacteria can be distinguished within genus by determining its cell wall type, LL-DAP or *meso*-DAP. Determination of cell wall composition was further studied on *Micromonospora*, which contain meso-diaminopimelic acid (Kawamoto *et al*., 1981). Further identification of actinobacteria cell wall diamino acids are listed at the Table 2.1.

Table 2.1 Identification of diaminopimelic acid in cell wall of actinobacteria

Genus	**Cell wall diamino acids**
Intrasporangium spp., *Kineosporia* spp., *Kitasatosporia* spp., *Nocardioides* spp., *Streptomyces* spp., *Streptoverticillium* spp., *Sporichthya* spp.	LL-DAP
Actinoplanes spp., *Actinosynnema* spp., *Actinopolyspora* spp., *Ampullariella* spp., *Amycolata* spp., *Amycolatopsis* spp., *Catellatospora* spp., *Dactylsporangium* spp., *Dermatophilus* spp., *Faenia* spp., *Frankia* spp., *Geodermatophilus* spp., *Glycomyces* spp., *Kitasatosporia* spp., *Kibdelosporangium* spp., *Micromonospora* spp., *Microbispora* spp., *Microtetraspora* spp., *Nocardia* spp., *Nocardiopsis* spp., *Pilimelia* spp., *Pseudonocardia* spp., *Planobispora* spp., *Planomonospora* spp., *Rhodococcus* spp., *Streptoalloteichus* spp., *Spirillospora* spp., *Streptosporangium* spp., *Saccharothrix* spp., *Saccharomonospora* spp., *Saccharopolyspora* spp., *Thermoactinomyces* spp., *Thermomonospora* spp.	*meso*-DAP
Actinoplanes spp., *Actinomadura* spp., *Oerskovia* spp., *Promicromonospora* spp.	No DAP

(Williams *et al*., 1989)

2.4.3 Screening of antimicrobial activities of actinobacteria

Actinobacteria are known as the major constituents in most soils and counts of over 1 million per gram are commonly obtained. The soil is considered as the most productive source of isolates, and many are found to produce antibiotics (Goodfellow, 1983) and secondary metabolites (Groth *et al*., 1996). Screening of antimicrobial activities of actinobacteria has been extensively undergone previously to identify their antimicrobial properties as they produce diverse secondary metabolites. Natural compounds produced by bacteria, especially from actinobacteria have been extensively used to produce most of the antibacterial drugs over the previous 50 years (Ginolhac *et al*., 2004).

Antimicrobial activities of *Streptomyces* sp. isolated from Brazilian tropical forest soil were performed using crude supernatant of the culture, extracts and fractions obtained by purification with a silica gel column. The actinomycete showed better antifungal activity compared to the antibacterial activity (Sacramento *et al*., 2004). Isolated actinomycetales from previous studies showed 48% antibacterial and 8% antifungal activity using diffusion method on Mueller-Hinton medium (Kitouni *et al*., 2005). Thakur *et al*. (2007) screened and studied *Streptomyces* strains by spot inoculation method and 65 strains showed promising antibacterial and antifungal activity. The active isolates were then subjected to submerged culture while 52 strains were only found to exhibit antibacterial activity in broth culture. Results showed that actinomycete isolates may not show antimicrobial activity on secondary screening.

The importances of actinobacteria lead to research on the evaluation of antimicrobial activity. Actinobacteria isolated from Philippines, Spain, Switzerland, Costa Rica, Sri Lanka and Mexico was tested for antimicrobial activity. In overall, *Streptomyces* group produce the highest number of antibacterial activities, which were 77% while 49% antibacterial activity was detected in non-*Streptomyces* species (Basilio

et al., 2003). Thus, microbial natural products screening are important in discovering novel bioactive and therapeutic chemicals (Nichols *et al*., 2002).

Studies on the antibacterial properties of actinobacteria were conducted and they contain active components which can be developed in the pharmaceutical era (Nedialkova and Naidenova, 2005; Moncheva *et al*., 2002). *Streptomyces* sp. which readily produces secondary metabolites are readily isolated from most aerobic soil and some aquatic environments in Antarctica (Nichols *et al*., 2002) leads to an interest in exploiting actinobacteria in the Antarctic regions. Investigation for antimicrobial activity of isolated actinobacteria strains from Antarctica against Gram-positive, Gram-negative bacteria and yeasts revealed as promising producers of antibacterial substances (Moncheva *et al*., 2002; Nedialkova and Naidenova, 2005). Reports also showed the ability of Antarctic actinobacteria strains to conduct keratinolytic enzymes (Gushterova *et al*., 2005) and produce cold adapted α-amylase (Zhang and Zeng, 2008) has proven that Antarctic is a presents valuable environment for biotechnological research.

