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ORIGINAL PAPER

Biodiversity of air-borne microorganisms at Halley station, Antarctica

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Abstract A study of air-borne microbial biodiversity over an isolated scientific research station on an ice-shelf in continental Antarctica was undertaken to establish the potential source of microbial colonists. The study aimed to assess: (1) whether microorganisms were likely to have a local (research station) or distant (marine or terrestrial) origin, (2) the effect of changes in sea ice extent on microbial biodiversity and (3) the potential human impact on the environment. Air samples were taken above Halley Research Station during the austral summer and austral winter over a 2-week period. Overall, a low microbial biodiversity was detected, which included many sequence replicates. No significant patterns were detected in the aerial biodiversity between the austral summer and the austral winter. In common with other environmental studies, particularly in the polar regions, many of the sequences obtained were from as yet uncultivated organisms. Very few marine sequences were detected irrespective of the distance to open water, and around one-third of sequences detected were similar to those identified in human studies, though both of these might reflect prevailing wind conditions. The detected aerial microorganisms were markedly different from those obtained in earlier studies over the Antarctic Peninsula in the maritime Antarctic.

Keywords Aerial · Air-borne · Antarctic · Biodiversity · Colonisation · Bacteria · 16S rRNA · Non-indigenous

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Introduction

Halley V Research Station (75°35′S, 26°34′W) is situated approximately 15 km from the Weddell Sea on the Brunt Ice Shelf (Fig. 1), 40 km from the grounding line where the ice begins to rise steeply to the Coats Land Plateau—ice which is moving westward by ~700 m each year (Rankin and Wolff 2003). Temperatures at Halley research station rarely rise above 0°C, although temperatures around −10°C are common on sunny austral summer days. Typical austral winter temperatures are below −20°C with extreme lows of around −55°C, and there is 24-h darkness for 105 days year^{-1}. Winds are predominantly from the east and strong winds usually pick up the dry surface snow reducing visibility to a few metres. Halley's isolation from marine biota varies greatly depending upon the season (from 10 km in the austral summer to ca. 1,600 km in the austral winter). As a result, in the austral winter Halley is surrounded by near-sterile snow and ice for hundreds of kilometres, and any biota in the air overhead is likely to have travelled several hundreds of kilometres. The nearest terrestrial ice-free areas to Halley are the Heimefrontfjella Mountains (400 km) and Theron Mountains (500 km) both of which have low biomass and low biodiversity flora and fauna, typical of continental Antarctica (Convey 2001). The ice and snow around Halley is accumulating at a rate of around 1.2 m year^{-1}. There is no terrestrial vegetation, and animal life is restricted to the occasional bird (Rankin and Wolff 2000), the human base population and any non-indigenous animal material imported with cargo. Halley Research Station has been occupied continuously since 15 Jan 1956, and in recent years, has had a maximum population of 70 during the austral summer and an average of 16 over the austral winter. Research activity is primarily focussed on atmospheric sciences, but also includes survey,

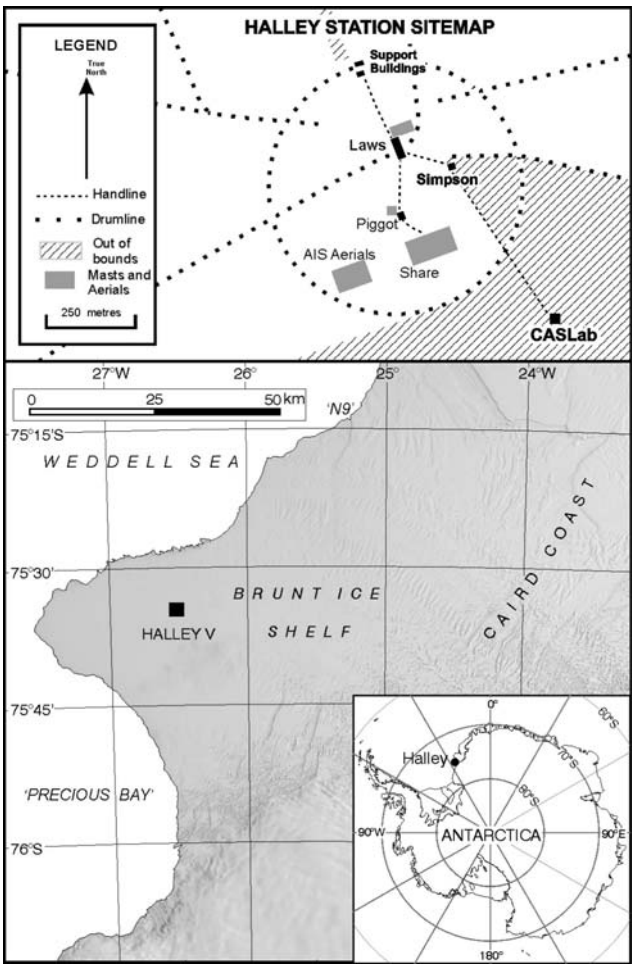

Fig. 1 The location of Halley Research Station and its proximity to the Weddell Sea

geology and glaciology. In this study, air samples were taken above the station during the austral summer and austral winter, in order to (1) establish the likely origin of air-borne microorganisms, (2) to investigate the effect of seasonal changes in sea ice extent and (3) to look for evidence of human impact.

The first studies of microbial biodiversity in the atmosphere over Antarctica were conducted by exposing plates to the atmosphere, particularly onboard early ship-based expeditions (Ekelöf 1908; Gazert 1912; Pirie 1912). Early work at Halley Bay using active sampling techniques showed maximum spore loads during sample periods of <24 h of 0.49 spores m^{-3}, although it was impossible to determine if these were natural or contaminants. More recent work near the Fosdick Mountains in Marie Byrd Land showed restricted air-borne biodiversity (limited to unicellular algae and fungal species) at a site 11 km from the nearest vegetation (Rudolph 1970). The study of microorganisms in the air over Antarctica was recently reviewed by Pearce et al. (2009). The present study

Fig. 2 The Clean Air Section Laboratory (CAS lab) at Halley research Station

adopted advances in the technology of both collection and identification in examining aerial transport of microorganisms in Antarctica.

