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Heterotrophic microbial colonization of the interior of impact-shocked rocks from Haughton impact structure, Devon Island, Nunavut, Canadian High Arctic

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Abstract: The polar desert is one of the most extreme environments on Earth. Endolithic organisms can escape or mitigate the hazards of the polar desert by using the resources available in the interior of rocks. We examined endolithic communities within crystalline rocks that have undergone shock metamorphism as a result of an asteroid or comet impact. Specifically, we present a characterization of the heterotrophic endolithic community and its environment in the interior of impact-shocked gneisses and their host polymict breccia from the Haughton impact structure on Devon Island, Nunavut, Canadian High Arctic. Microbiological colonization of impact-shocked rocks is facilitated by impact-induced fissures and cavities, which occur throughout the samples, the walls of which are lined with high abundances of biologically important elements owing to the partial volatilization of minerals within the rock during the impact. 27 heterotrophic bacteria were isolated from these shocked rocks and were identified by 16S rDNA sequencing. The isolates from the shocked gneiss and the host breccia are similar to each other, and to other heterotrophic communities isolated from polar environments, suggesting that the interiors of the rocks are colonized by microorganisms from the surrounding country rocks and soils. Inductively coupled plasma–atomic emission spectroscopy (ICP-AES), scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis were used to identify the chemical composition of the shocked materials and to document the *in situ* growth of microbes in their interiors. The identification of these heterotrophic communities within impact-shocked crystalline rocks extends our knowledge of the habitable biosphere on Earth. The colonization of the interiors of these samples has astrobiological applications both for considering terrestrial, microbiological contamination of meteorites from the Antarctic ice sheet and for investigating possible habitats for microbial organisms on the early Earth, and more speculatively, on Mars and other planetary bodies.

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Key words: endolith, Haughton impact structure, microbial colonization, shock-metamorphism, shocked gneiss.

Introduction

The polar desert is one of the least hospitable biomes on Earth, as it is characterized by ablative winds, low growth temperatures, freeze–thaw cycles, short growing seasons, high ultraviolet-B (UVB) flux, desiccation and oligotrophic (low nutrient) conditions (e.g. Wynn-Williams 2000). In response to these conditions, many microorganisms, known as endoliths or 'cryptoendoliths', inhabit the interior pore spaces of rocks (Friedmann & Ocampo 1976; Friedmann 1982). For endoliths, rocks serve two primary functions. First, they provide protection from many of the environmental stresses (fluctuating temperatures, wind ablation, intense UVB radiation) that threaten microbial existence in the polar environment (Nienow & Friedmann 1993; Wynn-Williams 2000; Cockell *et al*. 2001). Secondly, the rocks act as a reservoir for water, nutrients and heat, giving endoliths an advantage over organisms trying to survive outside the lithic environment (Hirsch *et al*. 1988; Vincent 1988; Nienow & Friedmann 1993). As a result, endolithic organisms are able to remain active for significantly longer periods than those living on the surface (Nienow & Friedmann 1993).

Despite inhabiting the interior of a rock, endolithic organisms are not completely isolated from the outside world. Atmospheric circulation entrains microbes and nutrients and subsequently redistributes them globally (Burckle 1995a, b; Burckle & Wasell 1995; Vincent 2000). Wind, flowing water or meltwater from ice and windblown snow can transport and deposit microorganisms and nutrients into cracks and crevices in a rock surface, allowing endolithic communities

to grow and develop (Wynn-Williams 2000). For example, examination of microfossils within four meteorites from the Allan Hills and Queen Alexandra Range, Antarctica has revealed the presence of modern terrestrial freshwater and saltwater diatoms, as well as specimens representing extinct diatom species (Burckle & Delaney 1999). The high degree of exogenous organic matter and microorganisms present in Antarctic samples, despite the relative isolation of Antarctica from global patterns of atmospheric circulation (Vincent 2000), suggests that there would be even greater deposition of nutrients and organisms in other, less isolated environments, including the Arctic.

Since polar endoliths were first characterized (Friedmann & Ocampo 1976), porous sedimentary rocks, such as the Beacon sandstone formation in Antarctica, have been identified as particularly suitable for endolithic communities, because their high porosity favours colonization by endolithic bacteria (Nienow & Friedmann 1993). Most studies have focused on phototrophic communities inhabiting the near-surface environment (Friedmann & Ocampo 1976; Friedmann 1982; Vincent 1988; Nienow & Friedmann 1993), although a few have noted the importance of heterotrophic bacteria in these near-surface endolithic communities (Hirsch *et al.* 1988; Siebert & Hirsch 1988; Nienow & Friedmann 1993). While crystalline rocks, such as gneiss and granite, have also been studied in Antarctica (Nienow & Friedmann 1993), their typically low porosities make them poor hosts for endolithic bacteria. However, the Arctic presents a unique environment in which endolithic organisms can readily colonize crystalline rocks: impact-shocked gneisses and their host polymict breccias from the Haughton impact structure on Devon Island in the Canadian High Arctic. The colonization of the heavily shocked gneiss by cyanobacteria in preference to unshocked or low-shocked gneisses at Haughton has been described previously (Cockell & Lee 2000; Cockell *et al.* 2002) and previous studies have determined the chemical compositions and shock levels of these rocks (Metzler *et al.* 1988; Osinski & Spray 2001). These rocks have undergone impact-induced shock metamorphism, one of the many effects of an asteroid or comet impact, are heavily fractured, and some of their constituent minerals have been partially volatilized (Dressler & Reimold 2001). This has generated habitats, which can be exploited by significant populations of endolithic organisms (Cockell & Lee 2000; Cockell *et al.* 2001).

In this study we describe the characterization of the heterotrophic communities within impact-shocked gneisses and their host breccia from Haughton and we discuss the implications of our findings for the contamination of highly shocked meteorites in Antarctica and the search for signatures of life on Mars and other planetary bodies.

Materials and methodology

Study site

The Haughton impact structure is located at 75° 22′ N, 89° 41′ W on Devon Island, Nunavut in the Canadian High Arctic (Fig. 1). The impact structure is approximately 24 km in diameter (Grieve 1988) and was created by the impact of an asteroid or comet 23.4 ± 1.0 Ma during the Early Miocene (Jessberger 1988). At the time of the impact, the Precambrian gneisses forming the crystalline basement on Devon Island were overlain by ~1750 m of Paleozoic sedimentary rocks, predominantly dolomite and limestone (Frisch & Thorsteinsson 1978). The presence of significant amounts of gneiss cropping out in the melt breccia deposits indicates that the excavation depth for the impact structure exceeded 1750 m.

Fig. 1. Geographical location. (a) Devon Island, Nunavut, in the Canadian High Arctic; (b) the Haughton Crater is located at 75° 22′ N, 89° 41′ W in the northwestern portion of Devon Island; (c) synthetic aperture radar image of the Haughton crater. The crater is approximately 24 km in diameter. Image obtained from Grieve (1988).

Haughton is set in arid polar desert, with less than 5% vegetation cover (Cockell *et al.* 2001). On Devon Island, the summer growing season is short, with an average number of 188 degree-days in July (Gold 1988). The soils at and around

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the Haughton impact structure are primarily dolomitic and oligotrophic (Bliss *et al*. 1994). Bacterial numbers within the soil average $3–4 \times 10^6$ colony-forming units per gram of dry-weight soil (Cockell *et al*. 2001).