2.4.4 Molecular characterization of actinobacteria

DNA extraction is a useful tool to identify the genetic composition of organisms. Soil DNA extraction enables to study the diversity of soil microorganisms (Clegg *et al*., 2005; Griffiths *et al*., 2000). Stach and co-workers (2003) proposed actinobacteria specific primers which had a perfect match with 82% of genera in the class actinobacteria, thus showing that the primers are appropriate for the detection of actinobacterial diversity. The specific primers were used to improve the detection and identification of actinobacteria, either those in culture or those represented in 16S rDNA clone libraries derived from DNA extracted from environmental samples (Stach *et al*., 2003). The availability of these actinobacterial primers can rapidly confirm the presence

of actinobacteria in environmental samples and even identify actinobacteria to its specific taxa (Stach *et al*., 2003).

2.4.4.1 Amplified Ribosomal DNA Restriction Analysis (ARDRA)

ARDRA analysis performed on variety of microorganisms such as the genera of *Clostridium* (Gurtler *et al*., 1991), *Bacillus, Paenibacillus*, *Bordetella, Alcaligenes* (Heyndrickx, 1996) were proven to be useful to distinguish different bacterial genus. Rapid identification and differentiation of *Mycobacteria* species was reported (Telenti *et al*., 1993). Subsequently ARDRA method was performed by Yoon *et al.* (1997) to identify *Saccharomonospora* strains. Similar approach has been carried out in identifying clinically significant aerobic actinobacteria species ranging from *Actinomadura, Gordonia, Nocardia, Rhodococcus, Saccharomonospora*, *Saccharopolyspora*, *Streptomyces* and *Tsukamurella* (Steingrube *et al*., 1997; Laurent *et al*., 1999, Wilson *et al*., 1998; Harvey *et al*., 2001).

Streptomyces, non-*Streptomyces* strains and rare genera of actinobacteria which exhibit similar colony morphologies were easily identified and differentiated by ARDRA could be identified to genus level by using four restriction enzymes (Cook and Meyers, 2003). ARDRA technique was reported as an effective, rapid and inexpensive technique (Cook and Meyers, 2003; Jiang *et al*., 2008; Zhang *et al*., 2006). ARDRA method had also been used for the classification and rapid identification of actinobacteria isolated from marine regions (Li and Liu, 2006; Zhang *et al*., 2006; Jiang *et al*., 2008). Zhang *et al*. (2006) used *Hha*I (5'-GATC3-') to digest the actinobacteria and they were able to effectively distinguish the groups of actinobacteria by the usage of *Hha*I while Jiang *et al*. (2008) used two restriction endonucleases, *Taq*I (5'-CCGG-3') and *Msp*I (5'-TCGA-3') to generate different actinobacterial genus. In this study, ARDRA method was employed to identify the isolated actinobacteria from Signy Island,

Antarctica which allowed unknown, non-*Streptomyces* soil isolates to be identified up to its genera.

2.4.3.2 Nonribosomal peptide synthetases (NRPS) genes

Microorganisms are the largest reservoir of potentially valuable natural compounds, such as polyketides, nonribosomal peptides, and alkaloids (Zhao *et al*., 2008). Many secondary metabolites with antimicrobial activity produced by actinomycete bacteria are synthesized by non-ribosomal peptide synthetases (NRPS) pathways (Bredholdt *et al*., 2007).

Nonribosomal peptide synthesis is carried out by nonribosomal peptide synthetases (NRPS) (Schwarzer *et al*., 2003). Nonribosomal peptide synthetases (NRPS) produce biologically active peptide compounds which contribute to the medical and agricultural field for example antibiotics, antifungals, antitumor agents and immunosuppressive agents (Ayuso-Sacido and Genilloud, 2005). Antibiotics such as balhimycin (Recktenwald *et al*., 2002), actinomycin D and cyclosporine A (Hahn and Stachelhaus, 2004) are synthesized by nonribosomal peptide synthetases.

Nonribosomal peptide synthetases (NRPS) were described to function in production of secondary metabolites in actinomycetes (Ayuso *et al*., 2005). Screening of NRPS is an important method to discover useful secondary metabolites or the production of natural products (Pathom-aree *et al*., 2006). Actinobacteria and Cyanobacteria are examples of producers of nonribosomal peptide (Dittmann, 2001; Zhang *et al*., 2008). Direct usage of nonribosomal peptide synthetases (NRPS) genes improved the screening for bioactive compounds in cyanobacteria (Dittman, 2001). One example is microcystin, a cyanobacterial hepatotoxin, was the first metabolite whose nonribosomal biosyntheis could be confirmed by knock-out mutagenesis (Dittman, 2001). The recognition of the diversity of NRPS in the environment is important for

future drug discovery and combinatorial biosynthesis efforts (Zhao *et al*., 2008). NRPS reveals the importance of producing secondary metabolites. Therefore, new primers of NRPS for actinomycetes were introduced (Ayuso-Sacido and Genilloud, 2005).