Materials and methods

Sample site

Air samples were collected above the Halley Clean Air Sector Laboratory (CASLab) (Jones et al. 2008a, b), which is positioned 1 km away from the main station infrastructure to avoid contamination from the base (Fig. 2). It is possible that black carbon from base generators and vehicles could be deposited onto the air sampling equipment (Wolff and Cachier 1998). However, the CASLab is south of the base generators, and as the wind blows mainly in an east–west direction, the research shows that pollution from the base only rarely reaches the laboratory.

Aerial sampling

Samples were collected using a Hivol sampler, designed to collect particulate matter (aerosol) from the air. It consisted of a large air pump (HI-Q Environmental products company, 3500 series automatic flow control high volume TSP w/ brushless blower) with a filter holder upstream of the pump. Units incorporated a maintenance-free, two or three stage centrifugal blower powered by a brushless, variable speed, maintenance free motor. The motor was controlled by an electronic mass flow sensor which detected changes in temperature, barometric pressure, and pressure drop due to dust loading on the filter media. All filters were handled wearing a laboratory coat and gloves inside a Bassaire laminar flow hood. Samples were collected over 2-week periods during 22 September–5 October 2004 (18,780.0 m^3;

austral winter) and 25 January–8 February 2005 (19,947.8 m^3; austral summer). In addition, one filter in November was changed after 1 week, without the pump being switched on to act as a control sample for operator contamination. Filters were sealed and sent back to the UK at −20°C.

Air mass back trajectory calculations

Air mass trajectory calculations were performed to establish the likely origins of any propagules identified from air samples using the back trajectory scheme at the British Atmospheric Data Centre (BADC) (http://badc.nerc.ac.uk/cgi-bin/mybadc/mybadc.cgi.pl). Five-day back trajectories were calculated for times that corresponded to the sample collection period. Careful consideration was given to error propagated in the trajectories and, for each sample time, trajectories were calculated for a cluster of starting points 1°N, S, E and W of the sample point to check how representative any given trajectory was likely to be of real flow.

Sample analysis

Bacteria were harvested from filter discs. For each analysis, 1 ml of cell suspension was drawn through a 0.2-µ polycarbonate filter (Poretics, Osmotics Inc, USA).

DNA was extracted from replicate filter sections using the Fastprep DNA extraction kit and the MoBio Soil DNA extraction kit according the manufacturer's instructions. Prokaryote species richness was determined through the construction and analysis of seven independent 16S rRNA clone libraries. Ribosomal 16S RNA genes were amplified by PCR using primers 8F (5′AGA GTT TGA TCC TGG CTC AG 3′) and 1492R (5′ TAC GG(C/T) TAC CTT GTT ACG ACTT 3′) in a reaction containing 5 ng DNA, 1 µl each primer (10 mM), 10 µl 10× NH_4-based reaction buffer, 2.5 µl $MgCl_2$ (50 mM), dNTPs (20 nmol each), 0.3 µl BIOTAQ polymerase (5 U μl^{-1}, Bioline Ltd.), and 0.8 µl bovine serum albumin (10 mg μl^{-1}, Sigma-Aldrich) then adjusted to a final volume of 30 µl using ultrapure, sterile water. Amplification was performed on a thermocycler under the following conditions: initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min 15 s, 72°C for 1 min and a final elongation step at 72°C for 10 min. Presence of product was checked by running agarose gels (2% w/v) stained with ethidium bromide (0.5 µl ml^{-1}). PCR products were cleaned using the Illustra GFX PCR DNA Purification Kit (GE Healthcare, Bucks, UK) and eluted into 30 µl sterile, nuclease-free water.

Ligation of DNA fragments was performed using the pGEM-T Easy Vector system (Promega Ltd, Hampshire, UK) according to the manufacturer's instructions. Ligated products were transformed into ultra efficient XL-2 blue MRF competent cells (Stratagene, CA, USA) with addition of 200 mM IPTG (isopropyl-beta-D-thiogalactopyranoside) and X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside, Bioline Ltd) and plated onto Luria agar containing ampicillin (100 µg ml^{-1}). Approximately 200 white/positive colonies per site were picked into 30 µl sterile water and subjected to two freeze–thaw cycles. 2–5 µl was used in a PCR reaction with primers M13F (5′-CGC CAG GGT TTT CCC AGT CAC GAC-3′) and M13R (5′-GAG CGG ATA ACA ATT TCA CAC AGG-3′) and reagents as previously described with the following cycle conditions: 95°C for 5 min followed by 29 cycles of 95°C for 30 s, 58.5°C for 30 s, 72°C for 1 min 10 s. The quality of the amplifications was checked by electrophoresis, and products were cleaned using purification columns. Sequencing reactions were performed with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc, CA, USA) using M13F (long) primer, and products were run on a MegaBace500 DNA Sequencing System.

Cloned sequences were checked for vector sequence using NCBI VecScreen protocol (http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html), for chimeric sequences using the Bellerophon chimera detection program (Huber et al. 2004) compared to sequences in the EMBL nucleotide sequence database and Greengenes (DeSantis et al. 2006) by BLAST-search and deposited in the EMBL database under accession numbers FN431986–FN432029.

Results

Weather conditions during sample periods

The winds during both periods are predominately easterly (Fig. 3), although during the January–February 2005 period the wind direction was more variable. During the September–October period, the average air temperature was −17.9°C, while during the summer period of January–February the average temperature was −8.15°C. The biggest difference between the two periods was in the number of precipitation events recorded; in the January–February period precipitation was recorded at 21 synoptic observations (18% of the total observations), while in the October–November period precipitation was recorded at only twice (1.8% of the total).

Sea ice images

The sea ice extent was much greater in September–October at ∼400 km and much lower in January–February at ∼15 km (Fig. 4).

