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Life in Darwin's dust: intercontinental transport and survival of microbes in the nineteenth century

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Summary

Charles Darwin, like others before him, collected aeolian dust over the Atlantic Ocean and sent it to Christian Gottfried Ehrenberg in Berlin. Ehrenberg's collection is now housed in the Museum of Natural History and contains specimens that were gathered at the onset of the Industrial Revolution. Geochemical analyses of this resource indicated that dust collected over the Atlantic in 1838 originated from the Western Sahara, while molecular-microbiological methods demonstrated the presence of many viable microbes. Older samples sent to Ehrenberg from Barbados almost two centuries ago also contained numbers of cultivable bacteria and fungi. Many diverse ascomycetes, and eubacteria were found. Scanning electron microscopy and cultivation suggested that *Bacillus megaterium*, a common soil bacterium, was attached to historic sand grains, and it was inoculated onto dry sand along with a non-spore-forming control, the Gram-negative soil bacterium *Rhizobium* sp. NGR234. On sand *B. megaterium* quickly developed spores, which survived for extended periods and even though the numbers of NGR234 steadily declined, they were still considerable after months of incubation. Thus, microbes that adhere to Saharan dust can live for centuries and easily survive transport across the Atlantic.

Introduction

Although ancient mariners experienced intercontinental dust storms that blew west-wards across the Atlantic Ocean from Africa (see Ehrenberg, 1849; Husar, 2004), one of the first scientific observations of these phenomena was presented by Charles Darwin (1845) who wrote 'On the 16th of January, 1832, we anchored at Porto Praya, in St. Jago, the chief island of the Cape de Verd archipelago. Generally the atmosphere is hazy; and this is caused by the falling of impalpably fine dust, which was found to have slightly injured the astronomical instruments. The morning before we anchored at Porto Praya, I collected a little packet of this brown-coloured fine dust, which appeared to have been filtered from the wind by the gauze of the vane at the masthead. Mr Lyell[1] has also given me four packets of dust which fell on a vessel a few hundred miles northward of these islands'. These samples were passed on to Christian Gottfried Ehrenberg, a pioneer of aerobiology (Krumbein, 1995) at the Royal Prussian Academy of Sciences in Berlin. Darwin further wrote that 'In five little packets which I sent him he (Professor Ehrenberg) has ascertained no less than 67 different organic forms' (Ehrenberg, 1845; Darwin, 1846). Shortly before Ehrenberg's death in 1876, this collection was donated to the Prussian Academy and it is currently housed in the Museum für Naturkunde der Humboldt-Universität Berlin (Lazarus, 1998; Lazarus and Jahn, 1998).

Dust that originates from deserts is now known to be a vehicle for the spread of microbial communities via natural atmospheric pathways (Griffin *et al*., 2002; 2006; Kellog *et al*., 2004; Weir-Brush *et al*., 2004; Prospero *et al*., 2005). Early in the 21st century, scientific curiosity about what dust storms may carry has been supplemented with worries about accidental or intentional spread of contaminants and diseases (Brown and Hovmoller, 2002). As Ehrenberg's collection provides snapshots of a more

[1]'I must take this opportunity of acknowledging the great kindness with which this illustrious naturalist has examined many of my specimens. I have sent (June, 1845) a full account of the falling of this dust to the Geological Society' (Darwin, 1846).

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Table 1. Origins, collectors, history and descriptions of historic aeolian dust samples.

Sample description/where collected	Date/place collected	Collector	No. in MfN[a]	Material/origin
May dust (aerial) over Barbados	1812	R.H. Schomburgk	938	Collected during a dust storm that blacked out the sun (*b*) over Barbados on 1 May 1812. Dust event described in Ehrenberg[5]
May dust (aerial) over Barbados	1812	R.H. Schomburgk	939b	
Passat dust, collected over the Atlantic Ocean, onboard a ship	10 March 1838	R.B. James, through C. Lyell to C.R. Darwin	2894a	Dust event described in Darwin (1846): '. . . numerous irregular transparent variously coloured particles of stone 1/1000th of an inch square and much fine matter'. Event lasted 4 days (7–10 March 1838)
Passat dust, collected over the Atlantic Ocean, onboard a ship	9 March 1838, 17°43′N 25°54′W 380 miles off African coast (*not* volcanic ashes)	R.B. James, through C. Lyell to C.R. Darwin, CRD handwriting – sent to C.G. Ehrenberg	2895	
Passat dust, collected over the Atlantic Ocean, onboard a ship	9 March 1838, 17°43′N 25°54′W 380 miles off African coast	R.B. James, through C. Lyell to C.R. Darwin, CRD passed on to C.G. Ehrenberg	2896(a + b)	
Passat dust, collected over the Atlantic Ocean, onboard a ship	7 March 1838, 21°40′N 22°14′W 330 miles off African coast (most coarse fraction)	R.B. James, through C. Lyell to C.R. Darwin and C.G. Ehrenberg	2897a	

a. Museum für Naturkunde (Natural History Museum) der Humboldt-Universität zu Berlin, Invalidenstraße, 10115 Berlin.
b. 'Tag in Nacht verwandelt' – original description of C.G. Ehrenberg (1845).

pristine world, we studied the microbiology of dust that was collected before the globalization of industry. Here we describe the microbiological properties of some of these samples and discuss their possible role in global seeding terms.

Results

Microbial characterization of the historic dust

Obviously, the Ehrenberg collection is irreplaceable, and for this reason sampling was restricted to the most abundant samples. Fortunately, sufficient material of two of the oldest accessions (which fell over Barbados in 1812) was available along with four others that were sent to Darwin (Table 1). Lieutenant R.B. James, in command of his Brig 'The Spey', was travelling south-south-west over the Atlantic Ocean in 1838, when he encountered a four-day dust storm. On three separate days, James and his crew collected four distinct samples, and sent them to Charles Lyell, a close personal friend of Darwin. Darwin examined the samples himself, recorded all relevant details and eventually passed the packets of dust on to Ehrenberg.