Sample collection

Samples of unshocked and shocked gneiss and host melt breccia considered in this study were aseptically collected from the exposed surface of the Haughton impact structure during the 1999–2001 summer field seasons of the NASA Haughton-Mars Project. All samples were between 7–15 cm in size and were retrieved from two locations within the impact structure: an isolated hill of impact melt rocks at 75° 24.53′ N, 89° 49.76′ W ('Wyle Labs Hill', at 'Lake Trinity'); and an escarpment of melt breccia deposits at 75° 23.9′ N, 89° 31.6′ W ('Bruno Escarpment'). In this study, the term 'unshocked' gneiss corresponds to those samples exposed to a maximum shock pressure of less than 5 GPa and 'shocked' gneiss to those samples exposed to between 25 and 45 GPa. Samples with shock levels in the 5–25 GPa range were not considered in this study. All samples were collected in sterile bags, transported in an ice cooler at approximately 0 °C, and curated at the British Antarctic Survey (BAS) where they were stored at −20 °C.

Physical characterization of samples

Inductively coupled plasma–atomic emission spectroscopy (ICP-AES) was applied to samples to determine their bulk composition using the Optima 3300RL inductively coupled plasma–atomic emission spectrometer (Perkin Elmer, Shelton, CT) at Royal Holloway, University of London. Three samples of unshocked gneiss, three samples of breccia and five samples of shocked gneiss were analysed in duplicate. Samples were prepared following the methods of Totland *et al*. (1992), which include a potassium hydroxide fusion to determine major element concentrations and a hydrofluoric acid (HF) digest to determine trace element concentrations.

Scanning electron microscopy (SEM) was used to examine interior fragments of samples to identify both the micron-scale effects of the impact-shock process on the samples and microorganisms living within fissures and cavities of the samples. Hand samples were broken open and interior fragments showing no sign of surface weathering were chosen for study. These fragments (with dimensions of approximately $0.5 \times 0.5 \times 0.5$ cm^3) were mounted on SEM stubs with epoxy adhesive after aseptic preparation in the laboratory. These were then coated with gold for secondary electron SEM imaging using a Stereoscan S360 scanning electron microscope (Cambridge Instruments (LEO), Cambridge, UK) fitted with a PCIT image capture system and stored as standard computer TIFF files.

Energy-dispersive X-ray (EDX) microanalysis was applied to samples of both unshocked and shocked gneiss to determine their average surface composition (over 10^4 μm^2) and its micron-scale variations (transects of ten points, each covering 1 μm^2 and separated by 5 μm). Hand samples of unshocked and shocked gneiss were cut to expose their interiors. Samples for EDX analysis were taken from the centre of the samples, away from any sign of surface weathering. These samples were then thin-sectioned, polished and carbon coated for X-ray analysis using an Oxford Instruments EDX/INCA energy X-ray microanalysis system (Oxford Instruments, Oxford, UK) fitted with a germanium detector.

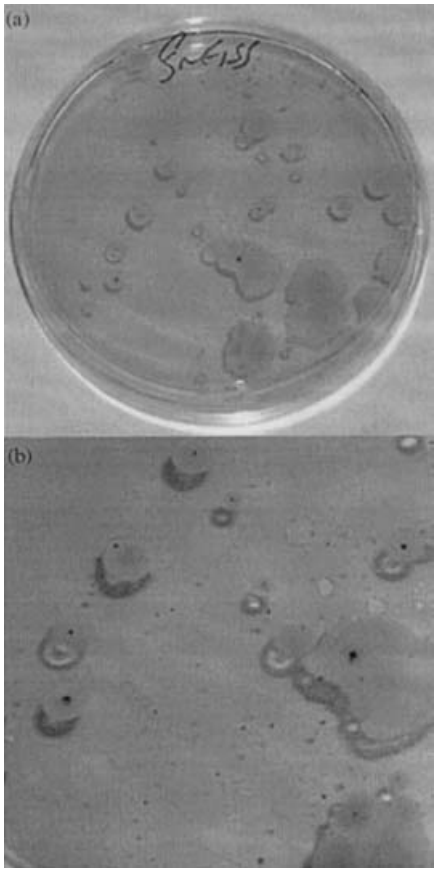

Fig. 2. Isolation of bacteria from interior fragments of shocked gneiss. (a) Cultivation of bacteria from the interior of shocked gneiss samples; (b) close-up showing colony formation only around fragments of the shocked gneiss.

Isolation and identification of heterotrophic bacteria

12 samples of shocked gneiss and of host breccia were broken open inside sterile bags using a rock hammer. Fragments from the interior of the sample were carefully removed under sterile conditions in a microbiological flow cabinet and scraped using a sterile blade into a Petri dish containing 4% tryptone-soy agar (TSA) (Difco Ltd, UK). After 2 days incubation at 20 °C, colonies had begun to grow around the pieces of rock fragments (Fig. 2). The colonies were subsequently streaked onto TSA plates until single isolates had been obtained. These colonies were maintained on TSA sloped agar tubes.

Polymerase chain reaction (PCR) was employed, using primers 8F (AGAGTTTGATCCTGGCTCAG) and 1500R

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Table 1. *Mineralogy of Unshocked Gneiss, Shocked Gneiss, and Breccia*

	Mineral Composition (% weight)										
Sample	Si_2O_3	TiO_2	Al_2O_3	Fe_2O_3	MnO	MgO	CaO	Na_2O	K_2O	P_2O_5	Total
Shocked Gneiss											
sg1	84.40	0.19	8.27	1.09	0.00	0.38	0.66	0.23	2.21	0.01	97.44
sg1a	85.46	0.18	7.70	1.07	0.00	0.38	0.66	0.23	2.14	0.01	97.83
sg2	77.95	0.07	10.03	0.60	0.00	0.26	2.35	1.81	4.20	0.00	97.27
sg2a	77.67	0.07	10.08	0.60	0.00	0.25	2.31	1.81	4.21	0.01	97.01
sg3	73.90	0.14	11.85	0.81	0.01	0.23	2.19	1.85	5.63	0.01	96.62
sg3a	73.87	0.14	11.76	0.82	0.01	0.23	2.18	1.85	5.64	0.01	96.51
sg4	73.95	0.13	11.01	0.53	0.00	0.14	2.58	1.63	5.49	0.01	95.47
sg4a	75.95	0.14	11.41	0.57	0.00	0.15	2.74	1.71	5.72	0.01	98.40
sg5	75.88	0.14	10.83	0.49	0.00	0.17	3.07	1.67	5.30	0.01	97.56
sg5a	75.89	0.14	10.81	0.48	0.00	0.16	3.04	1.65	5.22	0.01	97.40
Average	77.49	0.13	10.38	0.71	0.00	0.24	2.18	1.44	4.58	0.01	97.15
Unshocked Gneiss											
ug1	71.31	0.54	13.20	3.72	0.05	1.02	2.05	2.39	5.23	0.13	99.64
ug1a	71.37	0.55	13.11	3.74	0.05	1.03	2.07	2.37	5.24	0.13	99.66
ug2	67.52	0.65	14.71	5.36	0.03	2.45	2.57	3.03	3.41	0.24	99.97
ug2a	67.12	0.66	14.99	5.46	0.04	2.51	2.59	3.06	3.50	0.24	100.17
ug3	64.89	0.08	14.92	1.52	0.04	1.16	1.56	1.66	9.30	0.09	95.22
ug3a	65.61	0.08	15.46	1.54	0.04	1.19	1.61	1.74	9.67	0.09	97.03
Average	67.97	0.43	14.40	3.56	0.04	1.56	2.08	2.38	6.06	0.15	98.62
Breccia											
b1	21.67	0.19	4.34	1.64	0.03	8.50	24.26	0.10	1.17	0.03	61.93
b1a	21.70	0.20	4.26	1.61	0.03	8.37	23.29	0.10	1.16	0.04	60.76
b2	22.90	0.23	4.77	1.67	0.03	8.49	23.73	0.12	1.53	0.04	63.51
b2a	22.96	0.23	4.84	1.72	0.03	8.58	24.07	0.12	1.56	0.04	64.15
b3	24.82	0.21	4.83	1.85	0.03	9.68	21.89	0.14	1.33	0.04	64.82
b3a	25.87	0.21	4.79	1.79	0.03	9.53	21.63	0.14	1.31	0.04	65.34
Average	23.32	0.21	4.64	1.71	0.03	8.86	23.15	0.12	1.34	0.04	63.42

