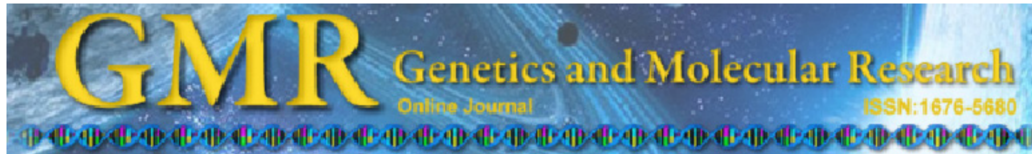

Identification of actinomycete communities in Antarctic soil from Barrientos Island using PCR-denaturing gradient gel electrophoresis

L. Learn-Han[1], C. Yoke-Kqueen[1], M.S. Shiran[2], C.M.W. Vui-Ling[3], A.M. Nurul-Syakima[1], R. Son[4] and H.M. Andrade[5]

[1]Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia
[2]Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia
[3]Biotechnology Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia
[4]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia
[5]Instituto Antártico Ecuatoriano, Guayaquil, Ecuador

Corresponding author: C. Yoke-Kqueen
E-mail: ykcheah@medic.upm.edu.my

Genet. Mol. Res. 11 (1): 277-291 (2012)
Received May 9, 2011
Accepted August 3, 2011
Published February 8, 2012
DOI http://dx.doi.org/10.4238/2012.February.8.3

ABSTRACT. The diversity of specific bacteria taxa, such as the actinomycetes, has not been reported from the Antarctic island of Barrientos. The diversity of actinomycetes was estimated with two different strategies that use PCR-denaturing gradient gel electrophoresis. First, a PCR was applied, using a group-specific primer that allows selective amplification of actinomycete sequences. Second, a nested-PCR approach was used that allows the estimation of the relative abundance of actinomycetes within the bacterial community. Molecular identification, which was based on 16S rDNA sequence analysis, revealed eight genera of actinomycetes, *Actinobacterium*,

 ©FUNPEC-RP www.funpecrp.com.br

Actinomyces, an uncultured *Actinomycete*, *Streptomyces*, *Leifsonia*, *Frankineae*, *Rhodococcus*, and *Mycobacterium*. The uncultured *Actinomyces* sp and *Rhodococcus* sp appear to be the prominent genera of actinomycetes in Barrientos Island soil. PCR-denaturing gradient gel electrophoresis patterns were used to look for correlations between actinomycete abundance and environmental characteristics, such as type of rookery and vegetation. There was a significant positive correlation between type of rookery and abundance of actinomycetes; soil samples collected from active chinstrap penguin rookeries had the highest actinomycete abundance. Vegetation type, such as moss, which could provide a microhabitat for bacteria, did not correlate significantly with actinomycete abundance.

Key words: Actinomycetes; Barrientos Island; Antarctic, PCR-DGGE; 16S rDNA

INTRODUCTION

Actinomycetes are a group of Gram-positive bacteria with high GC-content in their DNA. This bacterium order has been frequently found in Antarctic soils (Saul et al., 2005; Aislabie et al., 2006). Soil is an intensively exploited ecological niche for the inhabitants to produce numerous useful biologically active natural products like clinically important antibiotics (Thakur et al., 2007). Actinomycetes have been extremely useful for the pharmaceutical industry due to their seemingly unlimited capacity to produce secondary metabolites with diverse biological activities and chemical structure. The sequencing analysis of the chromosomal DNA of *Streptomyces griseus* showed the metabolic diversity of this bacterium family, which is due to their extremely large genome that has hundreds of transcription factors that control gene expression, thus allowing them to respond to specific needs (Goshi et al., 2002). The genus *Streptomyces* constitutes 50% of the total population of soil actinomycetes and about 75% of commercially and medicinally useful antibiotics that have been derived from this genus (Mellouli et al., 2003; Thakur et al., 2007).

To date, tens of thousands of actinomycete-derived compounds have been isolated and characterized, many of which have been develop into drugs to treat various diseases (Aislabie et al., 2008). Actinomycetes like *Streptomyces* isolated from different environments have produced the same compound due to frequent genetic exchange between species (Bredholt et al., 2008). Hence, this results in reduced chances of finding genuinely new biologically active molecules from *Streptomyces* and actinomycetes (Baltz, 2006; Busto et al., 2006). Therefore, the exploration of new habitats with unusual environment and poorly explored areas of the world has become important and useful to the discovery of novel compounds and actinomycetes (Nolan and Cross, 1988; Barakate et al., 2002; Saadoun and Gharaibeh, 2003). Consequently, the Antarctic, one of the harshest, coldest and poorly explored areas on Earth has now emerged as a highly potential area for the discovery of novel bioactive compounds from actinomycetes (Moncheva et al., 2000-2002).

Molecular techniques such as polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) has enabled researchers to study actinomycete diversity on molecu-

lar level and avoid the inadequacies of culture-based methods (Das et al., 2007; Nakatsu, 2007; Wenhui et al., 2007). PCR-DGGE has been used to resolve PCR-amplified region of small subunit ribosomal RNA genes (i.e., 16S rDNA or 18S rDNA) based solely on differences in the nucleotide sequence (Muyzer et al., 1998). From DGGE fingerprint, each DNA fragment in the profile is likely to be derived from one (or a few) phylogenetically distinct populations; hence one can readily acquire an estimation of species number and abundance based on the number and intensity of amplified fragments in the profile. Furthermore, it is possible to understand the phylogeny of community members by DNA sequence analysis of amplified fragments after they have been excised, cloned and sequenced (Muyzer et al., 1995; Ferris et al., 1996; Rölleke et al., 1996; Teske et al., 1996; Nakatsu, 2007). This study used two approaches to study actinomycete diversity. First, the selective amplification of actinomycete sequences (*Escherichia coli* position 226 to 528) for direct analysis of PCR products in DGGE. Second, a nested-PCR that amplifies actinomycete-specific fragments (*E. coli* position 226 to 1401) and used to serve as template for a PCR that uses conserved primers. These nested-PCR products (*E. coli* position 968 to 1401) were subjected to DGGE for separation (Heuer et al., 1997).

In this study, 17 soil samples were collected from different locations throughout Barrientos Island, Antarctica. The aims of this study were: i) To study the diversity and abundance of actinomycete communities from soils using the PCR-DGGE method; ii) To cluster soil samples based on PCR-DGGE profiles; iii) To determine a correlation between actinomycete abundance with soil characteristics, e.g., type of rookery and vegetation.