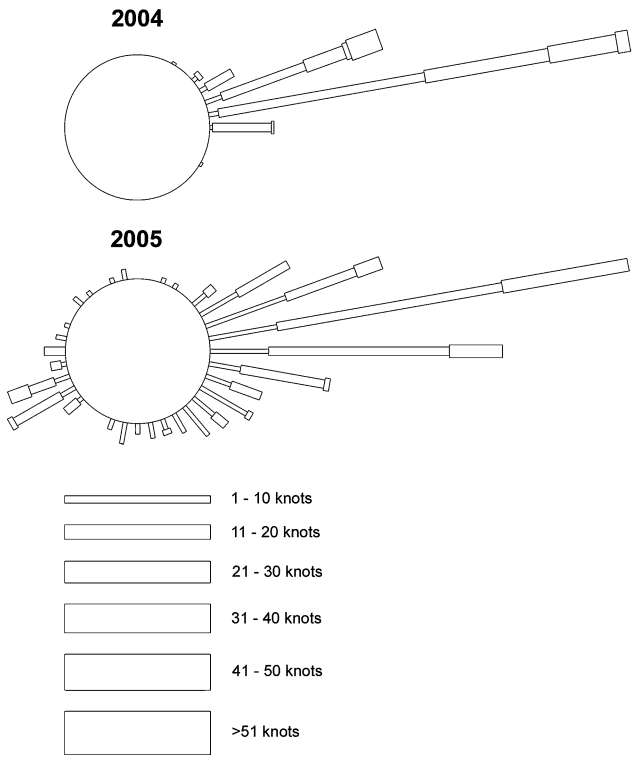

Fig. 3 Wind roses for 22 September–5 October 2004 (*top*) and 25 January–8 February 2005 (*bottom*). These show the distribution of the wind direction in the two periods. The width of the *bars* indicates the wind speed and the length the percentage time at each wind speed and direction (see key). The wind was calm for 0% of the time in September and 1.3% of the time in January. Figure courtesy of Steve Colwell (BAS)

Back trajectory

The back trajectories in Fig. 5 for the two periods show a large difference. For the September–October period the source of the air parcels, 5 days earlier, are mostly very close to the coast in Dronning Maud Land. In this earlier period, there was extensive sea ice and for the period that the back trajectories have been calculated, the air parcels remained over either sea ice or land ice. For the later period in January–February 2005 there was a much larger spread in the back trajectories. While some, in this later period, did seem to come from the interior of the continent, and many had a long track over the open ocean.

Microbial biodiversity

From a total of 373 clones constructed in seven independent clone libraries, only 31 clones (plus 12 identifiable fragments) could be matched with sequences in the 16S rRNA gene sequence databases. These consisted of 19 clones (plus 7 identifiable fragments) from the austral summer and 12 clones (plus 5 identifiable fragments) from the austral winter. A total of three identified sequences were common to both the austral summer and winter. The clone library constructed from the control filter did not generate any successful clones. In the austral summer *Acidovorax, Acinetobacter* (seven clones), *Cloacibacterium, Pseudomonas* (six clones) and *Sphingomonas* (11 clones) were identified. In the austral winter *Acinetobacter* (three clones), *Comomonas, Diaphorobacter, Imtechium, Pseudomonas* (five clones) and *Sphingomonas* (six clones) (Table 1). Coverage and diversity data are given in Table 2. Collectors' curves are given in Fig. 6.

In common with other microbial biodiversity studies, and in particular the Polar studies, many of the Halley sequences obtained matched as yet uncultivated organisms, although the majority cloned were from named groups. Two polar sequences were detected in the austral summer sample (Lin et al. 2008). Five marine sequences were detected (Halley austral summer sample three, winter two). One of the more common terrestrial sequences (detected thrice in summer and once in winter) was derived from deep sub-surface microorganisms from a mafic sill (Mislowack et al. 2005).

Discussion

Halley is an ideal site to study the long-range transfer of microorganisms in air due to its extreme distance from terrestrial ecosystems and its seasonal sea ice-dependent isolation from marine ecosystems, both of which would normally act as sources of microbiota for air-borne transfer. It is possible that the biota in air collected over Halley might represent a biodiversity 'baseline' for Antarctic (or indeed global) air, as local biological sources which may influence the level of air-borne biodiversity are lacking.

Comparison between locations

Only one previous study, conducted during the austral summer around Rothera Research Station (67°34′S, 68°08′W, Rothera Point, Adelaide Island, Antarctic Peninsula), has examined the microbial biodiversity of Antarctic environmental air samples (Hughes et al. 2004). During a 48-h sampling period (24 m^3 filtered air), this research found a range of species, including cyanobacteria (six sequences), actinomycetes (13 sequences) and other bacteria (13 sequences), which are commonly found in soils around the Antarctic Peninsula region, and clearly showed the influence of Antarctic terrestrial communities on air-borne microbial biodiversity. The Rothera sample site was on ice and snow free ground, and experienced periods where temperatures were above freezing which had