(AGAAAGGAGGTGATCCAGCC) (Invitrogen Life Technologies, Paisley, UK), to amplify the genes encoding the 16S rRNA (16S rDNA) from each isolate. The PCR amplicants were sequenced at the University of Cambridge, Department of Biochemistry on an ABI Prism 3700 DNA analyser (Applied Biosystems, Foster City, CA). Clone sequences were compared with the nucleotide database available at the National Center for Biotechnology Information's website (http://www.ncbi.nlm.nih.gov/blast) using GAPPED-BLAST (Altschul *et al.* 1997) to determine their closest phylogenetic neighbours.

Results

Characteristics of unshocked gneiss, shocked gneiss and host melt breccia

Inductively coupled plasma – atomic emission spectroscopy (ICP-AES)

ICP-AES analysis identified the bulk mineral composition and trace element concentrations within the samples (Table 1). When examining the shocked gneiss, a decrease in almost all metallic oxides, and a corresponding 10% increase in SiO_2, was observed relative to samples of unshocked gneiss. The following decreases were observed: Fe_2O_3 (4%); Al_2O_3 (3%); MgO (1.5%); K_2O (1.5%); Na_2O (1%); TiO_2 (0.3%); and P_2O_5 (0.14%). The decreases account for essentially all of the TiO_2 and P_2O_5, which were almost totally absent from the shocked samples. Of all the minerals identified, only the concentration of CaO in the shocked samples remained similar to its unshocked concentrations (increasing from 2.0% to 2.2%). The breccia composition was distinctive, having high percentages of MgO (9%) and CaO (23%), whereas these oxides constitute less than 3% of samples of unshocked and shocked gneiss. Also, the breccia samples contained up to 40% volatiles. In contrast, volatiles composed less than 4% of the samples of unshocked and shocked gneiss. Differences in the concentrations of trace elements within the samples were also observed (Table 2). All samples were observed to contain micronutrients (such as Zn, Cr and Ni), which are necessary to sustain bacterial communities (Egli 2000). However, in all cases, concentrations of each trace element in the unshocked gneiss were significantly higher than those of the shocked gneiss, and in the majority of cases (all elements except Cr, Cu, Li and Ni) concentrations were higher in unshocked gneiss than in the breccia. Concentrations were generally similar between shocked gneiss and breccia samples.

Scanning electron microscopy (SEM)

The surfaces of unshocked gneiss were quite homogenous, having few, if any, pore spaces for bacteria to inhabit (Fig. 3a). Images of shocked gneiss reveal the micron-scale effects that impact-induced shock has upon gneiss, particularly the generation of significant porosity within the samples, which

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Table 2. *Trace Element Concentrations of Unshocked Gneiss, Shocked Gneiss, and Breccia*

	Trace Element Concentrations (ppm)																			
Sample	Ba	Co	Cr	Cu	Li	Ni	Sc	Sr	V	Y	Zn	Zr	La	Ce	Nd	Sm	Eu	Dy	Yb	Pb
Shocked Gneiss																				
sg1	583	0	14	10	21	8	2	52	12	4	17	96	12	16	19	0	0.3	0.4	0.2	9
sg1a	570	0	14	9	19	8	2	51	12	4	20	91	11	18	16	0.3	0.3	0.4	0.2	20
sg2	1144	0	1	7	11	4	1	116	0	3	9	179	23	42	18	0.6	0.6	0.4	0.2	15
sg2a	1162	0	1	8	12	4	1	118	0	4	9	190	24	43	18	0	0.6	0.5	0.2	17
sg3	908	0	2	7	12	4	5	116	0	44	11	175	48	97	46	7.4	0.5	5.9	2.8	14
sg3a	882	0	2	6	11	5	5	112	0	39	11	171	52	100	50	7.5	0.6	5.7	2.7	14
sg4	659	0	2	5	12	5	3	164	0	17	7	206	76	139	66	7.3	0.6	3.3	0.6	16
sg4a	692	0	2	5	11	4	4	170	0	18	7	138	82	150	64	7.8	0.6	3.6	0.6	13
sg5	689	0	1	5	11	4	5	208	0	30	6	170	113	232	110	14.8	0.8	6.6	1.5	16
sg5a	706	0	1	5	11	4	5	213	0	27	6	160	104	208	96	13.2	0.7	6.1	1.4	12
Average	800	0	4	7	13	5	3	132	2	19	10	158	55	105	50	5.8	0.5	3.3	1.0	15
Unshocked Gneiss																				
ug1	1641	4	5	14	21	8	9	412	35	36	53	374	86	171	93	14.2	1.7	6.3	2.3	18
ug1a	1636	4	4	14	21	6	9	414	36	36	53	349	85	172	92	14.3	1.7	6.3	2.3	17
ug2	656	10	18	15	32	21	6	301	51	18	54	315	92	175	83	10.3	1.1	3.3	0.9	12
ug2a	666	10	18	14	34	22	7	309	52	18	56	263	99	187	84	10.8	1.2	3.3	0.8	10
ug3	5579	0	5	4	18	5	6	1474	14	26	40	16	263	619	337	44.7	11.1	6.7	0.9	25
ug3a	5738	0	5	4	18	5	6	1536	14	27	41	26	275	652	358	45.9	11.3	6.8	0.9	26
Average	2653	4	9	11	24	11	7	741	34	27	50	224	150	329	175	23.4	4.7	5.5	1.4	18
Breccia																				
b1	259	2	18	16	42	12	4	220	22	11	25	80	25	44	31	1.0	0.5	1.1	0.8	11
b1a	259	2	19	15	41	13	4	221	22	11	26	85	23	40	36	1.4	0.5	1.1	0.8	17
b2	265	2	18	16	42	13	4	195	26	11	26	91	21	35	36	1.0	0.4	1.2	0.8	14
b2a	273	2	19	16	43	13	4	201	28	11	26	93	25	38	38	0.3	0.5	1.0	0.8	17
b3	304	2	20	20	55	13	4	195	25	12	29	89	25	46	39	1.4	0.5	1.4	0.8	12
b3a	305	2	19	14	54	13	4	195	24	12	28	103	26	49	38	2.0	0.5	1.6	0.9	12
Average	278	2	19	16	46	13	4	205	25	11	27	90	24	42	36	1.2	0.5	1.2	0.8	14

could serve as bacterial habitats (Fig. 3b). Observations of the breccia show the complex topography that resulted from the impact shock to these samples (Fig. 3c). Unlike the surfaces present in samples of shocked gneiss, the breccia samples are a conglomeration of micron-scale features possessing a broad morphological variation, hindering visual identification of any bacteria. However, both rod-shaped and coccoid microbes were observed to inhabit surface cavities on the shocked gneiss. Rod-shaped microorganisms usually appeared singly or in small groups (Fig. 3d), whereas coccoid microorganisms tended to occur together in larger groups (Fig. 3e), often covering the entire surface of cavities (Fig. 3f). The coccoid microbes were always observed with a coating of extracellular polymeric substances (EPS), which usually connected all the individuals observed within a cavity.