MATERIAL AND METHODS

Environmental sampling

During the XI Ecuadorian Antarctic Expedition to the Research Station "Pedro Vicente Maldonado", Greenwich Island, South Shetland Islands, 2007, sampling for analysis of actinomycete communities from the soil was carried out at Barrientos Island (coordinates: S 62° 24' 18.7" to S 62° 24' 32.4" and W 59° 44' 13.2" to W 59° 45' 39.3"). Top soil samples of upper 20-cm layer (after removing the top 2-3 cm) were collected from 17 different sites within Barrientos Island. These sites have various interesting fauna and flora activities (Table 1). Soils were sampled into sterile plastic bags using an aseptic metal trowel, and kept in the dark for transport to Malaysia. Soils were subsequently stored at -20°C, with an aliquot stored at -80°C for molecular analysis like PCR-DGGE.

Soil DNA extraction

To minimize possible contaminants, all post-sampling manipulations were performed in a UV-sterilized laminar box hood, using sterile glass vials. Total soil DNA was extracted and purified from 1 g dry weight soil using the GF-1 Soil Sample Extraction kit (Vivantis, Selangor, Malaysia). The kit uses a specially treated silica-based material fixed into a column to efficiently bind DNA in the presence of high salt. This kit applies the principle of a mini-column spin technology and the use of optimized buffers to ensure that only DNA is isolated while cellular protein, humic acid and other low molecular weight impurities are removed during the subsequent washing stages (Cat. No: GF-SD-025). DNA yield and quality were

assessed by 0.8% (w/v) agarose gel electrophoresis following by DNA quantification using a Biophotometer (Eppendorf, Hamburg, Germany) and ratio A_{260}/A_{280} was measured. Pure DNA has an A_{260}/A_{280} ratio of 1.7-1.9.

Table 1. Soil sample characteristics.

Soil reference	Total bands[a]	Type of rookery/nest	Vegetation
442	22	Active chinstrap penguin	No moss
443	26	Abandoned Gentoo penguin	No moss
444	24	Abandoned Gentoo penguin	No moss
445	26	Abandoned Gentoo penguin	No moss
446	22	Active chinstrap penguin	No moss
447	21	Active chinstrap penguin	No moss
448	17	Active penguin	No moss
449	21	Active chinstrap penguin	No moss
450	18	Abandoned penguin	No moss
451	10	Gentoo penguin resting area	Moss
452	19	Abandoned penguin	Moss
453	18	Penguin resting area	Moss
455	14	Active Gentoo penguin	Moss
456	18	Abandoned penguin	No moss
457	17	Active Gentoo penguin	Moss
458	15	Near Seal colony	Thick moss
460	14	Giant Petrel nest	No moss

[a]Total number of bands detected by the Bionumerics Version 6.0 Gel Analysis Software for DGGE in Figure 1 (F243-R513GC).

PCR amplifications of 16S rDNA gene fragments

Primer pair F243-R513GC, as described by Heuer et al. (1997), was used for amplification of a specific actinomycete region by PCR using the Eppendorf Mastercycler (Eppendorf). The PCR mixture consisted of 100 ng soil bacterium DNA, 2.0 μL 10X optimized PCR buffer with 20 mM $MgCl_2$, 2.0 μL 10 mM dNTPs, 1 U Taq polymerase (Intron Biotechnology, South Korea) and 0.5 μL 100 nM F243 and 100 nM R513GC and sterile ultrapure water was added to final volume of 20 μL. The cycling parameters were 7 min at 95°C for pre-denaturation, 25 cycles each of 1 min at 95°C for denaturation, 40 s at 64°C for annealing, 40 s at 72°C for extension. The PCR amplification products were resolved by electrophoresis on 1.5% agarose gel (Promega, Madison, WI, USA), which was stained with ethidium bromide (0.5 μg/mL) and viewed under a gel documentation system (Alpha Imager, Alpha Innotech, USA).

For nested-PCR, primer F243 that specifically match and amplifies actinomycetes 16S rRNA genes was used with the reverse primer R1378 to amplify the fragment from position 226 to 1401. PCR products from primer pair F243-R1378 were used as a template for a second PCR with primer pair F984GC-R1378. This second PCR product contained a fragment from position 968 to 1401 that enabled DGGE to be performed. The reaction conditions for F243-R1378 followed the protocol of Heuer et al. (1997), while PCR conditions for F984-R1378 differed from those described above (F243-R513GC) in the use of a pre-denaturation time of 5 min, an annealing temperature of 70°C and final extension of 3 min at 72°C.

Denaturing gradient gel electrophoresis

The CBS DGGE 2000 system (CBS Scientific Company, Del Mar, CA, USA) was

utilized to analyze the PCR products of PCR (303 bp) and nested-PCR (433 bp). PCR products were loaded on a 0.75-mm thick vertical gel containing 7.5% (w/v) polyacrylamide (acrylamide-bisacrylamide, 37.5:1) gels in 1X Tris-acetate-EDTA (TAE). Linear gradient of 40-60 or 30-50% denaturant (100% denaturant correspond to 7 M urea and 40% de-ionized formamide) was used for the separation of 16S rDNA fragments 226-528 and 968-1401, respectively. One sample and a 1-kb molecular marker (Fermentas, Ontario, Canada) were used as reference to allow comparison between gels for normalization purposes. Electrophoresis was conducted for 9 h at 150 V. Gels were stained using 3 mL SYBR Gold (Molecular Probes, Invitrogen, Cergy Pontoise, France) in 15 mL TAE buffer for 30 min in the dark. Gels were rinsed with 500 mL TAE buffer and bands were viewed under a gel documentation system (Alpha Imager). In this study, repeated PCR and DGGE analysis consistently produced similar DNA banding profiles and nearly identical band intensities.

Sequencing of DGGE fragments

DNA fragments to be nucleotide sequenced were physically excised from well-defined DNA bands with a sterile razor blade and placed in sterilized vials, and 20 μL sterilized water was added. The DNA was allowed to passively diffuse into the water at 4°C overnight incubation. Eluate was quantitated to check for DNA concentration and purity. Then, 1 μL of the eluate was used as a template DNA for re-amplification using PCR with the primers and under conditions described above. Following amplification, the PCR product was purified using the GeneAll Expin Gel SV purification kit (GeneAll, Seoul, Korea) and proceed to molecular cloning using QIAGEN PCR cloning kit (Qiagen, Hilden, Germany) according to the manufacturer protocol. Through blue-white selection, insert was verified by colony-PCR and colonies with transformations were preceded to plasmid DNA extraction (Eppendorf) using the manufacturer protocol. Purified plasmid DNAs were served as templates for PCR to confirm the insertion of gene of interest. After confirmation, plasmid DNA was sequenced with an ABI PRISM® 3100 DNA sequencer.