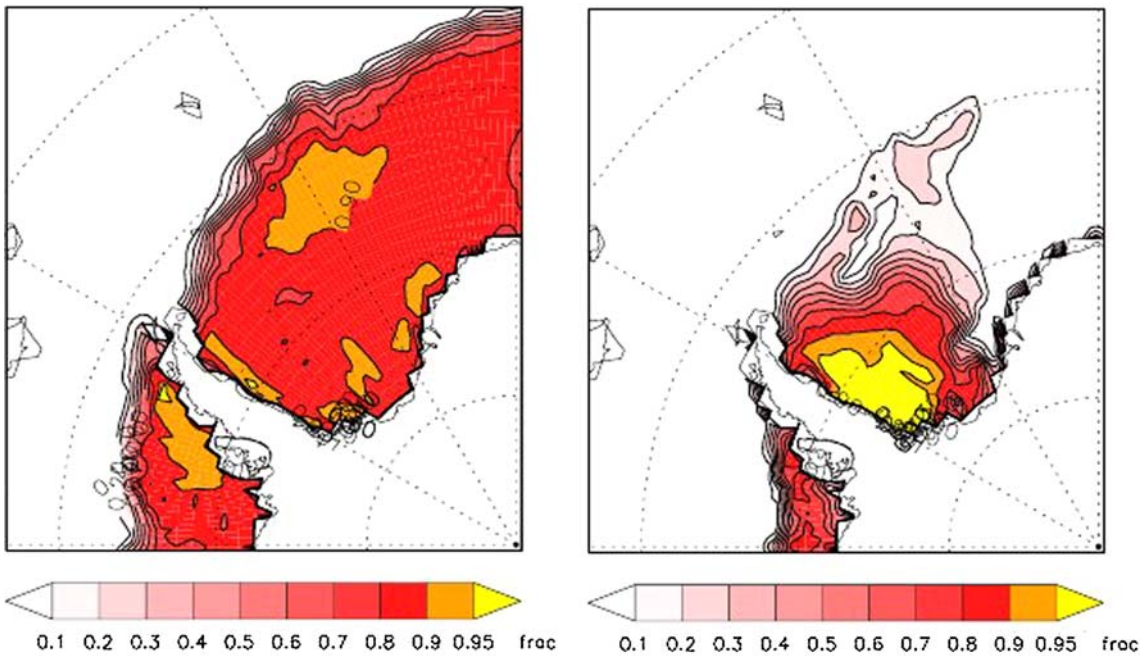

Fig. 4 Sea ice extent. These figures represent total monthly average ice concentration based entirely on satellite passive microwave data (*left* Sept 2004, *right* Jan 2005). Intensity scales are fractions (frac) of 1, where 1 represents 100% sea ice cover

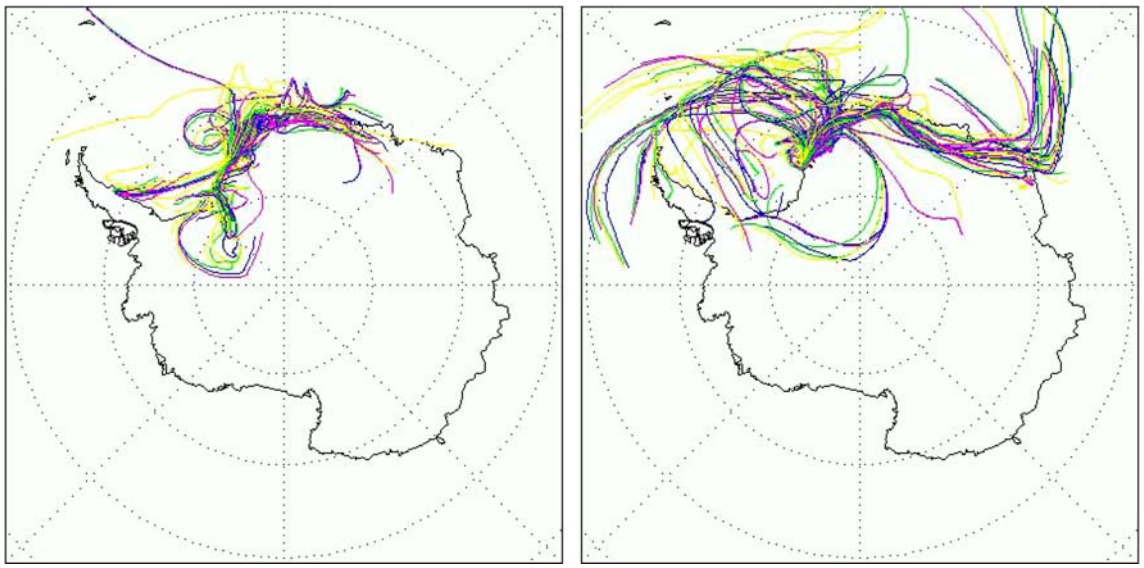

Fig. 5 Back track trajectories showing the origin of air arriving at Halley Research Station over the previous 14 days

led to the establishment of vascular plants and cryptogams and the formation of rudimentary soils. As air moves across these exposed soil areas, it is perhaps unsurprising that the majority of air-borne propagules are associated with this environment, for example, lichen associated with cyanobacteria and actinomycetes.

Terrestrial-associated microbial sequences

Eight of the Halley sequences (23.5% of those detected) were potentially of terrestrial origin, compared with over half of the Rothera sequences detected. The lower proportion of sequences of a terrestrial origin at Halley may

Table 1 Clones identified from aerial samples

Accession No.	BLAST match (with accession No.)	Origin (study)	Match (%)	Greengenes
Austral summer (January–February 2004)				
FN432004	Uncultured bacterium Oh3127A10C (EU137625)	Environ (Jones et al. 2008a, b)	99	Human, spacecraft assembly
	Acidovorax sp. R-24667 (AM084010)	Marine (Heylen et al. 2006)	99	Flea
FN431998	Uncultured *Acinetobacter* AV4RSG17 (EU341117)	Human (Osman et al. 2008)	99	Human, commercial airline cabin
	Acinetobacter sp. 1B3 (EU337120)	Fermented milk (Bauer et al. 2009)	99	Spacecraft assembly clean room
	Acinetobacter johnsonii (DQ911549)	Alga-lytic bacterium (Kim and Lee 2006)	98	
FN431991	Uncultured bacterium rRNA139 (AY958912)	Human (Hyman et al. 2005)	99	Human, commercial airline cabin
	Uncultured *Acinetobacter* sp. (EU341175)	Human (Osman et al. 2008)	99	Spacecraft assembly clean room
	Acinetobacter calcoaceticus 10C3 (AM157426)	Human (Martin et al. 2007)	98	
FN432003	Uncultured bacterium clone nbt09f05 (EU535688)	Human (Grice et al. 2008)	99	Human, spacecraft assembly
	Acinetobacter sp. PND-5 (EF494200)	Biotech (Dong et al. 2007)	99	River estuary sediment
FN431992	Uncultured bacterium clone rRNA139 (AY958912)	Human (Hyman et al. 2005)	99	Human, commercial airline cabin
	Uncultured *Acinetobacter* sp. (EU341175)	Human (Osman et al. 2008)	99	Spacecraft assembly clean room
	Acinetobacter calcoaceticus 10C3 (AM157426)	Not known (Martin et al. 2007)	98	
FN432001	Uncultured bacterium Sco2-20-228 (AJ575430)	Marine (Allen et al. 2006)	100	Spacecraft assembly clean room
	Cloacibacterium normanense (AM883185)	Marine (Lösekann et al. 2008)	99	Hydrothermal vent, activated sludge, biotech, marine, flea
FN431999	*Sphingomonas* sp. FZ016 (AY349412)	Human (Paster and Dewhirst 2003)	99	Oil field drainage water, human
	Sphingobium yanoikuyae (AY047219)	Biotech (Borde et al. 2003)	98	Deep sea
FN432002	*Sphingomonas* sp. 3B3 (EU337119)	Fermented milk (Bauer et al. 2009)	99	Bovine faeces, anchovy intestine
	Sphingomonas oligophenolica (AB365794)	Soil (Whang 2007)	99	Seafloor lavas, deep sea
FN432000	*Sphingomonas* sp. oral clone AV069 (AF385529)	Human (Paster et al. 2002)	99	Dairy cow rumen, geothermal mat
	Sphingomonas echinoides (DQ118270)	Biotech (Kim et al. 2005)	99	Peat soil, seafloor lavas, arsenic contaminated paddy soil
FN431997	*Sphingomonas* sp. 3B3 (EU337119)	Fermented milk (Bauer et al. 2009)	99	Soil, freshwater spring, Alps
	Sphingomonas oligophenolica (AB365794)	Soil (Whang 2007)	99	Bark, polluted soil
FN431990	*Sphingomonas* sp. FZ016 (AY349412)	Human (Paster and Dewhirst 2003)	98	Human
	Sphingomonas sp. KH3-2 (AF282616)	Soil (Chun 2003)	98	