Energy dispersive X-ray analysis

EDX analysis was used to investigate the chemical composition, with particular attention being paid to chemical signatures characteristic of bacteria, in the interior of samples of unshocked and shocked gneiss. The samples analysed came from the interior of larger hand samples, approximately 10 cm below the exterior surface, and showed no signs of surface weathering. Here, EDX observation characterizes the surface composition of fresh breaks and vesicle walls (found only in the shocked gneisses, see Fig. 3b) in the samples, and does not express surface weathering. Repeated (40) measurements were conducted, with individual measurements recording composition for an area of either 10^4 or 1 μm^2, to achieve an understanding of the average composition and its micron-scale variation. The analysis of the unshocked gneiss revealed that its surface was composed solely of Si, O, Al, Fe and trace amounts of Ti (Fig. 4a). Samples of shocked gneiss were observed to have highly heterogeneous surface composition, particularly along the edge and within the interior of impact-induced cavities. The surface of shocked gneiss distant from surface cavities resembled that of unshocked gneiss, although significant decreases in Al, Fe and Ti were observed, relative to the unshocked samples. The composition within surface cavities on shocked gneiss often changed dramatically and the presence of Mg, K, Na, P, Ca, S and Cl were detected (Fig. 4b).

The placement of these latter, biologically important elements in cavities within shocked gneiss correlates with SEM observations of microorganisms in the cavities. To observe the strength of a biological signal against a sterile mineral background, a sterile glass slide, representing an abiologic silicate background, and a slide with different concentrations of bacteria (isolate G20, cultivated from the sample of shocked gneiss), representing microorganisms inhabiting cavities within shocked gneiss, were analysed using EDX. The sterile glass slide (Fig. 4c) had a surface composition similar

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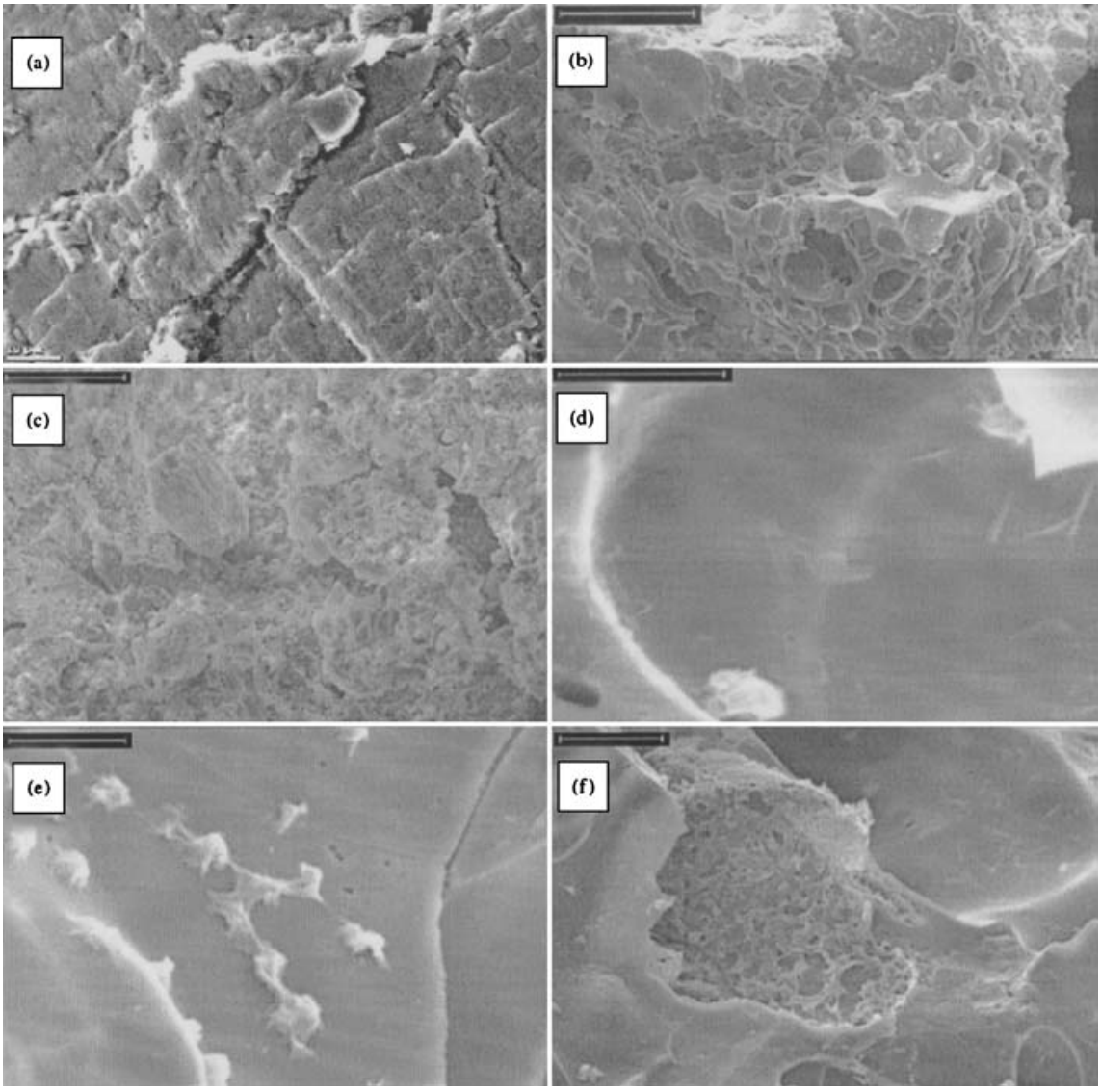

Fig. 3. SEM images. (a) Fragment of unshocked gneiss. Scale bar = 10 μm. (b) Fragment of shocked gneiss. Note the surface variation, particularly the abundant vesicles, which can serve as microbial habitats. Scale bar = 100 μm. (c) Fragment of breccia. Note the surface complexity drastically hinders visual identification of microbes. Scale bar = 20 μm. (d) Cluster of three rod-shaped bacteria. Scale bar = 5 μm. (e) Small group of coccoid-shaped bacteria. Note the EPS covering that seems to connect them. Scale bar = 5 μm. (f) Biofilm of coccoid bacteria completely covering the interior surface of an open cavity. Scale bar = 20 μm.

to the surface of unshocked gneiss (Fig. 4a) and the surface of shocked gneiss distant from surface cavities. The slides with isolate G20 had clearly biological spectra, for which the intensity relative to the silicate background increased in proportion to the thickness of the bacteria on the slide. The slide with the lowest bacterial density (Fig. 4d) and the surface cavities of shocked gneiss are observed to have approximately the same density of bacteria. The occurrence of chlorine in the spectra, observed even under the thinnest layer of bacteria, was the clearest and most sensitive indicator of the presence of bacteria using EDX.

EDX transects conducted along the surface of unshocked (Fig. 5a) and shocked (Fig. 5b) gneiss reveal impact-induced changes in surface composition and illustrate the microbial habitats created by micro-scale variations in the surface of the shocked gneiss. The surface of unshocked gneiss showed relatively little variation in surface composition, even on a micron scale. However, the surface composition of the shocked gneiss varied considerably even over a few microns, and dramatically near or inside a surface cavity (right of Fig. 5b), which again was characterized by high abundances of biologically important elements, including P, Mg, Cl and S.

