CHAPTER 5.0: DISCUSSION

5.1 Chemical analysis of soil and sediment samples

The values of pH (ranging from moderately acidic to moderately alkaline) varied between samples (Table 4.1, page 39). Beach, melt lake and marine samples were moderately alkaline while inland lake bank samples were slightly alkaline with an average pH 7.85. Lindstrom *et al*. (2005) had also reported the similar alkaline condition (pH 7.9 to pH 8.3) in Ny-Ålesund lakes in their study.

Terrestrial samples were slightly acidic to alkaline (pH 6.2 to pH 8.0) while periglacier samples were acidic to neutral (pH 5.9 to pH 7.3). Similar finding of acidic environment in soil samples collected from southwest Spitsbergen, Svalbard were also reported by Kastovska *et al*. (2007). Waita *et al*. (2005) had reported pH of samples from bird islands was significantly lower than samples from non-bird islands that due probably to soil acidification process by birds' guano. As Ny-Ålesund is inhabited by various animals (such as barnacle geese, eider ducks, terns, kittiwakes and reindeers), the acidic condition in samples studied might be due to the mineralization processes that acting on output of animals (such as guano) to produce nitric and sulphuric acids, as explained by Bolter *et al*. (1997) in their samples studied collected from King George Island, Antarctica. Simas *et al*. (2007) also reported that guano deposition by skuas had promoted soil acidification in Maritime Antarctica.

As for marine samples, increases in pH were found with the increase of the sediment depth. This indicates that a lower depth of marine sediment might have a higher pH value. Similar finding on higher pH in lower depth of soil in a studied site at Krasnoyarsk, Siberia was also reported (Menyailo *et al*., 2002).

The values of salinity (EC value ranging from 1.1 μS/cm to 1705.0 μS/cm) varied between samples (Table 4.1, page 39). The salinity of marine samples was found to be higher than terrestrial, inland lake bank, melt lake, beach and periglacier samples.

5.2 Extraction of genomic DNA from soils and sediments

The yield of genomic DNA extracted from the soil and sediment samples differs for all studied sites (Table 4.2, page 41). This might be due to varying amounts of plant DNA, animal DNA and microbial DNA that were found in different soil and sediment samples (Kastovska *et al*., 2007). However, the value of DNA yield could not indicate the abundance of microbial community (number of microorganisms) or the information on the bacterial diversity (different types of microorganisms) in the samples. This might be due to the genomic DNA extracted from samples has included DNA from plants and animals in the habitat (Kastovska *et al*., 2007).

The genomic DNA extracted from some marine samples have less protein contamination (A_{260}:A_{280} > 1.70) compared to samples from other studied sites. This might be due to the low input of animal droppings in marine samples, as compared to that of land samples. More animals were found on Ny-Ålesund land (such as birds, polar bears and foxes), and therefore there is a higher input of animal droppings, which increase the protein contamination in land samples. Bokhorst *et al*. (2007) had reported the potential importance of nutrient input from birds into terrestrial samples collected from Falkland Island and Maritime Antarctica. Higher nutrient input in soils from bird islands than to that of soils from non-bird islands was also reported (Waita *et al*., 2005).

Although the Ultra Clean Soil DNA Isolation Kit (MO Bio Inc., CA) that was used to extract the genomic DNA from soil and sediment samples has been reported to give a

pure and better DNA (Maarit Niemi *et al*., 2001), the purity of extracted genomic DNA from all samples were poor, with humic acid contamination (A_{260}:A_{230} < 2.0).

5.3 Amplification of 16S rRNA gene fragments

In this study, the presence of humic acid in extracted genomic DNA ($A_{260/230}$ = 0.7 < 2) was reduced ($A_{260/230}$ = 2.5 > 2) with a 10-fold dilution of DNA template in PCR (Table 4.3, page 41), resulting in a clearer DGGE banding pattern (Figure 4.4, page 48). This may be due to the presence of humic acid that was co-extracted with the DNA could inhibit the reaction of *Taq* DNA polymerase in PCR, as reported by Tsai and Olson (1992). Since DGGE is biased to PCR amplification, it is important to dilute the DNA template in PCR, in order to reduce the inhibitory effect of humic acid, and therefore get a better DGGE banding profile. The dilution of DNA template to reduce the humic acid in the samples and overcome the inhibitory problem in PCR was also suggested by Nakatsu (2007).