Light- and scanning-electron-microscopy (SEM) clearly showed that contemporary museum dust (data not shown) is full of pollen and other particulates not found in the ancient samples (Fig. 1B–E). To cultivate these microorganisms five different culture media were tried. All permitted colony development, but plating out on R2A gave rise to the largest numbers of different colonies (Table 2). The number of cultivatable microorganisms varied between 10^4 and 10^5 colony-forming units (cfu) g^{-1} historic dust (Table 2). Even some of the oldest samples in the Ehrenberg collection (#938 and #939b) that were collected over Barbados in 1812 (see Table 1) still possessed more than 10^4 cfu g^{-1} dust (Table 2). Plating out the re-wetted historic dust samples on nutrient-poor media resulted in 48 bacterial isolates belonging to 17 different positively identified species (based on 16S rRNA gene sequences) (Table 3). All were spore-forming bacilli (Fig. 1I–K). Most bacteria recovered were rod-like but with variable morphologies, with or without spores, and were generally Gram-positive (+) (or Gram variable). By sequencing about 750 bp of the 16S rRNA gene of the historic dust isolates we were able to classify three probable *Bacillus* species that it was not possible to name, along with nine distinct species of *Bacillus* (Table 3). *Brevibacillis*, *Cohnella* and *Paenibacillus* were also found, all of which are also capable of forming spores.

Surprisingly, we were only able to cultivate three, very slow-growing fungi belonging to two different species (*Aspergillus versicolor* and *Davidella tassiana*) from the historic dust (Fig. 1F and G; Table 4). This contrasts to the broad palette of fungi found in museum air/dust. The fungal isolates were closely related to normal, cosmopolitan, air-borne species however (Figs 1H and 2B). The dust aggregates themselves were extremely porous offering multiple attachment sites for microbes (Fig. 1B and E). Bacterial cells and fungal hyphae including characteristic spores (Fig. 1C and D) were attached to them (Fig. 1B–D).

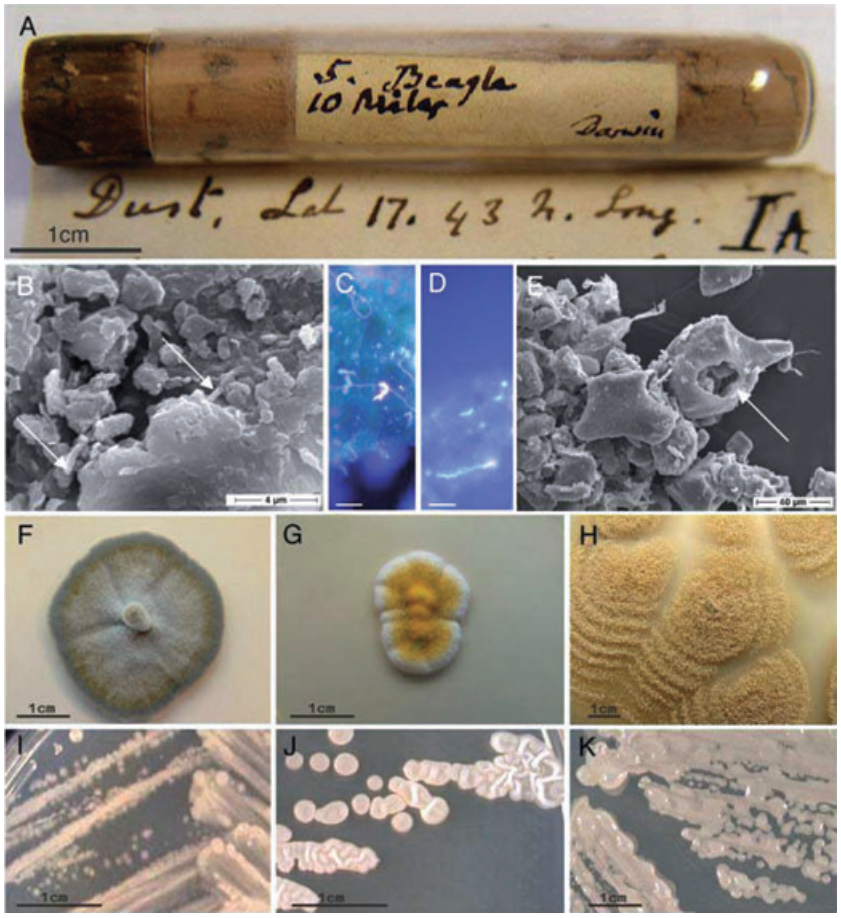

Fig. 1. A sample of historic aeolian dust, scanning electron micrographs of the dust and microorganisms cultivated from them.
A. Original glass vial from the collection at the Museum für Naturkunde, Berlin (vial # 2895, collected on 9 March 1838).
B and E. Microbes on mineral surfaces visualized by SEM (vial 2987a, coarse dust, collected on 7 March 1838).
C and D. Fluorescent micrographs of the samples shown in (B) after Calcofluor White staining (vial # 938).
F and G. Slow-growing colonies of the *Davidiella*/*Cladosporium*-like strain F6 (F) and *Aspergillus versicolor* (strains F7 and F8) (G).
H. Fast-growing *Aspergillus ochraceus* (F12) isolated from museum air and dust.
I–K. Bacterial colonies isolated from historic dust. (I and J) Different morphotype of *Bacillus subtilis*(I – isolate B27, J – isolate 3IIB7). (K) *Bacillus licheniformis* B37.

Excluding the possibility of contamination of historic dust in museums

As the Ehrenberg collection was passed from museum to museum during Berlin's turbulent past (Lazarus, 1998: Lazarus and Jahn, 1998), it was essential to check whether the re-packed samples shown in Fig. 1A and listed in Table 1 had been contaminated in Berlin. To do this, air and dust from the museum (collected on two separate occasions in 2006) was analysed microbiologically and compared with isolates from the original samples (see Tables 2–4). A number or striking observations were made, including:

(i) The density of cfu in museum dust was about 10 times higher than that found in the historical samples

Table 2. Number of microbes [in colony-forming units per gram dust (cfu g^{-1})] isolated from historic aeolian dust as well as from museum dust.

