Microbial Life in Volcanic Lakes

Francesca Mapelli, Ramona Marasco, Eleonora Rolli, Daniele Daffonchio, Stuart Donachie, and Sara Borin

Abstract

Lakes in the craters of active volcanoes and their related streams are often characterised by conditions considered extreme for life, such as high temperatures, low pH and very high concentrations of dissolved metals and minerals. Such lakes tend to be transient features whose geochemistry can change markedly over short time periods. They might also vanish completely during eruption episodes or by drainage through the crater wall or floor. These lakes and their effluent streams and springs host taxonomically and metabolically diverse microorganisms belonging in the *Archaea*, *Bacteria*, and *Eucarya*. In volcanic ecosystems the relation between geosphere and biosphere is particularly tight; microbial community diversity is shaped by the geochemical parameters of the lake, and by the activities of microbes interacting with the water and sediments. Sampling these lakes is often challenging, and few have even been sampled once, especially in a microbiological context. Developments in high-throughput cultivation procedures, single-cell selection techniques, and massive increases in DNA sequencing throughput, should encourage efforts to define which microbes inhabit these features and how they interact with each other and the volcano. The study of microbial communities in volcanic lake systems sheds light on possible origins of life on early Earth, or on extraterrestrial systems. Other potential outcomes

F. Mapelli · R. Marasco · E. Rolli · D. Daffonchio · S. Borin (✉)
Department of Food Science and Microbiology, Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy
e-mail: sara.borin@unimi.it

S. Donachie (✉)
Department of Microbiology, University of Hawai'i at Mānoa, 2538 McCarthy Mall, Honolulu, HI 96822, USA
e-mail: donachie@hawaii.edu

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include the development of microbial inocula to promote plant growth in altered or degraded soils, bioremediation of contaminated waste or land, and the discovery of enzymes or other proteins with industrial or medical applications.

Keywords
Volcanic lake · Microbial communities · Extremophiles · Microbial diversity

1 Introduction

If asked to define a lake, most people would probably describe a body of fresh water surrounded by land. Some might attempt to distinguish a lake from a pond. Few, however, would consider the origin or nature of the lake basin, or of the water therein. In all likelihood, most people view lakes as permanent features that only affect them in terms of recreation, commerce, and biological productivity. In such terms, lakes in the craters of extinct or dormant volcanoes probably differ little from most lakes on Earth, in that their hydrological conditions may largely reflect only the surrounding air temperature, amount and nature of meteoric water, inflowing streams or rivers, and host rock chemistry (Donachie et al. 2004). In short, volcanic or geothermal forces no longer drive their circulation and chemistry, or affect their flora and fauna composition; these are the neutral dilute volcanic lakes defined by Pasternack and Varekamp (1997). Lakes in the craters of active volcanoes present very different physical and chemical characteristics, with conditions spanning broad ranges defined largely by the proximity of magma, and origin and nature of the adjacent rock and water (Pasternack and Varekamp 1997; Takano et al. 1997; Martínez et al. 2000, 2002; Jóhannesson et al. 2007). Visitors to such lakes may be surprised to find life in what are, to humans, extreme conditions. In this respect, organisms that thrive in conditions markedly different from what we might consider 'normal' are defined by the generic term 'extremophile', and their presence in such environments should be expected (MacElroy 1974). Evidence for active microbial communities in volcanic systems emerged from geochemical studies, resulting in hypotheses describing microbial roles in the oxidation/reduction of particular chemical species. Studies of elemental and isotopic profiles along with direct measurements of microbial activity provided evidence for microbial participation in the stoichiometry of crater lake waters and sediments, and their importance in these systems (Takano et al. 1997; Wendt-Potthoff and Koschorreck 2002; Koschorreck et al. 2008; Parker et al. 2008).

Microbiologists until recently rarely visited such lakes (Takano et al. 1997; Donachie et al. 2002; Gaidos et al. 2004, 2008; Löhr et al. 2006a; Urbieta et al. 2012). Sampling strategies cannot always include boats, for obvious reasons, while remote sample-return vehicles have not been deployed, nor have automatic devices to collect samples at regular intervals as at hydrothermal vents (Reysenbach et al. 2000). As with other hydrothermal systems, however, the chemical composition of water in a crater lake depends on its interactions with the host rock and gas emissions. This chemistry, plus heat, generally shapes microbial community structure, so it is difficult to predict a universal microbiology for such features. One common trait of crater lakes and volcanic hot spring water is low pH, such that strong acids interact with the rock and mobilize potentially toxic metals. Here we focus on microbial diversity in volcanic acidic lakes, which represent one of the most common naturally acidic aquatic habitats on Earth (Löhr et al. 2006a). Little is known about the evolution and diversity of microbial communities in volcanic lakes, however, a fact that might be attributed to the lakes often being remote and difficult to

access. To simply establish that life is present in a lake in the crater of an active volcano requires overcoming significant logistic challenges (Woelfl and Whitton 2000; Gaidos et al. 2004, 2008; Thorsteinsson et al. 2008). The hypotheses to be tested will determine the methodology, but we should bear in mind that any sample may be the last before the lake vanishes.