A nested PCR: primary PCR and secondary PCR were conducted in this study (Chapter 3.2.4, page 27) to amplify the 16S rRNA gene fragments to achieve stronger PCR amplicons that could separate during DGGE, as recommended by Muyzer *et al*. (1993). A GC-rich clamp (341F-GC) that was used in secondary PCR stabilized the migration fragments of PCR amplicons during DGGE, in order to produce sharp bands on DGGE profile and to prevent complete strand separation during DGGE (Sheffield *et al*., 1989). The samples were also duplicated during PCR reaction to confirm reproducibility of banding patterns in DGGE profiles, as suggested by Pearce (2003).

In primary PCR amplicons, the faint intensity of bands in samples 8, 9 and 32 might have been due to a lower amount of bacterial DNA in the sample, while the bright intensity of band in sample 3 might indicating the sample has a higher amount of bacterial DNA. In

secondary PCR amplicons, the fragments observed being larger than 600 bp might have been due to the non-specific binding of primary products during secondary PCR and DNA smear. The non-specific binding of primary product in secondary PCR probably due to the PCR conditions were not optimized and were interfere by sample inhibitors (high humic acid content), as reported by Tebbe and Vahjen (1993). The inhibitors in samples might inhibit the reaction of *Taq* DNA polymerase during PCR (Tsai and Olson, 1992; Neef *et al*., 1998), and hence decrease efficiencies in DNA-DNA hybridizations (Maidak *et al*., 1996). Besides that, Wintzingerode *et al*. (1997) also reported that handling skill (too harsh) during DNA extraction could cause DNA degradation and lead to DNA smear in PCR products. However, the non-specific binding of primary product and DNA smear did not have any significant influences on the DGGE banding patterns (Figures 4.3a and b, page 47).

5.4 Statistical analysis of DGGE profile to compare bacterial diversity between samples from the eighteen studied sites

A total of thirty-seven DGGE banding positions were detected from all sampling sites (Table 4.4, page 54; Figure 5.1, page 76), and the identities of twenty-eight excised bands were determined (Table 4.7, page 64). Muyzer *et al*. (1995) reported that theoretically each distinct DGGE band at a specific position represents a different bacterial species. Therefore, band 17 (banding position 21) that showed 100 % homology to an uncultured *Polaromonas* sp. was also prevalent in terrestrial (samples 3, 9 and 34) and periglacier samples (sample 33) that have the same banding position, although it was excised and determined from tundra terrestrial sample (sample 17).

In the DGGE profile, the related species or same phylum were observed in different DGGE banding position. Two distinct bands (banding positions 22 and 34) showed 97 %

homology to an uncultured *Fusobacteria* bacterium, indicating that the theory of each distinct DGGE band that at a specific position represents a different bacterial species (Muyzer *et al*., 1995) might not be true. Jackson *et al*. (2001) also reported an rDNA fragment of closely related bacteria might produce two separate bands in DGGE profile. This phenomenon might be due to the natural microheterogeneity in the DNA sequence during DGGE, as reported by Van Hannen *et al*. (1998), Speksnijder *et al*. (2001) and Sekiguchi *et al*. (2001). Therefore, if the samples show different DGGE banding patterns, the bacterial communities in the samples are undeniably different. However, if they show a similar banding pattern, then they may or may not contain similar bacterial communities. Thus, further analysis such as sequence analysis and phylogenetic analysis were conducted in this study, as suggested by Jackson *et al*. (2000).

Artificial bands were observed near M2 in the DGGE profiles (Figures 4.5a, b and c, pages 50-52). However, no sequence was successfully obtained from these bands. Ohkubo *et al*. (2006) also reported similar artificial bands in their DGGE profiles and these bands were probably migrated independently due to both the concentration of the denaturant and the voltage in DGGE.

The DGGE band intensity was indicated to represent the abundance of the bacterial population (Muyzer *et al*., 1995). However, the DGGE bands 1, 10, 13, 20 and 22 with brighter intensity could not be explained by the higher abundance of bacterial population in the sample. This is because this study was not dependent on quantitative variable. Appropriate statistical analysis need to be conducted, in order to calculate the abundance of each DGGE band, as reported by Fromin (2002).

In the presence/absence binary matrix (Table 4.4, page 54), some of the samples might have the same number of detected DGGE bands. For instance, a total of 20 bands were detected in marine (samples 25, 26 and 28) and melt lake (sample 39) samples,

indicating that the number of bacterial species (species richness) existing in the marine samples is equivalent to that in melt lake sample. However, the similarity in the number of bacteria that exists in the samples does not represent the similarity in bacterial diversity (Chong *et al*., 2009b). For example, a beach sample (sample 1) that produces two DGGE bands (banding positions 2 and 11) related to a *Bacteroidetes* in DGGE profile will not showed any apparent difference (in terms of diversity index) to a melt lake sample (sample 39) that produces two DGGE bands (banding positions 33 and 37) related to *Betaproteobacteria*.