DGGE sequence analysis and phylogeny

In order to determine the phylogenetic position of microorganisms detected in PCR-DGGE, the partial 16S rRNA gene sequences analyzed were compared with the available National Center for Biotechnology (NCBI) GenBank database via non-redundant BLAST search to determine approximate phylogenetic affiliations (Altschul et al., 1990). Partial 16S rDNA gene sequences were compiled and aligned using the Bionumerics, version 6.0, software (Applied Maths, Kortrijk, Belgium). Trees were calculated for all target sequences and their close relatives using neighbor-joining (Saitou and Nei, 1987) and maximum parsimony (Kluge and Farris, 1969) methods in software.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences of DGGE bands determined in this study were deposited in the NCBI Genbank database. Both actinomycete and non-actinomycete bacterial sequences were deposited under accession numbers of GU549417 to GU549430 and AJ009642 to AJ009654.

DGGE pattern analysis

Each band in a denaturing gel was considered to be a discrete ribotype (Nikolcheva et al., 2003). By using the BioNumerics version 6.0 gel analysis software (Applied Maths), the positions of the markers on PCR-DGGE gels were normalized from lane-to-lane and gel-to-gel variation. This normalization enables comparison of banding patterns originating from different DGGE gels, provided there was a high degree of gel reproducibility based on migration of standards. Then, a binary matrix was constructed for each microbial community based on the presence and absence of bands. Jaccard's coefficient (a similarity measurement) was used to calculate the matrix and the data were subjected to clustering based on the unweighted pair group method using arithmetic averages (UPGMA) to identify samples that generated patterns similar to each other (Ibekwe et al., 2001; Boon et al., 2002). Results were displayed in a dendrogram form to illustrate the relationship between actinomycete communities from different soil.

RESULTS AND DISCUSSION

PCR-DGGE of actinomycetes and bacterial 16S rDNA

Expected DNA fragments of actinomycetes and bacterial 16S rDNA were amplified using PCR and the nested-PCR amplification technique. The PCR produced 303 bp while the nested-PCR, first and second rounds, produced 1175 and 433 bp of 16S rDNA fragments, respectively. The DGGE patterns of PCR and nested-PCR amplification products are shown in Figures 1 and 2.

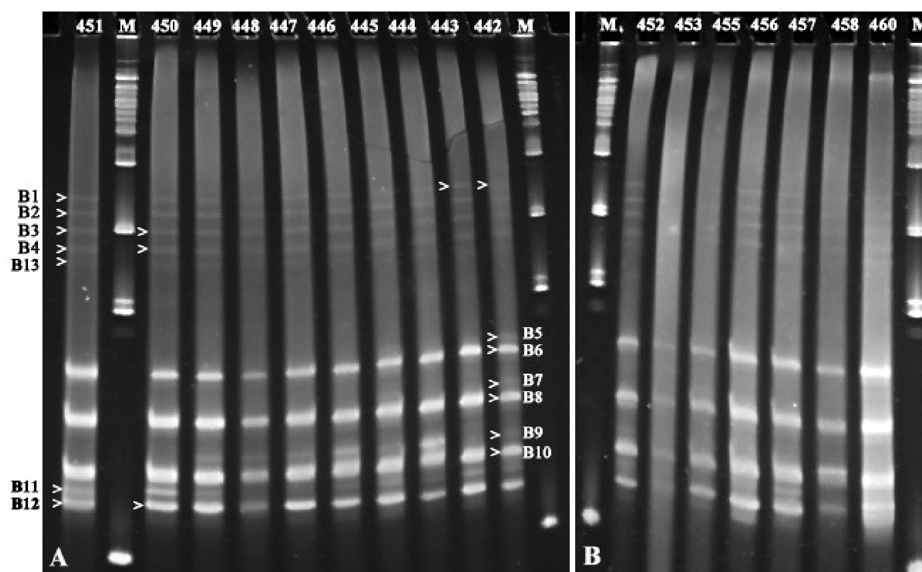

Figure 1. Separation of 16S rDNA fragments of actinomycetes spanning the region of position 226 to 528 (*Escherichia coli* positions) (PCR-DGGE) joined in a GC clamp in DGGE with gradient of 40 to 60%. For identification of different genera of *Actinomycetes*, see Table 2. *Lane M* = Molecular makers.

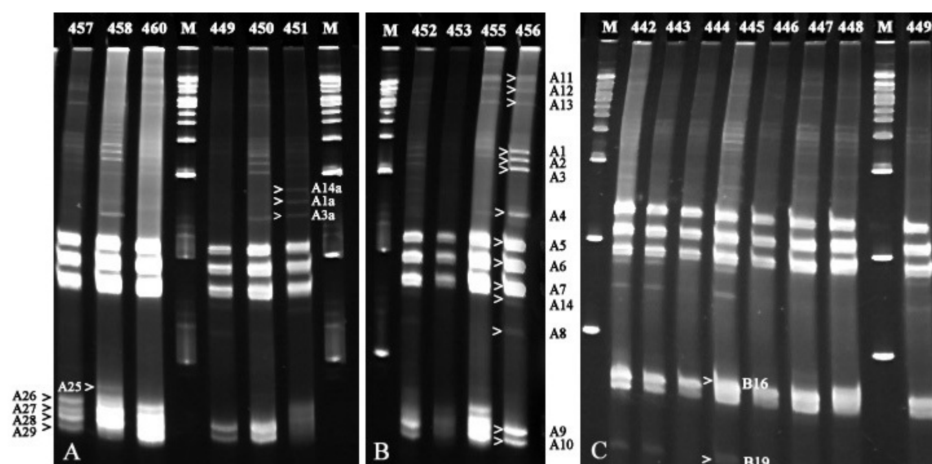

Figure 2. Separation of 16S rDNA fragments of actinomycetes and bacteria spanning the region of position 968 to 1401 (*Escherichia coli* positions) (nested-PCR-DGGE) joined in a GC clamp in DGGE with gradient of 30 to 50%. For identification of different genera of *Actinomycetes* and bacteria, see Table 2. M = molecular makers.