	TSA		R2A	
Medium/probe	20°C	37°C	20°C	37°C
938	3.4×10^2	3.0×10^3	3.8×10^4	4.8×10^4
939b	1.1×10^2	6.8×10^2	1.1×10^4	∝
2894a	9.3×10^5	3.2×10^4	∝	∝
2896	2.5×10^4	4.6×10^3	2.5×10^4	2.3×10^4
2897a	6.1×10^4	5.8×10^4	9.0×10^4	∝
1	8.2×10^4	n.d.	2.2×10^5	1.5×10^4
2	6.3×10^5	1.9×10^5	7.3×10^5	2.7×10^5
3	2.3×10^5	2.8×10^4	7.5×10^5	5.7×10^4

Duplicate Petri dishes containing one of two media were incubated at 20°C or 37°C for 12 days. Samples 1, 2 and 3 were collected from shelves and storage cabinets at the Museum für Naturkunde using a sterile brush on two separate occasions in 2006. Sample sizes of historic aeolian dust ranged from 71 to 257 mg, samples collected in the museum from 33 to 74 mg.
n.d., not detected. ∝, too many to count (colonies over-grew each other).

Table 3. Identity and description of bacterial isolates from historic aeolian dust samples, as well as contemporary museum air and dust.

Identification	Appearance in culture	Gram reaction, form	Sample	Isolation #
Historic dust				
Bacillus sp.	Thick, white	+ rods		
DQ448759 99% 1×			939b	1
EF522795 99% 1×			938	3
DQ993299 99% 1×			938	3
AM419753 99% 1×			938	4
DQ993299 100% 1×			938	4
Bacillus barbaricus	Clear	– rods		
AJ422145 99% 1×			938	1
AJ422145 98% 1×			938	3
Bacillus cereus	Thick, mat	+ large rods		
EF178440 99% 1×			938	3
Bacillus firmus	Cream-orange	+ rods		
AY833571 99% 1×			938	1
AY833571 99% 1×			938	4
Bacillus fusiformis	Small colonies, brown	Rods strangely stained		
DQ333300 99% 1×			938	3
Bacillus funiculus	Thick, white, mat	+ long rods in chain		
AB271137 98% 1×			938	4
Bacillus licheniformis	Thick, mat	+ rods central spore		
AY871102 99% 1×			938	1
EF059752 91% 1×			938	4
AY871102/EF059752 99% 1×			939b	1
AY871102/EF059752 99% 1×			2896	1
Bacillus megaterium	Yellowish, mat	+ large rods, central spore		
DQ660362 99% 1×			938	3
DQ660362 99–100% 3×			938	4
DQ660362 99% 1×			939b	1
Bacillus pumilus	Rough, thick	+ small rods, deforming spore		
AF526907 99% 1×			938	1
AF526907 99–100% 3×			938	2
AF526907 100% 2×			938	3
AF526907 99% 1×			938	4
AF526907 99% 1×			2897a	2
Bacillus simplex	Mat, pigmented brown-orange	± rods		
DQ275178 99% 1×			938	3
DQ275178 99% 2×			938	4
DQ275178 99% 1×			2897a	2
Bacillus subtilis	Thick, mucous	+ large rods		
AY881638 99–100% 2×	or		938	4
AY881638 99% 1×	Cratered, mucous	+ rods	2894a	1
AY881638 99% 4×			2896	1
AY728013 99% 1×			2897a	1
EF433403 90% 1×			2897a	1
Brevibacillus brevis	Thick, yellowish	– large rods		
AY591911 99% 1×			938	1
Cohnella ginsengisoli	Clear, veil-like	– fine rods		
EF368010 93% 1×			939b	1
Paenibacillus sp.	Fine, clear	– rods		
DQ512475 99% 2×			938	1
AM162326 96% 1×			938	1
Paenibacillus pocheonensis	Mucous, cream	– long, fine rods		
AB245386 96% 1×			938	1
AB245386 95% 1×			938	2
Paenibacillus panaciterrea	Mucous, clear	– long, fine rods, terminal spore		
AB245385 99% 1×			938	1
Paenibacillus chitinolyticus	Small colonies, clear	+ fine rods		
AB021183 94% 1×			938	2
Museum dust				
Arthrobacter sp.	Cream, thick	+ small rods		
AJ639830 98% 1×				
Bacillus subtilis	Cream, thick	+ rods, spores		
EF532601 99% 1×				
Cocci-like *Micrococcus luteus*	Yellow	+ small cocci		
Cocci	Rose	+ medium cocci		
Cocci	Cream, mucous	+ small cocci		

Table 3. *cont.*

Identification	Appearance in culture	Gram reaction, form	Sample	Isolation #
Museum air				
Arthrobacter sp. AJ785761 97% 1× AY512633 99% 1×	Mucous, cream	+ small rods		
Bacillus subtilis EF433403 99% 2×	Rough, white	+ large rods		
Curtobacterium sp. AM410688 99% 1×	Mucous, brown-rose	+ fine rods		
Labdella kawkjii DQ533552 99% 1×	Mucous, cream-orange	+ small rods		
Rhococcus sp. AJ244659 99% 1×	Orange	+ rods		

Isolations #1 and #2 were performed in Oldenburg, isolations #3 and #4 (after heating to 70°C) in Geneva. Suffixes (1×, 2 ×, 3 ×) represent the number of times a particular isolate was found in the sample.

(Table 2). Due to the limited availability of the historic probes, statistical comparisons could not be made however.

(ii) We were only able to isolate bacteria that are capable of forming spores from the historic samples, whereas museum air/dust carried a wider variety (Table 3), including *Arthrobacter* sp. and *Micrococcus luteus* (*Micrococceae*); *Curtobacterium* sp. and *Labdella kawkjii* (*Microbacteriaceae*); *Rhococcus* sp. (*Nocardiaceae*); as well as various unidentified cocci. All these bacteria, which are related to the Actinobacteria, are Gram-positive, and are unable to form spores.

(iii) Only one bacterial species, *Bacillus subtilis*, was common to dust of both the Ehrenberg collection and the museum (Fig. 2A, Table 3).

(iv) In comparison with museum air/dust which contains a

Table 4. Identity and description of fungal isolates from historic aeolian dust as well as contemporary air and dust from the Museum für Naturkunde.