Since the presence/absence binary matrix was transformed from DGGE profiles, the Shannon diversity index (*H′*) (Table 4.5, page 59), inferred from the DGGE presence/absence binary matrix might have the same indication as the total number of accumulated DGGE bands (species richness). For instance, the highest diversity (*H′* = 2.639) in terrestrial sample from tundra site (sample 17) was due to the highest total number of accumulated bands in the DGGE profile, while the lowest diversity (*H′* = 1.792) in freshwater inland lake bank sample (sample 35) was due to the lowest total number of accumulated bands in the DGGE profile. Hence, there is a limitation in interpretation of *H′* from presence-absence binary data, due to the inability to differentiate between samples with the same number of bands (Gafan *et al*., 2005). Besides that, *H′* does not correlate with the estimation of genetic distance. Chong *et al*. (2009b) also reported a studied site with the lowest *H′* showing the greatest genetic distance, compared to that with the highest *H′* showing only moderately genetic distance. Hence, in future studies, the analysis of similarity (ANOSIM) that could indicate a significant difference of DGGE bands across all sites and of the dominant bacterial community structure in all the studied locations, should be carried out to supplement *H′* (Chong *et al*., 2009b).

According to Kowalchuk *et al*. (2006), the banding pattern in DGGE profile is more accurately considered as the "structure of dominant bacterial populations" rather than a general measure of bacterial diversity, due to the fact that only numerically abundant bacteria will be detected (Nakatsu, 2007). Therefore, H' was used here to allow a coarse comparison of "dominant species richness" between samples studied. The highest H' value shown in the tundra terrestrial sample (sample 17) indicated the greatest bacterial species richness in tundra. The result was congruent to the studies conducted by Zhou *et al*. (1997) and Fierer and Jackson (2006) that reported high bacterial diversity was observed in tundra samples compared to other studied sites. Where as, the lowest H' shown in the freshwater inland lake bank sample indicated the least bacterial species richness in the sample. This might be due to the sample collected was mainly pebbles, and might not contain high microbial content compared to those of samples collected from other studied sites.

There was an increase of bacterial diversity ($H' = 2.197$ to $H' = 2.485$) in marine samples from shallower sediments (sample 24) to deeper sediments (sample 30), as shown by the number of bands (Table 4.5, page 59), indicating higher numbers of bacteria were found in deeper sediments. This is in a contrast to the theory stating that higher productivity of prokaryotes population exists in shallower waters, instead of in deeper waters, as reported by Parkes *et al*. (2000), Llobet Brossa *et al*. (1998) and Sahm *et al*. (1998).

The nMDS plots (Figure 4.6, page 57) and Hierarchical cluster analysis (Figure 4.7, page 58) of DGGE profiles were carried out to compare bacterial diversity between different studied sites. The samples were eventually clustered into two groups (marine and non-marine samples) and showed that the bacterial diversity in marine samples appeared differently to the non-marine (land) samples. Where as, the non-marine samples were clustered together with around 30 % similarity, suggesting that there might be a shifting of samples by an occasional movement of animals in the studied sites. In other words, animals

might carry some soils and sediments on their body to another place when they travel from one place to another. Chong *et al*. (2009a) had reported the similarity in bacterial community of two studied sites in Antarctica caused by occasional movement of penguins.

Furthermore, a trend of diversity overlap between samples from close vicinity was observed. For instance, highest similarity (> 90 %) was detected between beach soil (sample 1) and marine sediment (sample 24). This is expected because the beach sample was taken from the harbour, which is located just beside the sea. It is possible that either some of the beach samples were flushed out to the sea, or vice versus, thus influencing the bacterial composition in both sites. Zhang (2004) also reported the ocean basins might be closely correlated to many environmental factors such as terrestrial inputs from rivers, distance from the shore, circulation, topographical features and sea-ice transport.

The diversity of the melt lake sediment (sample 39) was approximately 40% similar to periglacier soils (samples 32 and 33). This perhaps supported the notion that local environmental influence played an important role in structuring the soil bacterial composition (Coleman and Whitman, 2005; Remenant *et al*., 2009; Chong *et al*. 2009b). Mindl *et al*. (2007) also reported that the runoff of glacier water during summer thawed having significant effect to the microbial community of a nearby lake.