2 Methods to Study Environmental Microbes

Defining which microorganisms are in a habitat has long challenged microbiologists. Indeed, we may yet be unable to say with certainty what is the absolute taxonomic diversity in a microbial community, regardless of the methods employed (Curtis et al. 2002). With the benefit of hindsight, supported by advances in technology, we can see how naïve we might have been when describing taxonomic diversity decades ago. For example, from the nineteenth and well into the twentieth centuries we were able only to determine which bacteria were in a sample by inoculating subsamples to nutrient media. Tweaking the concentrations of a medium's components, the gas atmosphere, adjusting the pH, or modifying the light regime, encouraged the growth of metabolically and taxonomically different bacteria. In essence, we 'knew' which microbes were in a sample by growing them on or in such media. However, after more than a century of offering what might seem 'infinite' media and incubation conditions, it became clear to microbiologists that they could not cultivate representatives of all species from most samples. This was underscored in the 1980s by a then new technique, the analysis of nucleotide sequences of specific genes in DNA extracted from the environment and amplified in polymerase chain reactions (PCR); this approach detected bacteria (*Archaea* and *Bacteria*) for which no cultivated representatives then existed (Stahl et al. 1984; Ward et al. 1990). Advances in microscopy also showed the number of bacteria we might cultivate ranges from far less than 1 to ~50 % of those counted by microscopy, depending on the sample type (Staley and Konopka 1985; Donachie 1996; Wilson et al. 1997; Handelsman 2004). It should be noted here that these statistics refer only to the number of cells present, and not the number of 'species' or any other taxonomic unit we might define (Donachie et al. 2007). Such observations, however, encouraged use of terms such as 'nonculturable' or 'unculturable' to describe those cells we could not grow (Xu et al. 1982; Colwell and Grimes 2000). These terms have taken on almost meme-like status. However, we might note that Amann et al. (1995) observed, perhaps wryly, "With the availability of innovative techniques, many more microorganisms will become culturable. […] After all, nature can cultivate all extant microorganisms."

That analysis of ribosomal gene sequences in environmental samples detected bacteria that had never been cultivated led many microbiologists to justify using only those techniques, now termed 'molecular approaches', in studies of taxonomic diversity (Schmidt et al. 1991; Barton et al. 2004; Valenzuela-Encinas et al. 2008). Such a single-method approach has been shown not to describe microbial diversity in toto; cultivation and molecular approaches detect very different bacteria in the same sample (Suzuki et al. 1997; Bowman et al. 1999; Kaiser et al. 2001; Donachie et al. 2002, 2004, 2007; Munson et al. 2002; Shawkey et al. 2005). Whatever might be the merits of one approach over another, the utility of both cultivation-based and molecular techniques in microbial diversity studies has been significantly enhanced with the development of high-throughput culturing methods and high-throughput DNA sequencing (Connon and Giovannoni 2002; Zengler et al. 2002; Donachie et al. 2007; Kalyuzhnaya et al. 2008; Kircher and Kelso 2010). The apparent unculturable nature of some microorganisms is surely in part attributable to the medium composition or growth conditions provided not suiting them. Conditions in extreme environments such as volcanic features render it difficult to exactly reproduce the correct concentrations of nutrients and growth factors in the laboratory (Rodríguez-Valera 2002). Characterizing geochemical and physical parameters is thus essential if one is to reproduce in situ conditions

in artificial systems, and highlights the importance of a multidisciplinary approach in microbiological studies of these environments (Fig. 1). In addition to extrinsic factors, microbes might also require intrinsic factors such as syntrophic interactions, a mutual relationship based on nutrient exchange, or quorum-sensing, intercellular communication mediated by small molecules only when a certain population density is attained.

