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Diversity and bioactivity of actinomycetes from Signy Island terrestrial soils, maritime Antarctic

Shing Yi Pan[1], Geok Yuan Annie Tan[1,2*], Peter Convey[2,3], David A. Pearce[2,3,4,5] & Irene K. P. Tan[1,2]

[1] Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia;
[2] National Antarctic Research Centre, B303, IPS Building, University of Malaya, Kuala Lumpur 50603, Malaysia;
[3] British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, United Kingdom;
[4] Faculty of Health and Life Sciences, University of Northumbria, Ellison Building, Newcastle Upon Tyne NE1 8ST, United Kingdom;
[5] University Centre in Svalbard, P.O. Box 156, Longyearbyen N-9171, Norway

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Abstract The Antarctic represents a largely untapped source for isolation of new microorganisms with potential to produce bioactive natural products. Actinomycetes are of special interest among such microorganisms as they are known to produce a large number of natural products, many of which have clinical, pharmaceutical or agricultural applications. We isolated, characterized and classified actinomycetes from soil samples collected from different locations on Signy Island, South Orkney Islands, in the maritime Antarctic. A total of 95 putative actinomycete strains were isolated from eight soil samples using eight types of selective isolation media. The strains were dereplicated into 16 groups based on morphology and Amplified Ribosomal DNA Restriction Analysis (ARDRA) patterns. Analysis of 16S rRNA gene sequences of representatives from each group showed that streptomycetes were the dominant actinomycetes isolated from these soils; however, there were also several strains belonging to diverse and rare genera in the class *Actinobacteria*, including *Demetria*, *Glaciibacter*, *Kocuria*, *Marmoricola*, *Nakamurella* and *Tsukamurella*. In addition, screening for antibacterial activity and non-ribosomal peptide synthetase genes showed that many of the actinomycete strains have the potential to produce antibacterial compounds.

Keywords **actinomycete diversity, Antarctica, rare actinomycetes, non-ribosomal peptide synthetase genes**

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1 Introduction

Actinomycetes, which are phylogenetically defined as a group within the high G+C Gram-positive Actinobacteria[1], are one of the major contributors to microbial communities in Antarctic soils[2-4]. Although several novel species of actinomycetes including *Friedmanniella antarctica*[5], *Modestobacter multiseptatus*[6], *Marisediminicola antarctica*[7], *Streptimonospora salina*[8] and *Pseudonocardia antarctica*[9] have been isolated from Antarctic samples, recent applications of molecular methods have revealed that a very wide diversity of actinomycete taxa in this region remains uncultured[3-4].

Signy Island (60°43′S, 45°38′W) is one of the South Orkney Islands located in the South Atlantic Ocean to the north of the Weddell Sea. The island has a maritime Antarctic climate with a mean annual air temperature of around −3.5℃[10]. About 50% of the surface of Signy Island is free from ice and snow during the summer and it has varied terrain including a lowland and coastal zone that provides a diversity of habitats[11-12]. Previous investigations of the microbial diversity on Signy Island have largely focused on its freshwater lake systems[13] or the direct application of molecular approaches[14-15]. In the present study, a culture-based method was used to isolate actinomycetes from

* Corresponding author (email: gyatan@um.edu.my)

soil samples collected from various sites on Signy Island. Actinomycetes are well known to have the capacity to synthesize bioactive compounds including antibiotics, antitumor agents and antimicrobial peptides[16]. Many of these peptides are produced using the non-ribosomal peptide synthetases (NRPS) biosynthetic system, which consists of multi-modular enzymes. Hence, screening for antibacterial activity and the presence of NRPS genes in the isolated actinomycetes was also carried out.

2 Materials and methods

2.1 Soil samples

Eight soil samples were collected from five sites on Signy Island representing various soil types. These included muddy soil from Backslope (BS7) and Elephant Flats (EF1 and EF2), ornithogenic soil from Gourlay Peninsula (G1, G2 and G4) and dry polygon soil from Three Lakes Valley (LV1) and Spindrift Col (SD1). Samples were collected between December 2005 and January 2006. At each location, approximately 10−30 g of surface soil were collected aseptically into sterile containers, returned to the research station on Signy Island and frozen at −20℃. Samples were kept frozen at this temperature throughout transport to the United Kingdom and then Malaysia until further processing. The soil pH was determined on thawed samples in the lab as the average of three readings of 1:2 (soil:water) suspensions using a waterproof pH tester 2 (Eutech Instruments, Malaysia).

