Metagenomic analyses of the dominant bacterial community in the Fildes Peninsula, King George Island (South Shetland Islands)

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Abstract

There is little information on the bacterial diversity of the Fildes Peninsula, King George Island. Hence, this study was conducted to determine the bacterial population of sediments and soils from the lakes, river, glacier and an abandoned oil tank area in the Fildes Peninsula, using a metagenomic approach. DNA was extracted from the sediment and soil samples, and analyzed using the 16S rDNA polymerase chain reaction—denaturing gradient gel electrophoresis (PCR—DGGE). A total of 299 DNA fragments resolved using the DGGE were sequenced. The results of the analysis provided an overview of the predominant groups of bacteria and the diversity of the bacterial communities. The most abundant phyla of bacteria in Fildes Peninsula were Bacteroidetes, Proteobacteria, Acidobacteria, Gemmatimonadetes, Nitrospira, Firmicutes, Actinobacteria, Chloroflexi, Cyanobacteria, Spirochaetes, Deinococcus-Thermus, WS3 and BRC1. All of the sediment samples from the lakes had different representatives of dominant bacterial species. Interestingly, 15% of the operational taxonomic units (OTUs) did not group into any of the existing phyla in the Ribosomal Database Project (RDP). One of the OTUs had a similarity of <0.90 when compared to the GenBank sequences and probably was a novel bacterium specific to that location. The majority of the bacterial 16S rDNA sequences were found to be closely related to those found elsewhere.

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Keywords: Bacterial diversity; DGGE fingerprinting; Sediment; Soil; Fildes Peninsula; Antarctica

1. Introduction

Many studies have been conducted on the bacterial diversity in Antarctica, for instance, in East Antarctica from the glacier meltwater lake sediment of Bratina Island (Sjöling and Cowan, 2003); cold desert mineral soils of Bratina Island (Smith et al., 2006); and soil in Victoria Land and Windmill Island (Aislabie et al., 2006; Chong et al., 2009a). Concurrently in the Antarctic Peninsula area, several studies have been conducted on the water, soil and sediment, and lakes of the sub-Antarctic Signy Island and King George Island (Chong et al., 2009b; Pearce, 2003; Xiao et al., 2007; Yergeau et al., 2007; Vinocur and Pizarro, 2000). There are also some previous reports on the diversity of bacteria of the Fildes Peninsula, an area with the strongest maritime influence and human activity in the Antarctic (Li et al. 2006; Xiao et al. 2007). These studies used bacterial DGGE fingerprinting to study soil samples. The scope of those studies was however, limited to soil samples from the vicinity of the Chinese Great Wall Station and lakes.
sediments from Ardley Island. The objective of this study was therefore to investigate the groups of bacteria that inhabit the sediments and soils of other parts of the Fildes Peninsula, King George Island.

2. Materials and methods

2.1. Sample collection

Sediment and soil samples were collected during the Austral summer (January–February) of 2007. The locations of the samples collected from the Fildes Peninsula were Antarctic lake (AL; S62°13′39.7″; W58°58′5.0″), GFZ lake (ZL; S62°09′55.5″; W58°55′32.2″), Estrellas lake (EL; S62°12′2.5″; W58°58′19.4″), Playa Elefantes (PE; S62°11′48.5″; W58°59′34.7″), Kitiesh lake (KL; S62°11′36.9″; W58°57′57.0″), Belen lake (BL; S62°13′45.4″; W58°58′53.9″), Geografos lake (GL; S62°13′24.7″; W59°00′22.6″), abandoned oil tank area (OT; S62°11′35.3″; W58°56′6.2″), Minas river (MR; S62°13′36.9″; W58°57′30.2″) and Collins Glacier (CG; S62°10′47.5″; W58°51′12.7″)(Fig. 1). The sediment and soil samples were stored at −20 °C and transferred to the laboratory. Sediment and soil pH were determined in a 1:5 sample-to-deionized water slurry ratio (Costello et al., 2009).