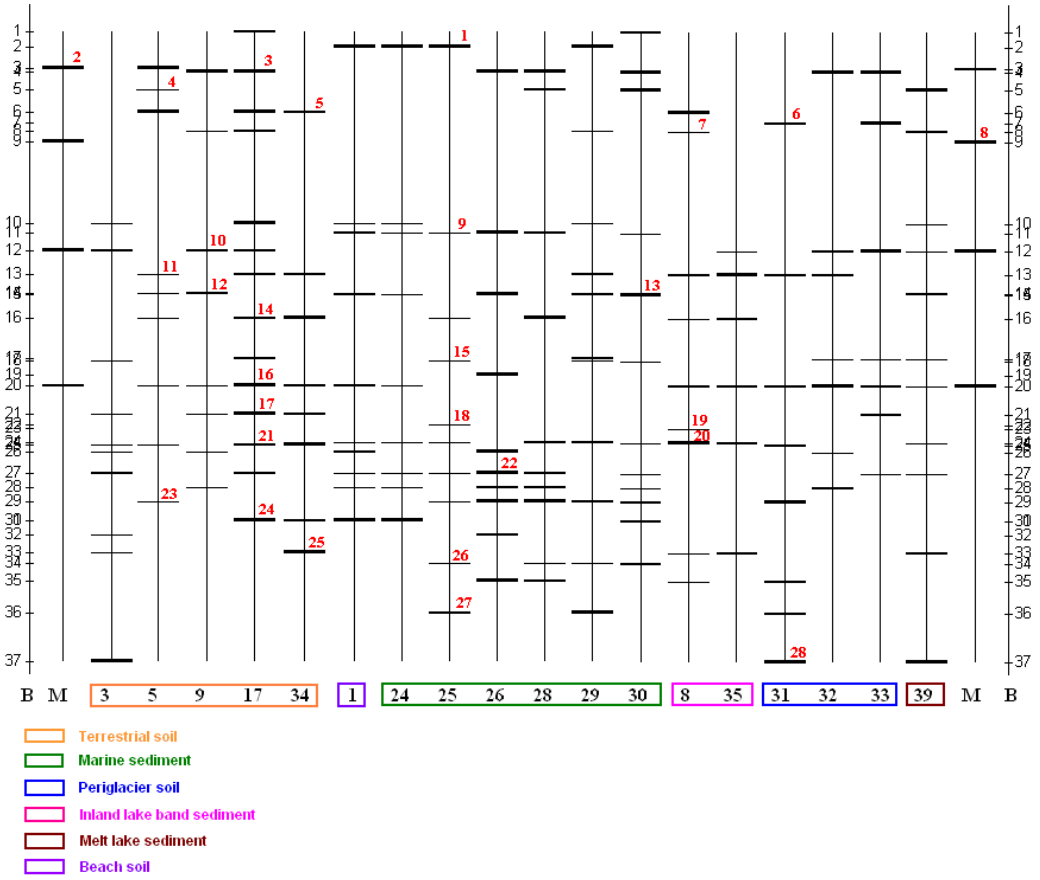

Figure 5.1: The schematic picture of DGGE profile for all samples studied in Ny-Ålesund. B refers to the banding position. M refers to bands with distinct melting position from the previous DGGE run as markers on the gel. Numbers (in red) against a band refer to well-defined bands excised for sequencing.

5.5 Statistical analysis of DGGE profile to correlate bacterial diversity with environmental variables

Spearman rank order correlation showed significant correlations (P = 0.001) between conductivity (r = 0.470) and pH (r = 0.294) with the bacterial diversity (Table 4.6, page 60). Environmental multiple variables (combination of pH and EC) showed higher correlation (r = 0.469) to the bacterial diversity compared to that of EC only. Similar findings of positive correlations between bacterial diversity with pH and salinity were also

reported by Yergeau *et al*. (2007), Giovannoni and Rappe (2000), Crump *et al*. (2004) and Lindstrom *et al*. (2005), due to their influences on the composition of microorganisms.

The pH of samples showed higher positive correlations (r = 0.470) with bacterial diversity. This indicates that pH played a more important factor in driving soil bacterial diversity, as reported by Fierer and Jackson (2006). Table 5.1 (page 78) showed a trend of increases in H' from slightly acidic samples (samples 9, 33 and 34; mean H' = 2.023) to slightly alkaline samples (samples 3, 5, 8, 17, 31, 32 and 35; mean H' = 2.107) and moderately alkaline samples (1, 24, 25, 26, 28, 29, 30 and 39; mean H' = 2.319). This observation suggesting that more bacteria prefer living in alkaline environment compared to acidic environment. This was congruent to the studies of Kim *et al*. (2007) that reported more bacteria could grow well in a range of pH 6.5 to 9.5. Similar finding of higher bacterial diversity in neutral soils and lower bacterial diversity in acidic soils were also reported by Fierer and Jackson (2006). Baath and Anderson (2003) indicated that lower bacterial diversity in acidic soils probably due to acidic soils favour fungal populations, and leading to increased competition between fungal and bacterial populations.