Closest GenBank similarity (partial SSU rDNA)	Similarity score	Morphological peculiarities	Isolated from:
Amylomyces rouxii AB250171	99%	Very fast growing, white mycelia, dark sporangia	Museum air and dust
Aspergillus niger NW_001594105	100%	Fast growing, black conidiophores, white mycelia	Museum dust
Aspergillus ochraceus AF548065	99%	Fast growing, sand-coloured, concentric sporulation structures (Fig. 1H)	Museum dust
Aspergillus versicolor AF548069	99%	Restricted growth, white growing edge, orange mycelia (Fig. 1G)	939b
Aspergillus versicolor AF548069	100%	White growing edge, orange mycelia, restricted growth	939b
Aspergillus versicolor/sylvaticus AF548069/8/7	99%	Fast growing, white growing edge, green sporulation structures, orange mycelia	Museum air
Aureobasidium pullulans DQ471004.1	98%	Yeast-like, glossy, beige submerged colony with brown patches	Museum dust
Chrysonilia sitophila	Morph[a]	Orange mycelia, flocculate, very fast spreading	Museum air
Davidiella tassiana DQ678022	99%	Dark brown colonies, velvet surface	Museum dust
or *Cladosporium cladosporioides* AF548071	98%		and air
Davidiella tassiana DQ678022	98% LSU[b]	Dark brown colonies, velvet surface, restricted growth (Fig. 1F)	938
Lecythophora mutabilis AJ496247	90%	Orange, slimy, hyaline mycelia	Museum dust
Penicillium sp.	Morph[a]	White mycelia, dark-green sporulation structures	Museum dust
Penicillium sp.	Morph[a]	Brown-beige, green sporulation structures	Museum dust
Penicillium sp.	Morph[a]	White mycelia, dark-green sporulation structures,	Museum dust
Penicillium sp. NS051-06 DQ810190	100%	Dark-green, smooth surface, abundant sporulation structures	Museum dust
Penicillium brevicompactum AF548085	99%	Dark-green, abundant aerial mycelia with spores	Museum dust
Several *Penicillium* sp., e.g. *P. italicum* AF548091	100%	White mycelia, fluffy, with bluish-green sporulation structures	Museum dust and air
Penicillium namyslowskii D88319	93%	Dark green, concentric sporulation zones	Museum air
Phoma sp. AB252869	98%	Greyish-white, fluffy mycelia	Museum dust
Trichoderma viride AF548104	95–99%	White mycelia, flocculate	Museum dust and air

a. Morphological identification.
b. Partial LSU rDNA. Shaded rows represent isolates unique to aeolian dust.

broad spectrum of fungi, the historic samples only contained two species – *A. versicolor* and *D. tassiana* (Fig. 2B, Table 4) which grew much slower than strains isolated from museum air/dust.

(v) All these differences are listed in Table 5 where it is apparent that the microbiological properties of the two sets of probes are so different that one cannot have contaminated the other.

Geochemical characterization, transport-connected fractionation and possible origins of historic dust

Winds transport huge amounts of desert material though the Sahara-Sahel dust corridor over the Atlantic Ocean and often onto the Americas (Moreno *et al.*, 2006). During these long flights, fractionation of the air-borne minerals occurs in which the heavier particles precipitate