2.1 Cultivation Approaches

Cultivating bacteria from environmental samples as a scientific objective dates back to the nineteenth century (Certes 1884). Detailed reviews of the myriad requirements and often creative techniques used in the pursuit of a 'pure culture' can be found throughout the literature. Today, data from molecular methods can be of use in developing media and incubation conditions to isolate new taxa (Palleroni 1997; Giovannoni and Stengl 2007). It remains statistically prohibitive to cater to all species that may occupy a particular habitat, but given sufficient information about the habitat and with supporting molecular data we can target specific genera or metabolic groups (Huber et al. 1995; Teske et al. 1996; Tyson et al. 2005). Although the exact concentration of every component in a volcanic habitat might be difficult to faithfully reproduce from scratch in the laboratory, anyone collecting samples from such sites should be able to collect enough material to provide the base of many culture media (Rodríguez-Valera 2002). We can thus address in part the need for specific components or combinations thereof found only in the native sample.

New cell selection and cultivation strategies have attempted to overcome the limitations of diluting samples to extinction, a procedure that statistically provides a suspension of the most abundant species, while diluting out those which are less abundant but perhaps more competitive (Fröhlich and König 2000). A drawback is that the least abundant taxa are unlikely to be present in the most dilute samples; they might also be outcompeted when inoculated to media with other species. Recent 'high-throughput' and 'gel microdroplet' techniques operate on the same principle of dilution to provide single to a few cells that are inoculated to media in microplates. These have enabled significant expansion of the number of

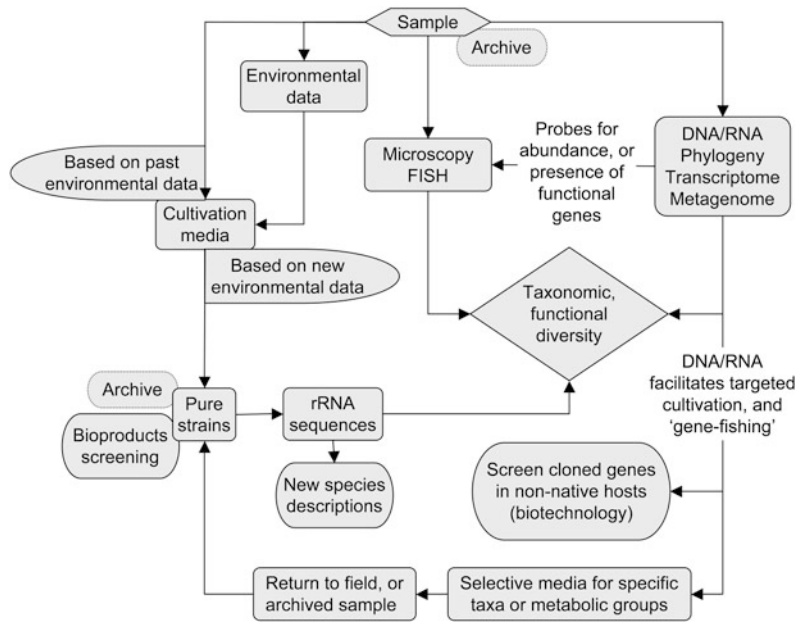

Fig. 1 Schematic representation of approaches used by microbiologists to investigate diversity, function and potential applications of microbes in environmental samples

samples that can be processed in a given time (Connon and Giovannoni 2002; Zengler et al. 2002). However, they do not allow direct selection of any particular cell type, and given there are perhaps billions of cells in a liter of most naturally occurring waters they would still require an unfeasibly high number of microplates to sample all cells per liter. And that is before one provides different media, temperatures, or any other such parameter. All is not lost, though. Cell sorting by flow cytometry can target specific cell types, but does not isolate single microbial cells that one directly observes. To collect single cells that one can see, and pass those cells from a sample to downstream analyses, or to media wherein they may grow free of competition from other taxa, a range of microfluidic, and laser microdissection and catapulting systems offer various capabilities (Krüger et al. 2002; Ho et al. 2005; Baret et al. 2009; Chen et al. 2009; Kang et al. 2011). Cells historically termed 'unculturable' will numerically dominate hundreds or even thousands of selected cells, but today's laser systems are combined with imaging capabilities that enable us to move beyond random selection. By establishing a library of cell images we might attempt to isolate visually distinctive cells that we have previously related to specific taxa through rRNA gene sequencing after whole genome amplification (sensu Morris et al. 2004; cf. Fig. 1); their phylogeny and physiology can thus be predicted and in subsequent selections they can be transferred to tailored growth media in microplates within which they can grow free of competition from more rapidly growing or antagonistic taxa (sensu Kovac and Voldman 2007). Laser-mediated methods have proved themselves in isolating novel microbes (Huber et al. 1995).