Out of 4 sequences that were related to *Bacteroidetes*, 83% of the sequences were found in more alkaline environment (samples 1, 3, 17, 24, 25, 26, 28, 29, 30, 32 and 39) whereas 17% of the sequences found in more acidic environment (samples 9 and 33). This indicates that *Bacteroidetes* were more prevalent in alkaline soils (Aislabie *et al.,* 2008).

According to Casamayor *et al*. (2002), a general trend of bacterial diversity clustering were observed when comparing microbial assemblage patterns from low salinity (4-15% salinity) solar saltern environment with high salinity (22-37% salinity) environment. The differences in salinity between terrestrial (ranged 72.7–506.0 μS/cm) and marine samples (ranged 1154-1705 μS/cm) might be the main factor contributing to the demarcating line between these two environments.

In order to study certain bacterial group favour a certain environment to live in, the pH value, that is one of the most distinct environmental factors, could act as a good tool to correlate with the bacterial diversity (Polz *et al.*, 2006). For better understanding, more soil chemical analysis, such as water, carbon, nitrogen and heavy metals (Chong *et al.*, 2009a), should be conducted for future studies, to give an indication of nutrient content as this could relate to the bacterial composition and density.

Table 5.1: Correlations of Shannon diversity index (*H'*) with samples pH and salinity (EC)

Sample	**pH**	**EC[a] (µS/cm)**	***H'*** **[b]**
35	7.9	273.3	1.792
33	5.9	50.6	1.946
32	7.2	323	1.946
31	7.3	1.1	1.946
9	6.2	506	2.061
34	6.4	95.6	2.061
5	7.5	156.1	2.079
8	7.8	130.4	2.166
3	7.9	116	2.181
24	8.1	1691	2.197
39	8.2	70.4	2.274
1	8.0	304	2.288
25	8.1	1696	2.303
26	8.1	1702	2.303
28	8.3	1154	2.303
29	8.2	1339	2.398
30	8.3	1705	2.485
17	7.9	72.7	2.639

[a] Electrical conductivity as measured in Table 4.1 (page 39)
[b] Shannon diversity index as measured in Table 4.5 (page 59)

5.6 16S rRNA gene fragment analysis and phylogenetic analysis to identify the bacteria represented by well-defined DGGE bands

The well-defined DGGE bands showed 71% to 100% similarities to a wide range of known sequences available in GenBank database (Table 4.7, pages 64-66). Out of 28 DGGE bands that were sequenced, four sequences each were related to *Bacteroidetes* and *β-proteobacteria*, two each were related to *Cyanobacteria*, *Firmicutes* and *Fusobacteria*, and one each were related to *Acidobacteria*, *ε-proteobacteria*, *δ-proteobacteria*, *Fibrobacteres* and *Nitrospira*. In additions, nine sequences were unable to classify into any known phylum.

According to Schloss and Handelsman (2005), ten sequences (bands 1, 3, 4, 8, 13, 14, 17, 18, 21 and 28) in this study that showed > 97 % homology to a known species in GenBank database were typically assigned to the same species, four sequences (bands 15, 19, 24 and 26) that showed > 95 % homology to a known sequence were typically assigned to the same genus, and thirteen sequences (bands 2, 5, 6, 7, 9, 10, 11, 12, 16, 20, 23, 25 and 27) that showed > 80 % homology to a known sequence were typically assigned to the same phylum (Table 4.7, page 64-66).

Sequences related to *Bacteroidetes* (banding positions 2, 4, 11 and 12) were found in all samples studied. Previous studies also showed that *Bacteroidetes* were found in a wide range of habitats, such as marine samples (Ravenschlag *et al.*, 2001; Crump *et al.*, 2004), terrestrial samples (Kobabe *et al.*, 2004; Nemergut *et al.*, 2005), glacier samples (Skidmore *et al.*, 2005) and melt lake samples (Larose *et al.*, 2010). *Bacteroidetes* that have been reported to possess the ability to degrade polymer (Aislabie *et al.*, 2008) were found in environmental samples that contain droppings of mammals (Dick and Field, 2004). This indicates that the samples studied might have a wide range of polymeric substances or animals' guano.

