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Lemese Ah Tow · Don A. Cowan

Dissemination and survival of non-indigenous bacterial genomes in pristine Antarctic environments

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Abstract Continental Antarctic is perceived as a largely pristine environment, although certain localized regions (e.g., parts of the Ross Dependency Dry Valleys) are relatively heavy impacted by human activities. The procedures imposed on Antarctic field parties for the handling and disposal of both solid and liquid wastes are designed to minimise eutrofication and contamination (particularly by human enteric bacteria). However, little consideration has been given to the significance, if any, of less obvious forms of microbial contamination resulting from periodic human activities in Antarctica. The predominant commensal microorganism on human skin, *Staphylococcus epidermidis*, could be detected by PCR, in Dry Valley mineral soils collected from heavily impacted areas, but could not be detected in Dry Valley mineral soils collected from low impact and pristine areas. Cell viability of this non-enteric human commensal is rapidly lost in Dry Valley mineral soil. However, *S. epidermidis* can persist for long periods in Dry Valley mineral soil as non-viable cells and/or naked DNA.

Keywords 16S rDNA PCR · Antarctica · *Staphylococcus epidermidis* · Microbial contamination

Introduction

The Dry Valleys of Eastern Antarctica harbor a variety of unique microbial biotopes, including lake systems, mineral soils, endolithic-bearing rock strata and seasonally inundated flush areas (Wynn-Williams 1990; Cowan and Ah Tow 2004). Culturing and microscopic studies, comprehensively reviewed by Friedmann (1993), have shown that mineral soils contain a variety of bacteria, fungi, yeast and protozoa. Culture-independent techniques such as DNA fluorescent dyes (for example, SYBR Green) (Weinbauer et al. 1998; Carpenter et al. 2000) and ssu rRNA gene PCR techniques (Carpenter et al. 2000; Glöckner et al. 2000; Gordon et al. 2000) have contributed significantly to the detection and identification of new microorganisms in these extreme environments, but so-called 'uncultured' phylotypes remain the dominant sequences isolated (Smith et al. unpublished results).

Various pathways for introducing microbes into Antarctica have been observed and these include atmospheric circulation (wind), oceanic currents, fish, migratory birds and marine mammals (Vincent 2000). While studies have shown that pollen and microorganisms are introduced into Antarctica via atmospheric circulation (Vincent 1988; Priscu et al. 1999), these can be considered as a means of natural dissemination. However, increases in human activities in Antarctica have accelerated the rate of introduction of non-indigenous microorganisms to the ice-free areas of the Antarctic continent. The durability or longevity of non-indigenous microorganisms in the Antarctic environment is also of concern. Numerous researchers have reported on the preservation of DNA in various environments, including amber (Cano and Borucki 1995), tundra soil (Stokstad 2003), permafrost (Willerslev et al. 2003) as well as in subfossil bones of Adelie penguins in Antarctica (Lambert et al. 2002).

Scientific, exploratory and touristic interest in this unique continent has led to a rapid increase in human activities. Research activities in the Dry Valleys alone, serviced by only two scientific bases (McMurdo station (US) and Scott Base (NZ)), typically imposes 500–1,000 individuals across a reasonably localised area for durations of 2–8 weeks each austral summer period. The results of human activity potentially include physical

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L. Ah Tow · D. A. Cowan (✉)
Advanced Research Centre for Applied Microbiology,
Department of Biotechnology,
University of the Western Cape, 7535 Bellville,
Cape Town, South Africa
E-mail: dcowan@uwc.ac.za
Fax: +27-219-593505

Table 1 Eastern Antarctic Ross Island and Dry Valleys mineral soils sampling sites

Site reference	Location	GPS reading	Designation[a]
CR1–2	Cape Royds, 20 m from front of historic hut	77°33.081′S 166°09.898′E	Medium impact area
CE1–2	Cape Evans, 20 m from ground in vicinity of front door to historic hut	77°38.292′S 166°24.414′E	Medium impact area
MAC1	McMurdo station, from Derelict Junction bus stop	–	High impact area
MAC2	McMurdo station, from pedestrian entrance to coffee shop	–	High impact area
SB1	Scott Base, from vicinity of rear entrance to Base	–	High impact area
SB2	Scott Base, from vicinity of side entrance to stores hanger	–	High impact area
SB(D)	Scott Base, floor dust from domestic vacuum cleaner	–	Positive control
BIC1–3	Bratina Island camp sites, from ground outside doors of huts 1–3	78°00.82′S 165°33.05′E	High impact areas
BIC4	Bratina Island, pristine sample from ridge S of Salt Pond.	78°03.991′S 165°32.753′E	Pristine site
MVT12	Myers Valley, from steep ridge above frozen lake; Altitude, 2689′ asl.	78°03.968′S 163°52.083′E	Pristine site
UWV1	Upper Wright Valley, pristine sample from elevated valley. Altitude; 4322′ asl.	77°30.674′S 160°40.164′E	Pristine site