From PCR-DGGE, a total of 13 ribotypes or bands were excised from DGGE gel and 4 bands (labeled B6, B8, B10, B12 in Figure 1) were dominant and ubiquitous, being observed in all soil from all sites. These bands have the greatest similarity to uncultured *Actinomyces* sp, *Rhodococcus* sp and *Streptomyces* sp, respectively (Table 2). The identification of these genera in all soil samples produced an important preliminary finding as genera like *Streptomyces* and *Rhodococcus* are extremely important due to their ability to catabolize a wide range of compounds and produce secondary metabolites with diverse biological activities and chemical structure (Arasu et al., 2008). Also these 4 ubiquitous bands (Figure 1) have the highest densities that reflect the relative high density of these genera from all soil samples. Other non-ubiquitous DGGE bands were closely affiliated with the *Mycobacterium terrae* and uncultured *Actinomycete*. Ribotypes B5, which occurred for soil samples 442 and 445, had the closest identity to *M. terrae* (Table 2). PCR-DGGE is straightforward and allows direct detection of actinomycetes up to the species level. This method enables quantitative estimation of specific actinomycete groups and provides rapid analysis to compare actinomycete communities from different soils in parallel (Heuer et al., 1997).

For nested-PCR-DGGE, a total of 22 bands were excised of which five were common bands (labeled A5, A6, A7, A9, A10 in Figure 2). The identities of five common bands were *Rhodococcus* sp except band A9 that was a *Frankineae* bacterium (Table 2). Sub-order *Frankineae* are important to fix nitrogen in nodules of non-leguminous plants (Heuer et al., 1997) and able to produce few important metabolites (Busto et al., 2006). Nested-PCR-DGGE enables group-specific pattern of the actinomycetes from one soil sample to be directly compared to the bacterial community pattern of the same sample (Heuer et al., 1997). Based on DGGE gel (Figure 2), bands with the highest intensities (A5, A6 and A7) are all from genus *Rhodococcus* sp, which indicated that the abundance of actinomycete communities like *Rhodococcus* sp was high in all 17 soil samples. This result could also be explained by the high diversity of bacterium communities that could have resulted in too little PCR product per

species to produce visible bands. This hypothesis is justified when comparisons of DGGE patterns were made between PCR and nested-PCR. The patterns were much more complex for the nested-PCR method, thus indicating a high bacterial diversity.

Table 2. DGGE bands, with accession number, percentage of similarity to known sequences in GenBank were retrieved from PCR-DGGE of Barrientos soil DNA using PCR (F243-R513GC) and nested-PCR (F243-R1378 and F984GC-R1378) approaches.

New references	Original references	Accession number	Closest relative	Sequence identity (%)	Primer
B1	442-Band-1	GU549417	Uncultured *Actinomyces* sp	98% (285/288)	1
B1	443-Band-1	GU549416	Uncultured *Actinomyces* sp	98% (285/288)	1
B2	442-Band-2	GU549418	Uncultured *Actinomyces* sp	100% (288/288)	1
B3	450-Band-3	GU549419	Uncultured *Actinomyces* sp	97% (281/288)	1
B4	450-Band-4	GU549420	Uncultured *Actinomyces* sp	100% (288/288)	1
B5	442-Band-5	GU549421	*Mycobacterium terrae* (N309)	97% (291/299)	1
B6	442-Band-6	GU549422	Uncultured *Actinomyces* sp	100% (288/288)	1
B6	442-Band-6A	GU549423	Uncultured *Actinomyces* sp	100% (288/288)	1
B7	442-Band-7	GU549424	*Streptomyces psammoticus* (KP1404)	99% (283/285)	1
B8	442-Band-8	GU549425	Uncultured *Actinomycetes* (PPS-4)	98% (282/285)	1
B9	442-Band-9	GU549426	*Rhodococcus* sp (5/3)	99% (283/284)	1
B10	450-Band-10	GU549427	*Rhodococcus* sp (5/3)	98% (280/284)	1
B11	451-Band-11	GU549428	*Streptomyces* sp (061337)	97% (279/285)	1
B12	450-Band-12	GU549429	*Streptomyces* sp (MI-3.3 Y1)	99% (283/285)	1
B13	451-Band-13	GU549430	*Streptomyces* sp (LK1331.2)	98% (282/285)	1
A1	456-Band-1	GU564576	*Rhodococcus* sp (5/3)	98% (426/434)	2
A2	456-Band-2	GU564577	*Rhodococcus* sp (5/3)	97% (423/433)	2
A3	456-Band-3	GU564565	*Leifsonia* sp (ODP61203by2)	98% (431/436)	2
A4	456-Band-4	GU564578	*Rhodococcus* sp (5/3)	97% (425/434)	2
A5	456-Band-5	GU564566	*Rhodococcus* sp (5/3)	97% (424/434)	2
A6	456-Band-6	GU564567	*Rhodococcus* sp (5/3)	97% (425/434)	2
A7	456-Band-7A	GU564569	*Rhodococcus* sp (5/3)	97% (423/434)	2
A7	456-Band-7B	GU564570	*Rhodococcus* sp (5/3)	91% (397/434)	2
A8	456-Band-8	GU564571	*Rhodococcus* sp (5/3)	97% (423/435)	2
A9	456-Band-9	GU564572	*Frankineae* bacterium (MI-1.2 V7)	97% (422/431)	2
A10	456-Band-10	GU564573	*Rhodococcus* sp (5/3)	97% (423/434)	2
A11	456-Band-11	GU564574	*Rhodococcus* sp (5/3)	97% (424/434)	2
A12	456-Band-12	GU564575	*Rhodococcus* sp (5/3)	96% (421/436)	2
A13	456-Band-13	GU564579	Uncultured bacterium clone 66	90% (396/437)	2
A14	456-Band-14	GU564580	*Rhodococcus* sp (5/3)	98% (426/434)	2
A25	458-Band-25	GU564581	Uncultured bacterium (FCPT53)	96% (423/438)	2
A26	458-Band-26	GU564582	Uncultured *Verrucomicrobia* bacterium (1Fe)	98% (432/440)	2
A27	458-Band-27	GU564583	Uncultured bacterium (Nera-L6-F12-T7)	93% (411/438)	2
A28	458-Band-28	GU564584	Uncultured *Gemmatimonadetes* bacterium (100M2-B10)	89% (396/442)	2
A29	458-Band-29	GU564585	Uncultured bacterium (Par-s-23)	98% (427/433)	2
A1a	451-Band-1A	GU564586	Uncultured bacterium (Pal146)	97% (430/440)	2
A3a	451-Band-3A	GU564587	Uncultured bacterium (Par-s-7)	98% (435/441)	2
A14a	451-Band-14A	GU564588	*Rhodococcus* sp (5/3)	97% (423/434)	2
A16	445-Band-16	GU564589	*Actinobacterium* (K4-07A)	96% (420/435)	2
A19	445-Band-19	GU564590	*Rhodococcus* sp (5/3)	97% (421/434)	2

Primer 1 = F243-R513GC; Primer 2 = F243-R1378 and F984GC-R1378.