Interestingly, *Fibrobacteres* (banding position 20), also known as rumen bacteria were found in all samples studied. *Fibrobacteres* were once reported their occurrence in tundra terrestrial samples (Zhou *et al.,* 1997). Since Drake and Marcus (2007) had reported that earthworm gut could act as a transient habitat for soil microbial biomass. There might also be a shifting of bacterial community from the animals output to the samples studied.

Acidobacteria (banding position 30) were observed in terrestrial and beach soils. This result was similar to the finding of Nemergut *et al*. (2005) that reported *Acidobacteria* as the dominant taxa in Arctic tundra samples. Although Jones *et al*. (2009) reported *Acidobacteria* are commonly isolated from soils but not from sediments, surprisingly *Acidobacteria* were also found in marine sediments in this study. *Acidobacteria* were absent in melt lake sample and this was in contrast to the finding of Larose *et al*. (2010), that reported observation of *Acidobacteria* in the melt lake water samples.

As for *Proteobacteria* group in this study, *β-proteobacteria* (banding positions 7, 21, 33 and 37) were found in non-marine group samples but were not observed in marine group samples. While *ε-proteobacteria* (banding position 15) were found in marine samples but were not observed in non-marine samples. Similar findings of *β-proteobacteria* in the Siberian tundra terrestrial samples (Kobabe *et al.,* 2004) and Kolyma lowland tundra terrestrial samples (Zhou *et al.,* 1997) had also been reported.

β-proteobacteria that present in non-marine samples are organotrophic and aerobic bacteria that might specialized in the degradation of complex macromolecules on land (Holmes, 1999). The *β-proteobacteria* group (sequences related to *Comamonadaceae* bacterium, *Polaromonas* sp. and *Burkholderiales* bacterium) were known as ammonium oxidizer and were prevalent in non-marine samples (Woese *et al*., 1984). On the other hand, *ε-proteobacteria* that function in the sulphur cycle were prevalent only in marine samples (Nakagawa *et al*., 2005).

δ-proteobacteria (banding position 36) were found in periglacier and marine samples studied. The observation of *δ-proteobacteria* in marine samples might be due to the important role of *δ-proteobacteria* that act as sulphur oxidizer and sulphate reducer in the marine sulphur cycle (Ravenschlag *et al.,* 1999). Ravenschlag *et al*. (1999; 2001) also reported observation of *δ-proteobacteria* in Spitsbergen marine samples. Although *δ-proteobacteria* were prevalent in tundra terrestrial soil collected from the Kolyma lowland (Zhou *et al.,* 1997) and Arctic (Nemergut *et al.,* 2005), there was no sequence related to *δ-proteobacteria* in this study.

Although *Cyanobacteria* were not usually detected in the DGGE profiles of environmental samples from Antarctica (Muyzer, 1999; Pearce, 2003; Dorigo *et al.*, 2005; Chong *et al.*, 2009a), Vincent *et al*. (2000) had reported the non-marine ecosystems within Ny-Ålesund were dominated by *Cyanobacteria*. However, results showed that the occurrence of *Cyanobacteria* (banding positions 5 and 16) were found not only in non-marine samples (terrestrial, inland lake bank, melt lake) but also in marine samples. The finding of *Cyanobacteria* in terrestrial samples was in congruent to the finding of Friedmann (1982) that reported *Cyanobacteria* as the dominant in a broad spectrum of terrestrial habitats.

The observation of *Cyanobacteria* in terrestrial samples might be due to they are important oxyphototrophic organisms that inhabiting terrestrial ecosystems both in the Arctic and Antarctic, as reported by Kastovska *et al*. (2005). For instance, they play important roles in soil formation and stabilization against wind, increasing soil organic matter and nitrogen content, and preparing substrata for subsequent succession stages of microorganisms (Belnap and Lange, 2001; Kubeckova *et al.*, 2002).

In contrast to the finding of Lindstrom *et al*. (2005), that reported the absence of *Cyanobacteria* in the Ny-Ålesund lake samples collected in the year 1997, *Cyanobacteria*

were observed in Ny-Ålesund lake samples studied collected in the year 2006. Surprisingly, *Cyanobacteria* were not observed in periglacier samples studied, although Vincent *et al*. (2000) had reported the non-marine ecosystems in Ny-Ålesund were dominated by *Cyanobacteria*. This was probably due to periglacier samples studied containing lower pH that may not be suitable for the growth of *Cyanobacteria*. Bauld (1984) had also reported that *Cyanobacteria* were prevalent in alkaline environments.