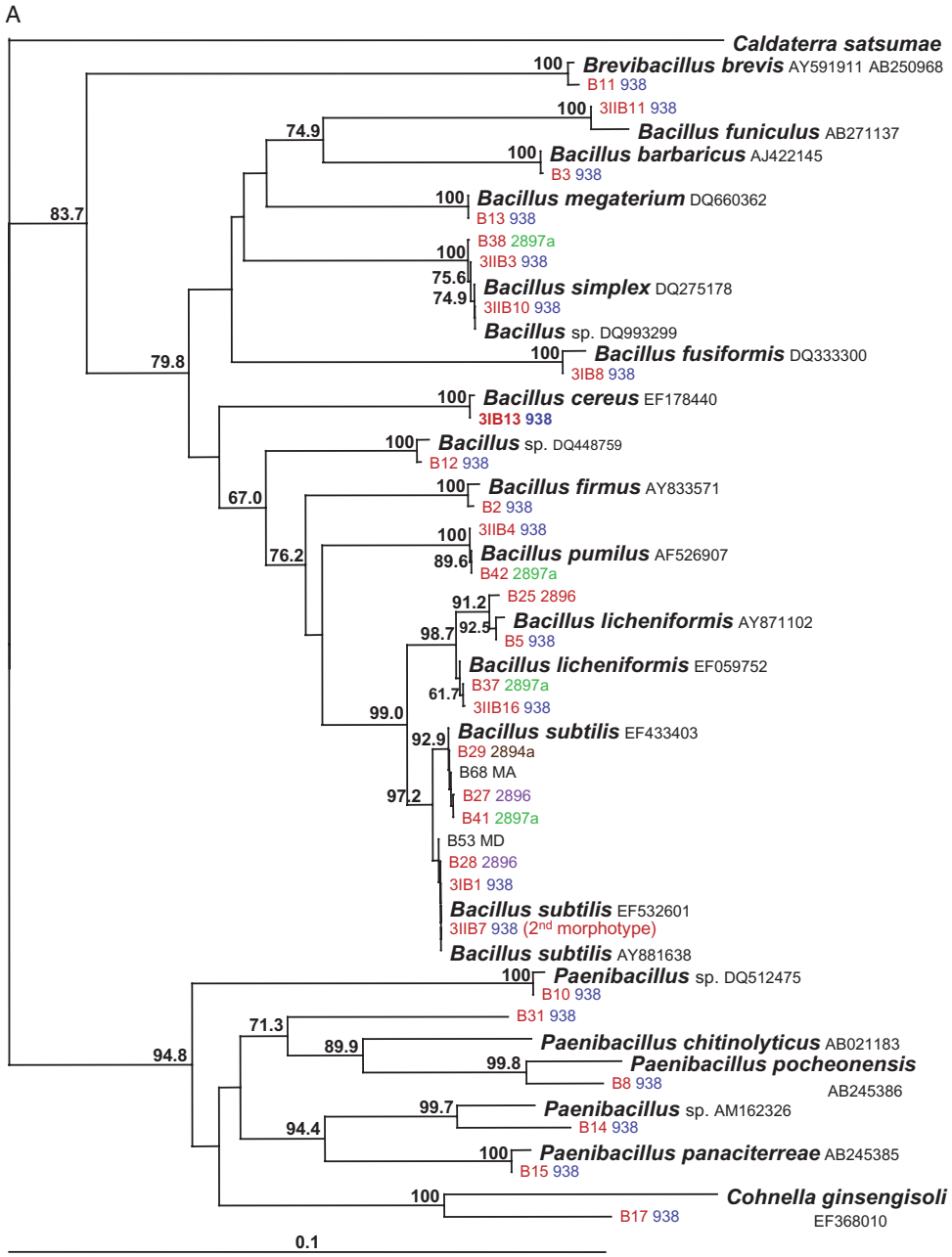

Fig. 2. Phylogenetic relationships of dust isolates presented as neighbour-joining bootstrap trees with Kimura correction.
A. Bacteria, based on 16S rDNA sequences (~750 bp) using *Caldaterra* as the out-group.
B. Fungi, based on 18S rDNA (~700 bp).
Each taxonomic name is followed by its GenBank accession number. The scale indicates the average number of substitutions per position.
MA, museum air; MD, museum dust.

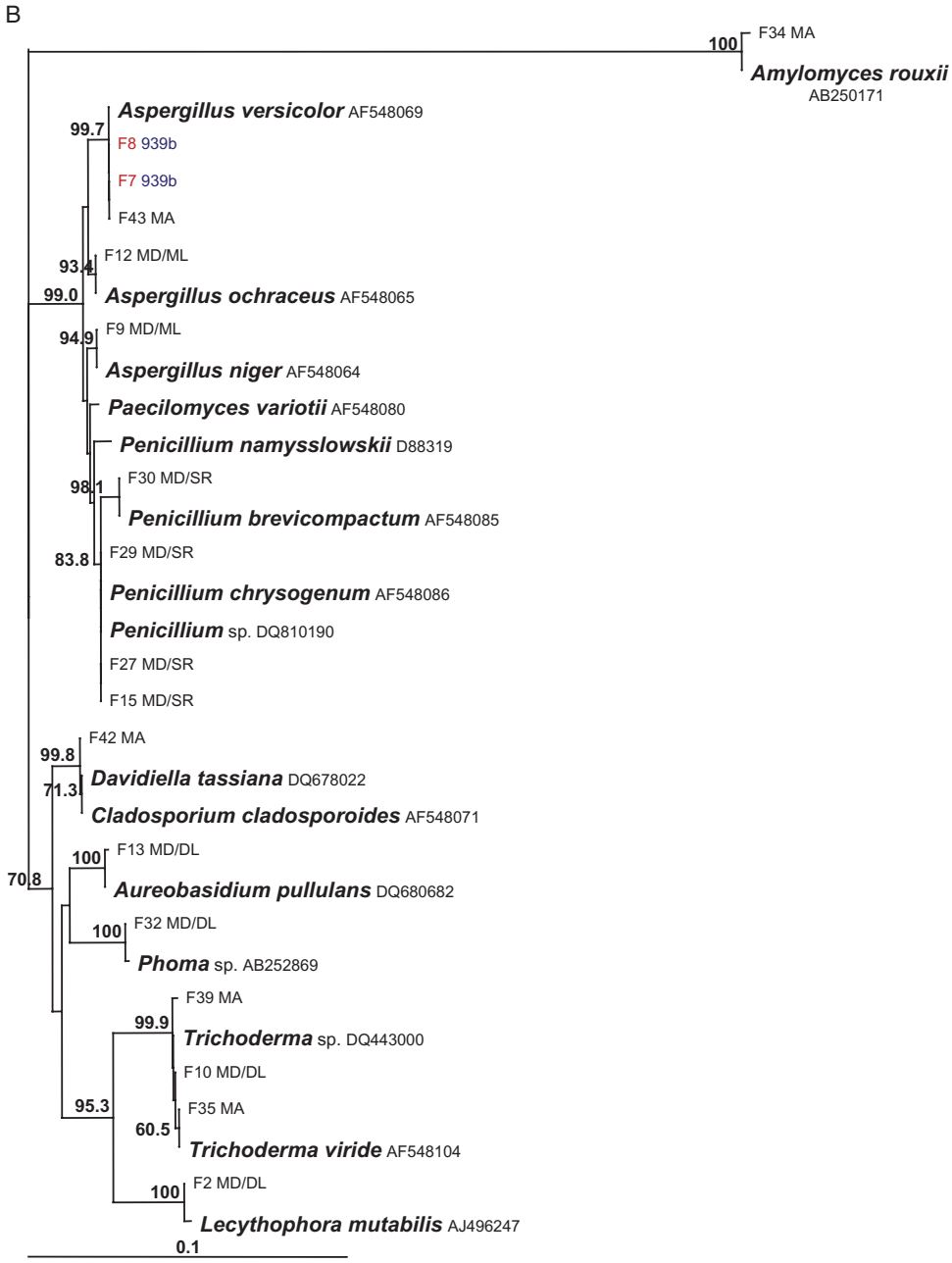

Fig. 2. *cont.*

Table 5. Microbiological differences between historic dust and that of the Museum für Naturkunde in Berlin.

Characteristic	Historic dust	Museum air/dust
Microbial density	~10^4 cfu g^{-1}	~10^5 cfu g^{-1}
Bacteria	Twenty species, all spore-forming	*B. subtilis* plus seven species, all non-spore-forming
Common bacterium	*Bacillus subtilis*	*B. subtilis*
Fungi	Two species	Seventeen species
Common fungus	*Davidiella tassiana*	*D. tassiana*