2.5 Importance of actinobacteria in healthcare industry

2.5.1 Antimicrobial and antitumor compounds

In 1955-1962, about 80% of the antibiotics were originated from actinobacteria which were patented and marketed every year (Labeda, 1990). Now, antibiotic derived from actinobacteria were increased to 90% in the market (Hamaki *et al*., 2005) in which most of the antibiotics produced were derived from the genus *Streptomyces* and *Streptoverticillium* (table 2.2). Since the discovery of streptomycin, there have been continued efforts towards the screening of novel antimicrobial compounds from the genus *Streptomyces* (Labeda, 1990; Watve *et al*., 2001). Munumbicins, a wide-spectrum antibiotics showed activity against methicillin-resistant *Staphylococcus aureus*, *Bacillus anthracis*, multidrug-resistant *S. a.*, multidrug-resistant *Mycobacterium tuberculosis*, malarial parasite *Plasmodium falciparum*, plant-pathogenic bacteria *Pseudomonas syringae* and various plant pathogenic fungi produced by *Streptomyces* NRRL 20562 was discovered by Castillo *et al*. (2002). A more recent discovery was a new antibiotic - Frigocyclinone produced by *Streptomyces griseus* strain from Antarctica (Bruntner *et al*., 2005).

Studies on rare genera of *Actinomadura, Actinoplanes, Amycolatopsis, Dactylosporangium, Kibdelosporangium, Microbispora, Micromonospora, Planobispora Streptosporangium and Planomonospora* were exploited and focused on the production of antibiotics (Table 2.2) (Lazzarini *et al*., 2000) which are promising sources in search for new drugs and might represent a unique potential for producing biologically active compounds (Bredholdt *et al*., 2007; Baltz, 2006). Bioactive

compounds have also been isolated in *Amycolaptosis* and *Actinomadura* species are were capable of producing vancomycin-type glycopeptide (Moncheva *et al*., 2002). Kigamicins, a new antitumor compound produced by *Amycolaptosis* sp. were able to inhibit the growth of various mouse tumor cell lines (Kunimoto *et al*., 2003). Production of Benanomicin and pradimicin by *Actinomadura* exhibit antifungal properties which prevent the fungal cell wall development (Debono and Gordee, 1994).

Compounds produced by actinobacteria are also used in agriculture mainly as pesticides, plant protecting agents and food additives (Moncheva *et al*., 2002). Actinobacteria was also known to contribute significantly to the turnover of complex biopolymers, such as hemicellulose, pectin, keratin, and chitin (Li and Liu, 2006). Actinobacteria could also be considered as one of the major cellulose decomposers in composting processes (Lamot and Voets, 1976).

Table 2.2 Examples of antibiotics produced by actinobacteria

Organisms	**Antibiotics**
Streptomyces spp.	Lipomycins, tetracenomycin D1, chromomycin A3, enterocin, Actiphenol, Maltophilin, Saphenic acid, 1-N-Methyl-(E,Z)-albonoursin, 1-6, dihydroxyphenazine, Pyridindolol, Elalomycin, Ankinomycin, Antimycin A Avermectin B_{1a}, Bestation, Bialaphos, ,Bleomycin A_2, Cyclotialidine, Cycolserine, Deoxylaidlomycin, Erythromycin, Esperamicin A_1, Globomycin, Helvecardins A and B, Herbimycins A, B, and C, , Kanamycin, Kasugamycin, Leucomycin A, Pyrrolomycin A, Pyrrolomycin B, Pyrrolostatin, Triacsin A, Tylosin A, Validamycin A, Sarkomycin, Streptomycin
Micromonospora spp.	Ikarugamycin, Astromicin,Mycinamicin II,
Saccharospora spp.	Hatomamicin A,
Actinoplanes spp.	Isohematic acid, Mycoplanecin A, Neplanocin A, 6-Thioguanosine,
Microbispora spp.	Propeptin
Actinomadura spp.	Thiazohalostatin
Amycolatopsis spp.	Vancomycin
Streptosporangium spp.	Sinefungin

(Fiedler *et al*., 2005, Midayoh *et al*., 1997)