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Bacterial Diversity of Weathered Terrestrial Icelandic Volcanic Glasses

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Short title: bacterial diversity in volcanic rocks

Abstract

The diversity of microbial communities inhabiting two terrestrial volcanic glasses of contrasting mineralogy and age was characterised. Basaltic glass from a <0.8 Ma hyaloclastite deposit (Valafell) harbored a more diverse *Bacteria* community than the younger rhyolitic glass from ~150-300AD (Dómadalshraun lava flow). *Actinobacteria* dominated 16S rRNA gene clone libraries from both sites, however *Proteobacteria*, *Acidobacteria* and *Cyanobacteria* were also numerically abundant in each. A significant proportion (15-34%) of the sequenced clones displayed <85% sequence similarities with current database sequences, thus suggesting the presence of novel microbial diversity in each volcanic glass. The majority of clone sequences shared the greatest similarity to uncultured organisms, mainly from soil environments, among these clones from Antarctic environments and Hawaiian and Andean volcanic deposits. Additionally, a large number of clones within the *Cyanobacteria* and *Proteobacteria* were more similar to sequences from other lithic environments, included among these Icelandic clones from crystalline basalt and rhyolite, however no similarities to sequences reported from marine volcanic glasses were observed. PhyloChip analysis detected substantially greater numbers of phylotypes at both sites than the corresponding clone libraries, but nonetheless also identified the basaltic glass community as the richer, containing approximately 29% unique phylotypes compared to rhyolitic glass.

1   **Introduction**

2   During volcanic eruptions, lava that cools quickly, for example by coming into
3   contact with ice or water, quenches rapidly to form glass. Depending on the silica
4   content the material may form basaltic glass or in the case of silica-rich material,
5   rhyolitic glass or obsidian. In the ocean environment alone the rate of crustal
6   production may be well over a billion tonnes a year [2] and much of this is in the form
7   of basaltic glass. Volcanic glasses are also common in terrestrial volcanic
8   environments [28].

9   Investigating the alteration or weathering of volcanic glasses has importance
10   for a number of key areas in Earth and planetary sciences including: 1) The chemical
11   weathering of rocks is well established [19, 37, 45], but less is known about the
12   biological contribution. As rock weathering contributes to the carbonate-silicate cycle
13   and long-term climate by consuming CO₂ in rock weathering reactions [7, 16, 17], it
14   is important to elucidate the diversity of organisms that might take part in these
15   reactions. An important first step is to understand the microbial diversity of volcanic
16   materials in relation to their geochemistry; 2) The weathering of volcanic rocks,
17   particularly glasses, contributes nutrients into the biosphere, providing highly fertile
18   agricultural soil and playing an important role in soil formation at the so-called
19   ‘critical zone’ [14]. Investigating the diversity of volcanic glasses will contribute both
20   to understanding the diversity of organisms that can be sustained by weathered
21   volcanic glass and the microbial taxa that might take part in weathering the material
22   during soil neogenesis.

23   The most intensive efforts to understand the microbial diversity of volcanic
24   glasses have focused on oceanic basaltic glass. Templeton et al. [50] showed the
25   presence of Mn-oxidising bacteria in basalts from Loihi Seamount, although

1 autotrophic Mn-oxidisers were not recovered, suggesting Mn oxidation in
2 heterotrophs is a secondary process [49]. Neutrophilic Fe-oxidizing bacteria were
3 isolated from oceanic basaltic glass [18]. In the study of the microbial community of
4 the basaltic glasses of the Knipovich Ridge, Arctic, Thorseth et al. [52] identified
5 heterotrophs and some chemolithotrophs including phylotypes belonging to the ϵ -
6 *Proteobacteria* and closely matching with sulphur-oxidisers. Stalk-like deposits
7 similar to those produced by *Gallionella* spp. were observed, but *Gallionella* spp.
8 were not identified by molecular methods. Iron-reducing organisms were cultured
9 from Arctic Ridge seafloor basaltic glasses by Lysnes et al. [32], suggesting the
10 possibility of an iron-cycle within seafloor basalts. They determined the presence of a
11 diversity of other organisms belonging to the *Proteobacteria*, *Chloroflexi*, *Firmicutes*,
12 *Actinobacteria* and Crenarchaeota of unknown physiology. Phylogenetic analysis of
13 the microbial inhabitants of seafloor basaltic glasses around Hawaii show high
14 diversity [38, 40] which is thought to be linked to the chemolithotrophic use of
15 basaltic glass alteration products [40].

16   Studies of the microbial communities associated with terrestrial volcanic
17 glasses are in comparison to marine studies relatively scarce. In a study of Icelandic
18 basaltic glass, Cockell et al. [10, 11] showed that most of the organisms corresponded
19 to heterotrophic taxa and there were no sequences obtained from chemolithotrophs,
20 suggesting that they are very rare, if they are present. The dominant phyla were
21 *Actinobacteria*, *Proteobacteria* and *Bacteroidetes*. They attributed these results to the
22 very different environmental conditions in terrestrial basaltic glasses compared to the
23 deep ocean, with the weathered terrestrial basaltic glasses being more similar to soils.
24 Organisms were pervasive in the basaltic glass and microbial abundance was on the
25 order of $\sim 10^7$ organisms/g.