2.2 Isolation, purification and preservation of actinomycetes

A sub-sample (1 g) of each sample was transferred into a universal bottle containing 9 mL of Ringer's solution and then serially diluted from 10^{-1} to 10^{-4} before 100 μL of each suspension was plated onto one of eight types of isolation media (10% strength): R2A[17], R2A with 0.4% (w/v) sodium propionate (modified from [15]), R2A with 50 μg·mL^{-1} rose Bengal (modified from [15]), Gauze's medium 2, starch casein nitrate (SCN) agar[18], tryptic soy agar (Difco laboratories), tryptic soy agar with 0.1% (w/v) starch and tryptic soy agar with 0.1% (w/v) colloidal chitin. Cycloheximide (25 μg·mL^{-1}) and nystatin (25 μg·mL^{-1}) were added to all isolation media to suppress the growth of fungi. The isolation agar plates were incubated at 15℃ for up to 4 months, and colonies of actinomycetes were purified onto yeast malt extract agar and preserved in glycerol (20%, v/v) at −20℃.

2.3 DNA extraction

Total genomic DNA was extracted from pure cultures of actinomycetes using a previously described method, with slight modification[19]. A loopful of cells was suspended in 150 μL of TE buffer containing glass beads (<106 μm; Sigma G-4649), 2.5 μL of lysozyme (50 mg·mL^{-1}) and 5 μL of Proteinase K (20 mg·mL^{-1}). The suspension was mixed by vortexing, incubated at 37℃ for 2 h and then centrifuged at 14 000 rpm for 10 min. Next, the supernatant was transferred into a new tube, incubated at 75℃ for 15 min and centrifuged. The quality of DNA preparations was checked by agarose gel electrophoresis (0.8%, w/v) for 30 min. The remaining DNA extracts were kept at −20℃ until further use.

2.4 Amplification of 16S rRNA genes

DNA extracts were used as template DNA (*ca*. 50 μg·μL^{-1}) in 50 μL reactions containing 0.2 mM of each of the four dNTPs, 0.2 μM of primers 27f and 1492r[20], 1.5 mM $MgCl_2$ and 1.25 U Bio*Taq* DNA polymerase (Fermentas, Lithuania) with the appropriate 1x reaction buffer. Controls without template DNA were included in each PCR run. Amplification was performed in a thermal cycler (Swift^{TM} MAXI, ESCO, Singapore) according to the following profile: initial denaturation for 5 min at 95℃ prior to the addition of Bio*Taq*, followed by 30 cycles of 1 min each at 94℃, 55℃ and 72℃, and then final extension at 72℃ for 10 min. The 1 500 bp fragments were analyzed by agarose gel electrophoresis (1.0%, w/v) for 45 min.

2.5 Amplified ribosomal DNA restriction analysis (ARDRA)

Two restriction enzymes, *Bss*MI and *Hha*I, were used to digest the amplified 16S rRNA fragment. The reactions were performed in final volumes of 20 μL containing at least 3 μg·mL^{-1} of 16S rRNA products at 37℃ for 3 h. The digestions were then analyzed by agarose gel electrophoresis (1.5%, w/v) for 1 h at 110 V followed by visualization of the banding patterns using a UV imager (Cleaver Scientific, UK).

2.6 16S rRNA gene sequencing and phylogenetic analysis

The amplified 16S rRNA gene products from representatives of each ARDRA group were purified using a QIAquick® PCR purification kit (Qiagen, California, USA) and then sent to 1st Base (Selangor, Malaysia) for sequencing, which uses the Sanger Big-dye deoxy-terminator method on an Applied Biosystems 3730xl DNA analyzer. The partial 16S rRNA gene sequences were compared against the GenBank database using the BLAST program[21] and the EZTaxon web-based tool[22]. The sequences were aligned with closely-related species and the neighbor-joining method was employed to construct the phylogenetic tree with bootstrap analysis of 1 000 replications using the Molecular Evolutionary Genetics Analysis software version 4 (MEGA4)[23].