2.2. Extraction of total DNA from soil and sediment samples

DNA extraction was conducted using the methods described by Zhou et al. (1996) with a minor...
modification. The incubation period of the SDS lysis mixture was increased to 3 h, and 0.5 g of soil or sediment sample was used for DNA extraction. DNA extraction from the sample was carried out in triplicate. The quality and quantity of DNA extracted were determined using agarose gel electrophoresis and a Nanovue Spectrophotometer (GE Healthcare). The MassRuler™ Express HR Forward DNA Ladder (Fermentas, Lithuania) was used as a DNA size and mass marker.

2.3. Amplification of partial 16S rDNA fragment

PCR amplification was carried out to amplify the bacterial variable V2–V5 regions of the 16S rRNA gene. The primers used were GC357f (5’-GC*CTT ACG GGA GGC AGC AG-3’) and 907r (5’-CCG TCA ATT CCT TTG AGT TT-3’) (Muyzer et al., 1995). GC*, which represents the GC-clamp (5’-CG CGC GCG GCG GGG GCG GGG GCA CGG GGG G-3’), was added to the 5’ end of the primer. The PCR reaction contained 1× PCR buffer, 2.5 mM MgCl₂, 200 μM of each deoxynucleoside triphosphate, 0.25 μM (each) primer (GC357f and 907r), 1.5 U Taq Polymerase (Promega, USA) and approximately 1–2 ng template DNA (5–100-fold dilution of extracted DNA). The PCR conditions were as follows: 95 °C for 5 min; 20 cycles of 95 °C 1 min, 66 °C for 40 s gradually decreased 0.5 °C per cycle (touchdown PCR), 72 °C for 40 s; 15 cycles of 95 °C for 1 min, 56 °C for 40 s, 72 °C for 40 s; and a final incubation at 72 °C for 30 min. Amplification products were analyzed first by electrophoresis in a 1.5% (wt/vol) agarose gel and stained with ethidium bromide. The size of the PCR amplicons (partial 16S rRNA gene) was approximately 550 bp.

2.4. Denaturing gradient gel electrophoresis (DGGE)

The DGGE was performed with the D-Code Universal Mutation Detection System (Bio-Rad, USA). Three or four independent PCR amplicons were pooled (400–500 ng) and applied directly onto a 6% (wt/vol) 16 cm × 16 cm polyacrylamide gel (ratio of acrylamide to bisacrylamide, 37:1) in a 1× TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA at pH 8.0) with a 35–75% denaturant gradient (where 100% denaturant was 7 M urea and 40% formamide). Electrophoresis was carried out at a constant voltage of 100 V for 18 h at a temperature of 60 °C in 0.5× TAE buffer. After electrophoresis, gels were stained with the SYBR Gold nucleic acid stain (Molecular Probes, PoortGebouw, The Netherlands) for 1 h, the image was observed on a SafeImager Blue light Transilluminator (Invitrogen, USA) and documented using AlphaImager 2000 Gel Documentation System (AlphaImager, San Leandro, CA).

2.5. Sequencing of DGGE bands

Dominant and well-defined bands were excised using a sterilized scalpel blade and incubated overnight in sterile distilled water at 4 °C. The eluate was re-amplified using the same primer set. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, The Netherlands) and cloned onto the pJET1.2/blunt vector using the CloneJET™ PCR Cloning Kit (Fermentas, Lithuania). Clones containing recombinant plasmids were grown overnight in the Luria-Bertani medium supplemented with ampicillin (100 μg mL⁻¹) at 37 °C. Plasmid DNA was extracted using the GeneJET™ Plasmid Miniprep Kit (Fermentas, Lithuania). DNA sequencing was carried out with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and ABI 3130 Genetic Analyzer. Partial 16S rRNA gene sequences were subjected to OTUs (operational taxonomic units) analysis with the MegAlign program (DNASTar, Madison, WI). The taxonomic identity of the OTUs was obtained using the Classifier tool from the Ribosomal Database Project II (RDP)-Release 10 website (http://rdp.cme.msu.edu/)(Cole et al., 2009). Relative abundance of the bacterial phyla from all the locations was depicted graphically in a pie chart. Average genetic distance of the sequences within each location was analyzed using the MEGA software version 4 (Tamura et al., 2007) and the Jukes Cantor model.