1   These results contrast to results by Herrera et al. [24, 25] who studied
2 Icelandic obsidian. Using FISH they showed that the organisms were highly localised
3 and were predominantly associated with phenocrysts of iron and pyroxenes. Microbial
4 enumerations were difficult on account of the localised communities, but
5 *Actinobacteria*, *Acidobacteria* and *Proteobacteria* were prevalent. They attributed this
6 localization to the high silica content of the rock and the likelihood that bioessential
7 cations were difficult for the biota to access.

8   In the present study our motivation was to further our understanding of the
9 composition of microbial communities within terrestrial volcanic rocks. We use 16S
10 rRNA gene clone libraries, DGGE and microarray analyses to characterise the
11 diversity and richness of *Bacteria* inhabiting terrestrial Icelandic obsidian and basaltic
12 glasses.

13 14 15 **Methods**

16 **Field site and sampling**

17 Sampling locations (Figure 1A) and methods have been described previously [24].
18 Briefly, obsidian samples were collected at obsidian outcrops formed during the
19 ~150-300 A.D formation of the Dómadalshraun lava flow (64°2.01'N, 19°7.75'W).
20 Naturally weathered obsidian samples were taken from a ~1 m³ block of rock using a
21 rock hammer (Figure 1B). Weathered hyaloclastite/basaltic glass produced during a
22 subglacial volcanic eruption in the Pleistocene [13] was collected some 20.8 km
23 distant, at Valafell (location 64°4.83'N, 19°32.53' W), near Mt. Hekla in southern
24 Iceland. Basaltic glass weathers to the clay-like material palagonite [46], which
25 imparts a brown colouration to the otherwise black basaltic glass. The material

1 examined here is therefore a mixture of unaltered basaltic glass and palagonite with its
2 associated organisms and is hereinafter referred to as basaltic glass/palagonite.
3 Samples were removed from blocks that lay on the ground (Figure 1C).

4 5 **Electron microprobe analysis (EMPA)**

6 The major elemental composition of the rocks was measured by electron microprobe
7 analysis on carbon-coated thin sections (obsidian) or carbon-coated resin-blocks
8 (palagonite) using a Cameca SX100 electron microprobe (Department of Earth
9 Sciences, Open University, Milton Keynes, UK), as previously described [11, 25].

10 11 **Porosity and surface area measurements**

12 Obsidian and basaltic glass/plagonite surface area was determined by BET (Brunauer-
13 Emmett-Teller) analysis (five point) (Imperial College, London, UK) [6] using ~3
14 mm³ fragments of material. Porosity was determined by mercury intrusion
15 porosimetry (MCA Services, Meldreth, Cambridge, UK). For both BET and mercury
16 intrusion porosimetry, fragments of rock pooled from six different samples were
17 examined.

18 19 **Extraction of total DNA from rock samples**

20 DNA was extracted from four (obsidian) or five (basaltic glass/palagonite) samples of
21 each glass type. For each sample 10 g of rock was crushed to a powder in a metal
22 cyclinder [23]. The total DNA was directly extracted from the crushed rock using the
23 PowerMax Soil DNA Isolation Kit (MoBio Laboratories, Cambridge, UK) according
24 to the manufacturer's instructions.

Denaturing gradient gel electrophoresis

Microbial communities in individual samples, in addition to a composite sample for each glass type (see below), was analysed by denaturing gradient gel electrophoresis (DGGE) analysis of the 16S rRNA genes using a nested PCR protocol. The first round of amplification was performed with primers pA [5] and com2 [43], targeting the V1-V9 hypervariable regions, while the second round of amplification was performed with the GC-clamped forward primer 338 [34] in combination with com2, targeting V3-V9. Amplification of the pA-com2 region was performed in 50 µl reactions with 0.2 µM each primer, 200 µM each dNTP (New England Biolabs), 2.5 U *Taq* DNA polymerase, 1.5 mM MgCl₂, 1X PCR buffer (200mM Tris-HCl (pH 8.4), 500 mM KCl) (Invitrogen Corporation, Paisley, UK), and 5 ng of template DNA. One microlitre of unpurified product was used as template in the second round of amplification with 0.4 µM each primer, 160 µM each dNTP, 2.5 mM MgCl₂, 2 U *Taq* and 1X PCR buffer (as described above). For each primer set, 25 amplification cycles were performed in a G-Storm GS1 thermal cycler (GRI Ltd, Essex, UK) as follows; 94°C for 1 min, annealing at 55°C and extension at 72°C, each for 40 s (pA-com2) or 30 s (338GC-com2). Initial denaturations and final extensions (at 94°C and 72°C respectively) were performed for 5 min. Each sample was amplified in duplicate with 338GC-com2 and products combined and purified (Illustra^{TM} GFX^{TM}, GE Healthcare, Buckinghamshire, UK). Three-hundred and fifty nanograms of each purified PCR product was resolved on a 6% polyacrylamide gel containing a 30-70% denaturant gradient, where 100% is defined as 7 M urea and 40% (v/v) formamide. The composite basaltic glass/palagonite product was included in multiple lanes as a reference. Gels were run using a D-Code Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA) at 75 V for 18 h in 1X TAE at 60°C. Bands were visualised

1 with SYBR® Green I (Invitrogen Corporation, Paisley, UK) and fingerprints analysed
2 with GelComparII® (Applied Maths, Sint-Martens-Latem, Belgium) based on
3 presence-absence of bands in each sample. A UPGMA dendrogram was constructed
4 using the Jaccard binary coefficient and 1000 bootstrap replicates. The DGGE
5 presence-absence matrix was used to generate a triangular similarity matrix based on
6 the Bray-Curtis index [31] using PRIMER5 software [9]. The analysis of similarity
7 (ANOSIM) routine was used to examine the statistical significance of differences
8 between the DGGE profiles (significance data reported as p-values). Spearman
9 correlation tests were performed to test for a correlation between bacterial community
10 structure and mineralogy.

