Pioneer microbial communities of the Fimmvörðuháls lava flow, Eyjafjallajökull, Iceland

Laura C Kelly[1,2*,§], Charles S Cockell[1, 3*], Thorsteinn Thorsteinsson[4], Viggó Marteinsson[5], John Stevenson[6]

[1]Geomicrobiology Research Group, CEPSAR, Open University, MK7 6AA, UK.

[2]School of Biology and Conservation Ecology, Manchester Metropolitan University, Manchester M1 5GD.

[3]School of Physics and Astronomy, University of Edinburgh, Edinburgh, EH9 3JZ, UK.

[4]Hydrology Division, National Energy Authority, Grensasvegi 9, IS-108 Reykjavik, Iceland

[5]Matís Idt./Food Safety, Environment & Genetics, Vínlandsleid 12, 113 Reykjavik, Iceland.

[6]School of Geosciences, University of Edinburgh, Edinburgh, EH9 3Jw, UK.

* Current addresses

§ Corresponding author

Laura C Kelly, School of Biology and Conservation Ecology, Manchester Metropolitan University, Manchester M1 5G, UKD.

Tel: +44 (0)161 2471190, Email: laurackelly@live.ie

Keywords: volcanic/basalt/pioneers/chemolithotrophs/diazotrophs/Iceland

ABSTRACT

Little is understood regarding the phylogeny and metabolic capabilities of the earliest colonists of volcanic rocks, yet these data are essential for understanding how life becomes established in, and interacts with the planetary crust, ultimately contributing to critical zone processes and soil formation. Here we report the use of molecular and culture-dependent methods to determine the composition of pioneer microbial communities colonising the basaltic Fimmvörðuháls lava flow at Eyjafjallajökull, Iceland, formed in 2010. Our data show that three to five months post eruption, the lava was colonized by a low-diversity microbial community dominated by *Betaproteobacteria*, primarily taxa related to nonphototrophic diazotrophs such as *Herbaspirillum* spp., and chemolithotrophs such as *Thiobacillus*. Although successfully cultured following enrichment, phototrophs were not abundant members of the Fimmvörðuháls communities, as revealed by molecular analysis, and phototrophy is therefore not likely to be a dominant biogeochemical process in these early successional basalt communities. These results contrast with older Icelandic lava of comparable mineralogy, in which phototrophs comprised a significant fraction of microbial communities and the non-phototrophic community fractions were dominated by *Acidobacteria* and *Actinobacteria*.

INTRODUCTION

Despite their global abundance and environmental and ecological significance, surprisingly little is known regarding the initial colonists of freshly-deposited volcanic rocks. Volcanic rocks play a significant role in the global carbonate-silicate cycle as they weather [1-5], while some of the most fertile soils in the world are of volcanic origin [6-8]. Understanding how newly-formed volcanic substrates become colonised by microbial communities, which may ultimately play a role in rock weathering, soil formation and plant ecosystem development, is an essential task in earth sciences.

The surface of unvegetated volcanic lavas are an extreme, but nevertheless viable habitat for microorganisms [9-12]. They can be subject to desiccation, exposure to UV radiation, temperature fluctuations and low organic and nitrogen availability. One of the first studies of microbial colonisation of freshly-deposited volcanic rock occurred on the island of Surtsey, Iceland, formed during the 1963-1968 volcanic eruptions off the southern Icelandic coast. These lavas provided a unique laboratory for the investigation of biological establishment and succession on newly deposited volcanic substrata. Phototrophs were already observed by 1968 [13] and subsequent culture-based and microscopy investigations reiterated the importance of chlorophytes, lichens and mosses to ecosystem development on the island [14-17]. A further study reported the presence of cyanobacteria, including *Anabaena* and *Nostoc* on the Icelandic Island of Heimaey, eighteen months after an eruption in 1973 [18].

Molecular-based studies on the microbiota of volcanic substrates have only emerged within the past few years, revealing that such habitats are capable of harbouring significant microbial diversity [11-12]. The study of a 1959 cinder deposit in Hawaii revealed a diverse community comprising *Cyanobacteria*, *Acidobacteria* and *Alphaproteobacteria* and the presence of organisms specifically capable of CO-oxidation [19-20]. On unvegetated volcanic substrates at the Mount St. Helens volcano, seventeen years after the eruption,

Ibekwe *et al.* [9] recorded the presence *Alpha-* and *Betaproteobacteria* and *Actinobacteria*, while Gomez-Alvarez *et al*. [11] found that in Hawaiian deposits formed in 1959, microbial communities were dominated by *Acidobacteria* and *Alphaproteobacteria*, with a large percentage of unclassified sequences. Recent molecular investigations of weathered, unvegetated Icelandic volcanic rocks revealed diverse microbial communities which, although differing in composition amongst volcanic rocks of different mineralogies, contained significant proportions of *Acidobacteria*, *Actinobacteria* and *Proteobacteria* [12, 21]. *Cyanobacteria* were abundant only in volcanic glasses [12].

In the aforementioned and indeed similar molecular studies of volcanic substrates, sampling began well after the establishment of microbial communities, with the substrates in these investigations ranging in age from seventeen years [9] to material deposited circa 0.8 Mya [12, 21-22], with no current existing reports of molecular analyses of freshly-deposited volcanic material. In the present study, we sought to redress this lack of in-depth characterisation of the earliest microbial colonists of freshly-deposited volcanic substrates.

In March and April 2010, eruptions of the Eyjafjallajökull volcano in Southern Iceland produced a new lava flow of basaltic composition, the Fimmvörðuháls flow, offering an opportunity to 1) identify the earliest microbial colonists of this globally abundant geological substrate and 2) compare these microbial communities with those of previous studies of older Icelandic volcanic substrates [10, 12, 21-22] less than 50 km away. In particular, we sought to test the hypothesis, using a combination of molecular and cultivation-dependent methods, that phototrophs are abundant colonists on the newly-available habitat offered by volcanic eruptions.

MATERIALS AND METHODS

Field site and sampling

Samples were collected from the Fimmvörðuháls lava flow, which was erupted from the Eyjafjallajökull volcano between 20 March and 12 April 2010 from the Magni and Móði craters (Fig. 1). The flow is located between the Eyjafjallajökull and Mýrdalsjökull glaciers in southern Iceland and comprises mildly alkalic olivine basalt lava (<2% phenocrysts of olivine, plagioclase and clinopyroxene; [23], forming lobes that cover an area of 1.3 km^2 with an average thickness of 10-20 m (estimated volume: 20 million cubic metres; [24]).

On 5 July 2010, nine readily accessible sampling sites (named 1 – 9) were established on the lava flow. The sites were located in three loose clusters of three along a transect, relatively close to the source of the flow, as shown in Fig. 1. The maximum distance between clusters was 0.48 km. The sampling sites described in this study are located within the area delineated by coordinates 63°38'13.20"N, 19°27'1.08"W and 63°38'23.10"N, 19°26'31.80"W. Replicate (three) samples of lava, weighing approximately 60-250 g, were taken from each site. The maximum distance between replicates was approximately 2 m. Further samples were taken on 31 August 2010 from the same sites.

The samples, taken from the surface of the lava flow, comprised the lava and associated eruption ash deposits contained within the fractures and pore spaces. Samples, removed using a rock hammer sterilized in ethanol, were broken directly into sterile plastic bags (Whirlpak, Fisher Scientific, UK) without handling, double bagged and boxed immediately after collection. Samples were frozen for molecular analysis (-20 °C), or subjected to culture-based analyses following collection. Additional samples of lava from each site were fixed in 2% formaldehyde for subsequent cell counts. Samples were labelled according to the month (J - July or A - August), site of retrieval and replicate number.

Geochemical analyses

Whole-rock major and minor element compositions were obtained using an ARL 8420+ dual goniometer wavelength-dispersive X-ray fluorescence (XRF) spectrometer (Thermo Scientific, USA). XRF analysis was carried out on glass discs (major element concentrations) prepared by fusing one part finely powdered lava sample, with five parts of FluXana flux (20% lithium tetraborate/80% lithium metaborate mix) [25], or on pressed powder pellets (trace elemental compositions) [26]. Four individual samples from each of the nine sampling sites were analysed.

Three finely-ground 25 mg samplesfrom each site in July were examined for their nitrogen, organic carbon and total sulfur concentrations. Carbon content was determined using a Europa ANCA-SL elemental analyser coupled to a continuous flow mass spectrometer (Europa GEO 20-20, Knutsford, UK) with a detection limit of 0.001%. Nitrogen analysis was carried out using a Carlo Erba NA2500 Elemental Analyser (Glasgow, Scotland) with a detection limit of 0.001%. Sulfur was measured using a LECO SC 444 (St. Joseph, MI, USA) instrument with a detection limit of 0.001%.