2.6. Statistical analysis

DGGE banding pattern analysis was conducted to (i) compare diversity by using the Shannon–Weaver index (Boon et al., 2002) and (ii) perform hierarchical cluster analysis via the unweighted pair group method with mathematical averages (UPGMA, Dice coefficient of similarity), using the GelCompar II software (Applied Maths, Belgium) which was expressed as a dendrogram. Band strengths (peak height) were estimated visually whereby weak bands were assigned a value of 1, intermediate bands a value of 2, and strong bands a value of 3 (Gafan et al., 2005). Shannon index (H) was calculated with the formula $H = \sum \left( \frac{n_i}{N} \ln \left( \frac{n_i}{N} \right) \right)$, where $n_i$ was the peak height and $N$ is the total height of the gel.
was the sum of all the peak heights of all bands (Fromin et al., 2002).

2.7. Phylogenetic analysis

Phylotypes were also compared with the current non-redundant sequence database at GenBank using NCBI's Basic Local Alignment Search Tool (BLAST) to determine phylogenetic relatedness (Altschul et al., 1990). Phylogenetic and molecular evolutionary analyses were conducted using the MEGA software version 4 (Tamura et al., 2007) and the ClustalW software (Thompson et al., 1994).

2.8. Nucleotide sequence accession numbers


3. Results

3.1. DNA extraction from environmental samples and PCR amplification

Direct soil and sediment community DNA extraction was conducted without prior enrichment or culturing steps. Intact genomic DNA was obtained (data not shown) with a yield that varied from 0.12 to 4.80 μg DNA per gram of sample. The DNA contained some contaminants, such as humic acid, that co-purified with the DNA. The DNA sample was diluted between 5 and 100-fold prior to PCR amplification to avoid the inhibitory effect of humic acid.

3.2. DGGE profiling and bacterial diversity

The results of the DGGE analyses (Fig. 2) showed that all locations produced a number of DNA bands, which distributed across the length of the gel. In general, each location had more than 15 strong (dominant) and weak (less dominant) DNA bands, showing that these locations have a relatively complex bacterial community. Dominant DNA bands were different among the 10 locations. Samples containing the greatest number of DNA bands were ZL (26) and CG (23), while sample OT (15) contained the least number of bands. These were reflected in the Shannon diversity index, ZL (3.15), CG (3.08) and OT (2.63) (Table 1).

The GelCompar II software generated a more informative interpretation of the DGGE banding patterns. The dendrogram constructed by the pairwise comparison using the Dice coefficient of similarity and UPGMA algorithm depicted the relatedness of the bacterial communities (Fig. 3). Samples AL and EL showed the highest similarity (70%) and thus both of them clustered together in the same branch. The same occurred with samples GL and PE (60.61%), BL and CG (51.29%) and OT and KL (45.17%) where they were clustered together respectively. Higher similarity or closely clustered samples would have relatively higher chance of sharing the same composition of bacteria. The remaining samples MR and ZL were relatively different from the other samples. However, this interpretation is based on the DGGE banding profiles which was done merely to obtain an overview. In order to get a better resolution of the dominant population of bacteria in these locations, dominant and discrete bands were excised from all the locations, cloned and sequenced. Positions of the excised DNA bands are shown in Fig. 4.

