Extremophiles
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ORIGINAL PAPER

Bacterial diversity in fumarole environments of the Paricutín volcano, Michoacán (Mexico)

Miguel Medrano-Santillana[1] · Elcia Margaret Souza-Brito[2] · Robert Duran[3] · Felix Gutierrez-Corona[1] · Georgina Elena Reyna-López[1]

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Abstract Active volcanoes are among the most extreme environments on Earth. The extreme temperatures, presence of toxic heavy metals and low nutrient bioavailability favor the development of extremophiles. We characterized the physical–chemical parameters of and bacterial communities (T-RFLP and 16S rRNA gene libraries) inhabiting fumarole niches of the Paricutín volcano located in Michoacán (Mexico). This volcano, which surged in 1943, is one of the youngest volcanoes on Earth and the microbial diversity in this area is yet to be characterized. The sampling stations were characterized in a pH range from 5.34 to 7.89 and showed different temperatures (soil, 27–87 °C; air, 13.6–56 °C) with high concentrations of metals such as iron and arsenic. The most abundant bacterial populations, confirmed by T-RFLP and 16S rRNA gene libraries, were related to members of Firmicutes and Proteobacteria phyla including sequences associated with thermophiles and sulfate reducing bacteria. Overall, the Paricutín volcano showed low bacterial diversity and its prokaryotic diversity was characterized by the impossibility of amplifying Archaea-related sequences.

Keywords Extreme environments · Bacterial communities · T-RFLP · 16S rRNA gene

Introduction

Volcanism may be defined as "the manifestation at the surface of a planet or satellite of internal thermal processes through the emission of solid, liquid, or gaseous products" (Francis 1993; Herrera and Cockell 2007). Environments resulting from volcanic activities are diverse, ranging from acidic hot springs to deep ocean basaltic habitats. Geothermal heating of groundwater near the surface and/or degassing of magma result in fumaroles characterized by the production of steam and volcanic gases (Costello et al. 2009). Since volcanic environments, especially active fumaroles (Hynek et al. 2011), are considered analogous to some of the earliest environments on Earth (Van Kranendonk and Pirajno 2004), the study of their microbial diversity may provide insights into the origin of life and extraterrestrial life as suggested by Benson et al. (2011). However, only a few studies revealing the Prokaryote (Bacteria and Archaea) diversity of soil fumaroles (Costello et al. 2009; Mayhew et al. 2007; Stott et al. 2008), steam deposits (Benson et al. 2011; Mayhew et al. 2007; Wall et al. 2015) and steam aerosols (Ellis et al. 2008) have been conducted. These studies have pointed to fumaroles as diversity hot spots for extremophile microorganisms with the potential to discover new taxa and novel metabolic capacities (Benson et al. 2011).

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✉ Miguel Medrano-Santillana
cuatro100@hotmail.com

✉ Robert Duran
robert.duran@univ-pau.fr

[1] Departamento de Biología, Universidad de Guanajuato, Guanajuato, Mexico

[2] Ingeniería Ambiental, División de Ingenierías, Universidad de Guanajuato, Guanajuato, Mexico

[3] Equipe Environnement et Microbiologie-MELODY group-UMR IPREM5254, BP 1155 Université de Pau et des Pays de l'Adour, 64013 Pau Cedex, France

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The Paricutín volcano (Michoacán, Mexico) began to emerge in 1943 with pyroclastic explosions, followed by volcanic bombs, which covered around 18.5, and 2 km^3 of lava fluxes. Nowadays it has an altitude of 2808 m a.s.l.; its cone is approximately 200 m high; and it features a parasitic volcano called *Sapichu* on the lateral north wall. It is formed mainly of andesites and basalts with little or no difference in its SiO_2 contents (Rodríguez-Elizarrarás et al. 1993). This volcano is not only interesting for volcanologists, but also offers a window to study biological colonization, especially the establishment of microbial communities (Sprent 1993). The Paricutín volcano fumaroles may represent a hot spot for extremophiles, inhabited by life forms adapted to the extreme environmental constraints, such as high temperatures inside the fumaroles in contrast with a cool atmospheric temperature, low water content, unstructured soil, high radiation, etc. In the present study we analyzed the bacterial diversity in several fumaroles showing different physical–chemical characteristics. Among the several small fumaroles the Paricutín volcano has, we selected six (three near the base, two in the middle and one at the top) corresponding to different habitats, with three of them featuring a thin microbial mat encrusted on the fumarole wall. Since fumaroles are considered as islands of biodiversity (Costello et al. 2009), we hypothesized that the microbial communities inhabiting fumaroles with different physical–chemical characteristics may provide information on the volcanoe's ecological niches for specific microorganisms. The bacterial diversity was characterized using culture-independent methods, terminal restriction fragment length polymorphism (T-RFLP) and 16S rRNA gene library analyses. The comparison of structures and composition of the bacterial communities between the diverse fumarole stations led to the determination of specific bacterial populations according to the physical–chemical conditions.

Materials and methods

Sampling site and chemical characterization

The Paricutín volcano (Supplementary Fig. S1) is located in San Juan Nuevo Parangaricutiro, Michoacán, Mexico. Six fumarole stations with a diameter of 50–60 cm were selected around the Paricutín volcano (Fig. 1): stations S1, S2 and S6, close to the base of the volcano; stations S3 and S4 on the crater slope and station S5 close to the crater rim. The characteristics of the stations are given in Table 1. The samples were collected in January 2010 (dry season). During sampling, the temperature of the vent walls and surrounding air as well as the relative air humidity were measured.

