Chapter 4

Discussion

4.1. Occurrence of soil microfungi in Maritime Antarctica

All locations samples yielded population of microfungi species recorded from Deception Island, Wilhelmina Bay and Yankee Bay. Deception Island was more diverse than others by harboring 13 fungal species, followed by Wilhelmina Bay harboring 8, then Yankee Bay harboring 6 fungal species. However, the suggestion of Malosso *et al.* 2006, "The environment is still too severe to support a diverse biota". And the study of Fell *et al.* (2006), where stated the number of micro-eukaryotes in Dry Valley soils are low compared to the other habitats. Nevertheless, this study cannot confirm the limited population number; since the potato dextrose agar (PDA) was the only isolating media, in addition to the uncultivable strains.

This work represents the occurrence of *Aspergillus*, *Mucor*, *Geomyces* and yeast species, were frequently recorded genera in Antarctic environment (Vishaniac, 1996). However, the majority of fungal species appeared as single colony on the plate surface. Ascomycete species were recorded to have higher frequency of occurrence in Maritime Antarctica followed by *Zygomycete*. For instant, *Ascomycetes* sp.4 and *Ascomycetes* sp.6 possessed 42.85% as the higher frequencies among the isolates of Maritime Antarctica. Moreover, it is generally accepted that Maritime Antarctica is known to be more diverse in terms of species richness compared to continental Antarctica, but yet less diversity than temperate regions (Wynn-Williams, 1996).

Conversely, since the incubation temperature known to be amongst the important factors in isolation of fungi, effort has to be made to incubate in deferent temperatures and this study significantly observed that. The present work indicate 15 out of the 27 fungal isolates were psychrophilic, followed by 9 mesophilic species then psychrotrophic comes third by only 3 species. Thus, psychrophiles are more abundant in Maritime Antarctica than psychrotolerants fungi. This results is supported by the study of Tosi *et al.* (2002), reported the majority of isolated species were psychrophilic. In contrast, this is totally opposite to many studies signifying that Antarctic microfungi are mostly psychrotolerant and less are psychrophilic (Onofri, *et al.* 2004), also Robinson (2001), suggest that the predominance of psychrotrophy, rather than psychrophily, in Antarctic environments. This is perhaps due to type of culture media or the sample collection time, which is seems to bias the frequency of isolation according to season of soil collection (Robinson, 2001).

Effort has been made to incubate the volcanic soil of Deception Island in 50°C to screen the thermophilic microfungi diversity. Unfortunately, there was no successful isolation of microfungi from Deception Island. However, to date there is no record of thermophiles microfungi form Deception Island available for comparison.

Generally, to date there is no published work studied the occurrence of microfungi in the soil of those Bays and Deception Island. However, the use of molecular techniques in Malosso *et al.* (2006) study on Fossil Bluff and Jane Col Maritime Antarctica, improved the characterization of microbial communities providing access to as yet uncultured microorganisms. Among those identified by Malosso *et al.* (2006) included species of yeasts *Cryptococcus* and *Rhodotorula,* filamentous fungi species such as *Geomyces, Penicillium, Cladosporium, Hypomyces, Raciborskiomyces, Aureobasidium,*

Discosphaerina, Pseudogymnoa, Ascozonus, Leotialubrica, Alternaria Ulocladium, Naohidea, Mucor and *Mortierella*.

Then again, it is difficult to detail on the fungal diversity based on one method, i.e identification based on morphology characterstic (Vishaniac, 1996 and Malosso, *et al.* 2006). In the present study, identification method relies solely on the microscopic morphology features of conidia, plate colony features and pigment production, and optimum growth temperature.

It is not easy to say those isolates are indigenous; because the biases of the conventional isolation method, since soil plating method cannot differentiate between indigenous and alien, count spores rather than hyphae and spores readily air-borne. In this study, the fungal isolates can be considered as indigenous, and this is supported by the fact that most of them are psychrophilic. Our recommendations for future work are to complete the identification of those isolates through molecular techniques (e.g. metagenomic), use of more than one culture media for cultivation of Antarctic fungi and well preserve of isolates for future biotechnological screening.

4.2. Antimicrobial activity of maritime antarctic microfungi

In the preliminary study, maritime Antarctic fungi were found to possess a variety of broad spectrum antibacterial potential (against negative and positive Gram bacteria), yet no antifungal potential possessed, and the majority exhibited weak to moderate activity. However, Deception Island isolates recorded the largest number of species with the antimicrobial activity, followed by Wilhelmina bay, then Yankee bay lastly. Again these sorting may due to the population number in each site. Moreover, such screening method was used by Nedialkova and Naidenova (2005), where 60% of antarctic isolates was found to exhibited antimicrobial activity against four test microorganisms. Plug assay method or Agar block method, has been used, as rapid test, for initial selection of fungi possess antimicrobial activity for further studies.