11

12 **PCR amplification, cloning and sequencing of bacterial 16S rRNA genes**

13 For both obsidian and basaltic glass/palagonite, equal amounts of DNA from four
14 individual samples were pooled to yield composite samples (samples Pal1, Pal2, Pal3
15 and Pal5 for basaltic glass/palagonite, and all four obsidian samples). These
16 composites were used to construct 16S rRNA gene clone libraries with primers pA
17 and com2. Conditions were similar to those described above, exceptions being an
18 increase in MgCl₂ (2 mM), cycle number (thirty), and an increase in the final
19 extension time to 10 min. Products were resolved on 1% agarose, gel purified
20 (Illustra™ GFX™, GE Healthcare, Buckinghamshire, UK) and cloned into pCR®4-
21 TOPO® vector before transforming into chemically-competent One Shot® TOP10
22 *Escherichia coli* (Invitrogen Corporation, Paisley, UK). Clones were selected from
23 plates at random and vector inserts were sequenced with primer pA (MCLAB,
24 California, US). Putative chimeras were identified in the clone libraries by submitting
25 sequences to the CHECK CHIMERA program of the RDP and to the Bellerophon

1 server (Huber et al., 2004). Phylogenetic searches were performed with SeqMatch
2 (RDP) and the Basic Local Alignment Search Tool (BLAST) [1]. Diversity and
3 richness estimators were computed with DOTUR [41]. Coverage estimates were
4 calculated manually [8, 21]. Clone libraries were compared at species (97%) and
5 genus (95%) sequence similarity levels using the Libshuff [44] function available in
6 MOTHUR [42]. Neighbor-Joining phylogenetic trees were constructed in MEGA4
7 [47] using the Jukes-Cantor [29] nucleotide substitution model and 1000 bootstrap
8 replicates.

9

10 **GenBank accession numbers**

11 16S rRNA gene clone sequences have been deposited in GenBank under accession
12 numbers GU219531 to GU219829.

13

14 **PhyloChip analysis**

15 The bacterial community present in obsidian and basalt glass/palagonite was
16 investigated by PhyloChip analysis of the composite samples. The 16S rRNA genes
17 (V1-V9 regions) were amplified using an 8-temperature gradient PCR and universal
18 primers 27f and 1492r [30]. Amplification was performed at each temperature, using
19 3 µl of template, 300 nM each primer, 1X Ex Taq buffer, 2 mM MgCl₂, 200 µM each
20 dNTP, 25 µg bovine serum albumin (Roche Applied Science, Indianapolis, IN, USA)
21 and 0.625 U Ex Taq (TaKaRa Bio, Fisher Scientific, Pittsburg, PA, USA). The
22 following thermocycling conditions were used in an iCycler (Bio-Rad, Hercules, CA,
23 USA): 95°C for 3 min, thirty cycles of 95°C for 30 s, 48-58°C for 30 s and 72°C for 2
24 min, with a final extension for 10 min at 72°C. Products from each annealing
25 temperature were combined and concentrated to 40 µl or less (Microcon YM-100

filters, Millipore, Billerica, MA) before DNA quantification on a 2% agarose E-gel using the Low Range Quantitative DNA Ladder (Invitrogen Carlsbad, CA, USA). PhyloChip (second-generation, G2) details are presented elsewhere [3, 4, 15]. Five hundred nanograms of purified PCR product was spiked with a mix of amplicons of known concentrations, fragmented with DNase I (Invitrogen, Carlsbad, CA, USA), and biotin-labelled according to the manufacturer's instructions for Affymetrix Prokaryotic arrays. Labelled products were hybridized overnight at 48°C and 60 rpm. Arrays were washed, stained, and scanned as previously described. Probe selection, scoring, data acquisition and analysis have been described elsewhere [3, 4, 15]. Data obtained from the cel files (produced from the GeneChip Microarray Analysis Suite, version 5.1) were normalized using the method described by Ivanov et al. [26], and log₁₀ transformed to compensate for slight variations in probe responses on different chips. A positive fraction (pf) cutoff value of 0.9 was used to select OTUs for further investigation. This value means that 90% of the probe pairs in a probe set had to be scored positive in order for the probe set intensity value (a trimmed mean) to be used further in data analyses. A probe pair was scored positive when the perfect-match probe intensity minus the mis-match probe intensity was at least 130 times greater than the squared noise value and when the perfect-match intensity was 1.3 times the mis-match intensity.

Results

Mineral analysis

The quantitative mineral composition of the rock samples was determined by EMPA (Table 1). Results support the basaltic nature of samples from Valafell, with high levels of CaO and MgO, and basaltic SiO₂ and K₂O concentrations. The mean

concentrations of these minerals in samples from the Dómadalshraun lava flow indicate a rhyolitic nature, with high K₂O and SiO₂ (>72%), and low MgO and CaO.

Porosity and Surface Area

The porosity of the basaltic glass/palagonite and obsidian material was 25.8 and 11.1% respectively, while the surfaces areas were approximately 109 and <0.3 m²/g respectively.