Direct isolations and enrichment cultures

To test for the presence of certain functional microbial groups, we attempted their direct isolation in the laboratory using samples from sites 1, 5 and 9. Unless otherwise stated, all samples from these sites and sampling period were processed identically. Subsamples of lava were crushed to a maximum size of 1 cm under sterile conditions in a laminar flow hood and shaken at 100 rpm at 21 °C for 1.5 h in 10 mL double distilled H_2O, before plating onto solid media (100 µL undiluted sample), or inoculating into 20 mL of liquid media (500 µL plus fragments).

To test for the presence of phototrophs, samples were incubated in BG11 broth (pH 7.2) [27] for enrichment. Chemolithotrophs capable of oxidising sulfur were enriched in sulfur-oxidizers (SOX) broth (pH 7.0-7.2) [28]. The presence of nitrogen-fixing organisms in samples collected in August 2010 was tested using nitrogen-free Norris agar (pH 7.2) [29], prepared using 1.5% Noble agar. Plates and broths were incubated at room temperature (21°C). Following 46 days growth in broth, cultures were plated in duplicate (100 µL) onto the corresponding solid media for isolation. Solid BG11 and SOX plates contained 2% Noble agar. BG11 broths and plates were incubated under natural light conditions. Solid media incubations were for 10-14 days. To test for the presence of heterotrophs, organisms were isolated on two solid media; nutrient agar (Oxoid, Fisher Scientific, UK) (pH 7.3 – 7.4) and a 1/100 nutrient agar (pH 6.7) prepared with 2% Noble agar (BD Biosciences, UK).

Identification of isolates

Colonies representative of the dominant morphologies present in each sample, in addition to a selection of some of the less common morphologies observed on the various media, were subcultured three to six times to obtain pure cultures. Selected bacterial isolates were subjected to colony PCR with universal eubacterial primers pA and pH [30-32] to amplify almost complete 16S rRNA genes, or in the case of cyanobacteria, pA and CYA781R [33], amplifying partial 16S rRNA genes. Primers 817f and 1536r [34] were used to amplify partial 18S genes from selected fungal isolates. Each 50 µL reaction mixture contained 0.2 µM each primer, 200 µM each dNTP (New England Biolabs, UK), 2.5 U *Taq* DNA polymerase, 2 mM $MgCl_2$ and 1X PCR buffer (200mM Tris-HCl (pH 8.4), 500 mM KCl) (Invitrogen Corporation, UK). Amplifications were performed in a G-Storm GS1 thermal cycler (GRI Ltd., UK), with an initial denaturation at 94 ºC for 5 min, followed by thirty-five cycles of 94 ºC for 1 min, 55 ºC for 40 s and 72 ºC for 40 s, with a final extension for 10 min at 72 ºC.

Sequencing of *Bacteria* and phototrophs was performed with pA. In instances where these sequences suggested new species, additional sequencing with com1 (as a forward primer) [35] and/or pH was performed (Mclab, USA or GATC Biotech, Germany) to provide a more complete gene sequence of at least 1 kb.

Direct cell counts

Microbial numbers were calculated per gram of dry weight of lava. Counts were performed on all replicate samples from sites 1, 5 and 9. To enumerate microbes, 100 µL of dd H_2O containing powdered lava (approximately 0.01 to 0.04 g, crushed as described below) was added to 900 µL of ddH_2O and 100 µL of a solution of 1X SYBR® Green I DNA binding dye according to the manufacturer's instructions (Invitrogen, UK). The solution was vacuum filtered onto black 0.2 µm Nuclepore polycarbonate filters (Whatman, UK). Microorganisms were enumerated under at least 30 fields of view on a Leica DMRP fluorescence microscope (Leica Microsystems, Germany) using an excitation waveband of 450-490 nm (Leica filter cube I3) and an emission long band cutoff filter of >515 nm. A two way analysis of variance (ANOVA) was performed on the data in Microsoft Excel.

DNA extraction

DNA was extracted from all samples from site 5 and one random chosen sample each from sites 1 and 9 at each sampling period. Lava was crushed to a powder in a laminar flow hood using a sterilized metal container and plunger as described previously [36]. Total DNA was extracted from all samples (~10 g each), using a PowerMax Soil DNA Isolation Kit (MoBio Laboratories, UK). Extraction was performed according to manufacturer's instructions, with the exception of an extended incubation period of 2.5 h after the addition of buffer C2

(designed for the removal of PCR inhibitors), and a 1 mL elution volume. DNA was quantified, in triplicate, by NanoDrop.

Bacteria 16S rRNA gene clone libraries

Amplification of 16S rRNA genes from basalt communities was performed with universal eubacterial primers pA and pH, and products purified before cloning into the pCR4® vector as previously described [12]. Inserts were sequenced with pA (GATC Biotech, Germany). Chimera detection was performed through greengenes [37]. Following chimera removal, all libraries were normalised to that containing the smallest number of sequences. Sequences were aligned over *E. coli* nucleotide positions 100 – 785 in MOTHUR (version 1.25.1) [38] against the greengenes core database set, and a distance matrix generated in Phylip [39] (version 3.6). This distance matrix was used in MOTHUR to group sequences into operational taxonomic units (OTUs) at 97% sequence identities. Following MOTHUR normalisation, richness and diversity estimates were calculated, and samples compared by Libshuff [40] using MOTHURr, and principal component analysis (PCA) using PRIMER 5 (version 5.2.0). Classification of clones was performed through the RDP [41] (release 10). Sequences representative of each OTU were also searched against those deposited in GenBank, through the NCBI blastn program, revealing the closest cultured and uncultured sequences.

Comparison of isolates and 16s rRNA gene clones

16S rRNA gene sequences from both isolates and clone libraries were aligned over *E. coli* nucleotide positions 134 – 730 and OTUs generated in MOTHUR at 97% sequence identities, as described above. Using these data, a bootstrapped (1000 iterations) Neighbor-Joining phylogenetic tree was constructed with MEGA4 [42] using representative sequences

from each OTU and related GenBank sequences from cultured and uncultured organisms. The process was repeated with the same sequences to generate a Maximum Likelihood tree.

RESULTS

Geochemical analyses

Average elemental compositions of lava from the nine sampling sites were as follows [equivalent oxide, mean (standard deviation)]: SiO_2, 47.37 (0.90); Al_2O_3, 14.88 (0.17); Fe_2O_3, 13.29 (0.34); MgO, 8.11 (0.39); CaO, 9.50 (0.39); Na_2O, 2.83 (0.27); K_2O, 0.72 (0.12); TiO_2, 2.90 (0.15); MnO, 0.19 (0.01); P_2O_5, 0.39 (0.01); total, 99.71 (0.35) These data show that the lava samples were of a basaltic composition according to the Total Alkali Silica (TAS) classification scheme of igneous rocks [43], having a mean Na_2O+K_2O content of 3.55%. These data are in agreement with Sigmundsson *et al*. [23].

Nitrogen was below detection using the method employed in all the samples analysed. The organic carbon content of the samples was determined to be less than 0.001% wt. The total sulfur concentration in all of the samples examined was 0.021% (±0.014) wt.

Identification of isolates

The identities of isolates relative to the closest 16S or 18S rRNA gene sequences of cultured organisms (GenBank deposits), are given in Table 1. Bacterial isolates were primarily members of the *Actinobacteria* and *Proteobacteria* phyla. Isolated fungi were members of the Ascomycota. Many bacterial isolates shared the greatest sequence identity with uncultured organisms from a variety of sources, many of these from Antarctic environments, glaciers and freshwater habitats. Among the isolates was a taxon represented by four isolates and displaying a maximum of 92% sequence identity (16S rRNA genes) to any sequence currently deposited in public databases. The closest cultured relatives of this taxon are *Alicyclobacillus*

spp., members of the phylum *Firmicutes*, and include an isolate from Antarctic geothermal soil (AJ607430). All isolate 16Sr RNA gene sequences have been deposited in GenBank under accession numbers JF417993 to JF418153 and JF706699 to JF706701.

Direct cell counts

The average cell counts (with standard deviations) for lava samples from July and August, per g of material dry weight, were 1.58 x 10^6 (1.27 x 10^6) and 2.58 x 10^6 (2.15 x 10^6), respectively. Averages for the individual sites, for each season, are given in Fig. 2. A two way ANOVA indicated a significant difference in counts among sites (P=0.009), while no seasonal effect of season was observed (P=0.138).

DNA extraction

Concentrations of DNA (ng/µ) in extracted samples from July and August (respectively) were as follows: site 1 (5.4 and 4.2), site 5 (4.4, 4.2, 4.7 and 4.0, 4.5, 5.0) and site 9 (4.7 and 4.0). Subsequent 1 ml elutions from the DNA extraction filters (see above) resulted in addition DNA recovery (not shown).