Table 1 continued

Accession No.	BLAST match (with accession No.)	Origin (study)	Match (%)	Greengenes
FN431986	Uncultured *Sphingomonas* sp. CSB6 (DQ069188)	Rock (Mislowack et al. 2005)	97	No matches
	Sphingomonas sp. 52-VN6-1 W (AB299569)	Biotech (Huong et al. 2007)	96	
	Sphingomonas paucimobilis EPA 505 (U37341)	Soil (Mueller et al. 1997)	96	
FN431989	Uncultured *Sphingomonas* sp. CSB6 (DQ069188)	Rock (Mislowack et al. 2005)	95	No matches
	Sphingomonas sp. (strain DhA-33) (AJ011505)	Soil (Mohn et al. 1999)	95	
FN431987	Uncultured *Sphingomonas* sp. CSB6 (DQ069188)	Rock (Mislowack et al. 2005)	96	No matches
	Sphingomonas sp. F M5-VN5-4 W (AB299577)	Soil (Huong et al. 2007)	95	
FN431993	*Pseudomonas* sp. LBUM636 (DQ788996)	Soil (Paulin et al. 2006)	94	No matches
	Pseudomonas putida G-4-1-2 (EF102847)	Not known (Tian et al. 2006)	93	
FN431988	*Pseudomonas* sp. 3(2008b) (FJ386496)	Polar (Lin et al. 2008)	99	Human
	Pseudomonas brenneri B6 (EU169172)	Marine (Li et al. 2007)	99	
FN431994	Uncultured bacterium rRNA215 (AY958988)	Human (Hyman et al. 2005)	97	Human
	Pseudomonas collierea PR212T (AM421016)	Polar soil (Rahman 2009)	97	
FN431995	Uncultured bacterium rRNA215 (AY958988)	Human (Hyman et al. 2005)	99	Human
	Pseudomonas fluorescens CICCHLJ (EF528294)	Unknown (Wang 2007)	99	
FN431996	Uncultured bacterium rRNA215 (AY958988)	Human (Hyman et al. 2005)	99	Human
	Pseudomonas fluorescens CICCHLJ (EF528294)	Unknown (Wang 2007)	99	
Sequence fragments				
FN432005	*Acinetobacter xiamenensis* KSL 4-102 (FJ477061)	Sewage plant (Lee 2008)	93	No matches
FN432006	*Acinetobacter* sp. SMCC B0261 (U87124)	Subsurface (Balkwill et al. 1997)	88	No matches
FN432007	Uncultured bacterium clone JH-YT23 (EF033214)	Soil (He et al. 2008)	96	No matches
	Acinetobacter sp. SMCC B0131 (U87112)	Subsurface (Balkwill et al. 1997)	95	
	Acinetobacter venetianus B753 (AY581132)	Bird feathers (Shawkey et al. 2005)	95	
FN432008	*Pseudomonas* sp. LBUM636 (DQ788996)	Soil (Paulin et al. 2006)	91	No matches
	Pseudomonas lurida (AY605697)	Soil (Paul et al. 2004)	90	
FN432010	Uncultured alpha proteobacteria APe4_19 (AB074601)	Aphid (Nakabachi et al. 2003)	97	No matches
	Sphingomonas oligophenolica WR95.(AB365794)	Soil (Whang 2007)	97	