3.3. Phylotypes sequence analysis and taxonomic grouping

A total of 99 DGGE bands consisting of 299 DNA fragments were sequenced (Table 1). All the amplicons (partial 16S rDNA sequences) were aligned using the ClustalW software and checked for sequence similarity using MegAlign software (DNASTAR). The OTUs (operational taxonomic units) were defined based on a cut-off value of 97% (in the sequence similarity). One hundred and seventy five (175) OTUs were found
in the 299 clones when all the sequences in the 10 sampling locations were compared.

Taxonomic classification of the OTUs using the Classifier in RDP II revealed that the dominant bacteria in those 10 locations were distributed into thirteen phyla with different compositions (Figs. 5 and 6). They were Bacteroidetes (27.4%), Proteobacteria (25.7%), Acidobacteria (13.1%), Gemmatimonadetes (4.0%), Firmicutes (4.0%), Actinobacteria (3.4%), Chloroflexi (1.7%), Nitrospira (1.1%), Cyanobacteria (1.1%), Cyanobacteria (1.1%), WS3

Table 1
pH, DNA concentration, diversity index, genetic distance, banding pattern information, number of sequenced clones, OTUs and phyla for all locations.

<table>
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<th></th>
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<th>EL</th>
<th>PE</th>
<th>MR</th>
<th>CG</th>
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</tbody>
</table>

ᵃ Shannon–Weaver diversity index.
ᵇ Sequence similarity of the partial 16S rDNA fragment with <97% as the cut-off value of different OTUs.
ᶜ Taxonomy classification of the sequences was analyzed using the CLASSIFIER in RDP II release 10.
ᵈ Genetic distance by Jukes Cantor Mode.
(1.1%), Deinococcus-Thermus (0.6%), Spirochaetes (0.6%) and BRC1 (0.6%). The remaining 15.4% of the OTUs were grouped into a cluster of unclassified bacteria with a threshold value of 80%. Comparison of the bacterial phyla composition between locations can be found in Fig. 6. Table 2 provides detailed information on the composition of each phylum at all locations.

In general, Bacteroidetes and Proteobacteria were the most prevalent phyla which were present at all the locations and often constituted the top two dominant groups of bacteria. MR and KL were exceptions as at MR, the most dominant bacterial phylum was the Gemmatimonadetes while at KL it was the Acidobacteria. GFZ and Kitiesh lakes contained the most diversified bacterial groups with a total of 8 and 9 different phyla respectively (Table 1). Collins Glacier had the greatest number of unclassified bacteria, which was 25.0% in a total of 32 OTUs. Samples from EL (23.5%), GL (23.5%) and BL (20.0%) also contained relatively high percentages of unclassified bacteria compared with other locations, such as PE which had no unclassified bacteria.

4. Discussion

The genomic DNA extracted from the sediment and soil samples in this study was relatively low (0.12–4.80 μg g⁻¹), indicating that a lower numbers of microorganisms were present in the samples. In contrast, the genomic DNA extracted from tropical soil samples ranged from 2.5 and 26.6 μg g⁻¹ (Porteous and Armstrong, 1991; Zhou et al., 1996). Spectrophotometric quantification of the DNA extracted from sediment and soil samples can be erroneously overestimated by the presence of contaminants such as humic acids, fulvic acids and polysaccharides (Cullen and Hirsch, 1998; Leckie, 2005). Therefore, in this study, quantification of DNA was conducted by comparing the intensity of the extracted genomic DNA on the agarose gel to a standard mass ladder to give a more accurate estimation. In such a case, only the concentration of the DNA that was stained with ethidium bromide was measured. Genomic DNA from PE and OT required a dilution of 80 to 100-fold prior for PCR amplification. This was probably due to the presence of contaminants such as humic acid in the DNA extract from the both locations. This was not surprising because both samples had a very high absorbance at 230nm of >5.0 (data not shown) and were weakly acidic (pH 6.4–6.6). It has been reported that the activity of the Taq polymerase is limited by the presence of humic substances that are normally present in soil samples, and a simple dilution step may attenuate the inhibition effect (Tebbe and Vahjen, 1993; Tsai and Olson, 1992).