Bacteria 16S rRNA gene clone libraries

Upon removal of chimeras (an average of 2.8% of sequences) up to 106 clone sequences remained in each library. However, for the purpose of meaningful comparisions, and statistical analyses, samples were randomly normalized to the smallest library, containing 82 clones.

Diversity and richness of Fimmvörðuháls bacterial communities was low (Fig. 3), as determined at 97% sequence identities (OTUs). The recovery of OTUs reached or approached saturation using <100 clones (not shown) andcoverage estimates for samples ranged from 87

to 99%. Shannon diversity indices ranged from 1.40 to 2.49 (average 2.07), while Chao1 [44] richness ranged from 8.0 to 46.5 (average 24.5).

Sixty-three individual OTUs (97% sequence identities) were identified amongst normalised libraries. The identities of the two most abundant OTUs in each library, accounting for nine OTUs due to shared OTUs between samples, are given in Table 2, relative to the nearest cultured and uncultured sequences deposited in GenBank. Together these OTUs accounted for almost 82% of sequenced clones and individually accounted for 2.0 – 29.7% of clones among all libraries. The most abundant library OTU (OTU63), accounting for up to 9.4 – 48% of sequences per library, was identified as belonging to the genus *Herbaspirillum*, a group known to possess diazotrophic members, while OTU59 and OTU54 (together accounting for 16.1% of sequences, were likewise affiliated with known nitrogen-fixing genera. OTU45 and OTU49, together accounting for 13.9% of library sequences, were affiliated with iron and H_2 oxiders, and OTU38 with a known sulfur-oxidizing chemolithotroph, *Sulfuricurvum* (Table 2). The remaining fifty-four library OTUs identified accounted for 0.3% of library clones on average (range 0.1 – 1.4%), and were affiliated with sequences recovered from a variety of habitats, particularly glacial and soil environments (not shown) and most closely related to taxa known for nitrogen-fixing and chemolithotrophic nutrition. Among the sixty-three identified clone OTUs, 52% were unique to one sample and were mostly representative of one or two clones. Clone 16S rRNA gene sequences have been deposited in GenBank under accession numbers HQ898914 to HQ900366.

Phylum *Proteobacteria* sequences and the *Betaproteobacteria* in particular, were numerically dominant in all libraries. The *Betaproteobacteria* comprised 57.7 – 97.1% of clones in each library (and 70% of sequenced clones combined) while *Epsilonproteobacteria* also contributed significantly (22 – 29%) to a number of samples (Fig. 4), corresponding to OTU38 described above. Despite the numerical dominance of *Betaproteobacteria* sequences

in all samples, Libshuff analysis comparing individual samples revealed significant differences in clone library composition, both within and between sampling sites. Sample pairings for which non-significant differences were observed are given in Supplementary Table S2. PCA performed on libraries also revealed variability among samples, with one sample from site 5 in July particularly different from all others, separated along the first component axis (Supplemental Material). The two principal components shown together accounted for 42.3% of the variability observed among lava samples.

Identification of cultured species also contained within clone libraries

Following alignment of isolate and clone 16S rRNA gene sequences, ninety-two OTUs were identified at 97% sequence identity, including twenty-eight represented by isolates only. Among these isolate-only OTUs, two were identified as *Arthrobacter* (represented by twenty-seven and seven isolates), most closely matching sequences from Svalbard soil (Arctic) and Livingston Island (Antarctica), respectively. A *Sphingobacterium* OTU was represented by 12 isolates most similar to sequences from temperate grassland (Fig. 4a). Other OTUs represented solely by isolates were affiliated with the genera *Rhizobium*, *Variovorax*, *Thiobacillus* and *Firmicutes*. Nine OTUs were represented by both clone and isolate sequences, including four of the abundant library OTUs (OTUs 49, 54, 60 and 63) described in Table 2. The most abundant OTU, affiliated with the genus *Herbaspirillum* and corresponding to OTU63 of clone libraries, comprised 282 clone and 10 isolate sequences. Other OTUs, their abundance in libraries and among isolate sequences and their phylogenetic affiliations are shown in Fig. 4.

DISCUSSION

The eruption of the basaltic Fimmvörðuháls lava flow from the Eyjafjallajökull volcano in southern Iceland, during March and April 2010, provided an opportunity to investigate the colonisation of fresh lava in the immediate aftermath of the eruptions. While investigations of colonists of newly deposited Icelandic lava has been previously undertaken on the island of Surtsey [13 – 17], revealing an abundance of phototrophs, our study is the first to undertake such a detailed analyses of lava flow microbial communities at such an early stage of colonisation. We sampled the lava flow on two occasions within the first three to five months post eruption, employing both traditional culture-based techniques in concert with molecular methods to characterise the pioneer bacterial inhabitants.

Contrary to our expectations based on Surtsey reports, both our molecular and culture-based results revealed that the early microbial colonists of the Fimmvörðuháls lava flow were not composed primarily of phototrophs but rather by organisms, primarily *Betaproteobacteria*, affiliated with known diazotrophs, chemolithotrophs and heterotrophs. Unlike other studies which have shown the abundance of phototrophs in early successional volcanic materials [18] and deglaciated soils [45, 46] and inferred the important role of phototrophs in the establishment of pioneer communities, we recovered only a few phototroph-related sequences from our clone libraries and only succeeded in isolating cyanobacteria or diatoms following prior enrichment. Early reports from Surtsey detected mosses, lichens and algae by microscopy and culture-based approaches [16, 17, 47]. However, these observations were generally made on substrata at least a few years post-deposition, rather than within months as in the present study, and methodological limitations precluded a detailed community molecular analysis. In contrast, our data show that while phototrophs were present in Fimmvörðuháls communities at the very early stages of basalt

colonization, they represented only a negligible proportion of the molecular diversity of organisms on the lava.

While our approach does not allow us to unequivocally prove the *in-situ* activities of isolated organisms or of taxa observed within 16S rRNA gene clone libraries, the abundance in each sample community of taxa affiliated with chemolithotrophs, heterotrophs and nitrogen-fixers, together with the available information regarding carbon, nitrogen and sulfur content of samples and the paucity of phototrophs, suggests that an early stage of chemolithotrophy and heterotrophy precedes an important role for phototrophs within the Fimmvörðuháls flow.

The accumulation of nitrogen in early primary successional environments can be a limiting factor in ecosystem development [46, 48-50] and nitrogen-fixing microorganisms are therefore often abundant colonizers in nitrogen-deficient terrestrial habitats [51]. Increases in nitrogen availability facilitate colonization by later successional species [48, 51]. Nitrogen was below detection in the Fimmvörðuháls lava flow following the eruptions, thus making diazotrophy a potentially important requirement for early pioneers in the nitrogen-limited basalt. The isolation of a diversity of nitrogen-fixing organisms on nitrogen-free agar plates, together with the abundance of library 16S rRNA gene sequences affiliated with diazotrophic taxa, suggests that diazotrophy was represented in phylogenetically diverse organisms.

Despite the low organic carbon content, the lava flow hosted a diversity of heterotrophs. We hypothesise that the Fimmvörðuháls heterotrophs may have used either organic carbon derived from chemolithotrophic metabolism within the lava, and/or the airborne input of low amounts of atmospheric organic carbon compounds [52]. Atmospheric inputs of microbes may also contribute to the organic carbon pool, via cell lysis and recycling of organic cellular constitutents. Taxa we isolated in the laboratory included representatives of the *Actinobacteria* (*Arthrobacter*), *Alphaproteobacteria* (*Sphingomonas*) and many

Betaproteobacteria including *Polaromonas*, *Variovorax* (Comamonadaceae) and *Duganella*, *Herbaspirillum* and *Massilia* (Oxalobacteraceae) species. These data are consistent with molecular sequencing data, which also revealed many heterotrophic *Betaproteobacteria*. The most abundant OTU was affiliated with *Herbaspirillum*, a genus which has previously been reported from 22-year-old volcanic deposits in Miyake-jima Island, Japan [53] and is known to contain representatives capable of growth under oligotrophic conditions.

Substrates supporting potential chemolithotrophic metabolism within the Fimmvörðuháls lava could have included the reduced iron available in basalt minerals such as olivines (detected as total iron in XRF data). Sulfur was also detected in our samples and exhalations of sulfur gases was shown to have occurred during the eruptions [54]. Chemolithotrophic potential within the Fimmvörðuháls lava is suggested by the isolation sulfur-oxidising *Thiobacilli*, and multiple independent isolations of a novel taxon on SOX media which failed to grow in the absence of thiosulfate. Furthermore, clone library OTUs affiliated with *Thiobacillus* and *Rhodoferax* were observedand an abundant OTU affiliated with the facultatively anaerobic genus *Sulfuricurvum* was particularly abundant in samples three months after the eruption, constituting up to 30% of clones. These data suggest the presence of other uncultured chemolithotrophic organisms capable of metabolising sulfur and/or iron.