2.7 Screening for antibacterial activity

Representative strains of each ARDRA group were tested for antibacterial activity against *Escherichia coli*

ATCC25922, *Proteus vulgaris* ATCC13315 and *Staphylococcus aureus* ATCC25923 using the agar plug method[24].

2.8 Screening for the presence of NRPS genes

DNA extracts were also used as template DNA for NRPS gene detection. The PCR reactions were prepared in a final volume of 50 μL containing 0.2 mM each of the four dNTPs, 0.4 μM of primers A3f and A7r[16], 10% DMSO and 1U Bio-*Taq* DNA polymerase with the appropriate 1x reaction buffer. The 700−800 bp amplified fragments were analyzed by agarose gel electrophoresis (1.0%, w/v) for 45 min and then visualized using a UV imager (Cleaver Scientific, UK).

3 Results and discussion

A total of 95 actinomycete strains were isolated from the eight soil samples using eight types of isolation media. The pH of the soil samples ranged from slightly acidic to neutral (Table 1). All media used supported the growth of actinomycetes; however, the medium best suited for isolation of actinomycetes could not be identified as different media was used for different soil samples. The largest diversity (54 strains) was isolated from the dry polygon soil sample from Three Lakes Valley (LV1).

Table 1 Sampling sites and average values of soil pH

Soil samples	Location (GPS coordinates)	pH
BS7	Backslope (60°42.6′S, 45°35.6′W)	5.41
EF1	Elephant Flats (60°42.2′S, 45°36.4′W)	6.60
EF2	Elephant Flats (60°42.3′S, 45°36.4′W)	6.80
G1	Gourlay Peninsula (60°43.9′S, 45°35.3′W)	5.80
G2	Gourlay Peninsula (60°43.9′S, 45°35.3′W)	5.10
G4	Gourlay Peninsula (60°43.9′S, 45°35.3′W)	6.81
LV1	Three Lakes Valley (60°41.9′S, 45°36.7′W)	7.54
SD1	Spindrift Col (60°41.1′S, 45°38.3′W)	5.61

The actinomycete strains were clustered into 16 different ARDRA groups based on the restriction patterns of *Hha*I and *Bss*MI. Phylogenetic analysis of the 16S rRNA gene sequences of representatives from several of the ARDRA groups showed 96%−100% similarity with sequences of previously described species including *Demetria terragena*, *Glaciibacter superstes*, *Kocuria palustris*, *Marmoricola aequoreus*, *Micrococcus luteus*, *Nocardia ninae*, *Rhodococcus yunnanensis*, *Streptomyces annulatus*, *Streptomyces beijiangensis* and *Tsukamurella tyrosinosolvens* (Table 2). Fifty-three strains were clustered in the same ARDRA group (Group 1), in which the 16S rRNA gene sequences were 99% similar to *Streptomyces beijiangensis*. These results indicate repeated isolation of the same species from different soil samples using different media (data not shown), suggesting that this streptomycete is readily cultivated from maritime Antarctic soils. Indeed, streptomycetes have previously been noted to be the most abundant actinomycetes readily isolated from most aerobic soils in Antarctica[3,16]. There were also several strains identified as belonging to rare actinomycete genera such as *Glaciibacter*, *Marmoricola* and *Tsukamurella*. In addition, six novel 16S rRNA gene sequences representing five genera, *Demetria*, *Glaciibacter*, *Mycobacterium*, *Nocardia* and *Rhodococcus*, were identified (Figure 1). Strain PSY032 could not be assigned to any genus (Table 2). These novel sequences likely represent undescribed species and/or genera, although further biochemical and genetic studies are required to confirm this.