Clostridia, in the phylum of *Firmicutes*, are anaerobic bacteria commonly isolated from Antarctic soils (Stackebrandt *et al.*, 2004). However, results showed that *Firmicutes* (banding positions 9 and 24) were prevalent in sediment samples (inland lake bank, melt lake and marine samples) but not in terrestrial soils. Previous study in Svalbard, Norway also showed that *Firmicutes* were found in melt lake sediments but were not observed in snow soils (Larose *et al.*, 2010).

Despite unclassified bacterial (26.53 %), *Bacteroidetes* were dominant in marine samples (22.45 %) (Figure 5.2, page 84). Previous studies also supported that Arctic marine samples were dominated by *Bacteroidetes* (Ravenschlag *et al.*, 2001; Crump *et al.*, 2004). Li *et al.* (2009) reported that *Proteobacteria* were the most cosmopolitan group that frequently found in Pacific Arctic sediments. However, results showed that *Bacteroidetes* were the more abundant group in marine samples compared to that of *Proteobacteria* (12.24 %). Llobet Brossa *et al.* (1998), and Cottrell and Kirchman (2000) also reported similar findings of *Bacteroidetes* were more abundant than *Proteobacteria* in the marine ecosystem.

Besides that, *Bacteroidetes* (28.55 %) were also dominant in beach samples studied. This is not surprising as *Bacteroidetes* favour more alkaline and higher salinity environments, as reported by Crump *et al*. (2004) and Aislabie *et al*. (2008). Furthermore, the pH and the salinity of beach samples were similar to the marine samples studied.

Figure 5.2 (page 84) shows that *β-proteobacteria* (15.85 %), regardless of unclassified bacteria (34.15 %), dominating the terrestrial samples studied. Similar findings also showed that *β-proteobacteria* were the dominant group in tundra terrestrial samples (Zhou *et al.*, 1997; Kobabe *et al.*, 2004). On the other hand, *β-proteobacteria* were dominant in melt lake samples studied. The results were in congruent to the finding of Larose *et al*. (2010) that reported *β-proteobacteria* (21.3%) dominating seasonal Arctic meltwater from Svalbard, Norway.

Despite unclassified bacteria (33.34 %), inland lake bank samples were eventually dominated by *Cyanobacteria, Fibrobacteres, Firmicutes* and *β-proteobacteria* with 14.81 % of each. Results showed a slightly different bacterial diversity compared to the Ny-Ålesund lake samples studied by Lindstrom *et al*. (2005). They found *β-proteobacteria* and *Actinobacteria* in Ny-Ålesund lake samples but *Cyanobacteria* were not observed in their samples studied.

Results also showed the periglacier samples studied were eventually dominated by *Bacteroidetes, β-proteobacteria* and unclassified bacteria with 21.05 % of each. The bacterial diversity in these samples was relatively similar to the bacterial diversity in Canadian glacier samples that was also dominated by *Bacteroidetes* and *β-proteobacteria* (Skidmore *et al.,* 2005).