Denaturing gradient gel electrophoresis (DGGE) comparison of bacterial communities

Three-hundred and fifty nanograms of purified PCR product was loaded for both composite samples and for each of the individual samples, to facilitate direct comparisons of bacterial community structure among glass types. Cluster analysis performed on the presence-absence of bands clearly separated the samples on the basis of mineralogy (Figure 2). Obsidian samples shared at least 58% similarity, while the basaltic glass/palagonite samples shared a minimum similarity of ~54%. Only one sample, Pal5 did not cluster with its counterparts. Analysis of similarity (ANOSIM) performed on the data also supported the separation of obsidian and basaltic glass/palagonite samples, where Pal5 groups most closely with other basaltic glass/palagonite samples (Supplemental Information Figure 1).

16S rRNA gene clone library analyses

One hundred and twenty-four and 175 chimera-free clones were sequenced from the obsidian and basaltic glass/palagonite 16S rRNA gene libraries respectively. One of the obsidian clones (deposited as GU219604) was determined to be of mitochondrial

1 origin. Rarefaction analysis at both 97% (species) and 95% (genus) sequence
2 similarity levels indicated that libraries were not sampled to saturation (Figure 2).
3 Coverage estimates for the obsidian library (0.66 and 0.73) were higher than for
4 basaltic glass/palagonite (0.48 and 0.66) at both levels of similarity. Diversity and
5 richness estimates for both libraries suggest a richer, more diverse community within
6 the basaltic glass/palagonite, with Chao1 richness estimates 147 and 111 in obsidian
7 compared to 216 and 131 in basaltic glass at 97% and 95% sequence similarities
8 respectively. The corresponding Shannon diversity indices were 3.93 and 3.80 for
9 obsidian and 4.28 and 4.08 for basaltic glass, derived using 120 sequences for each
10 library type aligned over 620 nucleotides. Non-parametric Shannon indices were also
11 calculated at 97% and 95% sequence identities. Values were higher than for Shannon
12 indices, but again indicated higher diversity at the Valafell site at both taxonomic
13 levels (not shown).

14   The distribution of clones within their respective phyla is represented in Figure
15 4. *Actinobacteria* dominated the basaltic glass/palagonite library (43% of clones
16 sequenced), followed by *Proteobacteria*, *Acidobacteria*, *Cyanobacteria* and
17 *Bacteroidetes*. The phylum *Actinobacteria* was also the largest phylum in the obsidian
18 library (25.6%), followed by *Cyanobacteria*, *Acidobacteria* and *Proteobacteria*. The
19 comparison of libraries by Libshuff analysis (10,000 randomizations) demonstrated
20 that they are significantly different ($P < 0.0001$). Libshuff analysis revealed a combined
21 116 OTUs at 97% sequence identities among libraries. Of these, 46 were unique to the
22 obsidian library, 57 unique to the basaltic glass/palagonite library, and the remaining
23 13 OTUs common to both. The phylogenetic relationship of these OTUs is
24 demonstrated in Figure 5, where each OTU is represented by one clone. All the phyla
25 identified in our libraries contained clones most similar to sequences from soil

environments. Indeed many of the phyla were dominated by sequences most similar to soil 16S rRNA gene clone libraries sequences deposited in public databases. The *Actinobacteria*, which are represented by two main clusters (Figure 5B) displayed the greatest sequence similarities to prairie, grassland, Antarctic, Hawaiian and Socompa Volcano soil sequences. Trembling aspen rhizosphere sequences also displayed great similarity to some of our *Actinobacteria* and to the *Gemmatimonadetes* and *Planctomycetes* clones in our libraries. Sequences from the Socompa Volcano study [12] and Hawaiian and Antarctic soils also feature among our *Acidobacteria*, *Bacteroidetes*, *Cyanobacteria* and *Proteobacteria* phyla. Sequences from prairie and polychlorinated biphenyl-contaminated soil also display great similarities to many of our *Acidobacteria*. A large proportion of clones within the *Cyanobacteria* and *Proteobacteria* phyla are most closely related to those retrieved from lithic environments elsewhere.

PhyloChip analysis and comparison with clone libraries

Bacterial communities from both glass types were compared using a high-density oligonucleotide microarray, the G2 PhyloChip. Thirty-six and 41 phyla were detected in obsidian and basaltic glass/palagonite respectively, representing 295 and 337 subfamilies within 1323 and 1710 OTUs. A comparison at the OTU-level revealed that the basaltic glass/palagonite contained a significantly higher proportion of unique phylotypes, more than twice that of the obsidian (Table 2). For a more direct comparison with PhyloChip results, clone library sequences were classified using the G2 chip taxonomy classifier provided through greengenes (http://greengenes.lbl.gov/cgi-bin/nph-classify_G2.cgi). In so doing, 85 and 60 OTUs were identified in basaltic glass/palagonite and obsidian libraries respectively (11

1 phyla in each). Within these OTUs 10.3% (obsidian) and 17.6% (basaltic
2 glass/palagonite) of subfamilies were unique to the clone libraries alone, being
3 undetected by microarray. Conversely, approximately 88% of subfamilies detected by
4 PhyloChip in each rock type were not detected by clone library analysis.