Compared to older (~1,750 yrs old), weathered crystalline Icelandic lava of comparable mineralogy previously studied [21], where microbial numbers were of the same order of magnitude as in the present study, the diversity and richness of bacterial communities within Fimmvörðuháls lavas, as determined by community 16S rRNA gene clone library analysis, were substantially lower. The Chao1 [44] and Shannon values we obtained were 24.5 and 2.07, as compared to 165 and 4.04 at the same sequence similarity cutoff (97%) on the older Icelandic basaltic lava flow [21] less than 50 km away. Low species diversity in

Fimmvörðuháls lava is not surprising given the very short time available for colonisation and community development. Diversity in successional communities from other environments has been observed to increase over time. For example, the Shannon index on newly exposed glacial forefields in Peru increased from 0.8 in newly exposed material to above 4.5 for 500 year old soils [44]. Increases in species richness have been observed in Arctic glacial soils covering a 150-yr time span [55]. Site 5, located to the south west of the Módi crater (Fig. 1), is closest to the craters; perhaps close enough that the elevation of Módi may provide some shelter to site 5, resulting in a reduced input of airborn microbes from the wind and a comparatively lower cell count from samples from this site.

The bacterial community composition of the newly colonised Fimmvörðuháls basalt, , being dominated by *Betaproteobacteria*, contrasts with that of older Icelandic rocks. Ancient Icelandic crystalline lava of basaltic and rhyolitic composition (~1,750 yrs old), analysed in the same manner as the present study, were dominated by *Actinobacteria* and *Acidobacteria* and harboured many other phyla, including *Cyanobacteria* and difficult-to-culture phyla such as *Verrucomicrobia* and *Gemmatimonadetes*. All of these rocks contained less than 5% *Betaproteobacteria* [21]. Similar results were reported for ancient Icelandic volcanic glasses [12]. While the Fimmvörðuháls flow was dominated by *Betaproteobacteria*, community variations were observed among samples, indicating community heterogeneity on small spatial scales, similarly to the ancient basalts. Such small-scale heterogeneity was also observed in weathered Icelandic lavas [12, 21].

Many of our isolates, including some of the most numerous species, remained undetected in clone libraries. Considering the high library coverage achieved by sequencing, and the use of the same primer set for both isolate identification and library construction, failure to detect isolated species within libraries may be due to incomplete extraction of community DNA, particularly in the case of sporulating species of *Arthrobacter* and

Firmicutes, and/or that cultivation resulted in significant bias towards rare organisms. Culturable fungi, which increased in abundance in August, could not be detected by fungal-specific PCR of community DNA and attempts to detect Archaea by molecular methods (FISH and PCR) also proved unsuccessful (not shown). Despite optimisation of a PCR protocol to detect algal 18S rRNA genes, we did not detect algae in our community DNA extracts (not shown), further evidence that despite their presence in many samples, as detected by enrichment culture, the abundance of algae was very low.

In conclusion, our data show that three to five months post deposition, the Fimmvörðuháls lava flow contained microbial communities characterized by low diversity and abundant *Betaproteobacteria* affiliated with diazotrophic, heterotrophic and chemolithotrophic taxa. Our observations show that even three months after their eruption, lava flows may be host to functionally distinct microbial communities of low diversity. Some of the most abundant OTUs observed in these samples could not be affiliated to a genus, evidence that the newly lava flows harbour novel organisms with unknown contributions to early biogeochemical cycles. We conclude that potentially biogeochemically active communities were established remarkably quickly after the Fimmvörðuháls eruptions and that future studies of primary succession on such volcanic materials must begin within days and months after the appearance of the new habitat.

Acknowledgements

This research was supported by a Natural Environment Research Council grant (NERC;NE/I007695/1. LiDAR data were collected on NERC Airborne Research and Survey Facility flight UR10/02. The open source GMT (Wessel and Smith, 1991) and GRASS GIS (Neteler et al., 2012) software packages were used in LiDAR processing. We thank Stephen

Summers (Dept. of Physical Sciences, Open University, UK) for assistance with July sampling.

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Table 1. Fimmvörðuháls isolates and their closest cultured relatives currently deposited in GenBank, as determined by partial 16S rRNA gene sequence comparison. In instances where the closest cultured organism or organisms are unclassified, the nearest classified cultured organism is also shown. Where multiple Fimmvörðuháls isolates returned the same hit (usually representing isolates within the same OTU; see text), the accession number of only one Fimmvörðuháls isolate is given. For ease of reporting, data for *Arthrobacter*, *Pseudomonas* and *Sphingomonas*, species isolated in abundance, are condensed. Accessions in bold font indicate those isolates which are cluster within within the most abundant clone library OTUs, as determined during Mothur analysis (see text and Table 2).

solate (this study)	**GenBank Accession**	**% Match**	**Phylum**	**Nearest Cultured Organism (GenBank)**	**Environment**	**Reference**
Photoautotrophs						
JF418020 (B)	FJ002185	97	Bacillariophyta	Pennate diatom sp, CCAP 1008/1		unpublished study
JF418123 (B)	D11348	92	Chlorophyta	*Chlorella saccharophilia*		Oyzizu *et al*. 1992
JF418116 (B)	EU912438	94	Chlorophyta	*Vaucheria litorea* chloroplast		Rumpho *et al*. 2008
JF418019 (B)	AM709632	98	Cyanobacteria	*Pseudanabaena* sp. PCC 6903		Marin *et al*. 2007
JF418021 (B)	GU935361	98	Cyanobacteria	*Pseudanabaenoideae* sp.	drinking water reservoir sediment	unpublished study
Bacteria						
JF418135 (S)	GQ477173	99	Actinobacteria	*Arthrobacter* sp. RKS6-6	Himalayan glacial soil	unpublished study
JF418068 (NA)	JQ977602	99	Actinobacteria	*Arthrobacter* sp. Alb6	root, Tianshan Mts, China	unpublished study
JF418119 (B)	KC236857	99	Actinobacteria	*Arthrobacter* sp. B2108	paddy soil	unpublished study
JF418087 (NA)	JX949837	99	Actinobacteria	*Arthrobacter* sp. TMT1-38	Chinese glacier	unpublished study
JF418081 (NA)	JQ977457	99	Actinobacteria	*Arthrobacter* sp. Zs11	rhizosphere soil, Tianshan Mts, China	unpublished study
JF418036 (N)	JX876867	99	Actinobacteria	*Frondihabitans* sp. GRS42	maple syrup	unpublished study
JF418063 (NA)	HQ728400	97	Unclassified	Bacterium M25	*Arabidopsis thaliana* phyllosphere	unpublished study
	EF540450	97	Actinobacteria	*Leifsonia* sp.	Semi-coke	unpublished study
JF418118 (B)	KC618504	100	Actinobacteria	*Rhodococcus erythropolis*	rhizosphere soil	unpublished study
JF418034 (N)	HQ113383	99	Alphaproteobacteria	*Caulobacter* sp. BSL1	*Daphnia magna* digestive tract	Martin-Creuzburg, Beck & Freese 2011
JF418035 (N)	AB531422	100	Alphaproteobacteria	*Mesorhizobium* sp. IV-10	grassland soil	Kasahara and Hattori, 1991
JF418133 (S, N)	DQ490353	99	Alphaproteobacteria	*Methylobacteriaceae* bacterium	volcanic deposit Kilauea volcano	unpublished study
JF418045 (N, NA)	JQ977299	100	Alphaproteobacteria	*Rhizobium* sp. Axs14	rhizosphere soil, Tianshan Mts., China	unpublished study
JF417995 (NA)	JQ396566	99	Alphaproteobacteria	*Rhizobium* sp. MN6-12	Arctic rhizosphere	unpublished study