Table 1 continued

Accession No.	BLAST match (with accession No.)	Origin (study)	Match (%)	Greengenes
FN432011	Uncultured alpha proteobacteria AI-2M_E07 (EF219786)	Antarctic (Yergeau et al. 2007)	93	No matches
	Sphingomonas sp. JFJ-Iso-Bact01 (AJ867722)	Mountain snow (Yuhana 2004)	93	
FN432009	Uncultured bacterium clone p_C08 (EU448649)	Leaf surface (Redford and Fierer 2008)	87	No matches
	Afipia sp. CZ332 (AJ299575)	Termite gut (Wenzel et al. 2000)	85	
	Sphingomonas oligophenolica WR95.(AB365794)	Soil (Whang 2007)	87	
Austral winter (September–October 2005)				
FN432022	*Acinetobacter* sp. Ic2 (DQ421391)	Biotech (Vasiliadou et al. 2006)	99	Human
	Alvinella pompejana (AF357182)	Hydrothermal vent (Campbell et al. 2001)	99	
FN432020	Uncultured *Acinetobacter* sp. 10V4 (AM157448)	Human (Martin et al. 2007)	99	Lignin enrichment culture, soil
	Acinetobacter sp. 1B3 (EU337120)	Milk (Bauer et al. 2009)	99	
	Acinetobacter johnsonii (DQ911549)	Alga-lytic bacterium (Kim and Lee 2006)	98	
FN432015	*Acidovorax* sp. 3DHB1 (AF458096)	Biotech (Mechichi and Fuchs 2001)	99	Arsenite oxidising enrichment
	Diaphorobacter nitroreducens NA10B. (AB064317)	Activated sludge (Khan and Hiraishi 2001)	99	
	Pseudomonas kilonensis 520-20 (AJ292426)	Soil (Sikorski et al. 2001)	99	
FN432013	Uncultured bacterium EV81/SAS75 (DQ256357)	Subsurface (Gihring et al. 2005)	100	North Atlantic gyre, ground water
	Comamonas sp. AM12 (EU252500)	Marine (Zhang 2007)	99	Spacecraft assembly room
	Comamonas aquatica strain F-1 (FJ493173)	Farmland (Liang and Li 2008)	99	
FN432012	Uncultured bacterium clone F6-62 (EU148648)	Terrestrial (Vasanthakumar et al. 2007)	99	Spacecraft assembly room, farm soil
	Uncultured beta proteobacteria AKYH610 (AY922140)	Environ(Tringe et al. 2005)	99	Water 20 m, prairie dog flea
	Imtechium assamiensis BPTSA16 (AY544767)	Spring (Saha and Chakrabarti 2004)	99	
FN432016	*Sphingomonas* sp. (strain DhA-33) (AJ011505)	Human (Mohn et al. 1999)	98	Human, bioremediation
	Sphingomonas sp. FZ016 (AY349412)	Human (Paster and Dewhirst 2003)	98	Subsurface groundwater
	Sphingobium yanoikuyae (AB109749)	Biotech (Hamada 2003)	97	House floor dust
FN432014	Uncultured bacterium clone PP4-22 (EU148965)	Terrestrial (Vasanthakumar et al. 2007)	99	Human oral, Baltic sea
	Sphingomonas sp. FZ016 (AY349412)	Human (Paster and Dewhirst 2003)	99	Subsurface water, deep sea
	Pseudomonas abikonensis IAM 12404T (AB021416)	Not known (Anzai et al. 2000)	96	

Table 1 continued

Accession No.	BLAST match (with accession No.)	Origin (study)	Match (%)	Greengenes
FN432018	Uncultured *Sphingomonas* sp. CSB6 (DQ069188)	Rock (Mislowack et al. 2005)	97	Human skin
	Sphingomonas sp. 52-VN6-1 W (AB299569)	Soil (Huong et al. 2007)	96	
FN432019	Uncultured *Sphingomonas* sp. CSB6 (DQ069188)	Rock (Mislowack et al. 2005)	96	Water 10 m
	Sphingomonas sp. (strain DhA-33) (AJ011505)	Soil (Mohn et al. 1999)	95	
FN432017	Uncultured bacterium rRNA215 (AY958988)	Human (Grice et al. 2008)	99	Spacecraft assembly clean room
	Pseudomonas sp. *Nj-55* (AM409368)	Antarctica (Gai 2006)	99	Human
FN432021	Uncultured bacterium215 (AY958899)	Human (Hyman et al. 2005)	100	Human, spacecraft assembly room
	Pseudomonas sp. *Nj-55* (AM409368)	Antarctica (Gai 2006)	99	Commercial airline cabin
FN432024	Uncultured bacterium nbt226g12 (EU537895)	Human (Grice et al. 2008)	89	Commercial airline cabin, marine
	Pseudomonas fluorescens strain B73 (EU169179)	Soil (Li et al. 2007)	89	Corroded concrete, Rio Tinto Hydrothermal vent
Sequence fragments				
FN432028	*Acinetobacter calcoaceticus* (M34139)	Not known (Woese 1990)	80	Spacecraft assembly clean room
FN432025	*Sphingomonas* sp. (strain DhA-33) (AJ011505)	Soil (Mohn et al. 1999)	74	Human
	Sphingomonas paucimobilis (X94100)	Subarctic (Nohynek et al. 1996)	72	
FN432026	Uncultured *Sphingomonas* sp. CSB6 (DQ069188)	Rock (Mislowack et al. 2005)	95	No matches
	Sphingomonas sp. FZ016 (AY349412)	Human (Paster and Dewhirst 2003)	94	
FN432027	Uncultured bacterium nbt210b10 (EU534732)	Human (Grice et al. 2008)	75	Human
	Pseudomonas sp. ps6-35 (AY303316)	Soil (Kwon 2003)	75	
	Pseudomonas putida strain HOT19 (AY738649)	Biotech (Flagan and Leadbetter 2006)	75	
FN432029	Uncultured bacterium nbt242e10 (EU539601)	Human (Grice et al. 2008)	72	Human, geothermal drainage
	Pseudomonas sp. *Nj-55* (AM409368)	Antarctica (Gai 2006)	72	
	Pseudomonas collierea PR212T (AM421016)	Polar soil (Rahman 2009)	72	

Clones are represented by the closest match to an existing sequence in the DNA sequence databases (which may not have any taxonomic information associated with it), then the closest match to a named genus and finally the closest match to a named genus and species

reflect the lack of terrestrial habitat close to the sampling point, the nearest rock outcrop being ca. 400 km away. *Sphingomonas* species were highly represented (six and three sequences during summer and winter, respectively) and whilst common throughout the world, this genus has been recorded from Antarctica environmental samples including the ice of Taylor Dome (77°47′S,158°43′E) situated close to the Transantarctic Mountains (Christner et al. 2000), from Antarctic freshwater lakes (Pearce 2000; Pearce et al. 2003) and cultured from air and dust over

Table 2 Clone library coverage and diversity data

Metric	Austral summer libraries	Austral winter libraries
Coverage	0.63 (0.89 by genus)	0.46 (0.77 by genus)
Chao1 diversity index	20.17	18.17
Simpson diversity index	0.74	0.89
Sorensen similarity coefficient	0.44	