2.2 Cultivation-Independent Approaches

Molecular approaches were first used in microbial ecology about three decades ago, based on the study of the nucleotide sequences of specific genes. They revolutionized the field and quickly became the most widely used methods to investigate microbial diversity in any sample. The most widely used methods are based on examination of so-called "molecular chronometers". These are ribosomal, or rRNA genes, e.g., 16S rRNA genes in *Archaea* and *Bacteria*, and 18S rRNA genes in the *Eucarya*, that encode for the production of ribosomes (Kimura 1968, 1983; Stackebrandt et al. 1985). The slow rate at which these genes' nucleotide sequences have mutated over evolutionary time renders them valuable in classification. Such sequences also form the basis of the reclassification of all life into the three currently recognized domains, *Archaea*, *Bacteria* and *Eucarya* (Woese 1987). Moreover, functional genes encoding proteins involved in specific metabolisms such as nitrogen fixation or sulphate reduction can be used as proxies for particular bacterial groups (Moisander et al. 2006; Wagner et al. 1998). Many DNA-based methods developed since the 1990s provide data on both taxonomic and metagenomic diversity in microbial communities. Furthermore, "-omics" approaches including metatranscriptomics and metaproteomics allow one to determine which proteins microbial communities in the environment express. Further advances now see these techniques being used at the single cell level, permitting evaluation of the role and spatial topology of each community member (Kang et al. 2011). Information about the presence and spatial distribution of specific populations can also be obtained through microscopic analyses that target specific phylogenetic groups. Among these, techniques such as fluorescence in situ hybridization (FISH) have been instrumental in both significant discoveries and routine counting of specific microbial groups (Amann et al. 1990; Karner et al. 2001). Enhanced FISH-based techniques including substrate-tracking autoradiography (STAR-FISH) and catalyzed reporter deposition (CARD-FISH) have been applied to the study of microbial communities in water, e.g., warm monomictic crater lake Alchichica (Mexico), and a subglacial volcanic lake beneath Iceland's Vatnajökull ice cap (Ouverney and Fuhrman 1999, 2000; Gaidos et al. 2008).

Molecular- or PCR-based approaches do have limiting factors, including the amount and quality

of DNA that can be extracted from the sample, and the amount of that DNA which can be sequenced in a given time at an acceptable cost. The latter is now a minor issue given that today's DNA sequencers can process, relatively cheaply, billions of nucleotides in a few hours. Molecular approaches today appear to offer the possibility of quickly detecting all genes, and thus all bacteria in a sample, as long as one can guarantee extracting all DNA from all cells. Just over a decade ago a multi-capillary Sanger-type sequencer was considered 'high-throughput.' Such a machine's performance pales into insignificance compared to that offered by today's platforms, which can complete millions of reads in an afternoon. Such performance revealed "unexpected" taxonomic diversity in the deep-sea (Sogin et al. 2006), while metagenomic analyses also showed an equally remarkable functional diversity (DeLong et al. 2006). These techniques have not yet been widely used in volcanic habitats, although Gaidos et al. (2008) showed the metagenome in anoxic water of a sub-glacial volcanic lake comprised an oligarchic microbial consortium suited to the lake's geochemical context. Further advances in sequencing technology, along with miniaturization and reduced cost per nucleotide sequenced, promise greater insights, with some caveats, of course (Kunin et al. 2010; Niedringhaus et al. 2011).

3 Microbial Diversity in Volcanic Lakes

3.1 Cultivated Microbes

Reports of microbes cultivated from crater lakes in active volcanoes are few and far between. Indeed, most of the effort to determine which microbes are in these lakes appears to have been based on molecular approaches. It also seems we might know more of the diversity, physiology and abundance of microbes, both cultivated and uncultivated, at submarine volcanic features than we do in their analogous terrestrial features (Karl et al. 1989; Donachie et al. 2003, 2004; Nakagawa and Takai 2008). In this respect, myriad reports of microbes in features related to volcanoes such as geothermal springs and effluent streams appear throughout the literature (Sonne-Hansen and Ahring 1997; Hugenholtz et al. 1998; D'Imperio et al. 2008). Through just a handful of papers, however, one can appreciate what challenging environments acidic crater lakes are for microorganisms.