solate (this study)	GenBank Accession	% Match	Phylum	Nearest Cultured Organism (GenBank)	Environment	Reference
JF418114 (NA)	NR029327	97	Alphaproteobacteria	*Sphingomonas asaccharolytica* Y-345	type strain	unpublished study
JF418101 (NA)	HQ825039	99	Alphaproteobacteria	*Sphingomonas* sp.	cryoconite hole, Austria	Lee *et al*.2011
JF418097 (N, NA)	Z23157	100	Alphaproteobacteria	*Sphingomonas* sp. BF14	air-handling system	Hugenholtz *et al*. 1995
JF418111 (NA)	JX949544	97	Bacteroidetes	*Mucilaginibacter* sp. MDB2-30	Chinese glacier	unpublished study
JF418079 (NA)	EF550172	99	Betaproteobacteria	*Acidovorax* sp.	arsenic contaminated soil, China	Fan *et al*. 2008
JF418028 (N)	GU213404	99	Betaproteobacteria	Beta proteobacterium 224	fresh granite, Damma Glacier	unpublished study
	JX304660	99	Betaproteobacteria	*Herbaspirillum* sp. JJ2206	Korean soil	unpublished study
JF418093 (N, NA)	FR682708	99	Betaproteobacteria	Beta proteobacterium R-36369	soil, Dronning Maud Land, Antarctica	Peeters, Ertz & Willems 2011
	JX304660	99	Betaproteobacteria	*Herbaspirillum* sp. JJ2206	Korean soil	unpublished study
JF418105 (NA)	AY561571	98	Betaproteobacteria	Beta proteobacterium RG-4	vadose zone sediment	Fredrickson *et al*. 2004
JF418103 (NA)	GQ354570	98	Betaproteobacteria	*Duganella* sp. HMD2171	mesotrophic artificial lake	unpublished study
JF418100 (NA)	AB495152	99	Betaproteobacteria	*Duganella zoogloeoides*		Tani *et al*. 2011
JF418127 (B)	EU130968	99	Betaproteobacteria	*Hydrogenophaga* sp.	water treatment filter	Magic-Knezev, Wullings & Van der Kooij 2009
JF418038 (N, NA)	D84572	99	Betaproteobacteria	*Janthinobacterium* sp. S21104		Mitsui *et al*. 1997
JF418117 (B)	JX177700	99	Betaproteobacteria	*Limnobacter* sp. 2D3	Baltic sea surface water	unpublished study
JF706699 (NA, B)	NR044274	98	Betaproteobacteria	*Massilia brevitalea*	type strain	Zul, Wanner & Overmann 2008
JF418121 (B)	GQ200828	99	Betaproteobacteria	*Massilia* sp. M1	raw milk	unpublished study
JF418065 (NA)	JX950006	99	Betaproteobacteria	*Massilia* sp. TMT2-56-2	Chinese glacier	unpublished study
JF418044 (N)	HM224491	99	Betaproteobacteria	*Massilia* sp. TPD44	permafrost headwaters, Urumqi River	unpublished study
JF418122 (B)	AB769202	99	Betaproteobacteria	*Methylibium* sp. UTPF84a	rice paddy field soil	unpublished study
JF418089 (NA)	FR682711	99	Betaproteobacteria	*Polaromonas* sp.	soil, Dronning Maud Land, Antarctica	Peeters, Ertz & Willems 2011
JF418129 (S)	AJ316618	96	Betaproteobacteria	*Thiobacillus plumbophilus* DSM6690		
JF418113 (NA)	JQ977458	99	Betaproteobacteria	*Variovorax* sp. Zs13	rhizosphere soil, Tianshan Mts, China	unpublished study
JF418026 (N)	CP000359	97	Deinococcus-	*Deinococcus geothermalis* DSM11300		unpublished study
JF418012 (NA)	EF093134	99	Deinococcus-	*Deinococcus* sp. VTT	Scottish stone monument	Suihko *et al*. 2007
JF418144 (S, B)	HE613268	92	Firmicutes	*Alicyclobacillus* sp.	human blood	unpublished study
	DQ999995	93	Firmicutes	Bacillales bacterium	opalinus clay	unpublished study
	AJ607430	92	Firmicutes	*Alicyclobacillus pohliae*	geothermal soil, Antarctica	Imperio, Viti & Marri 2008
JF418088 (NA)	KC865283	99	Firmicutes	*Staphylococcus warneri* 11BP	cherry (*Prunus avium)*	Serradilla *et al*. in press
JF417999 (NA)	FM955889	100	Gammaproteobacteri	*Pseudomonas moorei*	algal mat, Midre Lovenbreen	unpublished study
JF418010 (S, NA)	JQ977479	100	Gammaproteobacteri	*Pseudomonas* sp. Bma1	rhizoplane, Tianshan Mts,, China	unpublished study
Fungi						
JF418149 (NA)	JX982602	99	Ascomycota	*Cladosporium cladosporioides*	India	unpublished study
JF418153 (NA)	EU940042	100	Ascomycota	*Mniaecia nivea* M167	A bryosymbiont.	Stenroos *et al*. 2010
JF418150 (NA)	JX470336	100	Ascomycota	*Cladosporium cladosporioides*	Moon-1 Rover internal surface	unpublished study
JF418147 (NA)	JX303663	99	Ascomycota	*Aureobasidium pullulans* ZH1	Athletes foot-infected skin	unpublished study
JF418152 (NA)	AF548077	99	Ascomycota	*Microdochium nivale* UPSC 3273		Wu *et al*. 2003

Table 2. The most abundant OTUs (defined at 97% sequence similarity) among Fimmvörðuháls clone libraries. The two most abundant OTUs from each library, nine in total among all libraries due to abundance of certain OTUs in multiple libraries, are shown, and an accession number for a clone representative of that OTU. Number in parenthesis after OTU designation refers to alternative OTU designation in the combined clone library and isolate dataset (see text and Fig. 4; boxed branches). Cumultative contributions of each OTU to all libraries, their average and range of contribution, in addition to their nearest cultured and uncultured GenBank matches are also shown. Information regarding the known nutritional abilities of the associated cultured genera are also provided, and are discussed in the text.

OTU and accession	% in libraries (of total)	Average % per library (range)	Isolates	GenBank – closest uncultured			Genbank – closest cultured organism				
				Accession	% id	Source	Accession	% id	Genus/ species	Source (where disclosed)	Nutrition associated with genus
OTU63 (73) HQ899770	29.7	29.8 (9.4-48.0)	Yes	JQ684286 FM872918	99 99	Permafrost soil Floor dust	FR682708 JX304660	99 99	Beta proteobacterium R-36369 *Herbaspirillum* sp.	Antarctic soil	Diazotrophic
OTU45 (44) HQ899746	10.0	10.4 (0.0-36.3)	No	KC620647	98	Acid mine drainage sample	AJ316618	96	*Thiobacillus plumbophilus* DSM6690		Galena, H2 and iron oxidizer
OTU54 (71) HQ899721	9.8	9.7 (1.0-25.5)	Yes	JN178902	99	Kartchner Caverns, USA	NR074725	99	*Polaromonas* sp. JS666		Nitrogen fixation
OTU38 (37) HQ899891	9.0	9.0 (0.0-29.9)	No	DQ228366	99	Bench glacier	AB080643	97	*Sulfuricurvum kujiense*	Underground crude oil storage cavity	Sulfur-oxidizing chemolithotroph
OTU60 (76) HQ898929	7.5	7.5 (0.0-17.2)	Yes	JF703392	99	Root and rhizosphere soil	D84572	99	*Janthinobacterium* sp. S21104		
OTU59 (53) HQ899732	6.3	6.3 (0.0-23.4)	No	JF189275 KC286734	99 98	Human skin Glacial snow	EU636046 HQ699437 FJ812350	97 97 96	Antarctic bacterium GA0L *Actimicrobium antarcticum* *Herbaspirillum* sp.	Collins glacier Seashore water, Antarctica Soil	Diazotrophic
OTU49 (87) HQ899197	3.9	3.7 (0.0-28.4)	Yes	KC620646	98	Acid mine drainage sample	AJ316618	96	*Thiobacillus plumbophilus* DSM6690		Galena, H2 and iron oxidizer
OTU62 (55) HQ900023	2.2	2.2 (0.0-12.8)	No	AB488378	95	Rice paddy soil	HE613268 AB362268	95 94	*Alicyclobacillus* sp. *Alicyclobacillus* sp.	Human blood Subseafloor sediment	
OTU47 (45) HQ899794	2.0	1.9 (0.0-10.4)	No	KC110974	97	Soil microcosm	NR_074693	98	*Methylotenera versatilis*		Methylotroph

Fig. 1. Location map. Hillshade map generated from LiDAR data collected on NERC Airborne Research and Survey Facility Flight UR10/02 on 16 September 2010 and filtered with Sun's denoising algorithm (Stevenson *et al*., 2010).The sampling sites, denoted by numbers 1, 5 and 9, are located on the Fimmvörðuháls lava flow, between the Eyjafjallajökull and Mýrdalsjökull glaciers, in Southern Iceland. Flows (delineated by a broken line), originated from two craters, their positions as indicated by triangles (inset map; box denotes area of main map).