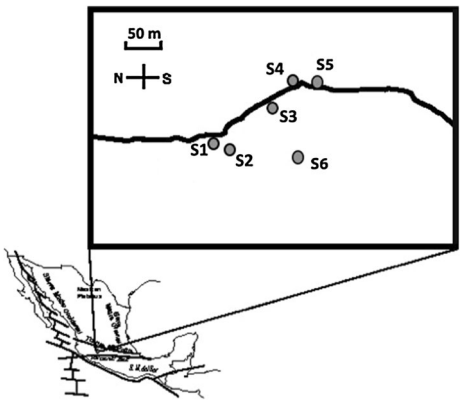

Fig. 1 Map showing the location of the Paricutín volcano and schematic position of the sampling stations. The sampling stations are indicated as follows: *S1* 19°29′27, 102°15′21′; *S2* 19°29′27′, 102°15′21′; *S3* 19°29′50′, 102°15′29′; *S4* 19°29′54′, 102°15′12′; *S5* 19°29′33′, 102°15′08′ and *S6* 19°29′25′, 102°15′23′

The internal surface layer (about 0.5 cm) of the fumarole walls was sampled with a sterile plastic spoon. For each station, approximately 20 g of soil were collected. A portion of the sample (approximately 10 g) was preserved using the LifeGuard Soil Preservation Solution® (MoBio) according to the manufacturer's instructions and stored at −20 °C until DNA extraction was performed for the molecular microbial analyses. The remaining portion of the sample was used for pH determination and chemical characterization following the Mexican norm NMX-AA-132-SCFI-2006 (2006). The metal analysis was carried out by flame atomic absorption (FLAA) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using standard methods (Soil Screening Guidance: User's Guide Publication 2nd edition 1996) at the "Centro de Investigacion en Materiales Avanzados S.C., Chihuahua, Mexico". The theoretical detection limits were based on a sample weight of 0.5 g with a volume of 100 ml.

DNA isolation and amplification of 16S rRNA gene fragment sequences

One to ten grams of soil sample were used to extract DNA with the Mobio Powersoil kit™. Extracted DNA was stored at −20 °C pending further analysis. The primers Arch21f and Arch958r (5′-TTCCGGTTGATCCYGCCGGA-3′ and 5′-YCCGGCGTTGAMTCCAATT-3′, respectively; DeLong 1992) were used for archaeal 16S rRNA gene amplification as previously described (Stauffert et al. 2014). The bacterial 16S rRNA gene was amplified with 8F and 907R primers (5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-GCCCCCGTCAATTCMTTTRAGTTT-3′, respectively; Lane 1991) in a PTC 200 Thermo-cycler (MJ research) as described in Guyoneaud et al. (2002). The

Table 1 Geographic coordinates of the stations and physical characteristics determined at the sampling time

Stations	S1	S2	S3	S4	S5	S6
Geographic data						
N coordinates	19°29″27′	19°29″27′	19°29″50′	19°29″54′	19°29″33′	19°29″25′
W coordinates	102°15″21′	102°15″21′	102°15″29′	102°15″12′	102°15″08′	102°15″23′
A (m.a.s.l.)	2634	2632	2680	2765	2763	2627
Physical data[a]						
ST (°C)	68.5	71.3	62	63	80	70.6
AT (°C)	55	55	56	41	40	48
RH (%)	20	72	75	73	67	73
SC	Red	Red, black	Red	Beige	Brown	Green, red, black
pH	6.42	6.58	6.11	5.34	7.98	7.89

[a]*A* altitude, *ST* soil temperature, *AT* air temperature, *RH* relative humidity, *SC* soil color

polymerase chain reaction (PCR) products were purified using a GFX PCR DNA purification kit (Amersham Biosciences) according to the manufacturer's instructions.

T-RFLP and 16S rRNA gene library analyses

T-RFLP analysis was performed with the 16S rRNA encoding genes amplified by labeled 8F [6-carboxifluorescein (FAM) label] and unlabeled 907R primers as previously described (Dias et al. 2008). Briefly, the purified PCR products were digested with 3 U of restriction enzymes *Hae*III or *Hin*P1I at 37 °C for 3 h. Approximately 50 ng of digested DNA were mixed with 20 µL of deionized formamide and 0.5 µL of TAMRA® ladder (Applied Biosystem®) and then denatured (94 °C for 5 min and cooled on ice). The lengths of the terminal restriction fragments (T-RFs) were determined by capillary electrophoresis on an ABI prism 310 (Applied Biosystem®) as previously described (Bordenave et al. 2004a). The T-RFLP profiles were analyzed using Gene Scan Software (Applied Biosystem®). Data sets were normalized as previously described (Brito et al. 2009). T-RFs representing less than 1% of total fluorescence were eliminated (Hewson and Fuhrman 2006) and then T-RFs with a length of ±1 bp were aggregated (Caretta and Brito 2011). Each T-RF was considered as an operational taxonomic unit (OTU; Said et al. 2010). Multivariate analyses of T-RFLP data were carried out with MVSP software (Multi-Variate Statistical Package 3.1, Kovach Computing Services).

For the 16S rRNA gene libraries, the amplified 16S rRNA gene fragments using unlabeled 8F and 907R primers were cloned in *E. coli* with the pCR2.1 TOPO® TA cloning kit (Invitrogen, Inc.) as described in Stauffert et al. (2013). At least 100 clones per library were analyzed. The cloned 16S rRNA gene fragments were amplified by PCR using M13 primers surrounding the insertion site as previously described (Pringault et al. 2008). Then the PCR products were sent to CINESTAV LangeBio (Mexico) for sequence determination. The obtained sequences were analyzed with BioEdit (Hall 1999) and the chimeras were removed with Pintail (Ashelford et al. 2005). Then, the online BLAST tool was used to compare the sequences with those deposited in the database of the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) and aligned using ClustalW program (Thompson et al. 1994) in BioEdit. To determine the phylogenetic relationship of the retrieved sequences from the Paricutín volcano fumarole environments, the aligned sequences were used to construct phylogenetic trees with MEGA 5.0 (Tamura et al. 2011) using the neighbour-joining method (Saitou and Nei 1987), with a bootstrap of 1000 replications. The OTU was defined as a group of clones with greater than 97% sequence similarity as previously recommended (Giloteaux et al. 2010). The coverage was used to estimate the exploration efficiency for each library (Chao and Lee 1992; Good and Toulmin 1956). The statistical significance of pairwise differences in OTU composition between libraries was assessed using LIBSHUFF (Singleton et al. 2001) (http://www.mothur.org/wiki/Libshuff).