Isolated heterotrophic bacteria

A total of 27 bacteria were isolated from the interiors of samples of shocked rocks (14 from shocked gneiss, 13 from breccia). These isolates were selected, along with three isolates from Antarctic soil as a comparison, to undergo sequencing

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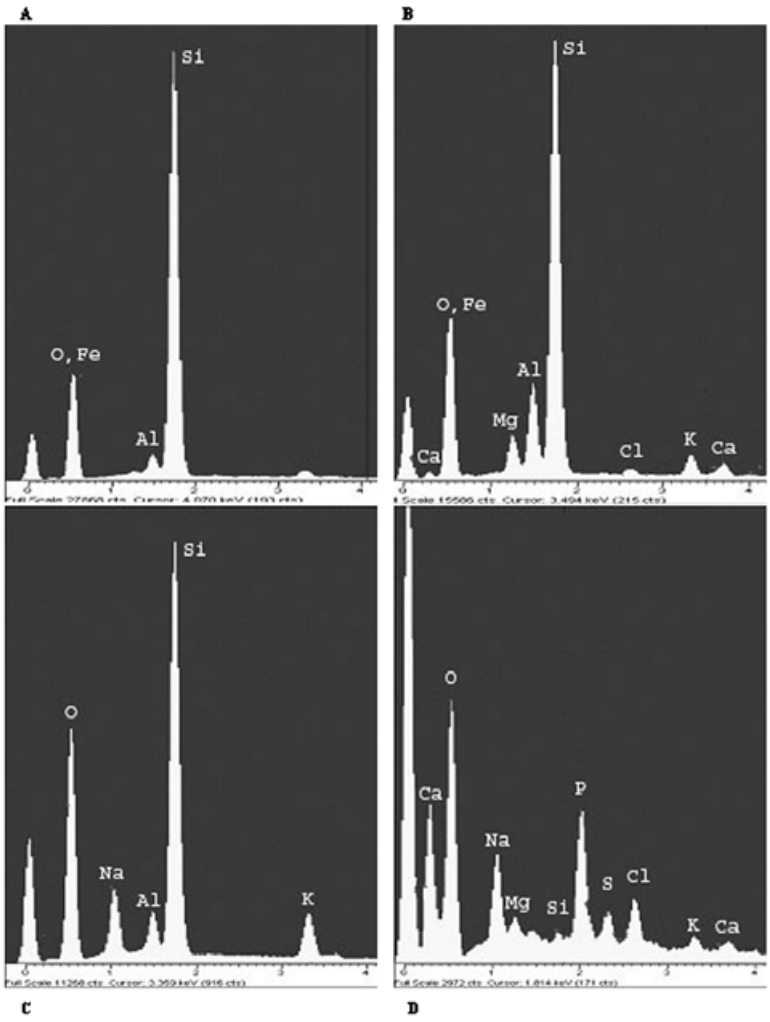

Fig. 4. X-ray spectra. (a) Surface of unshocked gneiss. The spectra are dominated by Si, O, Fe and Al peaks. (b) Interior of a cavity on the surface of shocked gneiss. The spectra contain the biologically important elements Ca, Cl, Mg and K, in addition to those found in (a). For comparison (c) the surface of a sterile glass slide and (d) bacterial isolate G20 streaked onto a glass slide. The elemental signature of the glass slide is still visible. Note the similarity between unshocked gneiss (a) and the glass slide (c) and between the shocked gneiss (b) and isolate G20 (d).

of their 16S rDNA genes. This sequencing process was successful for 13 out of 14 isolates from the shocked gneiss samples, for all 13 isolates from the breccia samples and for all three of the Antarctic soil isolates. Isolate G20 from the gneiss sample did not amplify properly during the PCR stage, preventing a proper sequence from being obtained. This isolate was observed to possess a thick waxy coating when observed under an optical microscope. This waxy coating is the likely cause of the failure of the amplification process because excess polysaccharides have been shown to inhibit the PCR reaction (Demeke & Adams 1992; Do & Adams 1991). Of the 30 isolates successfully sequenced, 24 were significantly similar to sequences found in the NCBI nucleotide database and have been identified to the species level (see Table 3). The sequences for the remaining six isolates (four from shocked gneiss (G11, G0, G13, G14) and two from breccia (B8, B11)) showed no significant similarity to any sequence within the database (having matches that, at best, were less than 20 base pairs in length).

The identification of our isolates with known species in the nucleotide database provides for the characterization of bacteria according to phylogeny, habitat and metabolism. Bacteria isolated from the shocked gneiss and host breccia were phylogenetically similar to each other, and to those isolated from Antarctic soil (Fig. 6). These isolates are prominently soil, ice or freshwater bacteria (Table 4). Members of the genera *Arthrobacter*, *Bacillus*, *Janthinobacter* and *Pseudomonas* are frequently found as soil bacteria. *Arthrobacter* species are common soil bacteria and all *Arthrobacter* species identified in this study have previously been isolated from soil as well as from glacial ice (Keddie *et al.* 1984). In addition, *A. nicotiana* has been found in cave silts, glacial silts, sewage, air and tobacco plants, and *A. sulfurous* has previously been isolated from oil brines (Keddie *et al.*

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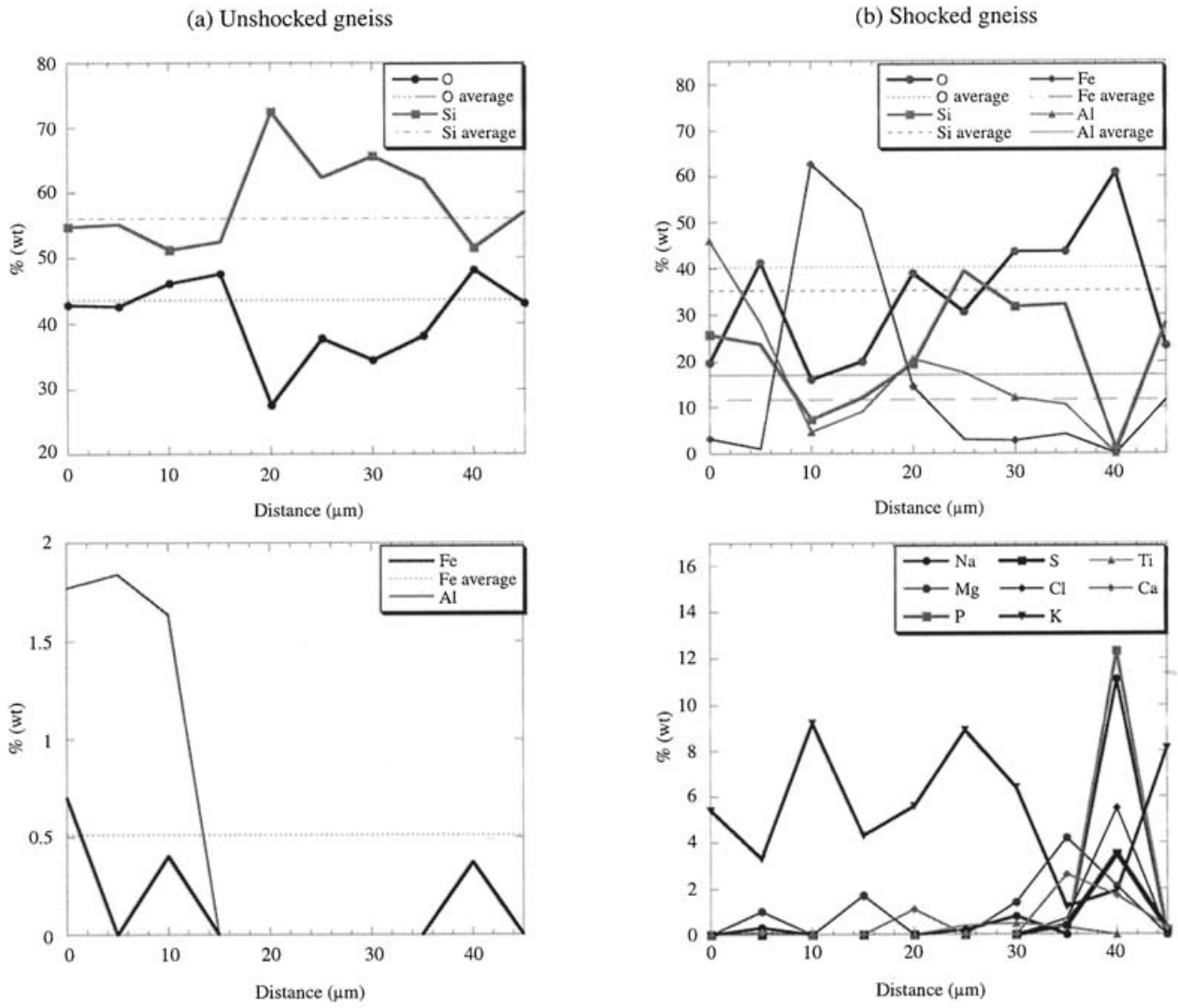