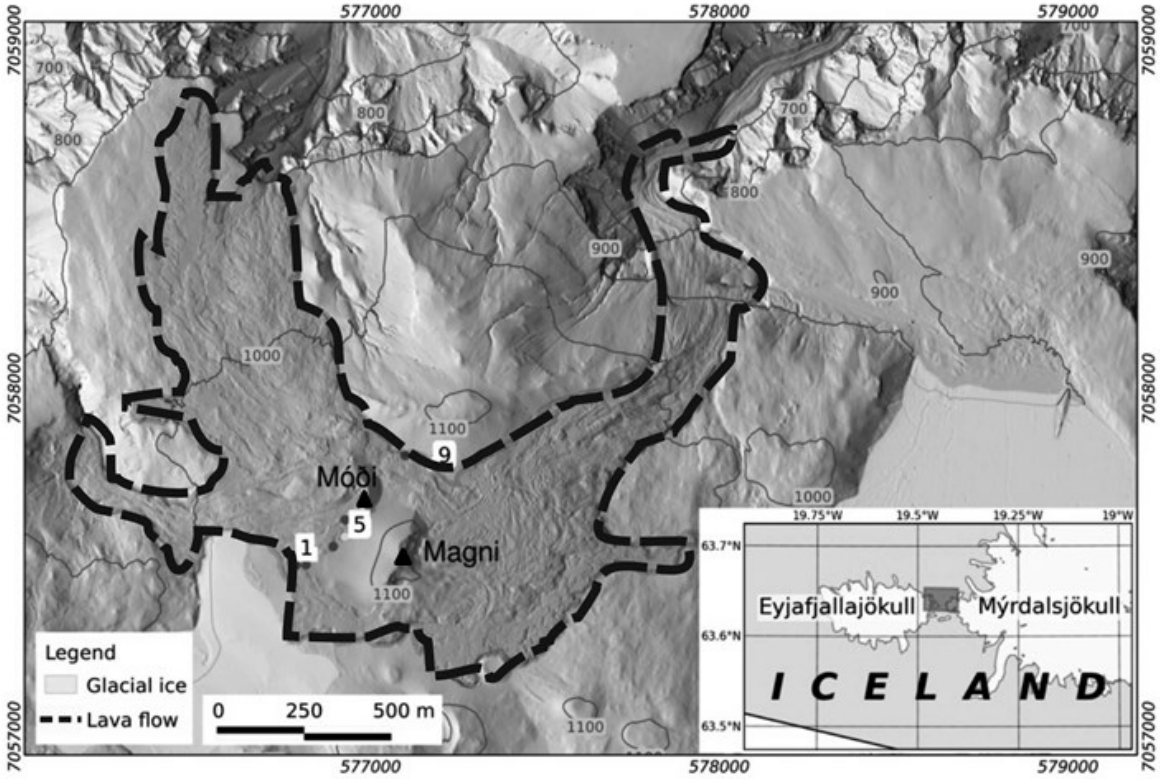


Fig. 2. Abundances of cells detected by fluorescent microscopy in lava samples (cells/g dry weight). Abundances correspond to the average for the three replicates for each site and season. Error bars represent standard deviations.

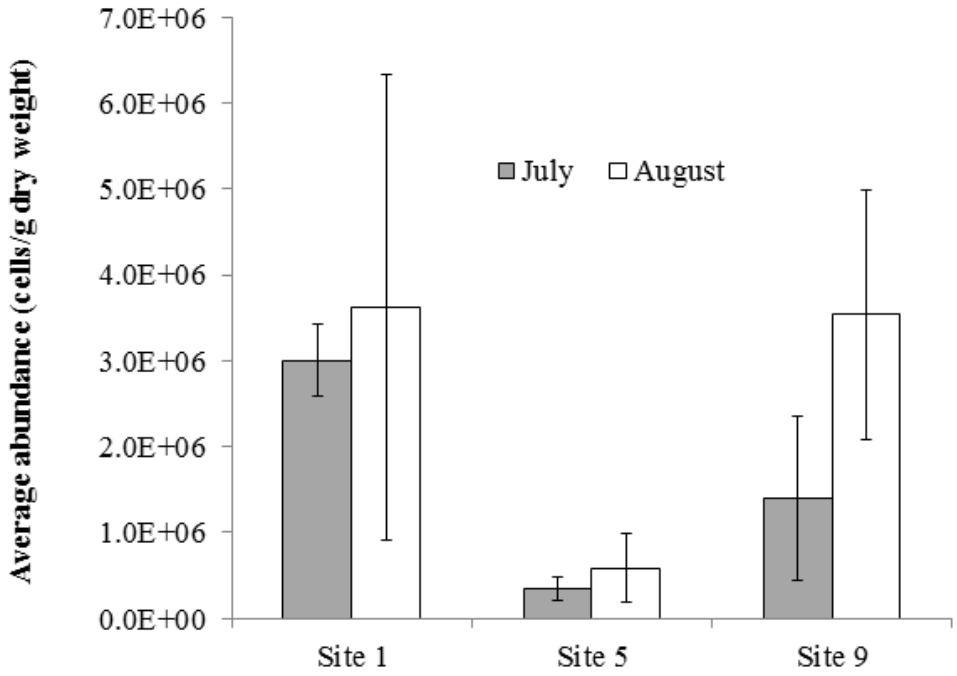


Fig 3. Richness and diversity of 16S rRNA gene clone libraries. Chao1 richness, and Shannon and Simpson diversity indices of individual Fimmvörðuháls lava samples, calculated in MOTHUR using 16S rRNA gene clone library sequences at 97% sequence identity. Upper and lower confidence intervals are also shown.

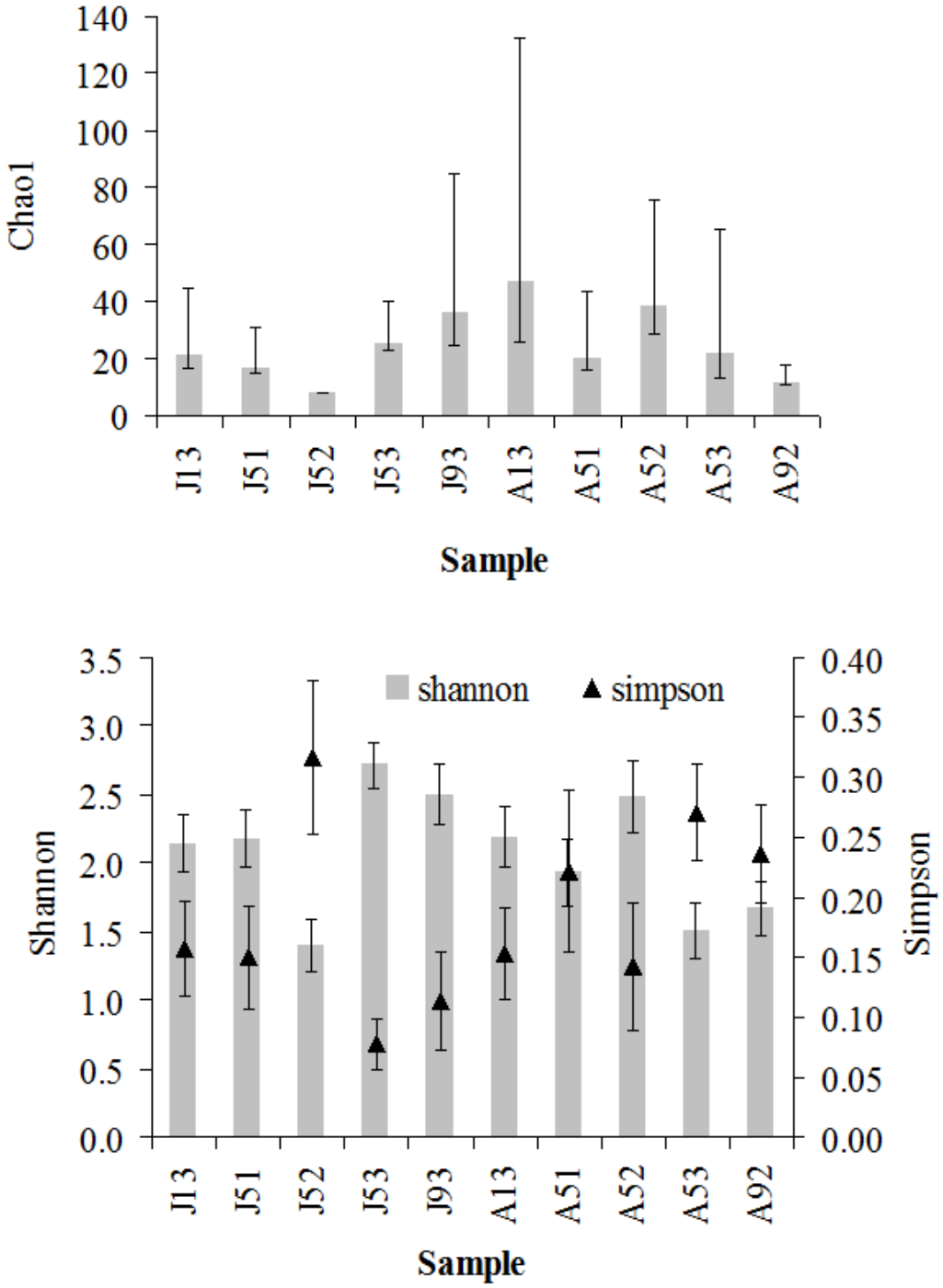


Fig. 4. Classification of 16S rRNA gene clones. Phylum- (and *Proteobacteria* sub-phylum) level classifications of bacterial 16S rRNA gene clones from July and August lava libraries. 'Weathered' represents basalt lava from the Icelandic Hnausahraun lava flow, erupted circa 150-300 A.D. (Kelly *et al.* 2011) and is given for comparison. All samples were analysed in the same manner**.** 'Others' refers to other phyla, each represented by a maximum of two clones in any Fimmvörðuháls library.