In general, the wide distribution of these actinomycete genera in different Antarctic soils showed their capability to thrive in a broad range of different soil environments. Microorganisms in these taxa can resist the stress of harsh environment as they could remain in the viable but dormant state for a long time in the form of a spore (Zhang et al., 2001). In this study, a total of 35 ribotypes from 10 genera were identified from 17 soil samples. Only three genera were non-actinomycete bacterium (uncultured bacterium, uncultured *Verrucomicrobia*, uncultured *Gemmatimonadetes*) and seven genera were actinomycetes (uncultured *Actinomy-*

ces sp, *M. terrae*, *Streptomyces* sp, *Rhodococcus* sp, *Leifsonia* sp, *Frankineae* bacterium, and *Actinobacterium*). Based on ribotypes, only 8 ribotypes were non-actinomycete bacteria while the other 27 ribotypes were actinomycetes (Table 2).

On the basis of multiple sequence analyses, the population represented by 450-Band-4 (GU549420) in Figure 3 has 16S rDNA sequences exactly identical to sequences from an uncultured *Actinomyces* sp (EU281967) previously detected in poultry litter in the USA. Sequences from 451-Band-11 (GU549428) were 97% (Table 2) closely related to *Streptomyces* sp 061337 sequences (GU130106) reported from an investigation into the actinomycetes and *Stretpomyces* diversity among mangrove soil from Wenchang, China (Figure 3). Sequences from 445-Band-16 (GU564589) had 96% similarity to *Actinobacterium* K4-07A (EF612290) sequences that were identified from soil collected from abandoned semiarid lead-zinc mine tailing site in Arizona, USA (Table 2). Sequences of 451-Band-3A were 99 and 98% closely related to uncultured bacterium from fresh water sediment (EF632909) from Chile and sub-glacially erupted basaltic rock from Iceland (FJ360656) (Figure 4).

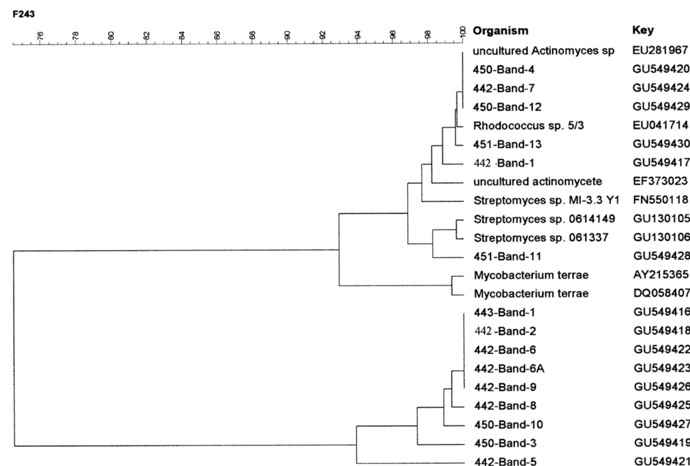

Figure 3. Neighboring-joining dendrogram showing the phylogenetic relationship between actinomycete sequences from PCR-DGGE bands to actinomycete-specific 16S rDNA sequences retrieved from the GenBank database (accession numbers on the right side).

Sequences of 450-Band-4 (GU549420), 442-Band-7 (GU549424) and 450-Band-12 (GU549429) formed a cluster with uncultured *Actinomyces* (EU281967) isolated from poultry litter in the USA and *Rhodococcus* (EU041714) isolated from Antarctic soil with similarity of 100 and 99.5%, respectively (Figure 3). This cluster showed that actinomycetes from the Antarctic and non-Antarctic regions share a high degree of similarity of 16S rDNA sequences. For bacterium from Antarctic and non-Antarctic regions, highly similar 16S rDNA sequences were also found. Sequences of 458-Band-29 (GU564585) formed a cluster with uncultured bacterium (EF632902) isolated from fresh water sediment from Chile and Antarctic bacterium (EU636020) isolated from Collins glacier of Antarctic with similarity of 98.5 and 97.5%, respectively (Figure 4).

Sequences from 442-band-9 (GU549426) and 450-Band-10 (GU549427) had sequences closely identical (99 and 98% similarity) to that of *Rhodococcus* sp 5/3 (EU041714)