6 **Discussion**

7 Igneous volcanic glasses are a major contributor to the global CO₂ cycle through
8 silicate weathering reactions, and to understand the potential role of microbes in this
9 rock weathering process requires knowledge of the microbial diversity contained
10 therein. While the microbial communities inhabiting marine glasses have been
11 comparatively well documented, information regarding the terrestrial glasses is
12 scarce. In the present study we sought to build upon earlier work on terrestrial
13 crystalline basalt and rhyolite [23] and characterise the bacterial communities in their
14 glassy counterparts.

16 **Bacterial diversity in terrestrial Icelandic volcanic glasses**

17 Analyses of the bacterial communities inhabiting basaltic and
18 rhyolitic/obsidian glasses not only revealed substantial bacterial diversity and
19 abundant novel 16S rRNA gene sequences, but furthermore that the bacterial
20 communities within each glass type differed quite substantially. Obsidian was
21 characterised by a lower diversity and richness, as observed by 16S rRNA gene
22 microarray and clone library analyses. Kelly *et al.* (in review) also observed lower
23 diversity and richness in crystalline rhyolite compared to crystalline basalt, thus
24 suggesting a possible link between mineralogy and community structure. DGGE

1 analysis of bacterial communities also clustered samples on the basis of glass
2 mineralogy, with the exception of one basaltic glass/palagonite sample (Pal5).

3   While all analysis methods point to differences in bacterial communities
4 existing between rock types, variability exists between the methods used and their
5 resolving power. DGGE analysis cannot fully determine richness or indeed diversity
6 [27, 36]. As observed in the current study, the number of bands present, averaging 34
7 for basaltic glass and 30 for obsidian, are substantially below any richness estimates
8 derived by other analyses. PhyloChip analysis detected up to a 9-fold higher
9 abundance of taxa (obsidian) than the corresponding predicted clone library richness
10 estimate, contrary to results obtained elsewhere, where good agreement between the
11 methods was generally observed [54], although agreement between microarray and
12 clone libraries is not always been observed [39]. Potential reasons for this discrepancy
13 have been discussed by Kelly et al. (in review) and include differences in DNA
14 amplification protocols, the use of substantially larger quantities of DNA for
15 microarrays compared to clone library construction and the use of a shorter segment
16 of the 16S rRNA gene for clone library construction, all likely contributing to the
17 underestimation of bacterial diversity by the clone libraries, factors also relevant in
18 the study of Rastogi et al. [39]. PhyloChip analysis also has methodological
19 limitations which it is prudent to consider. Firstly, the choice of an appropriate cut-off
20 or pf value (positive fraction) for OTU detection. This has been examined elsewhere
21 and our choice of 0.90 as a cut-off has been rigorously tested [3, 15]. Kelly et al. (in
22 review) examined the effects of an increased pf cutoff on the abundance of different
23 phyla and found only minor differences. The second and perhaps most important
24 consideration is the reduced capacity of the PhyloChip to detect novel taxa, as
25 previously discussed [15, 54]. In the present study 15% of obsidian and 34% of

1 basaltic glass/palagonite 16S rRNA gene clone sequences displayed <85% similarity
2 to sequences currently deposited within the RDP database. Additionally, as many of
3 our clones display the greatest similarities to more recent uncultured database
4 sequences, is it likely that the PhyloChip too has severely underestimated the true
5 phylogenetic diversity within these glasses. This supposition is also supported by the
6 presence in the clone libraries of up to 17.6% subfamilies unique to clone libraries.

7   The above considerations aside, PhyloChip and 16S clone libraries largely
8 agree in the presence-absence of the major bacterial phyla (Table 2). In the present
9 study, phyla representing at least 1% of the detected OTUs by PhyloChip were
10 generally detected by clone libraries, as also observed by Kelly et al. (in review) in the
11 comparison of bacterial communities from crystalline volcanic rocks. Notable
12 exceptions are the γ - and ϵ -*Proteobacteria*, and the *Spirochaetes*, each of which were
13 undetected in libraries. As clone libraries were not sampled to saturation, failure to
14 detect low abundance (<3%) phyla may be explained in terms of incomplete
15 sampling, however the failure to detect γ -*Proteobacteria* constituting >10% of
16 microarray OTUs is more difficult to account for and may be related to the failure of
17 the clone library primers to amplify these sequences. Kelly et al. (in review) detected
18 γ -*Proteobacteria* in crystalline rocks by PhyloChip and 16S rRNA gene clone
19 libraries, but used a different primer set for constructing the libraries. We have
20 examined a subset of the database sequences corresponding to the γ -*Proteobacteria*
21 OTUs which were detected by the PhyloChip in the basaltic glass/palagonite sample
22 and found suitable binding sites for both pA and com2 primers, thus suggesting that a
23 bias against the γ -*Proteobacteria* occurred during the clone library PCR, or that either
24 probe mis-hybridization occurred or an inappropriate pf cut-off was chosen (or a
25 combination of these) during PhyloChip analysis.