[a] Impact status: *Pristine site* < 0.1 h human presence within 50 m radius of sample site p.a., *Medium impact site* < 200 h human presence within 50 m radius of sample site p.a., *High impact site* > 1,000 h human presence within 50 m radius of sample site p.a. Note that positive control sample SB(D) represents approximately 15,000 h total impact (24 h p.d.×90 pax ×7d accumulation)

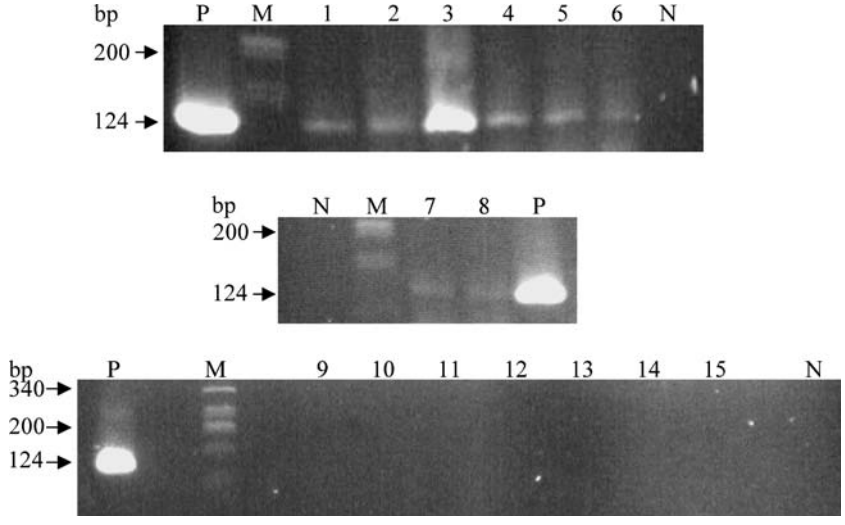

Fig. 1 Detection of *Staphylococcus epidermidis* in high impact, low impact and pristine sites in Antarctica. The *S. epidermidis*-specific PCR primers target a 124 bp DNA fragment which is specific to the *S. epidermidis* genome (Martineau et al. 1996). Approximately 10 ng of bulk genomic DNA, extracted from Antarctic Ross Dependency soil samples was used,as template DNA in *S. epidermidis*-specific PCR amplifications: SB1 (lane 1); SB2 (lane 2); SB(D) (lane 3); BIC1 (lane 4); BIC2 (lane 5); BIC3 (lane 6); MAC1 (lane 7); MAC2 (lane 8); CR1 (lane 9); CR2 (lane 10); CE1 (lane 11); CE2 (lane 12); BIC4 (lane 13); UWV1 (lane 14) and MVT12 (lane 15). N represents the PCR negative controls (sterile water as template); P represents the PCR positive controls (2 ng *S. epidermidis* genomic DNA as template); and M depicts the molecular weight marker (λ *Pst* I). PCR products were visualized on a 2% agarose gel containing 500 μg/ml Ethidium Bromide

disruption of surface soils, eutrofication of soils and water systems, and contamination by detritus, xenobiotic chemicals and non-indigenous microorganisms.

Microbial contamination has been detected at both the macroscopic (Eckford et al. 2002) and microscopic levels. For example, there are numerous reports on the isolation or detection of non-indigenous enteric bacteria derived from human faecal waste (McFeters et al. 1993; Edwards et al. 1998; Sjöling and Cowan 2000; Bruni et al. 1997; Baker et al. 2003). Here we show that non-enteric human commensal microbiota are rapidly disseminated into the Antarctic Dry Valley mineral soils tested, and that although cell viability is rapidly lost, non-viable cells and/or naked DNA persist for long periods.