Fig. 5. X-ray transects across gneiss samples. Major elements are shown on the top and trace elements on the bottom of each graph. Each point sampled an area of approximately 1 μm². Average values were obtained by integrating over a 100 μm square centred on the transect and are shown for each element (where no average is shown, the integration did not detect that element). (a) Unshocked gneiss: the composition is primarily of silicon and iron oxides and shows little variation. (b) Shocked gneiss: the composition varies significantly across the transect, particularly within a surface cavity (located at $x = 40$ μm on the distance axis). On flat surface areas the composition is primarily silicon, iron and aluminium oxides. Within the exposed surface cavity, peaks in the concentrations of sodium, chlorine, phosphorus and sulphur are observed. These elemental signatures are consistent with the X-ray spectra for biological samples (bacterial isolate G20 in Fig. 4d).

1984). *Bacillus* species are widely distributed in nature and *B. psychrophilus* has been isolated from soil and river waters (Claus & Berkeley 1984). *Janthinobacter lividum* is a common soil and freshwater organism, although it is most common in temperate climates (Sneath 1984). *Pseudomonas* species are common components of soil and freshwater bacterial communities. All *Pseudomonas spp.* isolated in this study have been found in freshwater habitats and *P. borealis*, *P. sp. IC038* and *P. psychrophilia* have also been isolated from soil environments (Palleroni 1984). Three bacteria were found that are not characteristically isolated from soil: *Caulobacter bacteroides*, typically isolated from freshwater habitats (Abraham *et al.* 1999); *Planococcus citreus*, a marine organism, frequently isolated from polar waters (Kocur 1984); and *Stenotrophomonas maltophilia*, which was isolated from both the shocked gneiss and the breccia, customarily isolated from clinical specimens and from milk, water and frozen foods (Palleroni 1984).

The three isolates from Antarctic soil samples (two species of *Arthrobacteria* and one species of *Bacillus*) were similar to those isolated from shocked gneiss and breccia (see Table 4). *Arthrobacter sp.* CAB1 (Morikawa *et al.* 2002) had previously been isolated from 200 year old glacial ice in China (Christner 2002) and from agricultural soils (Lukow 1999). *Arthrobacter*

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Table 3. *Phylogenetic Identification of Isolates from Shocked Gneiss, Breccia, and Antarctic Soil. The nearest phylogenetic species to our isolates is shown down to the species level, with the percent match between the sequence for our isolates and that found in the GenBank database. Novel indicates that there are no significant matches between the isolate sequence and any species in the database. N/A = sample G20 did not amplify properly in PCR*

	Phylum	Class	Genus	Species	% Match	Matches (bp)
Shocked Gneiss						
G4	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*nicotiana*	98.5%	460
G50	*Firmicutes*	*Bacilli*	*Planococcus*	*citreus*	99.3%	456
G21	*Firmicutes*	*Bacilli*	*Bacillus*	*psychrophilus*	97.2%	447
G23	*Proteobacteria*	β-*Proteobacteria*	*Janthinobacter*	*lividum*	99.4%	464
G10	*Proteobacteria*	γ-*Proteobacteria*	*Pseudomonas*	*rhodesiae*	99.8%	473
G22	*Proteobacteria*	γ-*Proteobacteria*	*Pseudomonas*	*borealis*	98.9%	465
G28	*Proteobacteria*	γ-*Proteobacteria*	*Pseudomonas*	*grimontii*	99.6%	781
G16	*Proteobacteria*	γ-*Proteobacteria*	*Stenotrophomonas*	*maltophilia*	99.3%	761
G15	*Proteobacteria*	γ-*Proteobacteria*	*Stenotrophomonas*	*maltophilia*	99.6%	465
G11			Novel			
G0			Novel			
G13			Novel			
G14			Novel			
G20			N/A			
Breccia						
B10	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*globiformis*	100.0%	474
B24	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*nicotiana*	98.9%	470
B16	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*nicotiana*	98.9%	470
B4	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*sulfureus*	99.2%	730
B20	*Proteobacteria*	α-*Proteobacteria*	*Caulobacter*	*bacteriodes*	96.8%	477
B12	*Proteobacteria*	γ-*Proteobacteria*	*Pseudomonas*	*IC038*	99.8%	479
B15	*Proteobacteria*	γ-*Proteobacteria*	*Pseudomonas*	*psychrophilia*	97.1%	706
B17	*Proteobacteria*	γ-*Proteobacteria*	*Pseudomonas*	*rhodesiae*	98.1%	471
B21	*Proteobacteria*	γ-*Proteobacteria*	*Pseudomonas*	*rhodesiae*	93.3%	431
B3	*Proteobacteria*	γ-*Proteobacteria*	*Stenotrophomonas*	*maltophilia*	99.0%	489
B6	*Proteobacteria*	γ-*Proteobacteria*	*Stenotrophomonas*	*maltophilia*	96.8%	479
B8			Novel			
B11			Novel			
Antarctic Soil						
S13	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*CAB1*	95.9%	772
S16	*Actinobacteria*	*Actinobacteria*	*Arthrobacter*	*S23H2*	95.8%	668
S21	*Firmicutes*	*Bacilli*	*Bacillus*	*psychrophilus*	98.0%	448

sp. S23H2 had previously been isolated from Antarctic sea ice brine and cryoconite holes (Junge *et al*. 1998). *Bacillus psychrophilus*, also isolated from shocked gneiss, had previously been isolated from soil and freshwater, including from a fiord in Greenland (Suzuki & Yamasato 1994).