The library coverage value was calculated as $C = 1 - (n_1/N)$, where n_1 is the number of clones which occurred only once in the library and N is the total number of sequences in the library (Good 1953). The diversity was estimated using the Simpson diversity index (Simpson 1949); $1 - \Sigma(P_i)^2$, where P is the proportion of each individual species (i) observed (number of that species/total number of species) and also the Chao1 estimator (Chao 1984; Lee and Chao 1994); $S_{\text{Chao1}} = S_{\text{obs}} + (n_1^2/2n_2)$ where S_{obs} is the total number of observed species, n_1 is the number of singletons (species captured once) and n_2 is the number of doubletons (species captured twice). Similarity between independent clone libraries was determined using the Sorensen coefficient; $S = 2a/(2a + b + c)$ where a is the number of sequences in common, b is the total in the first comparison library and c is the total in the second

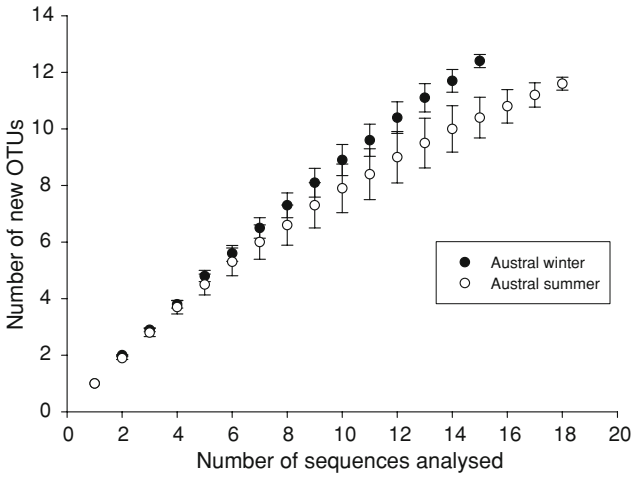

Fig. 6 Clone library collectors' curves

Antarctica (Busse et al. 2003). However, *Sphingomonas* spp. are also known to be dominant in the marine oligotrophic environment (Vancanneyt et al. 2001), highlighting the need for care when interpreting sequence data. *Sphingomonas* sp. and *Actinobacterium* sp. clones were identified in the air above Rothera (Hughes et al. 2004). The most prominent bacterium with the ice nucleation gene is reported as *Pseudomonas* spp. (Ahern et al. 2006). Elsewhere and on the basis of culture-dependent studies, it has been concluded that bacteria from Antarctic soils are probably cosmopolitan, similar to those found in soils elsewhere in the world. However, more recent molecular (DNA)-based investigations indicate that there are novel species and genera in Antarctica which may also extend to the eukaryotic microbiota (Lawley et al. 2004; Adams et al. 2006; Aislabie et al. 2006).

Marine-associated microbial sequences

Despite the Rothera air sample being collected ca. 70 m from open ocean, only three sequences (9.4% of those detected) were of marine origin, while only three marine sequences (14.7%) were detected from all Halley samples. Given the close proximity to the sea at Rothera (and to a lesser degree in the Halley summer sample), a higher proportion of marine sequences may have been expected. Oceans are important sources of microorganisms found in clouds (Amato et al. 2007), and aerosols around Halley are chemically dominated by marine influences (Wagenbach et al. 1998; Rankin et al. 2002: Rankin and Wolff 2003). Back track trajectory analyses from both Rothera and Halley samples suggested at least a partial marine origin. However, microorganisms from the terrestrial environment may be more resilient to physical stress and more able to maintain their DNA in an intact form for longer, which may explain the low representation of marine sequences. Models of aerosol droplet evaporation times under environmental conditions found at Rothera Research Station (1°C and 45% relative humidities) showed rapid evaporation to dryness with desiccation and osmotic stress on cell structure and oxidative damage to cell molecules including nucleic acids probable (Hughes 2003; Hatch and Dimmick 1966; Sawyer et al. 1996). Desiccation and freezing rates at Halley are likely to be much faster due to the colder and drier conditions generally found in continental Antarctica when compared to the maritime Antarctica.

Human-associated microbial sequences

Twelve sequences from Halley (35.3% of those detected) appeared to be related to microorganisms that had previously been identified in human studies (skin surface and excretions), i.e. human vaginal epithelium (Hyman et al. 2005), the human oral cavity (Paster and Dewhirst 2003), human skin (Grice et al. 2008), human breast milk (Martin et al. 2007), commercial airline cabin air (Osman et al. 2008) and municipal waste water (Allen et al. 2006). Humans were present at the experimental set up, so the potential for contamination existed. However, the lack of any successful

clones in the control filters would suggest that the sequences detected in the austral summer and austral winter air samples were part of the local aerial mictobiota being sampled. Evidence of a potential human influence on air-borne biodiversity was found in both austral summer and austral winter samples with seven and five sequences of human-associated bacteria, respectively. Apart from the aforementioned, there appeared to be no sequences derived from animals or plants, suggesting that microorganisms in the air were not derived from these sources. In comparison, air sampling at Rothera revealed no sequence matches for microorganisms that would be considered evidence of human association, even though the samples were taken within an area of significant human activity close to the station buildings. In areas with potentially exceptionally low levels of aerial microbial biomass and biodiversity, the influence of human-derived microorganisms could easily mask natural biodiversity. This is particularly true as long air sampling periods may be required to filter sufficient air to collect a representative sample of natural microorganisms. As a result of the apparently low biomass and biodiversity of air over the ice shelf and sea ice around Halley, it is unsurprising that any microorganisms derived from human sources in the local area (research station personnel, cargo and station infrastructure) would be strongly represented in the sequence list, as microorganisms from other environmental sources are likely to be scarce. Given the large amount of air filtered at Halley in this study (equivalent to the volume contained in a 200 × 200 × 200 m box), the detection of only 12 microbial sequences that can potentially be linked to human activity might reflect the success of the procedures to limit human influence on the samples. However, the prevalence of human-derived sequences in the sequence databases could be an artefact of predominant global research activity, and thus not necessarily reflect the source of Halley-derived sequences.