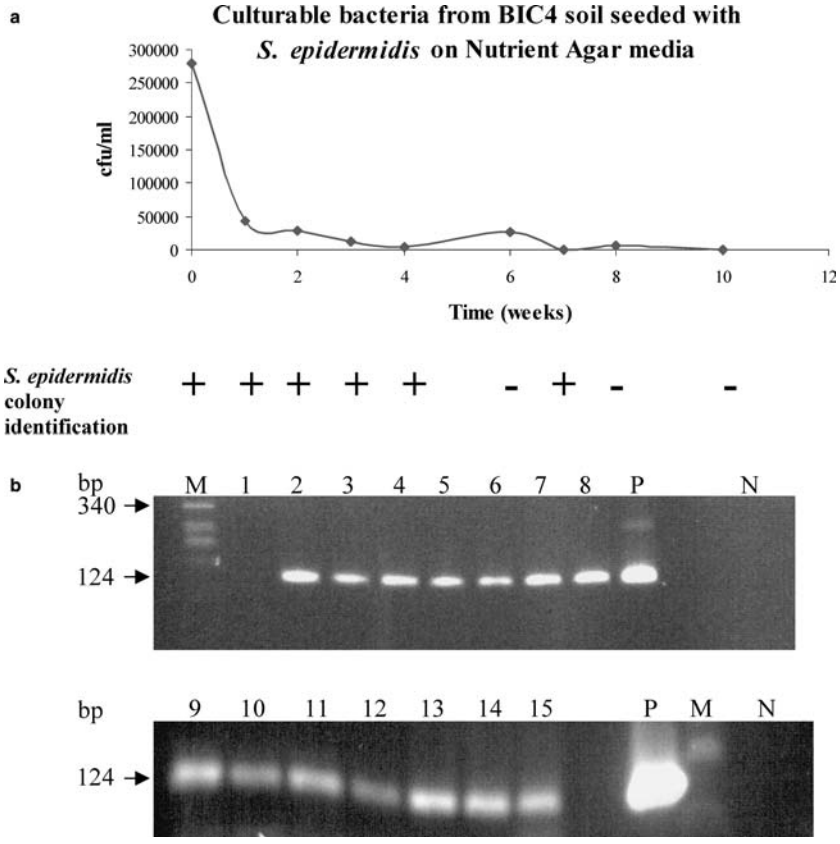

Fig. 2 *S. epidermidis* survival in BIC4 soil. **a** *S. epidermidis* isolation from BIC4 soil seeded with *S. epidermidis*. Plus signs represent isolation of *S. epidermidis* from the NA plates, while minus signs indicate failure to isolate *S. epidermidis*. **b** Detection of *S. epidermidis* in BIC4 soil seeded with *S. epidermidis* and incubated at 4°C for up to 54 weeks. DNA templates included total genomic DNA extracted from BIC4 soil (a pristine site) (lane 1); as well as BIC4 soil seeded with 1×10^5 cfu/ml *S. epidermidis* and incubated at 4°C for various lengths of time. Time of extractions, from the seeded samples were as follows: time 0 (immediately after inoculation with *S. epidermidis*) (lane 2); 1 week (lane 3); 2 weeks (lane 4); 3 weeks (lane 5); 4 weeks (lane 6); 6 weeks (lane 7); 7 weeks (lane 8); 8 weeks (lane 9); 10 weeks (lane 10); 15 weeks (lane 11); 21 weeks (lane 12); 30 weeks (lane 13); 40 weeks (lane 14); and 54 weeks (lane 15). N represents the PCR negative controls (sterile water as template); P represents the PCR positive controls (2 ng *S. epidermidis* genomic DNA as template); and M depicts the molecular weight marker (λ *Pst* I)

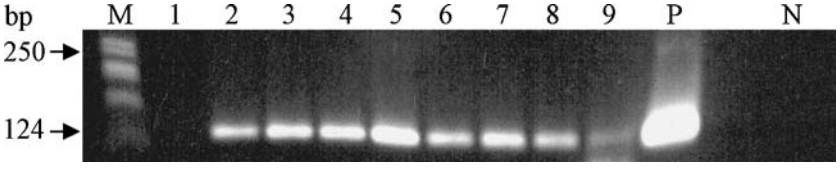

Fig. 3 Detection of *S. epidermidis* in BIC4 soil. Soil samples were seeded with *S. epidermidis* genomic DNA and incubated at 4°C for up to 23.5 weeks. DNA templates included total genomic DNA extracted from BIC4 soil (pristine site) (lane 1); as well as BIC4 soil seeded with *S. epidermidis* genomic DNA equivalent to 1×10^5 cfu/ml and incubated at 4°C for various lengths of time. Time of extractions, from the seeded samples, were as follows: time 0 (immediately after inoculation with *S. epidermidis* genomic DNA) (lane 2); 1.5 days (lane 3); 3.5 days (lane 4); 3 weeks (lane 5); 5.5 weeks (lane 6); 9.5 weeks (lane 7); 15.5 weeks (lane 8); and 23.5 weeks (lane 9). N represents the PCR negative control (sterile water as template); P represents the PCR positive control (2 ng *S. epidermidis* genomic DNA as template); and M depicts the molecular weight marker (λ *Pst* I)