Nucleotide sequence accession numbers

The sequences obtained in this study were submitted to the EMBL database and assigned accession numbers KM102547 to KM102646.

Results

Physical–chemical characterization

The samples were collected in the dry season, when the relative air humidity at the sampling stations ranged from 20 to 75% (Table 1). The temperature of the samples ("soil" in the case of the S5 or wall layer for the others) ranged from 62 to 80 °C, while the fumarole air temperature ranged

from 40 to 56 °C, placing the site between the thermophile and hyperthermophile levels. The samples were quite rich in heavy metals in comparison to non-volcanic soils but, compared to those observed in other volcanic soils (Aguilera et al. 2002; Amaral et al. 2006; Borie and Rubio 2003; Delfosse et al. 2003; Dœlsch et al. 2006; Taniai et al. 2012), the metal concentrations were similar for Fe, S, Pb and Cu; lower for Cr and Zn; and higher in the case of P (Table 2).

Bacterial community structure

First of all, it is important to indicate that we were unable to obtain Archaea 16S rRNA gene sequences after various attempts following previously described PCR procedures (Stauffert et al. 2014, 2015). Such difficulties have been previously described and were attributed to DNA extraction procedures being poorly suited to fumarole samples (Ackerman et al. 2007; Mayhew et al. 2007). As alternative DNA extraction procedures tested, including that proposed by Benson et al. (2011), were unsuccessful, we thus focused our study on bacterial diversity. The bacterial community structures were characterized by T-RFLP analysis with the *Hae*III and *Hin*P1I restriction enzymes (Supplementary Fig. S2). Each detected terminal restriction fragment (T-RF) was considered as representative of an operational taxonomic unit (OTU). In general, both enzymes showed similar richness for stations S1, S2 and S5 (between 31 and 44 T-RF for *Hae*III and between 20 and 34 T-RFs for *Hin*P1I) while OTU richness was higher for stations S3, S4 and S6 (between 55 and 72 T-RF for *Hae*III and between 47 and 65 T-RFs for *Hin*P1I). The canonical correspondence analysis (CCA) showing the correlation between T-RFLP and chemical data distributed the bacterial community structures into three clusters (Fig. 2). The bacterial community structures were clustered according to their position on the volcanic cone. First, bacterial communities from stations S1 and S2 at the base of the cone constituted a cluster driven by sulfur, phosphorus and air temperature. Second, bacterial communities from stations

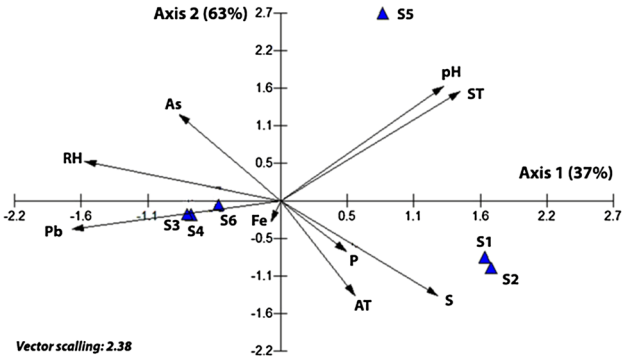

Fig. 2 Correlations between bacterial community structures (T-RFLP patterns) and the environmental chemical parameters by a canonical correspondence analysis (CCA). The bacterial communities from station S1 to station S6 are indicated by *triangles*. The *arrows* show the influence of environmental factors (pH, *ST* soil temperature, *AT* air temperature, *RH* relative air humidity (%), *P* phosphorus, *S* sulfur, *As* arsenic and *Pb* lead concentrations)

located on the volcano slope (S3 and S4) were clustered with those of station S6, which were strongly correlated with lead and relative air humidity. Finally, at the crater rim (S5) pH and soil temperature are the main factors driving the bacterial community structure. It is noteworthy that axis 1 and 2 explained 37 and 63% of data distribution, respectively.

Bacterial diversity

To determine the bacterial diversity we constructed five 16S rRNA gene libraries. The libraries S_3, S_4, S_5 and S_6 corresponding to stations S3, S4, S5 and S6, respectively, and the library S_{1+2} combining PCR products from stations S1 and S2 because they showed similar T-RFLP patterns for both enzymes (Supplementary Fig. S2) and close physical–chemical characteristics except for Fe (Table 2). After removing chimeras, 524 sequences (around 100 sequences per library) were analyzed. The sequences were dominated by sequences related to Firmicutes (33%) and

Table 2 Soil metal content of the fumarole stations at Paricutin volcano

Metal (mg/kg)	Stations					
	S1	S2	S3	S4	S5	S6
As	0.03	ND	2.04	4.41	4.23	0.95
Cr	ND	ND	ND	ND	0.005	ND
Cu	24.09	ND	ND	ND	4.03	3.05
Fe	20	34,546	34,546	2580	10,079	7876
P	395.12	259.78	401.15	136.12	198.92	192.35
Pb	22.09	7.32	51.52	25.61	14.72	50.56
S	256.28	193.23	184.06	98.36	94.17	120.88
Zn	ND	ND	ND	ND	ND	ND