In the study of air-borne biodiversity at the Rothera Research Station, air mass back trajectories showed that most of the air sampled had passed over the Antarctic Peninsula and Weddell Sea. However, it was impossible to tell which of the clones identified in the study were of local origin and which has undergone long-distance air-borne transfer. In contrast, with the Halley samples, we may have some more indication of the proportions of clones from local and more distance sources, as the local microorganisms may be the ones with human-associated sequences, with the microorganisms travelling further distances likely to have environmental origins. In this study, the ratio was 1:3 (local:distant), although it must be stressed that many other factors, including methodological biases, may have strongly influenced this figure.

Although the majority of Antarctic locations have been subject to little or no direct human impacts, some parts of Antarctica have been subject to long-term environmental damage (Tin et al. 2009). The introduction of non-indigenous biota into Antarctica is a major concern within the Antarctic Treaty System. In 2007, the Antarctic Treaty Consultative Meeting's (ATCM) Committee for Environmental Protection (CEP), which directs the management of environmental issues in Antarctica, assigned the introduction of non-indigenous species the highest priority for consideration over the following 5 years (New Zealand 2007). The increasing activity of scientists and tourists in Antarctica has the potential to introduce species from other areas of the Earth or redistribute species from distinct areas within Antarctica, leading to increased biological and genetic homogenisation (Frenot et al. 2005, 2008; Convey et al. 2006). Non-indigenous organisms, including microorganisms, can be transported into Antarctica associated with cargo, building and packing materials, personnel clothing and equipment, foods, vehicles and scientific equipment (Whinam et al. 2004; Lee and Chown 2009, Hughes 2006; Hughes et al. 2009). Our research has indicated that research stations may be releasing imported microorganisms into the local Antarctic environment. At Halley, this is not likely to have a noticeable impact as local indigenous life is absent. However, other areas of Antarctica are more suitable for biological colonisation with well-established local flora and fauna. It is not known what impact the release of microorganisms from research stations may have on indigenous biota close to existing Antarctic stations, though the introduction or spread of plant and animal pathogens is a concern (Kerry et al. 1999; Bridge et al. 2008; Tin et al. 2009). Other work has reported the air-borne microbial biota associated with Antarctic research stations. Van Houdt et al. (2008) showed the presence of many common microbial species, including *Staphylococcus* spp., *Bacillus* spp. and *Sphingomonas paucimobilis* within the Concordia Station on Dome C. Russian scientists reported α and β Protobacteriacae at many of their coastal research stations and pathogenic fungi at their research stations on King George Island (South Shetland Islands), and suggested they were probably introduced by human activities (Russian Federation 2006, 2009). Sampling for air-borne fungi at the Polish Arctowski Station (King George Island) revealed not only fungi common to local soils but also common contaminants of cold-stored foods (Czarnecki and Bialasiewicz 1987). Cameron et al. (1973) found higher concentrations of microbes (up to 100-fold) in areas experiencing human disturbance (McMurdo Station dump) compared to undisturbed areas (New Harbour, Taylor Valley, Dry Valleys). Benninghoff and Benninghoff (1978) found that the dominant particles collected by air samplers operated between McMurdo Station and Scott Base (i.e. ~2.5 km for each) were clothing fibres and wood cells produced as a result of

station activities. American researchers working in Antarctic interior (Beardmore Camp, 84°17′S, 162°22′E) found that air samples collected down-wind of the camp contained *Bacillus* sp. that were culturable at 37°C, and were therefore assumed to be contaminants (Lacy et al. 1970). However, researchers using PCR techniques at Halley Research Station previously failed to detect sequences for human commensals in the snow around the buildings (Upton et al. 1997).

Comparison between seasons

Both austral summer and austral winter air-sample clone libraries from Halley contained similar numbers of sequences (19 and 15, respectively) with matches for similar groups of organisms. Given the difference in distances to the open sea during the summer and winter sample periods (~15 km and up to 400 km, respectively), there was little difference in the number of potentially marine-associated sequences (see earlier discussion). When the air mass back trajectory data was examined, it was clear that air masses arriving at Halley came predominantly from an easterly direction, and had spent significant time over the continental land mass irrespective of their origin. Working in the Antarctic interior and other coastal sites, Lacy et al. (1970) found that bacterial counts in air samples increased as summer progressed. Marshall found that large increases in air propagule loads over Antarctica were associated with different origins of the air.

Methodological limitations

Sequences obtained from Halley air filters matched database sequences very well, with only five matches out of all those obtained being less than 97% and indeed >50% were ≥99%. Clone libraries were not sampled to extinction, though with such limited biodiversity and low numbers of cells, species accumulation curves were relatively good. Database comparisons do have their limitations; however, to allow for this, sequence identifications were monitored for consistency to at least 100 matches. Sample period will always be an issue in aerobiology; long flow periods with high volumes of air are good for representation but also have a disadvantage—two weeks running air through filters at high volume is likely to significantly reduce the viability of bacteria present and select for more resistant species such as Gram positives and sporulating species (another potential reason why the human input could be significant). It has been observed elsewhere, for example, that Gram-positive rods are often more prevalent than Gram-negative rods (Marafie and Ashkanani 1991). However, adaptations for survival in the Antarctic environment are likely to be similar to those for survival on a filter, so it may be necessary to compromise between long sample periods (to get a more representative sample) and short sample periods (to prevent harm to organisms already on the filter). In addition, long sample periods select for what might truly be regarded as Antarctic aerial extremophiles. Future experimental work could include a comprehensive time series and transect with distance from the coast, use a wider range of primers, including those specific for human bacterial contamination (Upton et al. 1997; Sjoling and Cowan 2000) or DNA extraction methodologies and spike an air sample to determine both the efficiency of detection and survival rates on filter membranes.

Conclusion

Air sampled from Halley Research Station, continental Antarctica, showed a low microbial biodiversity, which included many sequence replicates. No significant patterns were detected in the aerial biodiversity between the austral summer and the austral winter; however, many of the sequences obtained were from as yet uncultivated organisms, making detailed interpretation of the data difficult. Few marine sequences were detected irrespective of the season and distance to the open ocean.

Around one-third of sequences detected were similar to those identified in human studies, and whose origin may have been the nearby research station, indicating that the overall biodiversity may have a significant proportion of sequences of both local (research station) and distant (marine or terrestrial) origins.

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