 ©FUNPEC-RP www.funpecrp.com.br

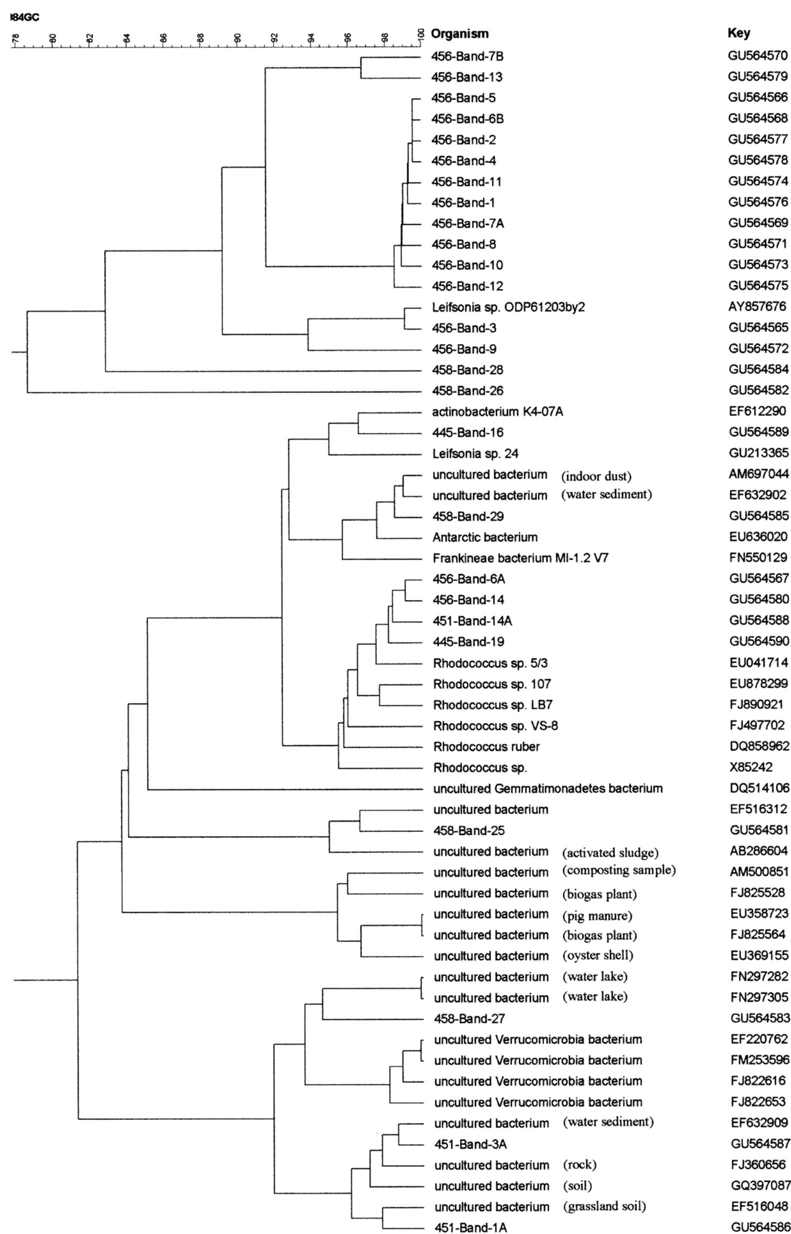

Figure 4. Neighboring-joining dendrogram showing the phylogenetic relationship between actinomycete and bacterium sequences from nested-PCR-DGGE bands to actinomycete and bacterial 16S rDNA sequences retrieved from the GenBank database (accession numbers on the right side).

identified in ornithogenic soil from Cape Hallet, Antarctica (Table 2). This showed that some *Rhodococcus* sp identified from Barrientos Island have a high similarity with *Rhodococcus* identified at other Antarctic regions.

UPGMA analysis of DGGE profiles

DGGE profiles were subjected to clustering based on UPGMA averages to identify soil samples that generated patterns similar to each other (Ibekwe et al., 2001; Boon et al., 2002). UPGMA of 17 soil samples of the actinomycete community (PCR-DGGE) revealed four clusters (a, d, e, h) and six single samples (b, c, f, g, i, j) at 55% similarity level (Figure 5). Within four clusters produced, two clusters (a, d) comprised the soil sample from a similar type of rookery, which is the abandoned gentoo penguin rookery and active chinstrap penguin rookery, respectively (Table 1). Nevertheless, some soil samples from different type of rookery were grouped together in a cluster, i.e., cluster 'e' and 'h', whereas UPGMA of both actinomycete and bacterial communities (nested-PCR-DGGE) revealed six clusters (I, IV, VI, VII, VIII, IX) and 5 single samples (II, III, V, X, XI) at 55% similarity level (Figure 6). Within six clusters, only two clusters (VI, VIII) comprised the soil sample from a similar rookery, which is the abandoned and active penguin rookery, respectively (Table 1).

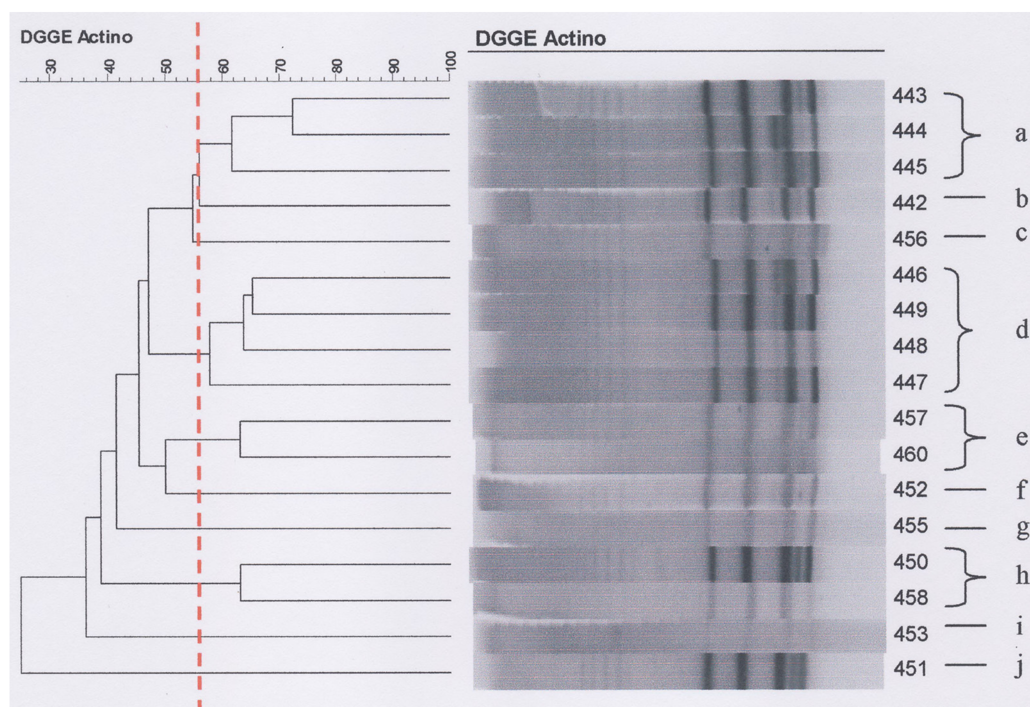

Figure 5. Cluster analysis and the unweighted pair group method using arithmetic averages dendrogram of the denaturing gradient gel electrophoresis (DGGE) from Figure 1A and B.

This dendrogram clustering indicates that there was significant influence of type of rookery towards the clustering of samples. DGGE pattern from PCR targeting actinomycete community is better at clustering samples according to type of rookery in comparison to nested-PCR.