The bacteria identified based on their sequence information all possess relatively similar metabolisms and physiological properties. All of the species identified are heterotrophs (chemoorganotrophs), although some species of *Pseudomonas* and *Bacillus* are known to be facultative chemolithoautotrophs (Claus & Berkeley 1984; Palleroni 1984). It has not been determined whether the *Pseudomonas spp*. or *Bacillus psychrophilus* identified in this study possess the ability to be facultative chemolithoautotrophs. All of the identified bacteria are motile, with the exception of the *Arthrobacter spp*., which are non-motile (Palleroni 1984). The remaining bacteria are motile through a combination of polar (*Pseudomonas*, *Stenotrophomonas*, *Planococcus*), polar and subpolar/lateral (*Janthinobacter*) or peritrichous (*Bacillus*) flagella (Claus & Berkeley 1984; Keddie *et al*. 1984; Kocur 1984; Palleroni 1984; Sneath 1984). Most of the bacteria isolated are obligate aerobes, notable exceptions include select species of *Pseudomonas*, which can replace oxygen with nitrate as a terminal electron acceptor, allowing anaerobic growth (Palleroni 1984), and species of *Bacillus*, which can replace oxygen with a variety of different terminal electron receptors, again allowing for anaerobic growth (Claus & Berkeley 1984). Facilities were not available to determine whether the *Pseudomonas spp*. or *Bacillus psychrophilus* identified in this study can function as facultative anaerobes. All of the isolated bacteria have strictly respiratory and never fermentative metabolisms (Claus & Berkeley 1984; Keddie *et al*. 1984; Kocur 1984; Palleroni 1984; Sneath 1984). All of the species are nutritionally non-exacting, either requiring no growth factors (*Pseudomonas spp*., *Janthinobacter lividum*, *Bacillus psychrophiles*, *Planococcus citreus*) or possessing basic requirements (*Stenotrophomonas maltophilia* usually requires methionine or cystine, *Arthrobacter spp*. require biotin) (Claus & Berkeley 1984; Keddie *et al*. 1984; Kocur 1984; Palleroni 1984; Sneath 1984).

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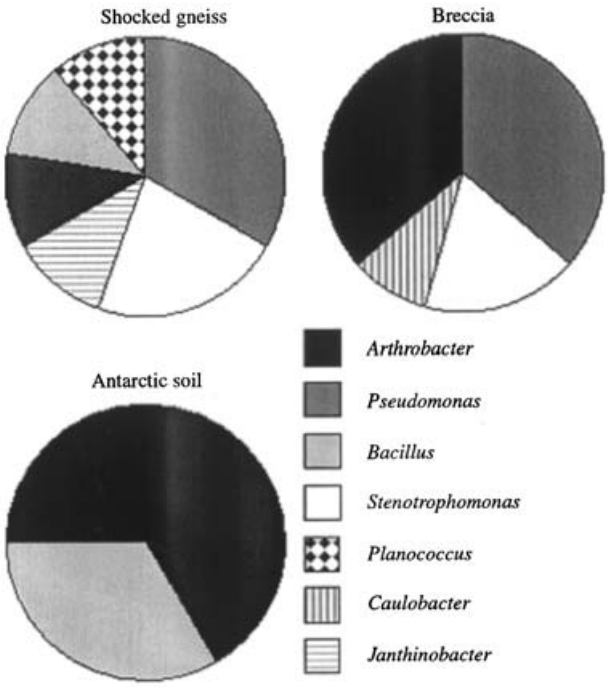

Fig. 6. Phylogenetic distribution of isolated bacteria. (a) Breccia; (b) shocked gneiss; (c) Antarctic soil. Bacteria of the genus *Arthrobacter* were isolated from all three types of samples. *Bacillus* and *Pseudomonas* species make up the majority of the remaining bacteria identified. *Stenotrophomonas* species, while found in both the shocked gneisses and breccia from Haughton, were not isolated from the Antarctic soil.

Discussions

Geological interpretation

An examination of the composition of gneiss yields insights into the effects of shock metamorphism. Overall, the surface composition as determined by EDX shows a significant disparity when compared with the bulk composition identified by ICP-AES. The ICP-AES bulk composition indicates that there are significantly higher concentrations of Fe_2O_3, Al_2O_3, MgO, K_2O, Na_2O, TiO_2 and P_2O_5, as well as of all trace elements, in samples of unshocked gneiss. The elevated concentration of MgO (9%) and CaO (23%) in the breccia and the high volatile concentration (up to 40%) agree with those measured by Metzler *et al.* (1988), and probably reflect the incorporation of dolomite into the breccia.

EDX spectra reveal the surface composition of unshocked gneiss to be composed almost entirely of silicon, oxygen, iron and aluminium, whereas the shocked samples have surface compositions containing less iron and aluminium and significant amounts of calcium, magnesium, phosphorus, potassium, sulphur and chlorine. This disparity may be explained by impact-induced shock metamorphism. First, as the shock wave resulting from the impact propagates through samples, darker, more refractory minerals are preferentially volatilized as they absorb a greater fraction of the energy incident upon them. This preferential volatilization tends to remove darker metallic oxides, particularly Fe_2O_3 and Al_2O_3 (Bunch *et al.* 1998). This process creates vesicles and fissures as near- and sub-surface pockets of these minerals volatilize. Furthermore, as magnesium, phosphorus, potassium and sodium oxides are shock-volatilized, they may have left behind thin and/or patchy coatings on the interior of these vesicles. This might account for the abundance of these latter minerals localized around vesicles walls of the shocked samples and their absence from surfaces of unshocked gneiss, despite the actual bulk percentage of these minerals being significantly less in shocked samples than in unshocked samples. Thus, the impact-induced shock metamorphism has a 'chemical gardening' effect on interior composition, resulting in the enrichment of biologically important elements in vesicle walls and fissures of the shocked gneiss. The relative bulk abundance of biologically important elements such as sulphur, calcium and chlorine, could not be determined between unshocked and shocked gneiss because the ICP-AES analysis was not sensitive to them. However, we suspect that such nutrients are deposited from meltwater into impact-generated cavities, which would encourage endolithic heterotrophic bacteria to inhabit them.

Biological interpretations

The heterotrophic bacteria isolated from samples in this study are similar to heterotrophic communities identified from other polar environments, including Antarctic soil samples (Roser *et al.* 1983), marine and sea ice samples (Delille 1992; Bowman *et al.* 1997), and Antarctic lacustrine samples (Franzmann *et al.* 1990; Nichols *et al.* 1995). Specifically, representatives of the genera *Pseudomonas*, *Arthrobacter*, *Bacillus* and *Planococcus*, which together constitute 70% of all bacteria identified in this study, were identified as major constituents of each of these other polar microbial communities. Two species, *Arthrobacter nicotiana* and *Planococus citreus*, were found both in this study and in marine and sea ice samples (Bowman *et al.* 1997).

The similarity between bacteria identified from these diverse environments raises the question of the ultimate origin of the communities. Are these bacteria truly cosmopolitan, or are they endemic to a particular environment and have been transported into the others? The identification of the bacterial isolates from the shocked gneiss reveals that the majority are species associated with soil, ice or freshwater and are commonly isolated from the Antarctic. The ready transport of soil bacteria to marine or sea ice environments has been demonstrated by studies of Antarctic soils (Delille 1987, 1990; Roser *et al.* 1993; Rotert *et al.* 1993; Bowman *et al.* 1997). Similarly, studies have also documented ice bacteria isolated from soil samples (Roser *et al.* 1993; Bowman *et al.* 1997). The common occurrence of genera and even species in ecologically and geographically diverse locations supports the notion of global transport and distribution of bacteria, by vectors such as atmospheric circulation and meltwater flow. As mentioned earlier, precipitation, wind and snowmelt have been identified as means of inoculating rock interiors with bacteria from the surrounding environment (Vincent 2000),

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Table 4. *Known Habitats of Identified Bacteria. This lists the species identified in this study through the sequencing of the genes for their 16S rRNA and whether they were found (+) or absent (−) in the samples of shocked gneiss, breccia, and Antarctic soil, and provides a list of other known habitats of the identified organisms*