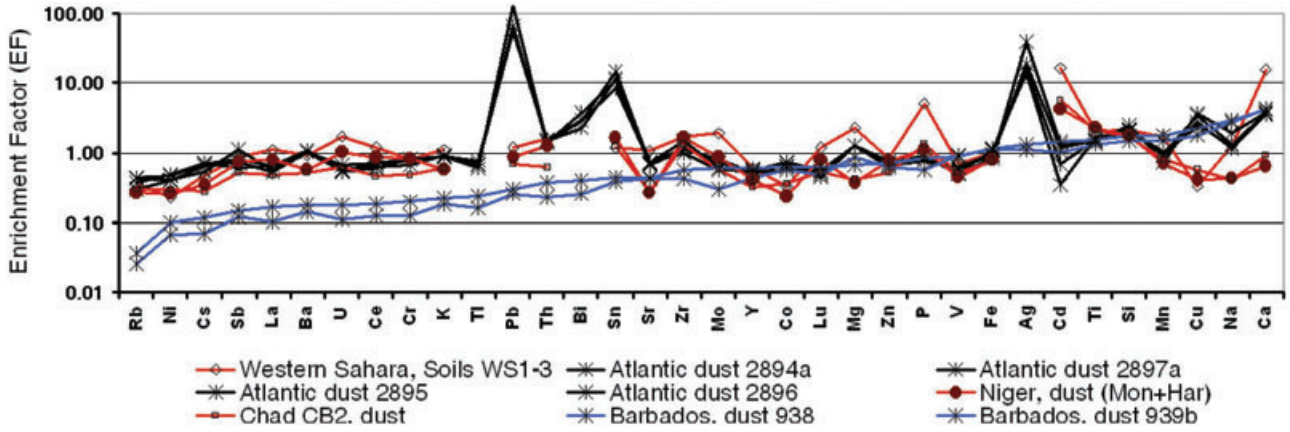

Fig. 3. Elemental compositions of historic versus modern aeolian dust along with those of soils from possible source areas (Moreno *et al*., 2006). Similar enrichment factors are shown for the four samples collected over the Atlantic in 1838. The Barbados samples from 1812 also plot close together and show a distinct fractionation of minerals caused by the long-range transport across the Atlantic Ocean.

first. Vertical fractionation also takes place (Husar, 2004), a process that is dependent on the water content of the atmosphere. In other words, the dust becomes more homogeneous the further and higher it travels. Fractionation is reflected in its chemical and mineral composition. Mineralogical comparisons of historical dust with samples taken recently as well as with soils from possible source areas were made. The four samples collected over the Atlantic Ocean in 1838 have comparable enrichment factors (EF) for a large number of elements (close to 1) indicating that little fractionation from average shale (Fig. 3) has occurred. Notable exceptions exist however. Although enrichment of Si is only moderate, SiO_2 is the major component of all the dust samples examined. Enrichment of quartz relative to source materials is the most important indicator of aeolian processes (e.g. in the genesis of loess – Schnetger, 1992). As the soils of the Western Sahara contain large amounts of Cretaceous carbonates (WS1-3, Fig. 3), they are the most likely source of the dust collected over the Atlantic. Other possible sources including the Hoggar Mountains (Algeria – sandstone) and the central Chad Basin (Cameroon, Chad, Niger, Nigeria – sand, silt, clay) do not contain significant amounts of carbonates (Moreno *et al*., 2006). As the samples collected in the 20th century do not contain high concentrations of Ag, Bi, Cu, Pb and Sn, it seems unlikely that soils in the source areas were rich in these elements. Several possible sources of Ag, Bi, Cu, Pb and Sn exist. Food on ships was preserved in tins that were sealed with solder, which probably contained all these metals. Bronze, a Cu-Sn-alloy, was widely used onboard sailing ships in bells, cannons, fittings, etc. Graphite pencils were invented towards the end of the 17th century. Flakes of lead from a pencilled note on the packet containing the dust could easily have contributed this element. Undoubtedly, one or more of these sources explains the presence of these elements in 19th century dust.

In contrast, the Barbados samples, which also have similar EFs, show a distinct fractionation of minerals due to the long-range transport across the Atlantic Ocean. Relatively, these samples were depleted in elements related to coarse grained K-feldspar (Ba, Cs, K, Rb, Tl) as well as to heavy minerals like cassiterite (Sn), chromite (Cr), monazite (Ce, La, U) and zircon (Zr). As expected of samples collected over land, neither of the Barbados samples was enriched in Ag, Bi, Pb or Sn, lending support to our suggestion that these elements found their way into the 1838 samples onboard 'The Spey'.

Survival of a Gram-positive and a Gram-negative bacterium on dry sand

Both cultivation and SEM suggested that *Bacillus megaterium*, a common soil bacterium, is attached to historic sand grains (Fig. 4B). As an experimental means of testing the long-term viability of microorganisms on inert substrates, a *B. megaterium* isolate from historic dust was inoculated onto dry sand, and its survival studied at regular intervals using both cultivation and microscopic methods (Fig. 4). In less than 1 week, all the vegetative cells of *B. megaterium* had developed spores (cf. Fig. 4A and D), which clearly cling to the grains of sand (Fig. 4C). As far as can be judged from this 10-week experiment, once formed the spores remained viable (Fig. 4D).

Obviously, controlled experiments in which soils of the Western Sahara are inoculated with genetically marked bacteria and their dispersal across the Atlantic Ocean followed are impossible to perform. As an alternative, we sought a common soil bacterium that is unable to form spores, and can therefore only survive in the vegetative