ND not detected

Proteobacteria (30%) (Fig. 3a). The coverage of the libraries ranged between 85 and 97% (Supplementary Table S1) indicating that a good representation of the diversity was collected. In agreement with the T-RFLP analysis, 16S rRNA gene libraries showed low bacterial diversity. The Simpson diversity index indicated that libraries S_{1+2} and S_5 had higher diversity than libraries S_3, S_4 and S_6. Library comparisons with the Jaccard similarity index and LIBSHUFF suggested that each station showed a different bacterial diversity (Supplementary Table S2). The distribution of the retrieved sequences at the species level illustrated the different bacterial diversities among the stations (Fig. 3b). Phylogenetic trees were constructed for Firmicutes (Fig. 4), Proteobacteria (Fig. 5) and the other phyla (Fig. 6). The fumaroles were characterized by the presence of sequences related to *Massilia* genera (nearly 60% in S_{1+2} and S_5, 10% in S_3 and 15% in S_6), which were found at most stations (Fig. 3b). The phylogenetic analyses revealed sequences affiliated to *Thermovenabulum* genera in S_4 (50%). The other relatively abundant sequences were related to *Pseudomonas contantini* (40% in S_5 and 12% in S_6), *Paenibacillus* (40% in S_{1+2}), *Naxibacter* and *Acidovorax* representing (25 and 20%, respectively in S_6). Sequences associated with thermophile and acidophilic species were detected in abundance, which is in agreement with the environmental conditions prevailing at the sampling stations.

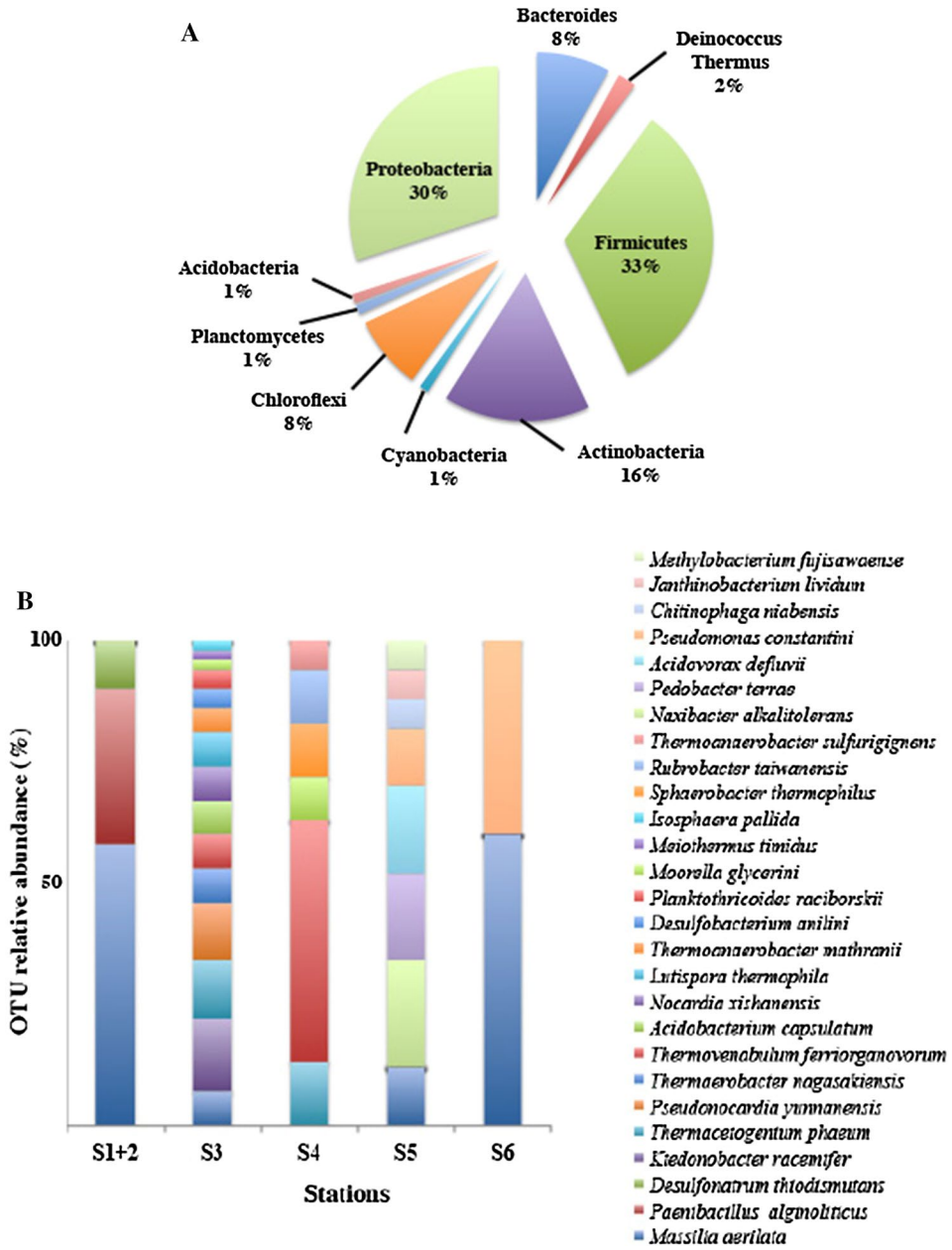

Fig. 3 Distribution of 16S rRNA gene sequences. **a** Distribution of the retrieved sequences within the bacterial phyla considering all the fumarole stations. **b** Distribution of the retrieved sequences in each fumarole station. The sequences were analyzed at the species level applying a threshold similarity of 97%