 ©FUNPEC-RP www.funpecrp.com.br

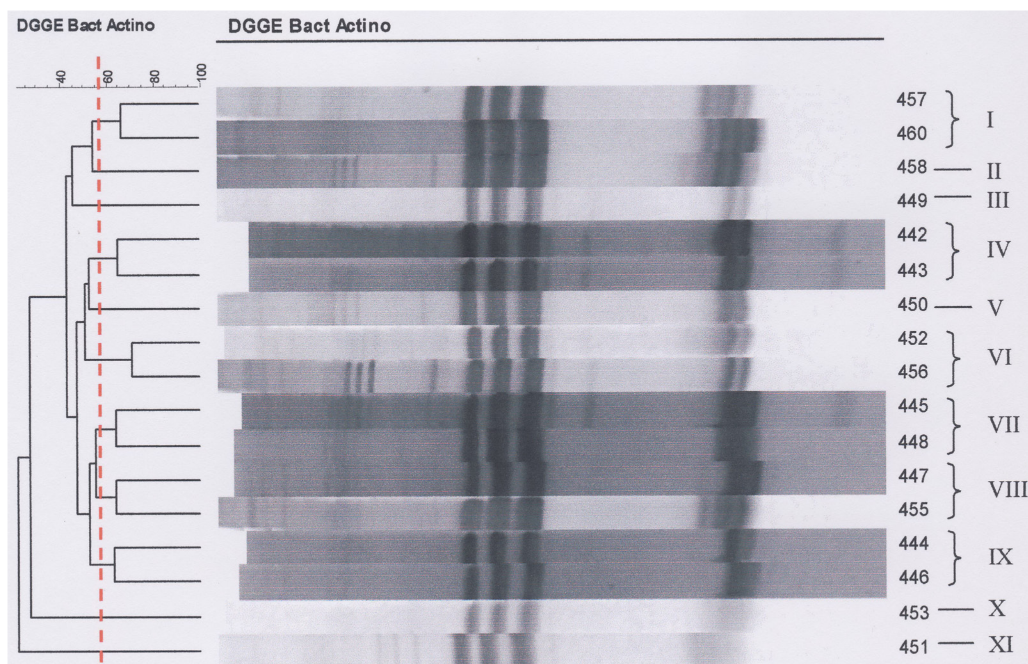

Figure 6. Cluster analysis and the unweighted pair group method using arithmetic averages dendrogram of the denaturing gradient gel electrophoresis (DGGE) from Figure 2A, B and C.

Actinomycetes abundance with soil characteristics

Soil richness with actinomycetes was estimated using the BioNumerics version 6.0 gel analysis software (Applied Maths) to determine total number of bands for each soil (Table 1). Soils with higher number of bands are considered to have higher abundance of actinomycete communities. From DGGE pattern of PCR, there was a significant correlation between types of rookery with abundance of actinomycetes. For example, the soil sample 451, a penguin resting rookery, comprised of low abundance of actinomycetes with only 10 bands detected, whereas soil samples 442, 446, 447, and 449, which were active chinstrap penguin rookeries, comprised of significant higher actinomycete abundance with 22, 22, 21, and 21 bands, respectively (Table 1). These results showed that actinomycetes are highly adaptable to extreme environments in the Antarctic regions.

Researchers have suggested that distributions of Antarctic bacteria are influenced by temperature patterns, plant cover and other soil characteristics (Christie, 1987; Tearle, 1987; Bölter, 1995; Bölter et al., 1997; Harris and Tibbles, 1997; Aislabie et al., 2008). The dense vegetation at Antarctic soils could aid to counter the effects of the extreme environment conditions to some extent (Kowalchuk et al., 2002; Yergeau et al., 2007). Also vegetation like mosses could provide soil microhabitats and influence bacterial abundance in soil. Polar soils with no vegetation generally support fewer microorganisms than soils associated with mosses (Kastovska et al., 2005). In this study, vegetation activity like mosses did not have significant correlation with actinomycete abundance. Soil samples (451, 452, 453, 455, and 458) covered by mosses con-

tain relatively low abundance of actinomycetes compared with no vegetation soils, while some soils (446, 447 and 449) without moss vegetation but active penguin activities contain relatively high abundance of actinomycetes (Table 1). As mosses provide buffering action to maintain soils beneath them at relatively constant water content and temperature, we hypothesized that abundance of actinomycetes were not influenced by these factors. As Gram-positive bacteria, particularly actinomycetes, are exceptionally well adapted for dispersal, as they could produce spores that are highly resistant to desiccation, extreme cold and heat (Wawrik et al., 2007).

To date, actinomycete community analysis across Barrientos Island was still lacking prior to this investigation. Therefore, this study provided the important baseline information regarding the distribution and diversity of actinomycetes throughout Barrientos Island. To conclude, all 17 soil samples from Barrientos Island contain actinomycetes. From PCR-DGGE pattern (Figure 1), relatively stable actinomycete communities were shown to exist across soil samples from different locations. The wide distributions of actinomycetes in Antarctica imply that actinomycete distributions are highly endemic, particularly in soil and sediment (Wawrik et al., 2007; Aislabie et al., 2008). Hence, to enable bioprospecting, this targeting of actinomycetes would benefit from sampling soil from a wide range of geographic locations, as in the Antarctic regions.

PCR-DGGE is cost-effective and enables microbiologists the means to examine and compare large number of samples within short time frame. This study demonstrated that PCR-DGGE can provide an estimate of actinomycetes richness and its diversity in Barrientos Island, Antarctica. Furthermore, PCR-DGGE enables us to determine subsequent analysis to be conducted and provided us a means of choosing samples representing unique or representative communities.

ACKNOWLEDGMENTS

Authors are grateful to Alex Foong Choon Pin for the helpful technical advices on PCR-DGGE. Authors would like to extend their deepest appreciation to Instituto Antártico Ecuatoriano (INAE) for its kind support during the XI Ecuadorian Antarctic Expedition to “Pedro Vicente Maldonado” Research Station, Greenwich Island, and South Shetland Islands. Research supported by the Malaysia Antarctic Research Program, the Academy of Sciences Malaysia and the Universiti Putra Malaysia Research Universiti Grant Scheme (#05-01-11-1219RU).

REFERENCES

Aislabie JM, Chhour KL, Saul DJ, Miyauchi S, et al. (2006). Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. *Soil Biol. Biochem.* 38: 3041-3056.

Aislabie JM, Jordan S and Barker GM (2008). Relation between soil classification and bacterial diversity in soils of the Ross Sea region, Antarctica. *Geoderma* 144: 9-20.

Altschul SF, Gish W, Miller W, Myers EW, et al. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215: 403-410.

Arasu VM, Duraipandiyan V, Agastian P and Ignacimuthu S (2008). Antimicrobial activity of *Streptomyces* spp. ERI-26 recovered from Western Ghats of Tamil Nadu. *J. Mycol. Med.* 18: 147-153.

Baltz RH (2006). Marcel faber roundtable: is our antibiotic pipeline unproductive because of starvation, constipation or lack of inspiration? *J. Ind. Microbiol. Biotechnol.* 33: 507-513.