Genus	Species	Shocked Gneiss	Breccia	Antarctic Soil	Other Known Habitats*
Arthrobacter	*nicotiana*	+	+	−	Soil/Glacial Ice
Arthrobacter	*globiformis*	−	+	−	Soil/Glacial Silts
Arthrobacter	*sulfurous*	−	+	−	Soil/Glacial Ice
Arthrobacter	*CAB1*	−	−	+	Soil/Glacial Ice
Arthrobacter	*S23H2*	−	−	+	Sea Ice Brine
Bacillus	*psychrophilus*	+	−	+	Soil/Freshwater
Caulobacter	*bacteriodes*	−	+	−	Freshwater
Janthinobacterium	*lividum*	+	−	−	Soil/Freshwater
Planococcus	*citreus*	+	−	−	Sea Ice/Glacial Ice
Pseudomonas	*borealis*	+	−	−	Soil/Freshwater
Pseudomonas	*grimontii*	+	−	−	Freshwater
Pseudomonas	*IC038*	−	+	−	Soil/Freshwater
Pseudomonas	*psychrophilia*	−	+	−	Soil/Freshwater
Pseudomonas	*rhodesiae*	+	+	−	Freshwater
Stenotrophomonas	*maltophilia*	+	+	−	Clinical/Frozen Foods

* See text for references.

and it is believed that a combination of these three factors is responsible for delivering the isolated bacteria to the interior of the samples discussed in this study.

The similarity between the bacteria isolated from within the breccia and the shocked gneiss, and to those isolated from soil, strengthens the argument that the bacteria inhabiting these rocks are derived from the surrounding soil. It is estimated that $3-4\times10^6$ colony-forming units of heterotrophic bacteria inhabit the soil in the area from which the samples were collected (Cockell *et al*. 2001). While the argument could be made that the bacteria isolated are evidence of surface contamination in the process of extracting the interior fragments, thereby explaining both the presence of complex heterotrophic communities in the interior of these rocks and the similarities between the bacteria inhabiting the breccia and the shocked gneiss, we believe this not to be the case for several reasons. First, careful attention was given to selecting interior fragments to be used for obtaining the isolates. The original rocks (approximately 10 cm in diameter) were broken into several centimetre-sized pieces, from which only fragments showing fresh break marks and with no evidence of surface weathering were chosen. These fragments were then broken open again, with millimetre or submillimetre fragments from the centre used to isolate bacteria from the samples. During the isolation process, growth was observed to occur only in those areas that surrounded these fragments (see Fig. 2). As a result, the likelihood that surface materials contaminated these fragments is deemed to be insignificant. A stronger argument against surface contamination comes from the lack of phototrophic organisms isolated in our study. The outer surfaces of both shocked gneiss and breccia were observed to have visible coatings of cyanobacteria and other photosynthetic organisms. Further, Cockell *et al*. (2002) identified endolithic phototrophs living in the upper few millimetres of similar rocks. Phototrophic bacteria would be present in any surface and near-surface contamination, and their complete absence in the isolates obtained from both the shocked gneiss and breccia samples indicates that surface contamination is not likely to have had a significant impact upon our results.

Relevance to meteorites

The microbial colonization of impact-shocked crystalline rocks has particular relevance to the study of meteorites, which are often highly shocked and in many cases covered by a fusion crust that is formed during entry into the Earth's atmosphere (e.g. Melosh 1989; Steele *et al*. 2000; Dressler & Reimold 2001). This fragile crust is frequently fractured and weathered, and may serve to trap dust particles (including microfossils) that have been transported into the proximity of the meteorites, as has been observed with Antarctic meteorites (Burckle & Delaney 1999; Steele *et al*. 2000). In addition to microfossils and microorganisms found on meteorite surfaces (Burckle 1995a, b; Burckle & Wasell 1995), detailed microscopic investigations of the interior of the Allan Hills 84001 Martian meteorite suggest that it may be contaminated by endolithic microorganisms of terrestrial origin (Steele *et al*. 2000). The entrapment of microorganisms by meteorites is likely to be a ubiquitous process in Antarctica (Burckle & Delaney 1999) and possibly elsewhere.

The presence of microfossils in the surface and near-surface environments of meteorites clearly demonstrates their susceptibility to terrestrial contamination. Our examination of shocked rocks from the Haughton impact structure provides insight into the extent to which, and the mechanism by which, their interiors have been colonized by heterotrophic bacteria. The prime factor facilitating this colonization appears to be the impact-induced porosity of the shocked rocks. The alteration of the chemical composition of vesicle walls to include higher abundances of biologically important elements, as a result of the partial volatilization of the constituent minerals, is also believed to favour colonization.

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As many meteorites present evidence of shock, often to levels well in excess of those exhibited in the samples examined in this study, they would be natural hosts for heterotrophic colonization. The source for microbial contamination in Antarctic meteorites could be either deposition of wind-transported microorganisms or, more indirectly, microorganisms derived from the ice immediately surrounding the meteorite. It has been observed that the Antarctic ice sheet contains a variety of heterotrophic bacteria (Hirsh *et al.* 1988; Franzmann 1996; Kellogg & Kellogg 1996). Furthermore, the albedo difference between the meteorite and the surrounding ice can cause the surrounding ice to melt when the meteorite is heated by solar radiation during the Antarctic summer. Low-albedo rocks, debris and organic material (cryoconite) have been observed to sink into Antarctic blue ice as a result of seasonal thawing and refreezing, becoming trapped in regelation portholes (Lee *et al.* 1999). Here, the seasonal availability of meltwater could help concentrate any heterotrophs present in the nearby ice, providing a mechanism to inoculate the interior of meteorites with heterotrophs.

Life on Mars

The observed impact-induced generation of habitats in crystalline rocks also has relevance to astrobiological questions of life on other planetary bodies with impact-shocked surface materials. This is particularly true of Mars, where as a result of the lack of global plate tectonics, the age of exposed surface materials could possibly exceed 4.5 Gyr (radiometric crystallization age of the ALH-84001 meteorite), a time when the impact rate was significantly higher than its present value. Were life to have evolved on Mars, impact-shocked rocks would have provided ready lithic habitats. As liquid water became increasingly scarce at the Martian surface, the interior of impact-shocked rocks could have served as a reservoir for water, heat and nutrients for any microbial colonies present. Thus, the interior of impact-shocked rocks in the Martian regolith, particularly that portion included at present in the Martian permafrost, might be among the best places to search for fossilized remnants of any past Martian life. Continuous permafrost, such as is found approximately 40 cm below the surface at Haughton and throughout much of the high Arctic, offers an environment that helps preserve an extended record of microbial endolithic colonization. Such preservation has been observed in Siberian permafrost, where ground ice and soils have been found to contain viable microorganisms that are probably 2–3 Myr old (Shi *et al.* 1997).

Recent observations from Mars Odyssey reveal concentrations of hydrogen in the upper few metres of large portions of the Martian subsurface, which may be water ice-rich layers on the scale of tens of centimetres (Mitrofanov *et al.* 2002). In such regions, ice may constitute $35\% \pm 15\%$ by weight of the subsurface layer (Boynton *et al.* 2002). If these regions of high hydrogen concentration represent water ice content, then they would be ideal places to search for organic preservation. On Mars and beyond, as impacts are a universal process of planetary formation and evolution, we speculate that the interior of impact-shocked rocks might offer a common environmental setting favourable for the development, survival and preservation of microbial life were it to arise on planetary bodies outside the Earth.

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