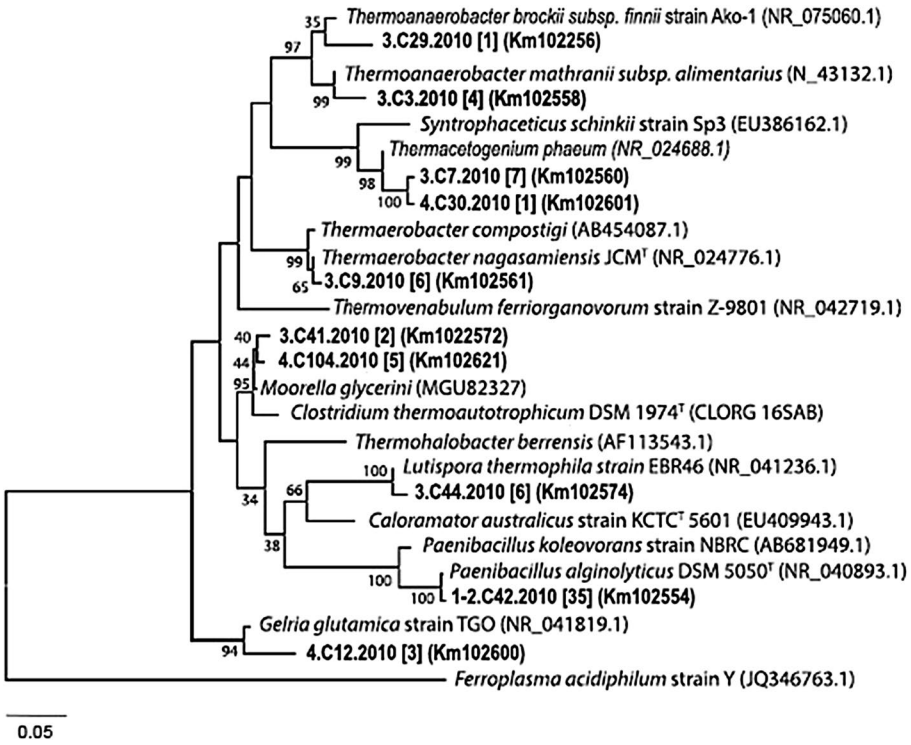

Fig. 4 Phylogenetic tree of sequences affiliated to Firmicutes. The neighbour-joining tree is based on 16S rRNA gene sequences. Bootstrap values (1000 replicates) over 40% are indicated at the nodes. Accession numbers are indicated in parenthesis. Numbers in brackets indicate the relative abundance. *Bar* 0.05 nucleotide substitution per site

Discussion

The Paricutín volcano is a young terrestrial volcano. Although it is not an active volcano, it still shows high fumarole activity, probably due to its location in the center of the neovolcanic Mexican axis. In this region, other volcanoes are still active such as Ceboruco, Colima and Nevado de Toluca for example (Espinosa-Rodríguez et al. 2014). Volcanic fumaroles are considered to be extreme environments due to their high temperature and high metal concentrations (Benson et al. 2011; Li et al. 2015). As expected Fe and S concentrations were found to be high (Table 2). They were similar to those found in other volcanoes (Aguilera et al. 2002) but quite above the Mexican norm (NMX-AA-132-SCFI-2006) for soils. The stations presented homogeneous phosphorus concentrations, which were higher than those reported for other volcanic soils (Borie and Rubio 2003). These elements are not considered toxic for humans, nor do they represent an environmental pollution problem, and are usually found in high concentrations in volcanic environments. In contrast Pb, Cu and As are classified as hazardous elements due to the risk they represent to ecosystems and human health. They were found in very high concentrations. The As content fluctuated from low concentrations for stations S1 and S2 ($\leq$0.03 mg kg^{-1}) to high concentrations for stations S4 and S5 where concentrations were 100 times higher (4.41 and 4.23 mg kg^{-1}, respectively). The Pb and Cu concentrations were far in excess of the environmental regulations (0.01 mg kg^{-1}) in accordance with reports for other volcanic sites (Delfosse et al. 2003; Taniai et al. 2012). It is noteworthy that Cr and Zn were not detected in any of the samples.

In spite of the harsh conditions prevailing in such environments, microorganisms probably develop taking advantage of their capacities to utilize metals for their development (Faure et al. 2015a, b; Rampelotto 2013). A recent study at the *Los Azufres* geothermal site (Mexico) revealed that such extreme Mexican environments are practically unexplored from a microbial diversity point of view (Brito et al. 2014). To further characterize the microbial diversity in unexplored extreme Mexican sites, the study aimed to examine the prokaryotic microbial diversity of different fumaroles in the Paricutín volcano and thus characterize the prokaryotic microbial colonization of such harsh environments. However, despite various trials we were unable to amplify archaeal 16S rRNA genes suggesting that Archaea were not present in the Paricutín volcano fumaroles studied. Further efforts are still required to exclude any analytical bias due to DNA extraction, primers used and PCR conditions (Nocker et al. 2007). In contrast, the bacterial 16S rRNA gene was efficiently amplified in all samples allowing the analysis of bacterial diversity. Although the methods (fingerprinting and cloning/sequencing) used to describe the bacterial community structures and compositions provide lower diversity coverage compared to high-throughput sequencing (HTS) methods, we were able to reveal the relationships between the most abundant bacterial populations and fumarole environmental conditions.