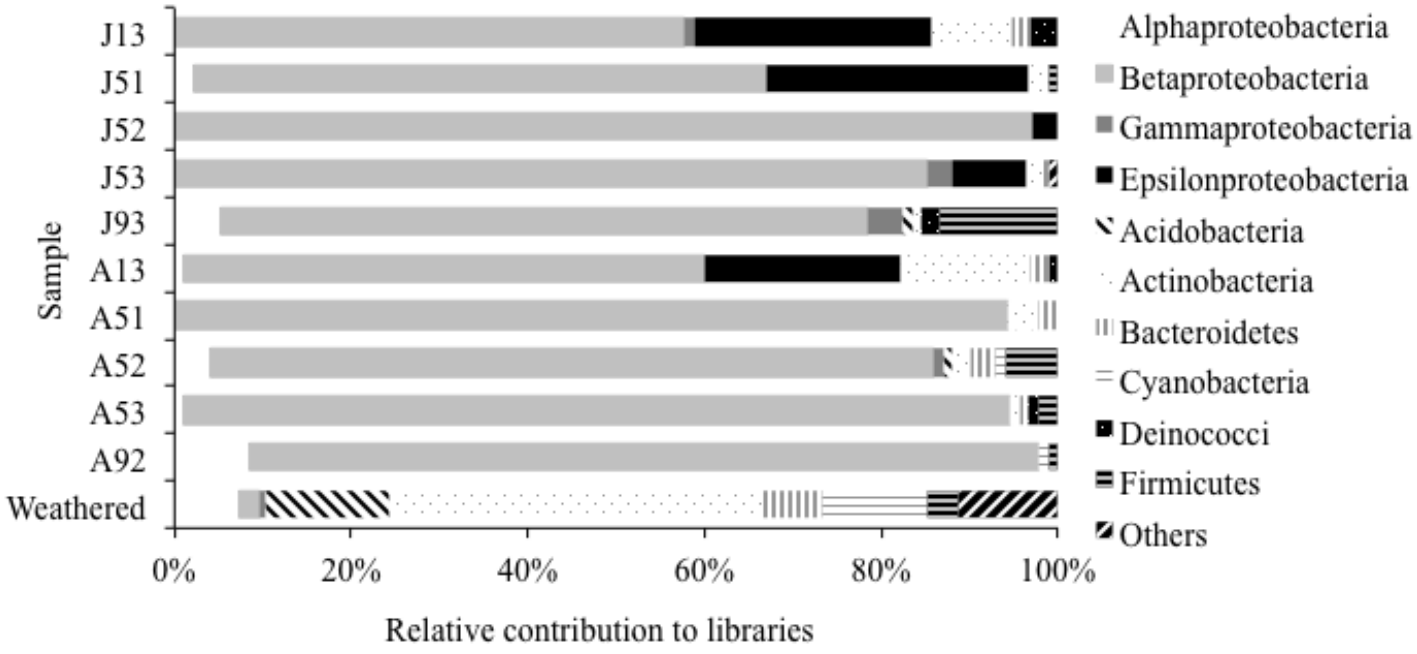


Fig. 5. Neighbor-Joining (NJ) phylogenetic tree, based on an alignment of 16S rRNA gene clones and isolate sequences from the Fimmvörðuháls lava flow. Numbers following OTU designations indicate the number of sequences represented by the OTU. **a**) full tree; **b**) expansion of *Betaproteobacteria* cluster of full tree. * indicates the OTU is represented by isolates only (number given in parentheses). ** Indicates the OTU is represented by both isolates and clones (numbers, respectively given in parentheses). The remaining OTUs are represented solely by clones (number given in parentheses). Highlighted OTUs represent those most abundant in libraries (Table 2). Values at nodes represent bootstrap values for the Maximum Likelihood (in parentheses) or Neighbour-Joining tree respectively, and are shown only where values exceeded 60% in one or both trees.

Figure 5 (a)

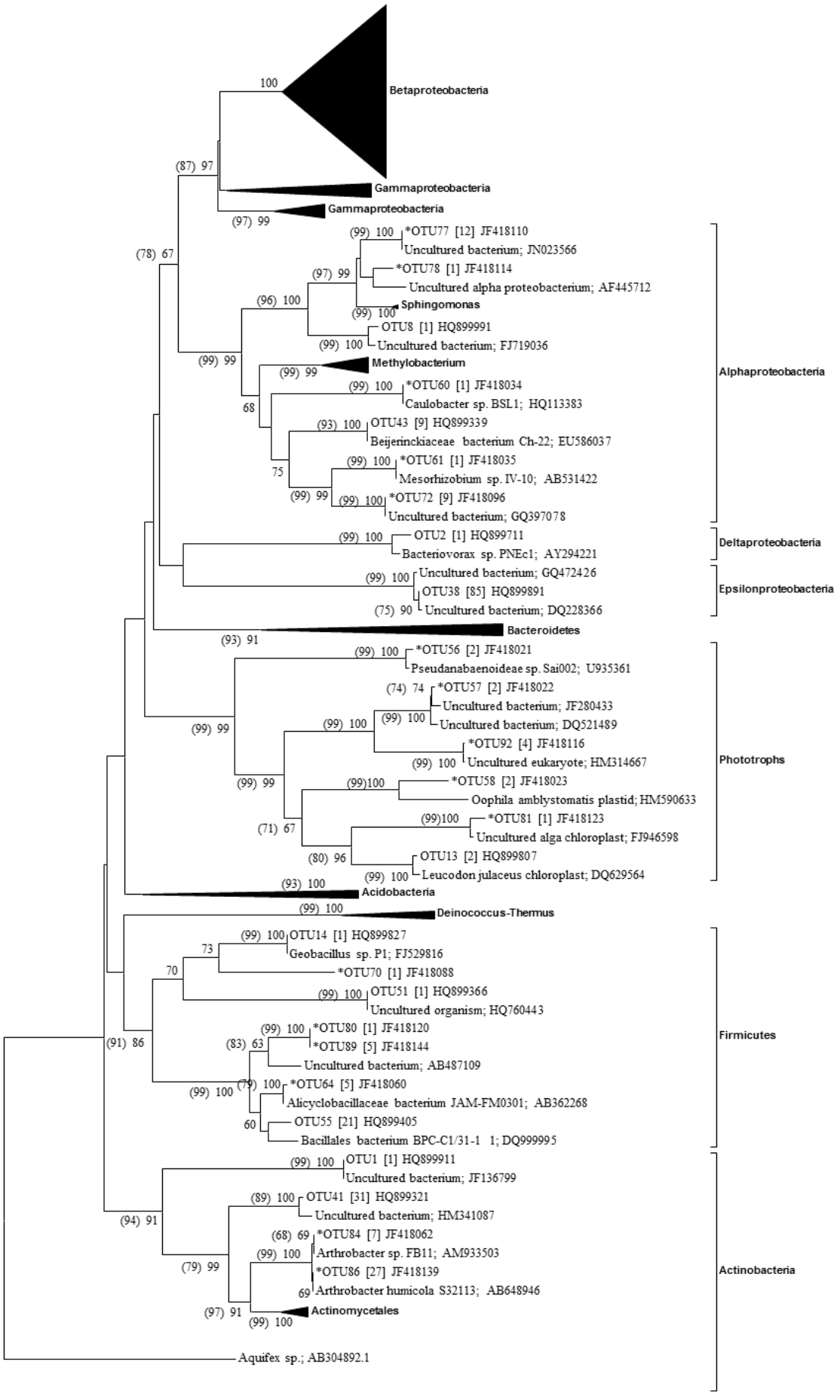
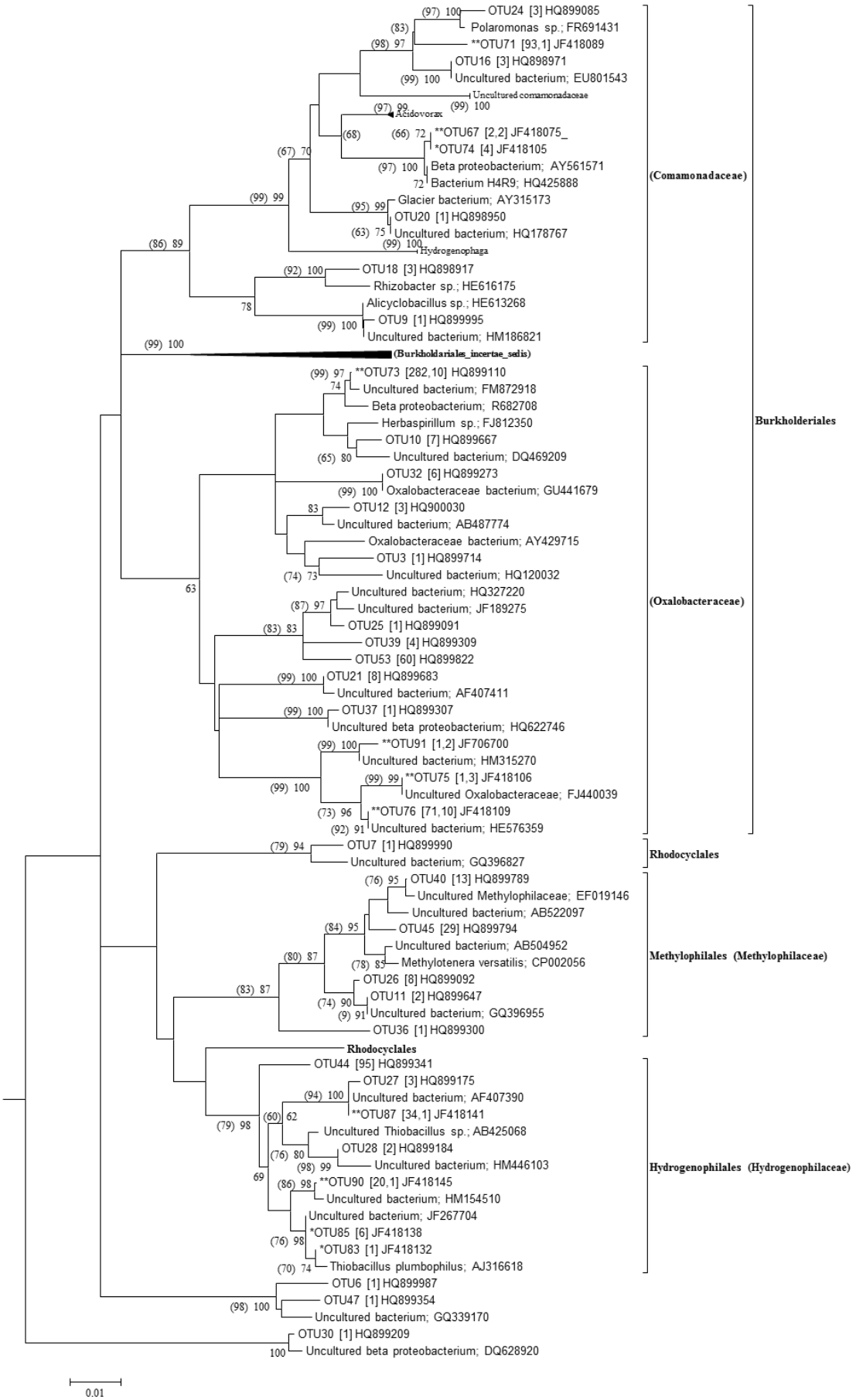

