Bacterial Diversity from Schirmacher Oasis, Antarctica

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ABSTRACT
Bacterial diversity from Schirmacher Oasis, Antarctica was assessed by 16S rDNA clone library analysis. The samples were collected from sites with obvious human impact and sites with low or non-obvious human impact during the austral summer of 2004/2005, winter of 2005 and austral summer of 2005/2006. Analysis of clones from high human impact sites showed higher bacterial diversity in comparison with clones from the pristine sites. About 74 different unique phylotypes have been identified. Most of the sequenced clones are related to the phyla \( \gamma \)-Proteobacteria, \( \alpha \)-Proteobacteria, \( \beta \)-Proteobacteria, Actinobacteria, and Bacteroidetes. Pseudomonas sp. from the \( \gamma \)-Proteobacteria phylum was the most dominant species found in the Schirmacher Oasis, occurring in the high as well as low impact sites. Bacterial community profiles from three consecutive seasons were analysed by denaturing gradient gel electrophoresis (DGGE).

INTRODUCTION
Maitri station (70°45’57"S, 11°44’09"E), the second Indian scientific station, is located in the Schirmacher Oasis, an ice-free land mass of 35 km\(^2\) area, situated in the Central Dronning Maud Land, East Antarctica. The station hosts summer and over-wintering scientific personnel conducting meteorological, geomagnetic, biological, geological, glaciological, medical, environmental, and physical research programs. Microbiologists are interested to study the diversity and ecological role of microorganisms in Antarctica. Total community DNA extraction and characterisation of 16S rRNA gene libraries using culture-independent methods has allowed a wide assessment on the composition of bacterial communities. Application of DNA-based methods such as denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene cloning has greatly contributed to the expanding knowledge of bacterial compositions in the
unique Antarctica ecosystems (Aislalie et al., 2006). This study incorporated both 16S rDNA clone library and PCR-DGGE to assess the bacterial diversity of environmental samples from Schirmacher Oasis. PCR-DGGE analysis was used to compare the environmental samples from three consecutive seasons from Schirmacher Oasis.

MATERIALS AND METHODS

Sampling

Samples were collected from impacted and pristine sites of Schirmacher Oasis, Antarctica. Sampling for this study was carried out during the austral summer of 2004/2005, winter of 2005 and austral summer of 2005/2006. Impacted areas include sites surrounding Maitri Station and the workshop area. The pristine sites include the Priyadarshini Lake and the western and eastern part of Schirmacher Oasis. The samples were collected in sterile containers and was stored at -20 °C.

Extraction of genomic DNA and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted using the UltraClean™ Soil DNA Isolation Kit (Mo Bio). 16S rDNA clone library was constructed using samples from austral summer 2004/2005. Universal primers 519f and 1492r (Bowman and McCuaig, 2003) was used to amplify the 16S rRNA gene fragment for clone library analysis. Samples from three consecutive seasons were used for DGGE analysis. Universal primers 27f and 1492r (Newberry et al., 2004) and 907r and 341f-GC (Powell et al., 2003) were used to amplify the 16S rRNA gene fragment for DGGE analysis.

Cloning and Restriction Fragment Length Polymorphism (RFLP)

PCR products of the clone library analysis were gel extracted, purified and cloned into pDrive cloning vector (Qiagen). This vector allows ampicillin and blue/white colony selection. Plasmids with positive inserts were extracted using the Miniprep Spin Kit (Qiagen). Clones were differentiated by RFLP analysis. Unique RFLP bands were reamplified, purified and sequenced. Sequences were aligned using ClustalW and compared to reference sequences obtained from the GenBank using Basic Local Alignment Search Tool (BLAST).

Denaturing Gradient Gel Electrophoresis (DGGE)

DGGE was performed using the D-Code Universal Mutation Detection System (Bio-Rad). The PCR product was analysed on 35-60% denaturant
Fig. 1: Research approach for the bacteria diversity from Schirmacher Oasis
polyacrylamide gel and viewed on an UV transilluminator. The banding patterns were converted into a presence/absence matrix. Multivariate approach using the PRIMER 6 statistical software (Plymouth Marine Laboratory, UK) was carried out to analyse the DGGE banding patterns. Similarity matrices were then generated by Bray-Curtis similarity measure with the presence and absence matrix. The non-metric multidimensional scaling plots (nMDS) in the PRIMER 6 package was used to show the relative similarities between sites and seasons (Powell et al., 2003). The approaches taken to achieve the above objectives are shown in Fig. 1.

RESULTS AND DISCUSSION

16S rDNA clone library analysis

16S rDNA clone library was used to assess bacterial diversity of impacted and pristine sites using samples collected in the austral summer of 2004/2005. 74 unique phylotypes were sequenced and were clustered into five major groups (Fig. 2). Phylum Proteobacteria with the subdivision of γ-Proteobacteria, β-Proteobacteria and α-Proteobacteria, phylum Actinobacteria and phylum Bacteroidetes. Similar results on Antarctic bacterial diversity has been reported earlier (Shivaji et al., 2004; Aislabie et al., 2006).

Fig. 2: Number of unique phylotypes from various phyla in soils from pristine and impacted sites of austral summer 2004/2005
The most dominant sequences of the 16S rDNA clone library of samples from the impacted and pristine sites were from the phylum Proteobacteria. Approximately 60.8% of the clone library was dominated by this phylum and about 68.9% of this phylum was dominated by subdivision γ-Proteobacteria (Fig. 2). Approximately 77% of the 16S rDNA clone library sequences were sequenced from impacted sites which appeared to have more diverse bacterial community in comparison to the pristine sites. The α-Proteobacteria was sequenced only from the impacted sites.

**DGGE analysis of three consecutive seasons samples**

DGGE profiles of soils from three consecutive seasons are shown in Fig. 3 (impacted sites) and Fig. 4 (pristine sites).

Fig. 3: DGGE profile of samples collected from impacted sites for three consecutive seasons
Fig. 4: DGGE profile of samples collected from pristine sites for three consecutive seasons.

L1: P. Lake (Summer 05/06)
L2: P. Lake (Winter 05)
L3: P. Lake (Summer 04/05)
L4: West (Summer 05/06)
L5: West (Winter 05)
L6: West (Summer 04/05)
L7: East (Summer 05/06)
L8: East (Winter 05)
L9: East (Summer 04/05)

Non-metric multidimensional scaling plots (nMDS) was derived by converting the banding patterns into a presence/absence matrix for statistical analysis. A score of one indicates the presence of a band and zero indicates the absence of the same band. The presence/absence matrix was used to compare positions of bands between gels and also banding patterns between lanes in the same gel. The overall similarity level between all the sites was about 20% with stress value of 0.01 (Fig. 5). The highest similarity (80%) was between the hydrocarbon site and the station soil. These two sites are geographically closer to each other and should have similar bacterial diversity a characteristic noted by Powell et al., (2003). All the other sites showed an nMDS value of 40% which showed lower similarity in bacterial diversity.
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