1   In addition to the differences in diversity and richness, the obvious difference
2   in the relative percentages or indeed phyla ranking observed between microarray and
3   clone libraries is noteworthy. This observation has also been made previously by
4   Kelly et al. (in review) and by Rastogi et al. [39] in their comparison of contaminated
5   and un-contaminated soil. Although the glass clone libraries were revealed to be
6   significantly different by Libshuff analysis and varied in the abundance of each
7   phylum, microarrays indicated a very similar phylum distribution among glass types.
8   At both OTU- and sf-level however, it is apparent that the basaltic glass/palagonite
9   contained a significant fraction (19 & 29.2% respectively) of unique phylotypes. We
10   suggest however that given the likelihood that many additional novel phylotypes went
11   undetected by the PhyloChip, as discussed above, these figures may not be
12   representative of the true difference in glass bacterial communities

13   The vast majority of terrestrial Icelandic glass clones share the greatest
14   sequence homologies with uncultured organisms, principally from soil environments,
15   consistent with the observation that they represent very different organisms than those
16   inhabiting deep-ocean basaltic glasses [10]. The levels of diversity and richness
17   reported here in volcanic glasses is comparable to those reported in soil environments
18   and higher than richness reported in oceanic basalt [40]. The richness of basaltic glass
19   bacteria is also higher than that reported for Icelandic crystalline basalt (Kelly et al. in
20   review).

21   In the present study, sequences from volcanic soil habitats such as the
22   Socompa Volcano in the Andes [12] and Hawaiian volcanic deposits [20] are
23   abundant among the *Actinobacteria*, *Acidobacteria*, *Cyanobacteria*, *Bacteroidetes*
24   and to a lesser extent, *Proteobacteria*. Sequences from these Andean and Hawaiian
25   studies also displayed homologies to the crystalline basalt and rhyolitic clones within

1 *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, *Proteobacteria* and *Verrucomicrobia*
2 phyla identified by Kelly et al. (in review) and may represent ecotypes adapted to
3 volcanic environments.

4 In contrast to the *Actinobacteria*, *Proteobacteria* clones from glass 16S rRNA
5 gene libraries display little overlap in sequence homologies with sequences from
6 volcanic environments, being dominated by 15 α -*Proteobacteria* clones most similar
7 to a cryptoendolithic sandstone clone (EU751317). None of the *Proteobacteria* clones
8 from the crystalline Icelandic rocks of Kelly et al. (in review) share appreciable
9 similarities with those from the present glass libraries, suggesting that crystalline and
10 glassy rock types harbor different *Proteobacteria* ribotypes. Many of the other glass
11 phyla however do contain sequences similar to crystalline basalt and rhyolite clone
12 sequences from Iceland (Kelly et al. in review), although the rhyolite clones are not
13 included in Figure 5 due to their comparatively shorter lengths.

14 The *Cyanobacteria* cluster of Figure 5 similarly contains abundant endolith-
15 type sequences, similar to those from sandstone (EF522285), quartz (FJ790633) and
16 an underground tomb (FN298014) suggesting that the cryptoendolithic way of life
17 may be an important determinant of cyanobacterial community composition in
18 terrestrial volcanic glass. Furthermore, > 50% of clone sequences within the
19 *Cyanobacteria* cluster are more similar to algae. The presence of algae in Icelandic
20 volcanic glass has been observed by us previously by microscopy (unpublished). The
21 presence in both glass types of significant proportions of phototroph-derived
22 sequences (~12-17%) suggests a potential role of algae and cyanobacteria in
23 supporting the diverse heterotrophic bacterial communities in these low organic
24 carbon habitats. It should be mentioned here that some of the 16S rRNA gene clone
25 sequences from basaltic glass and obsidian displayed great similarities to those

retrieved by Herrera et al. [25] and Cockell et al. [10] in libraries generated from a single sample of each of the present glass types, from the same sites as the present study. Those clone libraries however were generated from smaller 16S fragments and as such, to enhance the phylogenetic resolutions in the present study were not included in the phylogenetic trees here. Furthermore, the use of longer sequences and their generation from a composite rather than a single sample, resulted in differences in the relative abundances of phyla observed between libraries from the same rock type.

Possible factors influencing microbial community composition in volcanic glasses

The greater diversity observed in the older, weathered basaltic glass cannot be attributed to any one cause, however we suggest that apart from differences in age and location (the latter undoubtedly resulting in differences in environmental conditions), mineralogical differences and porosity may have contributed to the elevated richness of basaltic glass compared to obsidian. Basaltic glass weathers to palagonite [46, 51], a soft secondary weathering material whose cations may be more amenable to microbial access compared to the solid rock. Individual samples may well have different quantities of palagonite, which could influence the microbial communities within the rocks. This could be one factor that might account for why Pal5 clustered differently from the other basaltic glass/palagonite samples by DGGE analysis. Two additional key differences between the glasses relating to mineralogy are silica and bioessential cation contents. Wolff-Boenisch et al. [53] showed that at 25°C and pH 4, basaltic glass weathers approximately an order of magnitude faster than silica-rich glass. As the cations are bound within the glass silica matrix, the high silica concentrations in obsidian would be expected to limit the availability of bioessential

1 cation, which is reflected in the calculated release rates of elements from glass based
2 on silica content [53]. Basaltic glass contains a higher percentage of the bioessential
3 cations, Mg, Ca and Fe than obsidian, but lower concentrations of Na and K, which
4 might influence the types of microorganisms that thrive by selecting for those with
5 nutrient requirements that can be met within each rock type. As discussed by Kelly et
6 al. (in review), rock mineralogy also influences albedo, thereby influencing
7 temperatures experienced within the rock [22]. Obsidian is darker than the weathered
8 basaltic glass and small differences in temperatures experienced on the rock surface
9 and within the rock might well influence the communities. An additional, indirect
10 effect of mineralogy is the difference in rock porosity. At more than double the
11 porosity of obsidian and >300-fold larger surface area, basaltic glass offers a
12 substantially larger area for microbial attachment and colonisation. Porosity will also
13 affect the uptake and retention of water, thus influencing the microbial communities
14 within.