During the Disc diffusion method, 5 species (out of 10 species with good antimicrobial potential from the preliminary result) were observed to lose totally or partially their inhibition potential. This is perhaps due to inappropriate fermentation circumstances as many factors may affect such batch fermentation, such as growth medium, temperature, incubation time, aeration, or even inoculums size, or could be due to unextractable compounds with high polarity or sensitivity of metabolites to solvent evaporation temperature. Also such results has been reported by other scientists (Nedialkova and Naidenova, 2005; Moncheva, *et al.* 2002).

Use of Dimethyl Sulfoxoid (DMSO) to dissolve the crude extract was confirmed to not have any antimicrobial activity as used as negative control and did not show any activity among the eight test microorganisms. However, during the disc diffusion method all the 5 ethyl extracts found to exhibit wide range from weak, moderate to excellent

antibacterial activity from 8-28mm in diameter zone of inhibition. Compared to the antibiotic chloramphenicol they were significantly weaker on *B. cereus*, *B. subtilis*, *S. aureus* and *E. coli*. Whilst on *P. aeruginosa* they were very close to chloramphenicol activity except *Hyphomycetes* sp.8 was better than Chloramphenicol activity. On the other hand, the antifungal activity was only recorded by *Hyphomycetes* sp.8 against *S. cerevisiae* with 17mm inhibition zone. Even Chloramphenicol possessed no antifungal activity up on all yeast species. In general, *Hyphomycetes* sp.8 observed to be the best among the fungal extract with broad spectrum antimicrobial activities and could be used in antibiotic development.

Comparing the others scientist results, our extracts seems to possess higher activity than Antarctic *Actinomycetes* of Nedialkova and Naidenova (2005) when performed against *P. aeruginosa*, *E. coli* and *S. cerevisiae*. While relatively lower compared to results obtained against *B. subtilis* and *S. aureus*. However, the study of Corte *et al.* (2000) found that there is no differences between the antibacterial activity of antarctic *Penicillium* sp. compared to the none antarctic *Penicillium* sp.

Unsurprisingly, when compared the our results of disc diffusion method to agar block method, some extracts showed activity on bacterial strains was not observed in plug assay, and some were weakly active in the initial screening showed better activity in disc diffusion method. For example, *Aspergillus fumigatus* was observed to produce limited activity only on *B. subtilis* during the initial screening. On the contrary, when disc diffusion method applied, showed moderate to good activities on all the bacterial strains confirming the necessarily of using disc diffusion method in the qualitative screening.

Disc diffusion is known to be only qualitative assay with limitations that cannot determine the Minimum inhibitory concentration (MIC), in addition to do not distinguish between the bacteriostatic and bactericidal effects (Ncube, *et al* 2008). Therefore, Micro-titer plate or broth microdilution method was performed as offer to determined MICs of large number of sample against variety of test microorganisms with reproducible results. Beside could easily subculture to determine the MBC or MFC (Ncube, *et al* 2008).

In the present study, the MICs and MBCs of fungal extracts wherever they active on test microorganisms. Generally, MICs values were varied among the extracts from 3.13-12.5mg/ml against *E.coli*, 1.56-6.25mg/ml on *B.subtilis* and *P.aeruginosa*, and 0.78-3.13mg/ml on *B.cereus.* Whilst the lowers MICs recorded 0.78-1.56mg/ml was on *S.aureus*. However, low MIC value does not correlate to high activity on disc diffusion assay (Ncube *et al.* 2008). Furthermore, 50% of MBC values were observed to have same values of MIC. These results explained that, most of fungal extracts possessed relatively low lethal dose. Again comparing to others results, Paudel *et al.* (2008) studied the MICs values of 5 Antarctic lichens against Gram-positive pathogens. The MIC against *B. subtilis* was recorded between 0.037 – 0.954 mg/ml whereas against *S. aureus* was recorded between 0.069 - >1 mg/ml.

All in all, there were a few problems encountered with bioactivity assays compared to the biodiversity studies, such as mentioned above of residue extraction method was tedious and time consuming, beside the obtained crude was very low in amount. Moreover, we proposed to do further studies on the four extracts to determine the bioactive compounds using HPLC after study their cytotoxicity up on tissue culture cells.

Ultimately, the following recommendations could be useful for the future of such research:

- Use of more than one isolation method, will probably provide more large and diverse fungi.
- Better if use another, not tedious, method to get the fungal extract. And consider the use of more than one solvent.
- When enough extract available, well diffusion method will be an advance to evaluate the bioactivity.
- Purify and identify the bioactive compound of *Hyphomycetes* sp.8 extract, may provide good novel bioactive compound.
- Test the isolates for other biotechnological valuable metabolites that may produce, such as enzymes.