Barakate M, Ouhdouch Y, Oufdou Kh and Beaulieu C (2002). Characterization of rhizospheric soil streptomycetes from Moroccan habitats and their antimicrobial activities. *World J. Microbiol. Biotechnol.* 18: 49-54.

Bölter M (1995). Distribution of bacterial numbers and biomass in soils and on plants from King George Island (Arctowski Station, Maritime Antarctica). *Polar Biol.* 15: 115-124.

Bölter M, Blume H-P, Schneider D and Beyer L (1997). Soil properties and distributions of invertebrates and bacteria from King George Island (Arctowski Station), maritime Antarctic. *Polar Biol.* 18: 295-304.

Boon N, Windt W, Verstraete W and Top EM (2002). Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiol. Ecol.* 39: 101-112.

Bredholt H, Fjaervik E, Johnsen G and Zotchev SB (2008). Actinomycetes from sediments in the Trondheim fjord, Norway: diversity and biological activity. *Mar. Drugs* 6: 12-24.

Busto E, Monciardini P, Cavaletti L and Bamonte R (2006). Antibiotic-producing ability by representative of a newly discovered lineage of antinomycetes. *Microbiology* 152: 675-683.

Christie P (1987). Nitrogen in two contrasting Antarctic bryophyte communities. *J. Ecol.* 75: 73-93.

Das M, Royer TV and Leff LG (2007). Diversity of Fungi, Bacteria, and Actinomycetes on leaves decomposing in a stream. *Appl. Environ. Microbiol.* 73: 756-767.

Ferris MJ, Muyzer G and Ward DM (1996). Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. *Appl. Environ. Microbiol.* 62: 340-346.

Goshi K, Uchida T, Lezhava A, Yamasaki M, et al. (2002). Cloning and analysis of the telomere and terminal inverted repeat of the linear chromosome of *Streptomyces griseus*. *J. Bacteriol.* 184: 3411-3415.

Harris JM and Tibbles BJ (1997). Factors affecting bacterial productivity in soils on isolated Inland Nunataks in continental Antarctica. *Microb. Ecol.* 33: 106-123.

Heuer H, Krsek M, Baker P, Smalla K, et al. (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl. Environ. Microbiol.* 63: 3233-3241.

Ibekwe AM, Papiernik SK, Gan J, Yates SR, et al. (2001). Impact of fumigants on soil microbial communities. *Appl. Environ. Microbiol.* 67: 3245-3257.

Kastovska K, Elster J, Stibal M and Santruckova H (2005). Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (high arctic). *Microb. Ecol.* 50: 396-407.

Kluge AG and Farris FS (1969). Quantitative phyletics and the evolution of annurans. *Syst. Zool.* 18: 1-32.

Kowalchuk A, Buma DS, De Boer W and Klinkhamer PGL (2002). Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Antonie Van Leeuwenhoek* 81: 509-520.

Mellouli L, Ben Ameur-Mehdi R, Sioud S, Salem M, et al. (2003). Isolation, purification and partial characterization of antibacterial activities produced by a newly isolated *Streptomyces* sp. US24 strain. *Res. Microbiol.* 154: 345-352.

Moncheva P, Tishkov S, Dimitrova N and Chipeva V (2000-2002). Characteristics of soil actinomycetes from Antarctica. *J. Culture Collections* 3: 3-14.

Muyzer G, Teske A, Wirsen CO and Jannasch HW (1995). Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Arch. Microbiol.* 164: 165-172.

Muyzer G, Brinkhoff T, Nubel U, Santegoeds C, et al (1998). Denaturing Gradient Gel Electrophoresis (DGGE) in Microbial Ecology. In: Molecular Microbial Ecology Manual (Akkermans ADL, Van Elsas JD and Bruijn FJ, eds.). Kluwer Academic Publishers, Dordrecht, 3.4.4/1-3.4.4/27.

Nakatsu CH (2007). Soil microbial community analysis using denaturing gradient gel electrophoresis. *Soil Sci. Soc. Am. J.* 71: 562-571.

Nikolcheva LG, Cockshutt AM and Barlocher F (2003). Determining diversity of freshwater fungi on decaying leaves: comparison of traditional and molecular approaches. *Appl. Environ. Microbiol.* 69: 2548-2554.

Nolan R and Cross T (1988). Isolation and Screening of Actinomycetes. In: Actinomycetes in Biotechnology (Goodfellow M, Williams ST and Mordarski M, eds.). Academic Press, London, 1-32.

Rölleke S, Muyzer G, Wawer C and Wanner G (1996). Identification of bacteria in a biodegraded wall painting by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl. Environ. Microbiol.* 62: 2059-2065.

Saadoun I and Gharaibeh R (2003). The *Streptomyces* flora of Badia region of Jordan and its potential as a source of antibiotic-resistant bacteria. *J. Arid. Environ.* 53: 365-371.

Saitou N and Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406-425.

Saul DJ, Aislabie JM, Brown CE, Harris L, et al. (2005). Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. *FEMS Microbiol. Ecol.* 53: 141-155.

Tearle PV (1987). Cryptogamic carbohydrate release and microbial response during freeze-thaw cycles in Antarctic fellfield fines. *Soil Biol. Biochem.* 19: 381-390.

Teske A, Wawer C, Muyzer G and Ramsing NB (1996). Distribution of sulfate-reducing bacteria in a stratified fjord

(Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. *Appl. Environ. Microbiol.* 62: 1405-1415.

Thakur D, Yadav A, Gogoi BK and Bora TC (2007). Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. *J. Mycol. Med.* 17: 242-249.

Wawrik B, Kutliev D, Abdivasievna UA, Kukor JJ, et al. (2007). Biogeography of actinomycete communities and type II polyketide synthase genes in soils collected in New Jersey and Central Asia. *Appl. Environ. Microbiol.* 73: 2982-2989.

Wenhui Z, Zucong C, Lichu Y and He Z (2007). Effects of the long-term application of inorganic fertilizers on microbial community diversity in rice-planting red soil as studied by using PCR-DGGE. *Acta Ecol. Sin.* 27: 4011-4018.

Yergeau E, Bokhorst S, Huiskes AH, Boschker HT, et al. (2007). Size and structure of bacterial, fungal and nematode communities along an Antarctic environmental gradient. *FEMS Microbiol. Ecol.* 59: 436-451.

Zhang X, Yao T, Ma X and Wang N (2001). Analysis of the characteristics of microorganisms packed in the ice core of the Malan Glacier. *Tibet. China Sci. China Ser. D. Earth Sci.* 44: 396-374.