15 Another confounding factor in the present study is the contrasting age of the
16 parent rocks. Substrate age is known to have an influence on microbial communities.
17 Mason et al. [33] analysed the microbial communities within marine basalts of
18 varying age and locations from the East Pacific Rise. T-RFLP analysis revealed that
19 the old samples (ranging in age from a few thousand to approximately 3 Ma) clustered
20 together, while the younger basalts formed a separate cluster. Elsewhere, Lysnes et al.
21 [32] examined marine basalts from the Arctic spreading ridges, also of varying ages.
22 The authors found that *Actinobacteria* were absent in the youngest samples but
23 demonstrated increasing abundance with sample age, being the most abundant group
24 in the oldest basalt (1 Ma). Even on shorter timescales, an increase in soil Chao 1
25 diversity indices from 111 to 378 was observed at 99% sequence similarities [48],

1 while Nemergut et al. [35] observed an increase of approximately 60% in the
2 phylogenetic diversity of soils from <1 yr to 1-4 yr, levelling off in 20 yr soils. In the
3 current study, the older age of the basaltic glass may have resulted in increased
4 diversity on two levels that are intrinsically linked to glass composition, namely
5 succession and weathering rates. Studies such as those of Nemergut et al. [35] and
6 Tarlera et al. [48] attribute the observed increases in soil microbial diversity over time
7 to successional changes in the microbial communities. Succession may indeed
8 contribute to the increased diversity in the present study, however the older age of the
9 basaltic glass means that it has been subjected to weathering for a longer period, thus
10 facilitating an increase in the surface area for microbial attachment and nutrient
11 leaching, thereby providing a more clement microbial environment.

12 In conclusion, this study shows that terrestrial volcanic glasses host
13 remarkably diverse bacterial communities. Rock composition and thus weathering
14 rate are likely to play an important role in influencing this diversity. However, this
15 study highlights the potentially complex involvement of other factors such as age and
16 porosity in determining the endolithic communities in volcanic terrains.

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9 **Figure 1** Location of Valafell (star) and Dómadalshraun (circle) sampling sites in
 10 Southern Iceland (A). Obsidian samples were retrieved from the Dómadalshraun lava
 11 flow (64°1.76'N, 19°6.37'W), formed ~ A.D. 150-300 (B), while basaltic
 12 glass/palagonite samples were taken from hyaloclastite deposited at Valafell
 13 (64°4.83'N, 19°32.53'W) <0.8 Myr (C).

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1 **Figure 2** Cluster analysis of glass bacterial communities based on DGGE (30-70%
2 denaturant) community fingerprints. The Jaccard UPGMA dendrogram was generated
3 from the presence-absence matrix in GelComparII®. Values at nodes represent
4 percentage similarities. Basaltic glass/palagonite samples from Valafell and rhyolitic
5 glass/obsidian samples from Dómadalshraun are represented by Pal and Obs
6 respectively. Samples ending with C are the composites for the given site.

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8 **Figure 3** Rarefaction curves of basaltic glass/palagonite (pal) and obsidian/rhyolitic
9 (obs) glass clone libraries at genus (95%) and species (97%) sequence similarity
10 levels.

1 **Figure 4** Phylogenetic affiliations at the phylum level (or *Proteobacteria* class) of
2 16S rRNA clone sequences from basaltic glass/palagonite (A), and obsidian (B) 16S
3 rRNA gene libraries, based on RDP classification.

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8 **Figure 5** Neighbor-Joining phylogenetic tree of 16S rRNA gene clone sequences
9 from basaltic-glass/palagonite and obsidian libraries. The tree was generated using
10 nucleotide positions 112-770 (*E.coli* numbering). A) full phylogenetic tree, B)
11 *Actinobacteria* phylum as represented by wedge in A. Clones are represented at the
12 OTU-level (defined at 97% sequence similarities) by one sequence from each
13 Libshuff-identified OTU. OTU designations are followed in parenthesis by the
14 number of clones represented by that OTU in basaltic-glass/palagonite and obsidian
15 libraries respectively. OTUs are highlighted in bold, and database sequences from
16 other from Icelandic clone libraries are in bold italics. Bootstrap values (1000
17 replicates) are shown where they exceed 60%. The scale bar represents 2% estimated
18 sequence divergence. *Aquifex* sp. was used as an outgroup.

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Supplemental Information

Figure 1 ANOSIM analysis of DGGE profiles. Pal represents basaltic glass/palagonite samples from Valafell, Obs represents rhyolitic glass/obsidian samples from Dómadalshraun. Samples ending in C are composites for the given glass type.

1 Table 1 Mean element concentrations for each rock type as obtained by electron
2 microprobe analysis (EMPA). Values are the average of 10 (obsidian) or 6 (basaltic
3 glass/palagonite) samples.