Table 2 Identification based on 16S rRNA gene sequences and bioactivity of selected actinomycete strains

ARDRA Group	Representative strains	Closest phylogenetic affiliation	Identity	Number of strains	Antibacterial activity	Presence of NRPS genes
1	PSY097	*Streptomyces beijiangensis*	99%	53	Yes	Yes
2	PSY019	*Rhodococcus yunnanensis*	99%	8	No	Yes
3	PSY065	*Mycobacterium aromaticivorans*	98%	6	No	Yes
4	PSY066	*Demetria terragena*	96%	3	No	No
5	PSY027	*Rhodococcus corynebacterioides*	97%	10	No	Yes
6	PSY085	*Kocuria palustris*	100%	3	No	Yes
7	PSY095	*Glaciibacter superstes*	97%	1	No	No
8	PSY079	*Nakamurella panacisegetis*	99%	1	No	No
9	PSY096	*Rhodococcus triatomae*	96%	1	No	No

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10	PSY086	*Microbacterium testaceum*	99%	1	No	No
11	PSY016	*Streptomyces anulatus*	99%	1	No	Yes
12	PSY093	*Marmoricola aequoreus*	99%	3	No	Yes
13	PSY024	*Tsukamurella tyrosinosolvens*	100%	1	No	Yes
14	PSY031	*Micrococcus luteus*	99%	1	No	No
15	PSY032	*Actinobacterium*	97%	1	No	No
16	PSY021	*Nocardia ninae*	97%	1	No	Yes