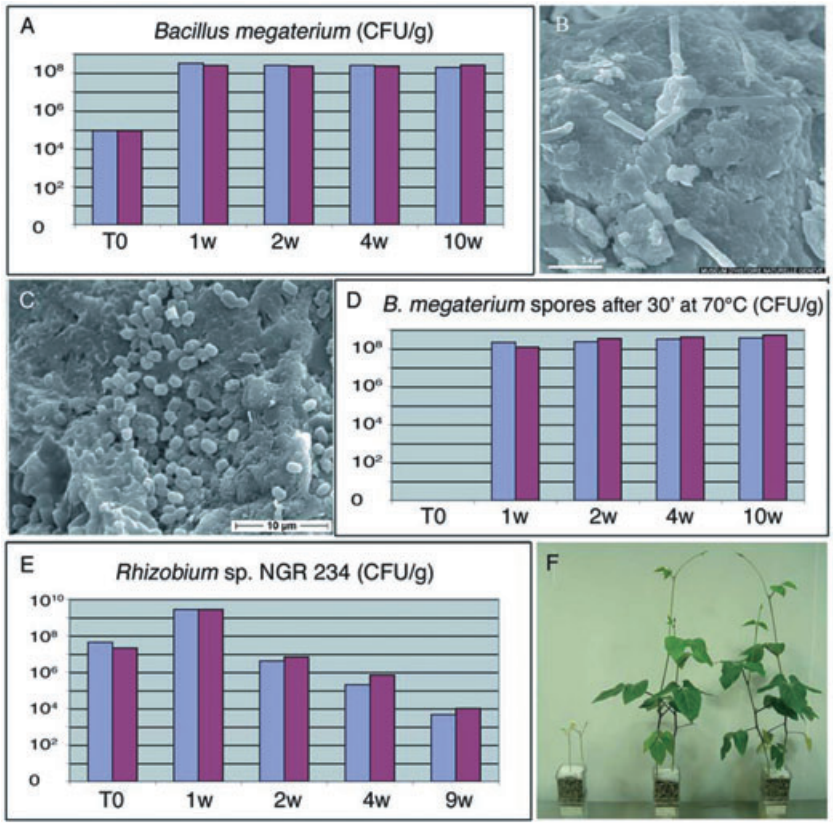

Fig. 4. Survival of *Bacillus megaterium* B13 and *Rhizobium* sp. NGR234 on dry sand.
A, D and E. Vertical axes – number of bacteria in cfu g^{-1} sand; horizontal axes – time in weeks (w). Blue and burgundy colours represent two separate replicates.
A. Numbers of *B. megaterium* as a function of time.
B. *B. megaterium*-like cells on the surfaces of a historic sand grain (vial # 2987a).
C and D. After 1 week of desiccation, spores dominate on dry sand (C – SEM, and D – numbers after heating to 70°C and plating out).
E and F. Non-spore-forming Gram-negative bacteria (here *Rhizobium* sp. NGR234) survive extended periods of desiccation without loosing their capacity to form functional nitrogen-fixing symbioses with legumes.
E. Numbers of rhizobia on the sand grains.
F. *Vigna unguiculata* inoculated with wild-type NGR234 (centre), and NGR234 taken from sand grains held for 9 weeks at 20% relative humidity (pot at left – non-inoculated control).

state. Gram-negative rhizobia (Rhizobiales) are ubiquitous symbionts of legumes including the *Acacia* species that dot the Sahara/Sahel. *Rhizobium* sp. NGR234 is known not only for its ability to nodulate many legumes, including *Acacia* spp. (Pueppke and Broughton, 1999), but also for an inherently unstable genome (Flores *et al*., 2000), stocked with insertion and mosaic sequences (Freiberg *et al*., 1997) as well as complex, repeated elements (Perret *et al*., 1997). It was selected as a negative control for these reasons, but especially because a genetically unstable bacterium would not be expected to survive long periods of desiccation. In a similar experiment to that performed with *B. megaterium*, the numbers of NGR234 steadily declined with time, but the dry sand still contained almost 10^4 viable cells g^{-1} after 9 weeks of desiccation (Fig. 4E). Even more surprisingly, given the instability of NGR234 mentioned above, these bacteria maintained perfect symbiotic competence when cultivated in sterile pots containing all essential elements except nitrogen (Fig. 4F).

Discussion

Ehrenberg himself wrote 'Probably even in 100 years research will find interest in carefully collected (dust) material be it on behalf of meteorology or of the study of organic life within' (Ehrenberg, 1851). Although the exact beginnings of the industrial revolution are hard to pinpoint, in 1812 they were mostly confined to the UK, and the textile industry. Thus, dust that was blown from the Western Sahara to Barbados early in the 19th century was unlikely to be affected by manmade pollution. As we were able to positively identify most of the isolates and align them with modern-day species, the types of bacteria and fungi cannot have appreciably changed over the centuries. Rather, any differences that exist must represent subtle changes in genomes but unfortunately, there is no simple way to ascertain whether and how much the modern day microbial variants have evolved.

Other aspects of this intercontinental transport of dust and accompanying microorganisms have changed little too. Our geochemical data show that the most likely source of the samples collected over the Atlantic Ocean and Barbados is the Western Sahara. Then as now, long-range aeolian transport strongly fractionated the dust, resulting in depletion of coarse grains and heavy minerals. Just as dust is depleted in certain elements and large grains during long-range transport, adherent microbial populations are also 'fractionated' both because of varying sensitivities to travel and due to the attachment abilities of individual microbes.

The largest, single source of dust on the planet is the Bodélé Depression in Northern Chad (Giles, 2005; Engelstaedter *et al*., 2006; Todd *et al*., 2007). There, a gap between two mountain ranges funnels winds onto the chalky white diatomaceous desert. Unlike other sources of dust, the Bodélé is active all the year, and the diatomaceous dust clouds can stretch for thousands of kilometres. Such a source fits well with our findings of strong enrichment in calcium.

Like Sneath (1962) who studied the microbiology of soil attached to roots of ancient herbarium species and Nicholson's group who isolated different bacilli including *B. subtilis* from granite (see Nicholson *et al*., 2000; Fajardo-Cavazos and Nicholson, 2006), our analyses of authentic samples, gathered by pioneers of modern biology, prove beyond doubt that members of the *Bacillales* can live for centuries. Assuming a distance of about 7500 km from the Bodélé to Miami and moderate winds of 30 km h^{-1}, the travelling time from Africa to the Americas would be ~10.5 days. Intercontinental-scale transport of dust mostly occurs in the free troposphere (2–10 km elevation) however, where the winds are much stronger (Husar, 2004), and the journey is quicker. Rhizobia and similar microorganisms present in the topsoil of the Sahara/Sahel could thus probably be transported to, and survive in the Americas.

Prospero and colleagues (2005) wrote 'There is, however, only anecdotal indirect evidence for the long-range transport of viable microorganisms on intercontinental scales'. By trapping air over Barbados during a dust storm, by isolating fungi from it and by correlating African dust plumes with the appearance of microbes in Barbados, these authors contributed several pieces to the puzzle of intercontinental transport of microbes. Like Lyell 169 years ago, Griffin and colleagues (2006) sampled a probable African dust storm onboard a ship anchored over the mid-Atlantic Ridge. Using polymerase chain reaction (PCR)-based methods, they were able to identify both fungi and bacteria, adding the latter to the puzzle. Only by combining geochemical, microbiological, microscopic, modelling and molecular methods to analyse almost 200-year-old samples, were we able to show beyond doubt that dust, which clearly originated from West Africa, transported viable microorganisms across the Atlantic Ocean, at least as far as the Caribbean. Obviously, part of this longevity is due to the microbes' ability, under adverse conditions, to quickly form spores, but we suggest that fine inorganic dust also aids survival. Much of the historic calcareous dust is porous (Fig. 1E) and/or possesses convoluted surfaces that provide a refuge for microorganisms, especially from desiccation and UV radiation. Once established within the pores or between the grains, bacteria and fungi have probably always hitch-hiked their way across oceans.