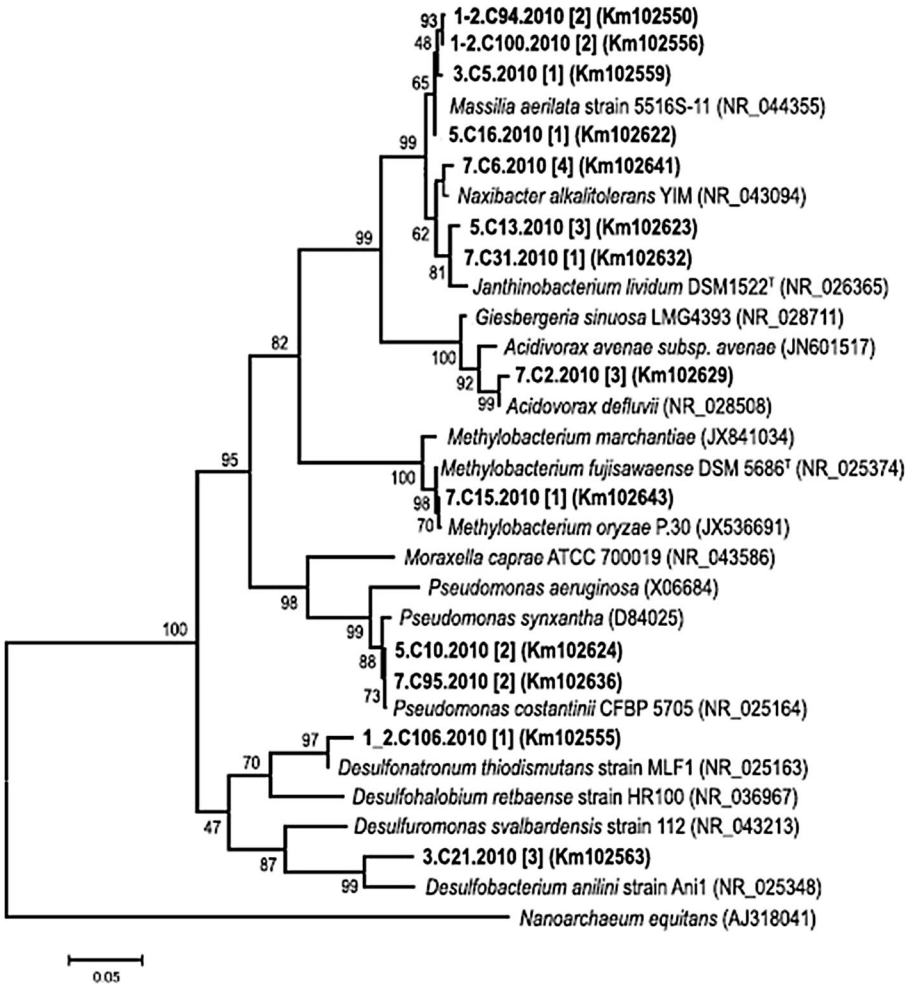

Fig. 5 Phylogenetic tree of sequences affiliated to Proteobacteria. The neighbour-joining tree is based on 16S rRNA gene sequences. Bootstrap values (1000 replicates) over 40% are indicated at the nodes. Accession numbers are indicated in *parenthesis*. *Numbers* in *brackets* indicate the relative abundance. *Bar* 0.05 nucleotide substitution per site

In spite of its intrinsic drawbacks, T-RFLP has proven to be a valuable method for comparing bacterial communities (Bordenave et al. 2004b; Nocker et al. 2007; Schütte et al. 2008), especially in volcanic environments (Curtis et al. 2013; Emerson and Moyer 2010; Mayhew et al. 2007). The cloning/sequencing approach results in longer sequences than the HTS's short reads, thereby providing a more robust phylogenetic affiliation. It also helps to supplement the prokaryotic 16S rRNA gene sequence databases, which is an essential step to improve the census of prokaryotic 16S rRNA sequences (Schloss et al. 2016). Both T-RFLP and 16S rRNA gene library analyses showed a low bacterial diversity as described for extreme environments such as acid mine drainage (Bruneel et al. 2008; Fahy et al. 2015), geothermal vents (Brito et al. 2014) and volcanic environments (Kelly et al. 2014; Mayhew et al. 2007). However, underestimation of the bacterial diversity due to the inherent bias of PCR-based analysis (Nocker et al. 2007) cannot be excluded, particularly considering the primers' specificity which could limit the targeted sequences (Weisburg et al. 1991). Nevertheless, the comparison of bacterial diversity between the stations revealed lower OTU richness for the hottest stations S1, S2, S5 and S6 than that observed for stations S3 and S4 with lower temperatures (Table 1, Supplementary Table S1). Sequence analyses revealed the presence of thermophile- and acidophile-related species, which are usually found in volcanic environments such as crystalline volcanic rocks (Kelly et al. 2011), lava flow (Kelly et al. 2014), volcanic glass (Cockell et al. 2009) and fumaroles (Costello et al. 2009). The differences between bacterial communities from the fumarole stations were revealed by CCA based on T-RFLP patterns (Fig. 2) and comparisons of 16S rRNA libraries (Supplementary Table S2). These observations were consistent with previous studies showing that the structure of prokaryotic communities in volcanic environments was dependent on local physical–chemical characteristics as demonstrated for Bacteria (Tobler and Benning 2011) and Archaea (Perevalova et al. 2008) in thermal waters. Similarly, Costello et al. (2009) described fumaroles as biodiversity islands emphasizing specific bacterial communities for each fumarole condition. In our study, the comparison of the bacterial