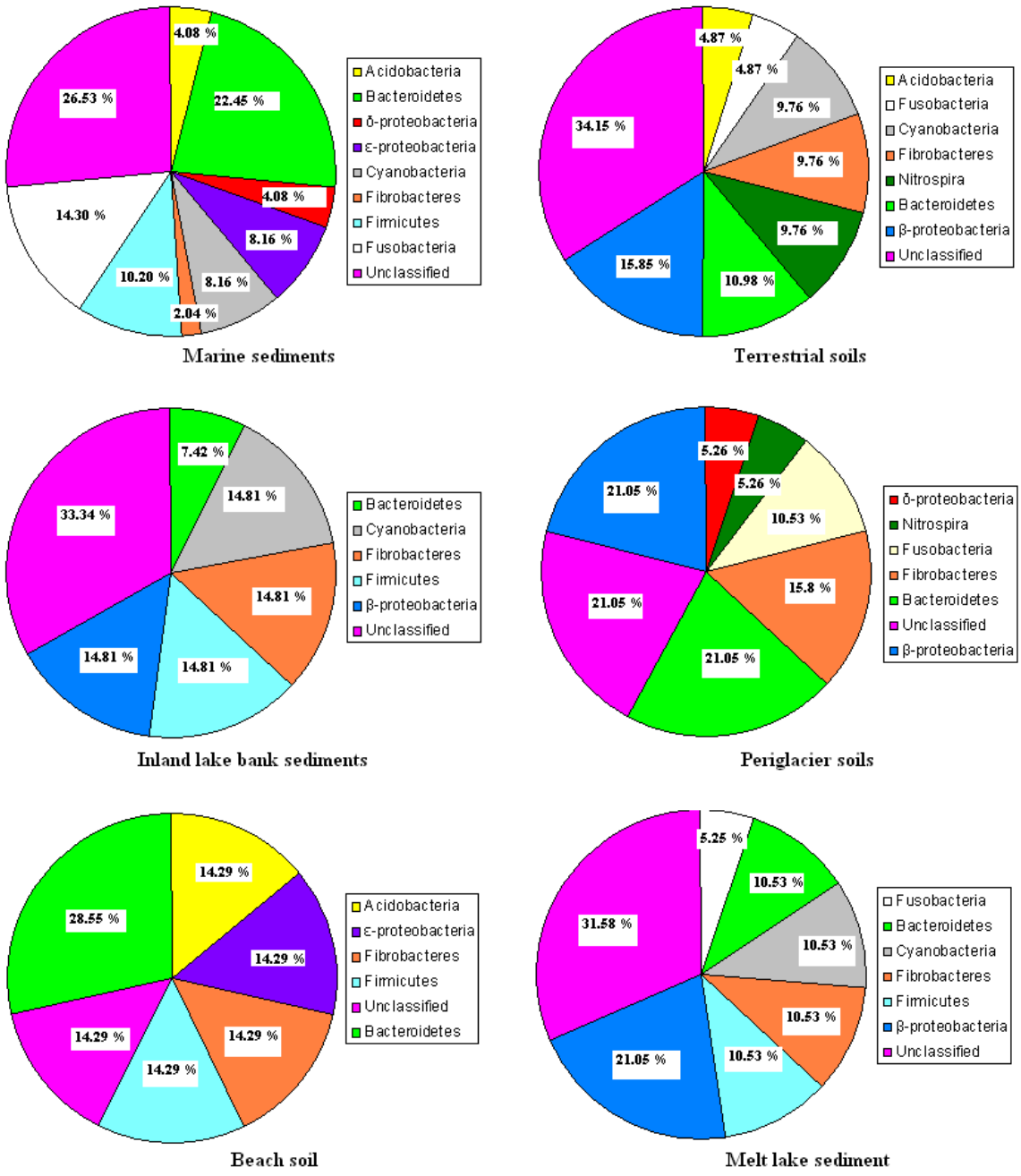

Figure 5.2: The composition of bacteria in all samples studied in Ny-Ålesund.

There were difficulties in studying and identifying a sequence as an ecologically distinct population. According to Polz and co-workers (2006), the nearest matching (> 97 % homology) of a sequence to a known taxonomic from GenBank database was not good enough to study a sequence; probably due to a closely-related strain of bacteria might

have a diverse gene sequence (Hacker and Carniel, 2001). Therefore, in this study, phylogenetic analysis of the sequences was carried out to compliment the BLAST results from GenBank database.

The phylogenetic analysis of the well-defined DGGE bands (using Neighbor-Joining algorithm of 1000 replicates with a scale length of 0.05) showed four distinct clades displayed in the phylogenetic tree, which consisted of (i) members of *Bacteroidetes* and *Cyanobacteria* (ii) members of *Firmicutes*, *Nitrospira*, *Fibrobacteres*, *Acidobacteria*, *ε-proteobacteria* and *δ-proteobacteria*; (iii) members of *β-proteobacteria*; and (iv) members of *Fusobacteria* (Figure 4.9, page 67).

β-proteobacteria were not closely related with *δ-proteobacteria* and *ε-proteobacteria*. *β-proteobacteria* were present in non-marine samples while *ε-proteobacteria* and *δ-proteobacteria* were present in marine samples, indicating their distinct role in the distinct environment. In this study, *β-proteobacteria* group that related to *Comamonadaceae* family, act as ammonium oxidizer (Woose *et al*., 1984) probably involved in the nitrogen removal process on Ny-Ålesund land. While *δ-proteobacteria* and *Sulfurovum* species (sulphate reducing bacteria) in the *ε-proteobacteria* group might play important role in the sulphur cycle in Ny-Ålesund marine habitat (Nakagawa *et al*., 2005).

There was a distinct clade of unidentified representative (banding positions 8, 13, 14, 22, 23, 27 and 29) that was not closely related to any known bacteria sequences in GenBank BLAST. These sequences probably represent a potential gene pool of new species. So, a combination of conventional culture-dependent and culture-independent molecular approach that could provide better information is suggested for future studies.