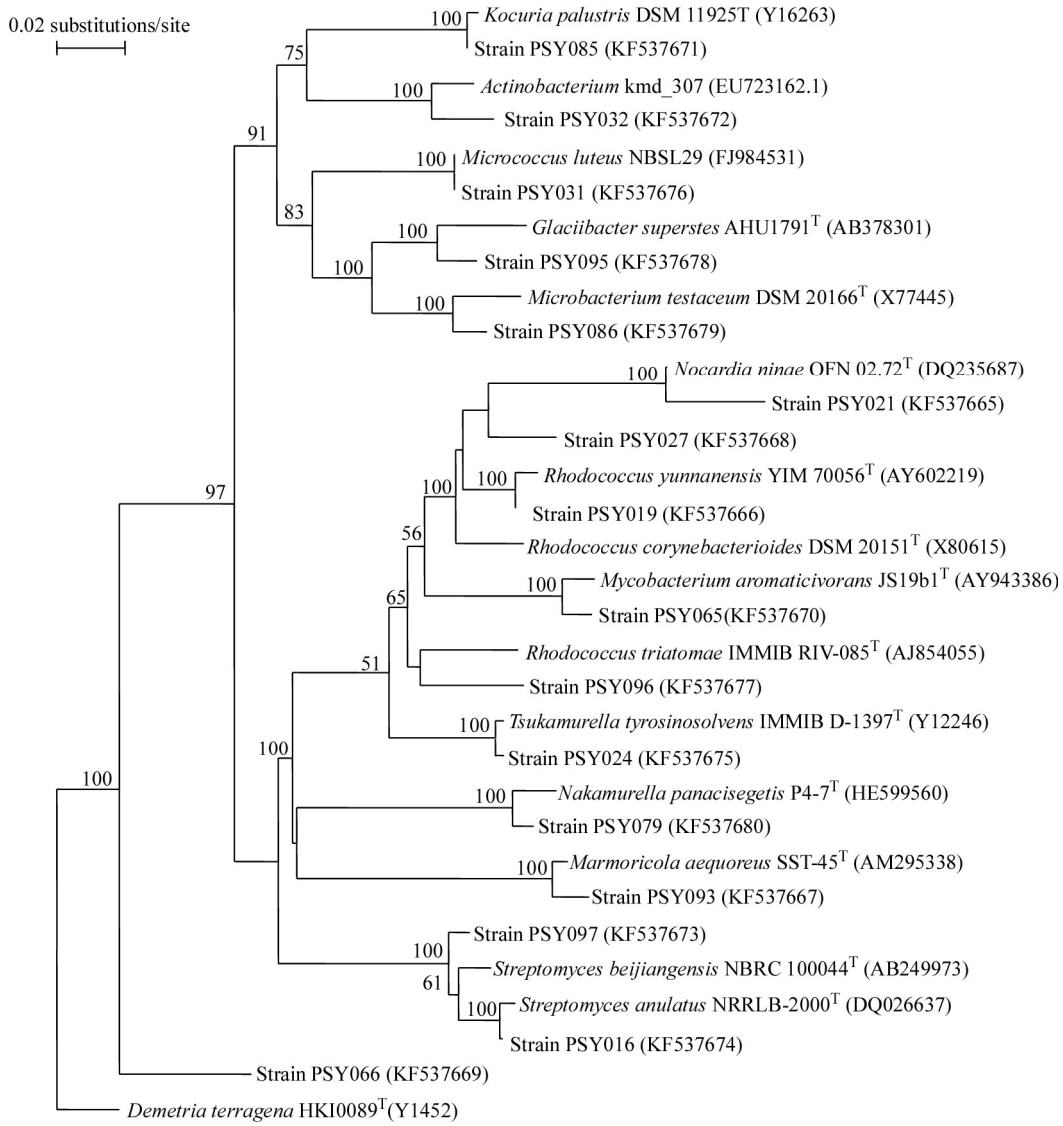

Figure 1 Neighbor-joining phylogenetic tree showing relationship of selected actinomycete strains and their close relatives based on partial 16S rRNA gene sequences. Numbers at the nodes indicate levels of bootstrap support based on 1 000 resamplings, only values ⩾ 50% are indicated.

Forty-six of the 53 strains from the ARDRA group 1 (*S. beijiangensis* group) showed antibacterial activity against *P. vulgaris* and *S. aureus*. In addition, all 53 strains were found to contain the NRPS genes. To the best of our knowledge, this is the first report indicating that *S. beijiangensis* (a psychrotolerant actinomycete initially isolated from soil in Beijiang, Xinjiang, the most-westerly province of China) possesses antibacterial activity and NRPS genes. NRPS genes were also detected in several of the novel strains including strains PSY085 (*Kocuria* sp.) and PSY024 (*Tsukamurella* sp.) (Table 2). There was no inhibitory activity against *E. coli* in any of the actinomycetes.

Previous studies[3,25-29] have shown that there is a very high density of new or unknown actinomycetes in Antarctic environments. In our phylogenetic analysis, cultures were obtained from a wide range of existing genera that are mostly cosmopolitan. However, several of the cultures recovered may represent previously unknown species and/or genera. This preliminary investigation of terrestrial soils from Signy Island demonstrated that there is much untapped potential in maritime Antarctic terrestrial environments and that they are rich sources of both novel species and rare genera of actinomycetes with the ability to produce bioactive compounds.

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References

1 Stackebrandt E, Rainey F A, Ward-Rainey N L. Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. Int J Syst Bacteriol, 1997, 47(2): 479-491.

2 Aislabie J M, Chhour K L, Saul D J, et al. Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. Soil Biol Biochem, 2006, 38(10): 3041-3056.

3 Babalola O O, Kirby B M, Roes-Hill M L, et al. Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. Environm Microbiol, 2009, 11(3): 566-576.

4 Cary C S, McDonald I R, Barrett J E, et al. On the rocks: the microbiology of Antarctic Dry Valley soils. Nature Rev Microbiol, 2010, 8(2): 129-138.

5 Schumann P, Prauser H, Rainey F A, et al. *Friedmanniella antarctica* gen. nov., sp. nov., an LL-diaminopimelic acid-containing actinomycete from Antarctic sandstone. Int J Syst Bacteriol, 1997, 47(2): 278-283.

6 Mevs U, Stackebrandt E, Schumann P, et al. *Modestobacter multiseptatus* gen. nov., sp. nov., a budding actinomycete from soils of the Asgard Range (Transantarctic Mountains). Int J Syst Evol Microbiol, 2000, 50(1): 337-346.

7 Li H R, Yu Y, Luo W, et al. *Marisediminicola antarctica* gen. nov., sp. nov., an actinobacterium isolated from the Antarctic. Int J Syst Evol Microbiol, 2010, 60(Pt 11): 2535-2539.

8 Cui X L, Mao P H, Zeng M, et al. *Streptimonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*. Int J Syst Evol Microbiol, 2001, 51(Pt 2): 357-363.