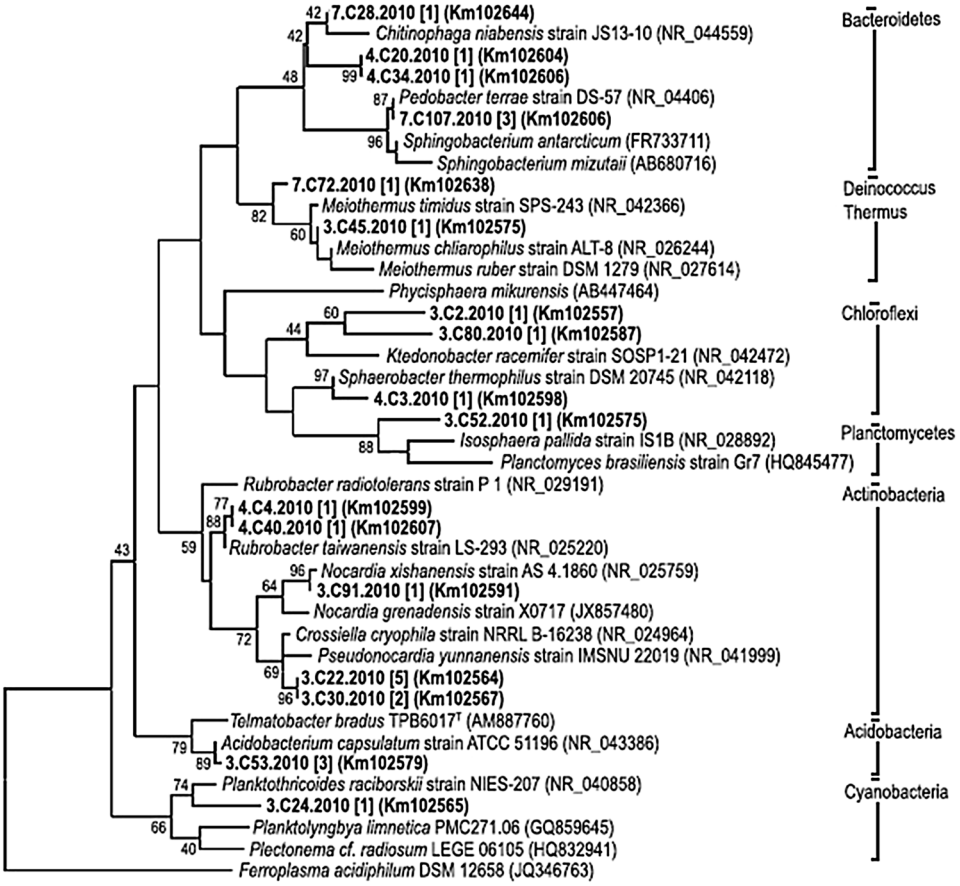

Fig. 6 Phylogenetic tree of sequences affiliated to other Bacteria phyla. The neighbour-joining tree is based on 16S rRNA gene sequences. Bootstrap values (1000 replicates) over 40% are indicated at the nodes. Accession numbers are indicated in *parenthesis*. *Numbers in brackets* indicate the relative abundance. *Bar* 0.05 nucleotide substitution per site

communities pointed to differences in bacterial composition between the fumarole stations (Fig. 3b). The S_{1+2} (stations S1 and S2) library analysis revealed three OTUs associated with *Massilia aerilata, Paenibacillus alginoliticus* and *Desulfonatronum thiodismutans* (representing 58, 32 and 10% of relative abundance, respectively). Sequences related to the genus *Massilia*, aerobic and non-spore-forming rod-shaped bacteria were related by in silico restriction analyses to T-RF 61 with *Hin*P1I revealing that related members were found at all the stations. *Massilia*-related species have been isolated from diverse environmental samples, such as air, soil, water and ice core (Gallego et al. 2006; Orthová et al. 2015; Shen et al. 2013; Weon et al. 2008) revealing their versatility and capacity to cope with a wide spectrum of environmental conditions. Moreover, *Massilia*-related species have been isolated from pioneer microbial communities of basaltic lava flow suggesting that they play a crucial role in colonizing volcanic environments (Kelly et al. 2014). Members of the genera *Paenibacillus*, facultatively anaerobic spore-producing bacteria excreting extracellular polysaccharide-hydrolyzing enzymes, have been isolated from soil and grow optimally at 30 °C (Shida et al. 1997; Uetanabaro et al. 2003). Members of the *Desulfonatronum* genera were classified as thermophile sulfate-reducing bacteria (Cheng et al. 2014).

In station S6, two OTUs associated with *M. aerilata* and *Pseudomonas constantinii* were observed, representing 60 and 40%, respectively. *P. constantinii*, which is not considered to be a thermophile bacterium, has been isolated from mushroom (*Agaricus bisporus*) sporophores (Munsch et al. 2002).

Station S4 revealed 6 OTUs, whose sequences were related to thermophile bacteria: *Thermovenabulum ferriorganovorum* (50%), *Thermacetogenium phaeum* (13%), *Sphaerobacter thermophilus* (11%), *Rubrobacter taiwanensis* (11%), *Moorella glycerini* (9%) and *Thermoanaerobacter sulfurigignens* (6%). It is important to note that only *T. sulfurigignens* had previously been isolated from a volcanic environment in New Zealand (Lee et al. 2007).

At station S5, 8 OTUs were observed. None of them have been found in extreme conditions but rather they were isolated from soil, for instance *Naxibacter alkalitolerans* (23%; Xu et al. 2005), *Pedobacter terrae* (18%; Yoon et al. 2007), *M. aerilata* (12%; Weon et al. 2008), *P. constantinii* (12%; Munsch et al. 2002), *Chitinophaga niabensis* (6%; Weon et al. 2009), *Janthinobacterium lividum* (6%; Lincoln et al. 1999) or from activated sludge, in the case of *Acidovorax defluvii* (18%; Schulze et al. 1999), or from plants, as for *Methylobacterium fujisawaense* (6%; Green et al. 1988; Madhaiyan et al. 2007).

Finally, 15 OTUs were detected at station S3. Most of them were related to thermophile bacteria including *Thermaerobacter nagasakiensis* (7%), *T. ferriorganovorum* (7%), *M. glycerini* (2%), *Meiothermus timidus* (2%) and *Isosphaera pallida* (2%) isolated from hot springs and hydrothermal marine vents (Carlier et al. 2006; Giovannoni et al. 1987; Nunoura et al. 2002; Pires et al. 2005) and *Lutispora thermophile* (7%) isolated from an anaerobic bioreactor (Shiratori et al. 2008). Interestingly, *I. pallida*-related strains have a cell wall similar to Archaea with a high muramic acid content, which offers an advantage for growth in thermophile conditions (Giovannoni et al. 1987). *Acidobacterium capsulatum* (7%) was isolated from mineral environments (Kishimoto et al. 1991) while the other bacteria (*Ktedonobacter racemifer*, 15%; *T. phaeum* and *Pseudonocardia yunnanensis*, 12%; *M. aerilata* and *Nocardia xishanensis*, 7%; *Thermoanaerobacter mathranii*, 5%; *Desulfobacterium anilini* and *Planktothricoides raciborskii*, 4%) were isolated from soils or non-extreme environments. Only *T. phaeum* had been studied for a biotechnological purpose, specifically methane production in a bacterial consortium with Methanothermobacter thermautotrophicus (Hattori et al. 2000).