Results and discussion

Dry Valley mineral soils were collected aseptically in January 2002 from various sites in the Ross Dependency region, Eastern Antarctica, namely (i) pristine sites; (ii) low impacted areas such as historic huts; and (iii) heavily impacted areas (Table 1). Samples were kept frozen from the time of collection and stored at −80°C. Bulk DNA was extracted directly from the Antarctic Dry Valley mineral soils (Table 1) according to the modified Zhou method (Stach et al. 2001). Genomic DNA concentrations ranged between 0.09–8 ng/g w.w. soil. *S. epidermidis*, the predominant commensal microorganism found on human skin

(Kloos et al. 1992), was used as a target organism for studies of dissemination and longevity. Using species-specific PCR (Martineau et al. 1996), we were able to detect *S. epidermidis* in Dry Valley mineral soils collected from sites that are heavily impacted by human activity (Table 1, Fig. 1). The presence of *S. epidermidis* cells in these high impact areas presumably results from the continuous dissemination of human skin and hair particles. In contrast, *S. epidermidis* could not be detected from mineral soils collected from low impact and 'pristine' sites (Table 1, Fig. 1). The sensitivity of the *S. epidermidis*-specific primers (Martineau et al. 1996) was tested on DNA extracted from Antarctic volcanic ash seeded with serially diluted *S. epidermidis* cells (ranging from 1×10^7 cfu/g to 1×10^2 cfu/g). The level of detection of *S. epidermidis* in Antarctic soil was determined to be 1×10^5 cfu/g (data not shown). This would imply that either *S. epidermidis* is not present in the low-impact areas (e.g., pristine sites); or that *S. epidermidis* is present in the low-impact areas in numbers below detection levels.

It should be noted that *S. epidermidis* contamination in Antarctic mineral soils has been documented as early as 1978 (Friedmann 1993). It is therefore important to quantify the period that non-indigenous species such as *S. epidermidis* survive in the harsh Antarctic conditions. To address this issue, we inoculated 0.5 g aliquots of pristine Antarctic soil (BIC4) with 1×10^5 cfu/ml *S. epidermidis* and incubated at 4°C for an extended period. At various time intervals, aliquots of seeded soil were serially diluted in Ringer's solution (Merck), plated on Nutrient agar (NA) media (in duplicate) and incubated at 37°C for at least 24 h. The colony forming units per millilitre (cfu/ml) were calculated and plotted against time. Single colonies were randomly selected from the NA plates and tested using *S. epidermidis*-specific PCR (Martineau et al. 1996) primers. *S. epidermidis* could be isolated from the seeded soil for no more than seven weeks from the time of inoculation (Fig. 2a), with an estimated t_{50} of approximately 4 days. However, *S. epidermidis*-specific PCR of DNA extracted from these seeded soil samples continued to show a positive amplification signal after 54 weeks (Fig. 2b). Whether this result indicates that *S. epidermidis* continues to exist in a viable but non-culturable (VBNC) state, as non-viable but non degraded cells, or merely as exposed DNA from lysed cells is not clear.

To investigate DNA stability, we seeded 0.5 g aliquots of BIC4 soil with ~0.3 ng *S. epidermidis* genomic DNA (equivalent to 1×10^5 cfu/ml) and incubated at 4°C. At various time intervals, bulk DNA was extracted (Stach et al. 2001) from aliquots and used as template DNA in *S. epidermidis*-specific PCR reactions (Martineau et al. 1996). *S. epidermidis* DNA could be detected in soil DNA extracts (Fig. 3) at the termination of the experiment (23.5 weeks). Although we cannot rule out the possibility that *S. epidermidis* survives in a VBNC state, this result suggests that naked genomic DNA is well preserved in Antarctic soil, possibly due to the low temperatures and water activities typical of Dry Valley soils (Wynn-Williams 1990).

The survival of *S. epidermidis* genomic DNA in these harsh conditions has significant implications for the survival of any other contaminating microorganisms, including those less benign than *S. epidermidis* (Vincent 2000). Regardless of whether *S. epidermidis* survives in the VBNC state or purely as naked DNA, these results suggest that in the desiccated mineral soils of the Antarctic continent, incident microbial cells of any source are probably not subject to the rapid lysis and degradation, as is assumed to occur in more temperate and moist environments. Our findings therefore potentially impact both on the interpretation of microbial diversity studies and on the results of gene mining in these 'so called' pristine sites.

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