9 Prabahar V, Dube S, Reddy G S, et al. *Pseudonocardia antarctica* sp. nov. an actinomycetes from McMurdo Dry Valleys, Antarctica. Syst Appl Microbiol, 2004, 27(1): 66-71.

10 Guglielmin M, Ellis-Evans C J, Cannone N. Active layer thermal regime under different vegetation conditions in permafrost areas. A case study at Signy Island (Maritime Antarctica). Geoderma, 2008, 144(1-2): 73-85.

11 Holdgate M W. Signy Island. Phil Trans R Soc Lond B Biol Sci, 1967, 252(777): 173-177.

12 Smith R I L. Signy Island as a paradigm of biological and environmental change in Antarctic terrestrial ecosystems // Kerry K R, Hempel G. Antarctic ecosystems: ecological change and conservation. Berlin Heidelberg: Springer Verlag, 1990: 32-50.

13 Pearce D A, van der Gast C, Lawley B, et al. Bacterioplankton community diversity in a maritime Antarctic lake, determined by culture-dependent and culture-independent techniques. FEMS Microbiol Ecol, 2003, 45(1): 59-70.

14 Chong C W, Tan G Y A, Wong R C S, et al. DGGE fingerprinting of bacteria in soils from eight ecologically different sites around Casey Station, Antarctica. Polar Biol, 2009, 32(6): 853-860.

15 Chong C W, Pearce D A, Convey P, et al. High levels of spatial heterogeneity in the biodiversity of soil prokaryotes on Signy Island, Antarctica. Soil Biol Biochem, 2010, 42(4): 601-610.

16 Ayuso-Sacido A, Genilloud O. New PCR Primers for the screening of NRPS and PKS-I systems in actinomycetes: Detection and distribution of these biosynthetic gene sequences in major taxonomic groups. Microb Ecol, 2005, 49(1): 10-24.

17 Ronald M A. Handbook of microbiological media. New York: CRC Press, 1993.

18 Küster E, Williams S T. Selection of media for isolation of streptomycetes. Nature, 1964, 202(4935): 928-929.

19 Sambrook J F, Russell D W. Molecular cloning: A laboratory manual, 3rd edn. New York: Cold Spring Harbor Laboratory Press, 2001.

20 Lane D J. 16S/23S rRNA sequencing // Stackerbrandt E, Goodfellow M. Nucleic acid techniques in bacterial systematics. New York: Wiley & Sons, 1991: 115-175.

21 Altschul S F, Gish W, Miller W, et al. Basic local alignment search tool. J Mol Biol, 1990, 215(3): 403-410.

22 Chun J, Lee J H, Jung Y, et al. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int J Syst Evol Microbiol, 2007, 57(10): 2259-2261.

23 Tamura K, Dudley J, Nei M, et al. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol, 2007, 24(8): 1596-1599.

24 Nedialkova D, Naidenova M. Screening the antimicrobial activity of actinomycetes strains isolated from Antarctica. J Cult Collect, 2005, 4(1): 29-35.

25 Nichols D S, Sanderson K, Buia A, et al. Bioprospecting and biotechnology in Antarctica // Jabour-Green J, Haward M. The Antarctic: Past, Present & Future. Hobart: Antarctic CRC, 2002: 85-103.

26 Teng H B, Tang H X, Xiao H, et al. Isolation and identification of actinobacteria from rhizospheric soil in the Arctic Yellow River Station. Chinese J Polar Res, 2009, 21(1): 33-42 (in Chinese).

27 Sanyika W T, Stafford W, Don Cowan A. The soil and plant determinants of community structures of the dominant actinobacteria in Marion Island terrestrial habitats, Sub-Antarctica. Polar Biol, 2012, 35(8): 1129-1141.

28 Peeters K, Hodgson D A, Convey P, et al. Culturable diversity of heterotrophic bacteria in Forlidas Pond (Pensacola Mountains) and Lundström Lake (Shackleton Range), Antarctica. Microb Ecol, 2011, 62(2): 399-413.

29 Pearce D A, Newsham K K, Thorne M A, et al. Metagenomic analysis of a southern maritime Antarctic soil. Front Microbiol, 2012, 3: 403.