4.1. Banding pattern-based analyses

DGGE profiles were useful in providing a rapid overview of the richness and the differences between the bacterial communities in the sediments collected from the various locations. In this study, it was found that all 10 locations had relatively high bacterial diversity, comprised of different dominant bacterial taxa. Minor members (less-abundant taxons) in the community, however, might be undetected or underestimated by DGGE because it has an abundance detection limit of 1% (Fromin et al., 2002). High-levels of complexity in the prokaryotic community of cold sediments and soils have been reported previously from Antarctic meltwater lake sediments (Bowman et al., 2000), microbial mats in East Antarctic lakes (Brambilla et al., 2001), and glacial meltwater lake sediment of East Antarctic, Bratina Island (Sjöling and Cowan, 2003). In general, the samples with a lower pH (weakly acidic) in this study such as PE and OT had a lower Shannon diversity indices of 2.86 and 2.63 and a smaller number of phyla (4; PE was absent of unclassified bacteria) respectively (Table 1). In contrast, higher pH (weakly...
alkaline) samples such as ZL and CG had higher Shannon diversity indices (>3.0) and a greater number of phyla (8; CG had high proportion of unclassified bacteria, 25%). Fierer and Jackson (2006) stated that bacterial diversity is related to pH where acidic soils have a lower diversity of bacteria. The same trend was observed by Aislabie et al. (2006) where bacterial diversity (total microbial count) increased as the pH increased.

4.2. Sequence-based analyses

Generally, the taxonomic classification of bacteria will be more accurate if near-full-length sequences of the 16S rRNA gene are used. However, the partial 16S rDNA sequences used here were sufficient to provide enough information on the bacterial taxonomical classification to the genus level with 92% accuracy for 400-base segment (Wang et al., 2007).

Fig. 4. Schematic of the DGGE banding patterns from Fig. 1. Bands with red circle were those excised for subsequent sequence analysis. Color of the bands: Strong = Brown; Intermediate = Orange; Weak = Yellow.
A total of 299 clones from the 10 locations were sequenced. Sequence alignment and similarity checked using the MegAlign software showed that 175 different bacterial OTUs were present. The most dominant phylum was the Bacteroidetes (27.4%), followed by the Proteobacteria (25.7%) and Acidobacteria (13.1%). About 15% of the OTUs were not classifiable at the phylum level in the RDP II database. The composition of each sub-division was also included in the results due to the large sub-division of the Proteobacteria group. Betaproteobacteria was found in all locations and was the most dominant sub-division of the Proteobacteria (except for GL) (Table 2). Most of the Betaproteobacteria, Sphingobacteria, Acidobacteria, Gemmatimonadetes, and Nitrospira clones were highly similar in their 16S rDNA sequences (≥97%) after the OTU analysis.

The dominant phyla found in the Fildes Peninsula which included Acidobacteria, Actinobacteria,
Bacteroidetes, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Firmicutes, Proteobacteria and Spirochaetes, were consistent with previous reports from Antarctic soil and sediment samples (Aislabie et al., 2006, 2008; Bowman and McCuaig, 2003; Chong et al., 2009b; Li et al., 2006; Sjoling and Cowan, 2003; Smith et al., 2006; Xiao et al., 2007). The phyla Gemmatimonadetes, Nitrospira, BRC1 and WS3 were the only exceptions as they were rarely found in the Antarctic region. However, Gemmatimonadetes is among the most common bacterial groups found in soil and sediment samples from other parts of the globe (Janssen, 2006).

Members of the Bacteroidetes phylum (sometimes referred to as Cytophaga-Flavobacteria-Bacteroides or CFB in the literature) are abundant in cold marine habitats and tundra soil of Siberia (Liebner et al., 2008). The same phyla were also reported from soil in Victoria Land (Aislabie et al., 2006, 2008), surface lake sediments of Ardley Island (Li et al., 2006) and soil from the Windmill Islands (Chong et al., 2009a). Many strains were cold-adapted, psychrophiles or psychrotrophs (Shivaji et al., 1991). CFB were capable of degrading a wide range of polymeric substances (Aislabie et al., 2006, 2008; Li et al., 2006) and utilized a large number of carbohydrates (Liebner et al., 2008).