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	SiO ₂	TiO ₂	Al ₂ O ₃	FeO	MgO	CaO	Na ₂ O	K ₂ O	Total
<u>Obsidian</u>									
Mean	72.66	0.24	14.77	2.44	0.17	0.76	4.88	4.71	100.63
SD	0.45	0.02	0.14	0.06	0.01	0.03	0.06	0.04	0.48
<u>Basalt glass/palagonite</u>									
Mean	46.10	2.63	15.31	13.2	7.09	10.71	2.57	0.39	98.00
SD	0.22	0.03	0.04	0.06	0.13	0.04	0.03	0.01	0.27

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11 Table 2 Phyla and *Proteobacteria* classes detected in terrestrial Icelandic glasses by
12 PhyloChip analysis. Numbers represent the contribution of each phylum at the
13 operational taxonomic unit (OTU) or subfamily (sf) level, as a percentage of the total
14 at the indicated sites and are rounded to the nearest decimal. Numbers in parentheses
15 refer to the percent contribution of OTUs or sfs that are unique to that glass type. The
16 detection of phyla in the corresponding 16S rRNA gene clone library (using clone
17 classifications determined using the G2 chip taxonomy classifier in greengenes) is
18 indicated by bold figures at the OTU level.

Phylum	Dómadalshraun		Valafell	
	OTU	sf	OTU	sf
<i>Acidobacteria</i>	3.9 (0.2)	3.4 (1.0)	3.7 (0.8)	2.1
<i>Actinobacteria</i>	15.3 (1.2)	12.8 (2.0)	13.3 (2.4)	11.3 (1.8)
<i>AD3</i>	0.1	0.3	0.1	0.3
<i>Aquificae</i>	0.2	0.7	0.2 (0.1)	0.6
<i>Bacteroidetes</i>	5.7 (1.1)	4.7 (0.3)	5.6 (1.9)	5.9 (2.1)
<i>BRC2</i>	0.2	0.7	0.1	0.6
<i>Caldithrix</i>	0.2	0.7	0.1	0.6
<i>Chlamydiae</i>	0.1	0.3	0.1 (0.1)	0.6 (0.3)
<i>Chlorobi</i>	0.5 (0.1)	1.7	0.4 (0.1)	1.5
<i>Chloroflexi</i>	2.6 (0.6)	5.8 (0.7)	1.6	4.5
<i>Coprothermobacteria</i>	0.1	0.3	0.1	0.3
<i>Cyanobacteria</i>	4.7 (0.5)	5.1 (0.3)	3.9 (0.6)	5.0 (0.9)
<i>Deferribacteres</i>			0.1 (0.1)	0.3 (0.3)
<i>Deinococcus-Thermus</i>	0.4 (0.1)	1.0	0.2	0.9
<i>Dictyoglomi</i>	0.0		0.1 (0.1)	0.3 (0.3)
<i>DSS1</i>	0.1	0.3	0.1 (0.1)	0.6 (0.3)
<i>Firmicutes</i>	14.7 (1.7)	8.8	17.8 (7.8)	11.0 (3.3)
<i>Gemmatimonadetes</i>	0.5	0.3	0.4	0.3
<i>LD1PA group</i>			0.1 (0.1)	0.3 (0.3)
<i>Lentisphaerae</i>	0.2	0.3	0.2	0.3
<i>Marine groupA</i>	0.2	0.3	0.2 (0.1)	0.3
<i>Natronoanaerobium</i>	0.2	0.3	0.2 (0.1)	0.3
<i>NC10</i>			0.1 (0.1)	0.3 (0.3)
<i>Nitrospira</i>	0.2	0.3	0.5 (0.3)	0.9 (0.6)
<i>OD1</i>	0.1	0.3	0.1	0.3
<i>OP10</i>	0.4	1.0	0.3	0.9
<i>OP3</i>	0.2	0.7	0.2 (0.1)	0.9 (0.3)
<i>OP8</i>	0.1	0.3	0.1 (0.1)	
<i>OP9/JS1</i>	0.4	0.3	0.3	0.3
<i>Planctomycetes</i>	0.7 (0.1)	1.4	1.1 (0.6)	2.1 (0.9)
<i>Proteobacteria</i>	41.1 (2.7)	36.5 (2.4)	42.0 (12.3)	36.6 (6.5)
α	14.4 (0.7)	12.5 (1.0)	14.4 (3.8)	12.2 (2.1)
β	8.2 (0.2)	4.4	8.9 (2.7)	4.7 (0.9)
γ	10.2 (1.0)	10.8 (1.0)	10.6 (3.5)	10.7 (2.1)
δ	5.2 (0.5)	6.4	5.3 (1.6)	6.8 (1.2)
ϵ	2.9 (0.2)	1.4	2.5 (0.5)	1.2
<i>SPAM</i>	0.2	0.3	0.1	0.3
<i>Spirochaetes</i>	2.2 (0.2)	1.0 (0.3)	1.9 (0.3)	0.9 (0.3)
<i>SR1</i>	0.1	0.3	0.1	0.3
<i>Synergistes</i>	0.4	0.3	0.3	0.3
<i>Termite group 1</i>			0.2 (0.2)	0.3 (0.3)
<i>Termodesulfobacteria</i>	0.1	0.3	0.1	0.3
<i>TM7</i>	0.5	0.7	0.5 (0.2)	0.6
Unclassified	2.2 (0.3)	4.1 (0.3)	2.0 (0.6)	2.7
<i>Verrucomicrobia</i>	1.7	3.7	1.7 (0.4)	3.6 (0.3)
<i>WS3</i>	0.2	0.7 (0.3)	0.1	0.6
<i>WS5</i>	0.1	0.3	0.1	0.3
Total unique	(8.5)	(7.1)	(29.2)	(19.0)