Satellite imaging has shown the global extent of these storms in real time (Washington *et al*., 2003). As the amounts of fine particles carried are enormous (e.g. Free, 1911; Shinn *et al*., 2000), this means that those regions where dust lands are both extensively fertilized with minerals and inoculated with desert microorganisms. In other words, dust has probably always played a role in global microbial ecology. Given this constancy of transport, induced changes in the seeding areas (Shinn *et al*., 2000; Gardner *et al*., 2003; Weir-Brush *et al*., 2004) must reflect differences in the types of microbes carried (Brown and Hovmoller, 2002). As much of the dust carried across the Atlantic Ocean comes from the Bodélé depression in Northern Chad (Goudie and Middleton, 2001; Giles, 2005), we will examine whether soils from this area contain known pathogens.

Experimental procedures

Historic dust

Posterity demands that the Ehrenberg collection, which is both irreplaceable and of extreme scientific interest, should only be sampled when technological progress permits a vast increase in understanding of its contents. We felt that this was the case and small samples were taken aseptically (in April 2006) and subdivided for: (i) light as well as fluorescence microscopy, (ii) scanning SEM and (iii) microbial characterization. In addition, the air in the museum rooms that housed the collection was sampled on two occasions (in April and December 2006) using a Merck MAS-100 air-sampler (Darmstadt, Germany). On the same occasions, a sterile brush was used to collect dust that had settled on shelves and storage cabinets.

Microbiological techniques

Subsamples (< 50 mg) of historic dust were aseptically weighed and suspended in physiological saline containing 0.001% (v/v) Tween 80. The tubes were shaken (1 h, 160 rev min^{-1}, 26°C), kept overnight at 4°C, and the next day shaken for 2 h. Portions were streaked out on 10 different media [CzD, DG18, DRBC, ECA, Maxted, MEA, Plate Count Agar (PCA), PYGV, R2A and Tryptone Soya Agar (TSA)]. Afterwards, the suspensions were incubated at 70°C for 30 min; then portions were plated on R2A and TSA. Different fungal types were studied morphologically and further identified by partial sequencing of the nuclear rRNA genes. Polymerase chain reaction primer pairs: (i) nSSU97b (Kauf and Lutzoni, 2002) + NS22 (Gargas and Taylor, 1992) or (ii) LR0R (5′-ACCCGCTGAACTTAAGC-3′) (R. Vilgalys, http://botany.duke.edu/fungi/mycolab) + IR5 (Vilgalys and Hester, 1990) were used. Universal prokaryote primers (27F-1385R) were used to amplify almost the entire 16S rDNA gene fragment (*c*. 1400 bp) and sequenced by Fasteris SA, 1228 Plan-les-Ouates, Switzerland (info@fasteris.com). Sequences were blasted against the EMBL and GenBank accessions (NCBI BLAST: http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Microscopy

Fungal isolates were classified according to typical colonial and conidial morphologies using a stereomicroscope (Samson *et al*., 1996). Samples for SEM (Hitachi S-2300 N, Tokyo, Japan) were air-dried, and coated with platinum (Gorbushina *et al*., 2004).

Geochemistry

Triplicate acid digestions of 25 mg dust were analysed for Al, Ba, Ca, Cr, Fe, K, Mn, Na, P, Sr, Ti, Y, Zn and Zr by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). Sc was used as an internal standard for all elements except K and Na. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) was used to analyse the trace elements Ag, Bi, Cd, Ce, Co, Cu, Cs, La, Li, Lu, Mo, Ni, Pb, Rb, Sb, Sn, Tl, U, V, Y using In as an internal standard (Schnetger, 1997). Cu, Ni and V were measured at medium resolution. All other isotopes were analysed at low resolution which necessitated correcting ^{107}Ag (^{91}Zr), ^{109}Ag (^{93}Nb), ^{111}Cd (^{95}Mo) and ^{114}Cd (^{98}Mo) for oxide interference (interfering isotopes in brackets). Oxide formation was calculated from blanks containing only the interfering isotope. Due to the cooled double-spray chamber used, oxide formation for Mo, Nb and Zr was typically low (0.3–0.8%). Analytical precision was better than 3% relative standard deviation for all elements and both methods. Accuracy was $\leq$ 7% for Cd, Cu, K, Na, Ni, P, Pb, U, V, $\leq$ 11% for Ag and Sn and $\leq$ 5% for all other elements. Owing to the low amount of material available, SiO_2 was calculated as the difference of all other constituents to 100%. An EF, calculated as the ratio of the element to Al in the sample divided by the ratio of the element to Al in shale, was used to cancel-out dilution effects caused by, for example, carbonate and quartz (Brumsack, 2006). Average shale was used as the standard as it is a well-proven, terrestrial mineral with high carbon content. An EF > 1 indicates relative enrichment of the element, whereas samples in which the element has been depleted have an EF < 1.

Bacterial survival on sand

To test whether *B. megaterium* (and as a control *Rhizobium* sp. NGR234) are capable of surviving extended periods of desiccation, 1 g of quartz sand was weighed into 3 cm diameter watch glasses, and autoclaved. Then, the watch glasses containing sand were individually transferred to multiwell plates, inoculated with or without 10^9 cfu ml^{-1} bacteria. After 1, 2, 4 and 10 weeks of incubation (at 26°C, first week at 100% relative humidity, thereafter at 20% relative humidity), two replicate watch glasses representing three different treatments were removed, and divided into two portions – one to count the numbers of viable cells (and spores), the other for observation under both the scanning electron and fluorescence microscope. Similar experiments were performed with *Rhizobium* sp. NGR234. Symbiotic competence was tested by inoculating *Vigna unguiculata* (Pueppke and Broughton, 1999).

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