All of the OTUs from this study had their closest matches with the uncultured bacteria in the NCBI database (data not shown) indicating that most of the bacteria from the Fildes Peninsula have not been cultured yet. Some of the OTUs had their closest identities with sequences retrieved from other sources in Antarctica, such as from the sediment samples from the Onyx River, Wright valley, Victoria Land; penguin dropping sediments from Ardley Island; microbial mat and ice core from Lake Fryxell and Lake Vida ice cover in McMurdo Dry Valleys, Southern Victoria Land. Aislabie et al. (2006) reported that bacteria from some soils were closely related to those from other Antarctic environments, indicating that they were probably brought there by the wind. The presence of unclassified bacteria in this study is similar to observations reported from other parts of King George Island and Antarctica (Li et al., 2006; Smith et al., 2006).

The majority of the OTUs retrieved in this study were similar to bacteria found outside the Antarctic region. For instance, from Himalayan glaciers (Roopkund, and Kafni), Kuytun Glacier (China), Arctic subglacial water (Canada), alpine tundra soil (France), Lake Washington, Lake Michigan and Crate Lake (USA), Lake Hibara and Lake Biwa (Japan), Nam Co Lake (Tibet), Illinois River, Lahn River (Germany), fumarole soil (Andes),

### Table 2
Summary of the CLASSIFIER (RDP II database) analysis results for the dominant OTU in the Fildes Peninsula, King George Island.

<table>
<thead>
<tr>
<th></th>
<th>AL</th>
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\* Threshold value of 80% was selected as the default in the CLASSIFIER RDP II analysis
rhizosphere, prairie soil, hydrocarbon-contaminated soil, municipal wastewater sludge, membrane bioreactor, human skin and even from clean rooms. This is not surprising as the CFB group has been found in the inner layers of biofilms in wastewater treatment systems (Koch et al., 2000; Egli et al., 2003), intestine and faeces of mammals (Dick and Field 2004; Flint et al., 2007) and in early stages of guano decomposition (Zdanowski et al., 2004). Vincent (2000) reported that many cosmopolitan species of microorganisms are able to survive in the Antarctica. Fierer and Jackson (2006) stated that similarity between soil bacterial communities was unrelated to geographic distance and Finlay (2002) suggested that prokaryotes are unlikely to be endemic because of their high abundance, small size, short generation time and high dispersal rates. They are able to overcome large geographical barriers but their ability to survive depends on their capacity to adapt to the environment and many of them may survive locally for long periods in a “cryptic” instead of “active” state (Findlay, 1998; Staley and Gosink, 1999). Out of 175 OTUs, there was only one sequence that may represent a taxonomically novel group of bacteria (similarity to GenBank sequence of <0.90). This indicates that large numbers of higher-level prokaryotic groups have been discovered using the techniques available currently (Bowman and McCuaig, 2003).

One of the more interesting locations in this study was sample OT, which was from an abandoned oil tank area. This location has weakly acidic soil, a low Shannon diversity index and a low number of bacterial phylum. The same observations were found by Saul et al. (2005) from hydrocarbon-contaminated soil from Ross Island and by Chong et al. (2009a) from an oil spill site in the Windmill Islands.

The results of this study gave an overview of the various types of bacterial groups that were present in the sediments samples of lakes, river and glacier in the Fildes Peninsula. The most significant finding was that the dominant bacterial population of a geographical location differed only slightly from other geographical locations within the Fildes peninsula and that most of the bacteria were ubiquitous. Hence, in future it would be interesting to find out what are the environmental parameters that determine the dominant bacteria population in a specific location, how this community contributed to the food webs and how they are affected by global warming.

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