The characterization of bacterial communities inhabiting different fumaroles around the volcano allowed hypotheses to be formulated on bacterial colonization processes. The presence of sequences related to cosmopolitan plant-associated *Massilia, Methylobacterium, Pseudomonas costantinii* and *C. niabensis* strains suggested that air transportation plays an important role in bacterial colonization. The Paricutín volcano is surrounded by pine forest that may represent a source of such bacteria, which enter the atmosphere as aerosol particles, carried by air currents and deposited by rainfall as previously reported (Burrows et al. 2009; Polymenakou 2012). The presence of thermophile anaerobic spore-forming bacteria was also noticeable. They represented 32, 44 and 86% of the bacterial abundance at stations S1/S2 (S_{1+2} library), S3 and S4, respectively (Fig. 3b). Spore production provides significant adaptive advantages for colonizing adverse environments including spreading and resistance to acid, alkaline and extreme thermal conditions. However, further studies are required to determine whether the retrieved sequences correspond to active forms in the fumaroles. Additionally, it is noteworthy that the bacterial diversity collected encompasses several metabolic groups including sulfate and thiosulfate reducers (*D. thiodismutans, D. anilini* and *T. sulfurigignens*), iron reducers (*T. ferriorganovorum*), syntrophic acetate-oxidizers (*T. phaeum*), chemo-organotrophs (*N. alkalitolerans*), phototrophs (*P. raciborskii*), acetogens (*Moorella thermoacetica*) and methanogens (M. thermautotrophicus). These metabolic groups may interact with each other to colonize the fumarole and ensure the ecosystem functions. Deciphering the metabolic networks that operate in fumaroles is of paramount importance to understand the microbial processes involved in colonizing such adverse environments. Metagenomic shotgun sequencing involving high-throughput sequencing technology is a promising approach (Cravo-Laureau and Duran 2014) to describe metabolic networks and explore novel microbial capacities.

Because the selected fumaroles represented diverse spatially distributed habitats with different physical–chemical characteristics, they showed specific bacterial communities organizations, which provide useful information to further understand the colonization processes. At station S6, situated in hot soil located at the top of the volcano, the observed bacterial diversity is strongly influenced by weather conditions (wind, rain) and solar radiation. The bacterial populations detected at S6 were cosmopolitan bacteria that can be transported by air, or even introduced by human activities (the S6 station is located on a tourist route). This observation is in agreement with previous studies which have shown the impact of human activities on fumarole bacterial communities (Costello et al. 2009). These bacterial populations can be considered as pioneer species (possibly exhibiting high grow rates), but due to the fumaroles natural extreme conditions a more structured bacterial community could not be established.

At stations S1 and S2, two fumaroles 2 m apart, cosmopolitan bacterial genera were also detected such as for example, *Massilia* sp. as well as sulfate reducers and anaerobic bacteria. In these fumaroles with surface temperatures above 68 °C, the development of a thin microbial biofilm layer was observed. In comparison with the bacterial diversity observed at stations S3, S4 and S5 where highly developed microbial mats were observed, the microbial biofilm might be associated with the beginning of a microbial mat formation. However, to prove this hypothesis, further analyses are required over a long period. A relatively high bacterial diversity was observed at stations S3, S4 and S5, characterized by the dominance of Fe, S and acetate oxidizers, in addition to the presence of thermophiles and acidophiles. Station S3 showed the highest bacterial diversity, dominated by two populations representing 14 and 11%, and the remaining populations representing less than 2% each. Such a structuration is typically observed for a community that has reached its climax (Fierer et al. 2007) and is well structured and comprises populations already adapted to the environmental conditions and fluctuations as described in volcanic environments (Mayhew et al. 2007). The fumaroles at stations S4 and S5 presented half the species richness than that observed at station S3. The bacterial populations were more or less at the same abundance for the station S5 while only one abundant population was observed for station S4. The bacterial communities from stations S1–2 were characterized by the presence of pioneer

populations while the bacterial community from station S4 could be considered at a secondary stage, with a majority of pioneer bacterial populations competing with invader bacterial populations well adapted to the environmental conditions and mineral resources. It is likely that the more structured bacterial communities were observed at station S5, dominated by few bacterial populations and at station S3 exhibiting many low abundant populations. These bacterial populations are probably representatives of specific species well adapted to the resources and conditions prevailing in fumarole environments as described by Mayhew et al. (2007).

In summary, the Paricutín volcano, like other young volcanoes, are interesting places for studying the biological colonization, not only by Eukaryotes (i.e., plants) but also by Prokaryotes. Research in this environment should be encouraged because studies describing the bacterial communities colonizing and inhabiting such extreme environments are scarce. The study presented here constitutes the first ecological approach revealing the niche complexity at the Paricutín volcano fumaroles. The results provide useful information for understanding not only the bacterial phylogenetic diversity but also obtaining insights into microbial colonization. However, we have described the bacterial community structures at one site at one specific time, and thus further analysis is still required involving appropriate replicates to investigate both the seasonal variations and the spatial heterogeneity to decipher the microbial metabolic networks involved in the different fumarole ecosystems. Such studies may also allow the exploration and the exploitation of the metabolic bacterial potential in extreme environments.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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