Betaproteobacteria
Gammaproteobacteria
Gammaproteobacteria
*OTU77 [12] JF418110
Uncultured bacterium; JN023566
*OTU78 [1] JF418114
Uncultured alpha proteobacterium; AF445712
Sphingomonas
OTU8 [1] HQ899991
Uncultured bacterium; FJ719036
Methylobacterium
*OTU60 [1] JF418034
Caulobacter sp. BSL1; HQ113383
OTU43 [9] HQ899339
Beijerinckiaceae bacterium Ch-22; EU586037
*OTU61 [1] JF418035
Mesorhizobium sp. IV-10; AB531422
*OTU72 [9] JF418096
Uncultured bacterium; GQ397078
Alphaproteobacteria
OTU2 [1] HQ899711
Bacteriovorax sp. PNEc1; AY294221
Deltaproteobacteria
Uncultured bacterium; GQ472426
OTU38 [85] HQ899891
Uncultured bacterium; DQ228366
Epsilonproteobacteria
Bacteroidetes
*OTU56 [2] JF418021
Pseudanabaenoideae sp. Sai002; U935361
*OTU57 [2] JF418022
Uncultured bacterium; JF280433
Uncultured bacterium; DQ521489
*OTU92 [4] JF418116
Uncultured eukaryote; HM314667
*OTU58 [2] JF418023
Oophila amblystomatis plastid; HM590633
*OTU81 [1] JF418123
Uncultured alga chloroplast; FJ946598
OTU13 [2] HQ899807
Leucodon julaceus chloroplast; DQ629564
Phototrophs
Acidobacteria
Deinococcus-Thermus
OTU14 [1] HQ899827
Geobacillus sp. P1; FJ529816
*OTU70 [1] JF418088
OTU51 [1] HQ899366
Uncultured organism; HQ760443
*OTU80 [1] JF418120
*OTU89 [5] JF418144
Uncultured bacterium; AB487109
*OTU64 [5] JF418060
Alicyclobacillaceae bacterium JAM-FM0301; AB362268
OTU55 [21] HQ899405
Bacillales bacterium BPC-C1/31-1 1; DQ999995
Firmicutes
OTU1 [1] HQ899911
Uncultured bacterium; JF136799
OTU41 [31] HQ899321
Uncultured bacterium; HM341087
*OTU84 [7] JF418062
Arthrobacter sp. FB11; AM933503
*OTU86 [27] JF418139
Arthrobacter humicola S32113; AB648946
Actinomycetales
Actinobacteria
Aquifex sp.; AB304892.1
0.05

Figure 5 (b)


OTU24 [3] HQ899085
Polaromonas sp.; FR691431
**OTU71 [93,1] JF418089
OTU16 [3] HQ898971
Uncultured bacterium; EU801543
Uncultured comamonadaceae
Acidovorax
**OTU67 [2,2] JF418075_
*OTU74 [4] JF418105
Beta proteobacterium; AY561571
Bacterium H4R9; HQ425888
Glacier bacterium; AY315173
OTU20 [1] HQ898950
Uncultured bacterium; HQ178767
Hydrogenophaga
OTU18 [3] HQ898917
Rhizobacter sp.; HE616175
Alicyclobacillus sp.; HE613268
OTU9 [1] HQ899995
Uncultured bacterium; HM186821
(Comamonadaceae)
(Burkholdariales_incertae_sedis)
**OTU73 [282,10] HQ899110
Uncultured bacterium; FM872918
Beta proteobacterium; R682708
Herbaspirillum sp.; FJ812350
OTU10 [7] HQ899667
Uncultured bacterium; DQ469209
OTU32 [6] HQ899273
Oxalobacteraceae bacterium; GU441679
OTU12 [3] HQ900030
Uncultured bacterium; AB487774
Oxalobacteraceae bacterium; AY429715
OTU3 [1] HQ899714
Uncultured bacterium; HQ120032
Uncultured bacterium; HQ327220
Uncultured bacterium; JF189275
OTU25 [1] HQ899091
OTU39 [4] HQ899309
OTU53 [60] HQ899822
OTU21 [8] HQ899683
Uncultured bacterium; AF407411
OTU37 [1] HQ899307
Uncultured beta proteobacterium; HQ622746
**OTU91 [1,2] JF706700
Uncultured bacterium; HM315270
**OTU75 [1,3] JF418106
Uncultured Oxalobacteraceae; FJ440039
**OTU76 [71,10] JF418109
Uncultured bacterium; HE576359
(Oxalobacteraceae)
Burkholderiales
OTU7 [1] HQ899990
Uncultured bacterium; GQ396827
Rhodocyclales
OTU40 [13] HQ899789
Uncultured Methylophilaceae; EF019146
Uncultured bacterium; AB522097
OTU45 [29] HQ899794
Uncultured bacterium; AB504952
Methylotenera versatilis; CP002056
OTU26 [8] HQ899092
OTU11 [2] HQ899647
Uncultured bacterium; GQ396955
OTU36 [1] HQ899300
Methylophilales (Methylophilaceae)
Rhodocyclales
OTU44 [95] HQ899341
OTU27 [3] HQ899175
Uncultured bacterium; AF407390
**OTU87 [34,1] JF418141
Uncultured Thiobacillus sp.; AB425068
OTU28 [2] HQ899184
Uncultured bacterium; HM446103
**OTU90 [20,1] JF418145
Uncultured bacterium; HM154510
Uncultured bacterium; JF267704
*OTU85 [6] JF418138
*OTU83 [1] JF418132
Thiobacillus plumbophilus; AJ316618
Hydrogenophilales (Hydrogenophilaceae)
OTU6 [1] HQ899987
OTU47 [1] HQ899354
Uncultured bacterium; GQ339170
OTU30 [1] HQ899209
Uncultured beta proteobacterium; DQ628920
0.01