Takano et al. (1997) elegantly describe how sulfur-oxidizing bacteria affect the sulfate budget of the Yugama crater lake (pH 1–1.5), part of the andesitic Kusatsu-Shirane strato-volcano, Japan, noting also how pH controls distributions within the crater of two *Thiobacillus* species, *T. thiooxidans* and *T. ferrooxydans*. Members of the *Thiobacillus,* subsequently reclassified as *Acidithiobacillus*, are known to colonize extremely acidic environments characterized by high levels of reduced sulfur, which they oxidize (Kelly and Wood 2000). In the Yugama crater lake a molten sulfur pool at a then active subaqueous fumarolic vent was discharged into the water column, while sulfur particles dispersed throughout the lake as aqueous sulfur dioxide and hydrogen sulfide reacted (Takano et al. 1994b); hydrogen sulfide, polythionates, and elemental sulfur were consumed by bacteria, while reduced sulfur compounds were converted by these bacteria to sulfuric acid. An acidophilic diatom, *Pinnularia braunii* var. *amphicephala*, on the floor of a stream in the crater was identified by microscopy, showing that eukaryotes also colonize crater lake environments.

The first report of microbial communities in acidic waters of the White Island andesitic stratovolcano applied both cultivation and molecular methods (Donachie et al. 2002). The water here is a dilute mix of sulfuric and hydrochloric acids containing dissolved andesite rock and other components. Sub-samples of water emanating from a hot spring in the crater floor were inoculated to a range of media (pH 1.29 to ~8) to cater to expected metabolic groups, and incubated at one of five temperatures between 30 and 60 °C for up to one year. Four pure cultures affiliated with the heterotrophic and acidophilic mesophile, *Acidiphilium* sp. (*Alphaproteobacteria*), which requires only low concentrations of organics and

cannot use sulfur or Fe^{2+} (Harrison 1981); with *Nocardia* sp., and *Nocardioides* sp. (*Actinobacteria*), and with *Cyanidium caldarium* (*Eucarya*, Rhodophyta), a photoautroph (Doemel and Brock 1971). The success of the *Eucarya* in volcanic crater lakes is likely controlled by temperature, even if they can tolerate the extremely low pH (Tansey and Brock 1972). For example, *Cyanidium caldarium* was absent from the lake in the White Island volcano, where pH was ~0 but temperature was 58 °C, yet it was isolated from slightly cooler 'soil' just meters from the lake (Fig. 2) (Donachie et al. unpubl.). This photosynthetic eukaryote does not grow above 56 °C (Kleinschmidt and McMahon 1970).

A crater lake whose microbiology was investigated in austral summer 2007 by both cultivation and molecular approaches is the summit lake on Simba volcano (Demergasso et al. 2010), also referred to as Lake (cf. Laguna) Aguas Calientes (Escudero et al. 2007; Cabriol et al. 2009). This is an unusual lake because of its high altitude (5,870 m) and concomitantly high UV radiation exposure, yet rather moderate pH for a crater lake. Although water and sediment samples were inoculated to enrichment media, no growth was detected in the incubation period, while several bacteria from just two phyla were cultivated from nearby Salar Aguas Calientes and Laguna Lejía. In November of 2006, *Bacteria* were cultivated from the same lake, but their diversity was limited to just a couple of *Gammaproteobacteria* (*Pseudomonas* spp.) and a *Firmicutes* (*Staphylococcus epidermidis*). *S. epidermidis* is probably not part of the autochthonous microbiota since it is generally found on the human skin and membranes, and is a frequent contaminant in laboratory tests (Queck and Otto 2008). Molecular methods showed a higher phylogenetic diversity, revealing the presence of taxa reported from other high altitude lakes rather than acidic lakes per se, and belonging in the *Bacteria* and *Archaea* (Demergasso et al. 2010). This work demonstrates the value of coupling cultivation-dependent and molecular methods.

Wendt-Potthoff and Koschorrek (2002) determined the abundances of various physiological groups of bacteria along the Rio Agrio on the Copahue volcano (Argentina), an andesitic stratovolcano, and its recipient lake, Caviahue. Iron- and sulfur-oxidizers, and sulphate-reducers were enumerated in enrichment media used in other acidic environments. The presence of fermentative bacteria was determined by gas production at pH

Fig. 2 Sampling at White Island, New Zealand. Acidic gases require use of respirators. The water's green coloration is due to elemental sulfur. (*Photo* SPD)

2.0. All metabolic groups were detected, although abundance was low, and the authors posited that distribution varies with rate of water flow, sediment texture, and light availability rather than solely with pH. Lavalle et al. (2005) worked along the same river, but also sampled from the crater lake (Laguna del Volcán) itself. In a targeted approach with specific media, acidophilic, chemolithotrophic and ferrous-oxidizing bacteria were cultivated only from the river, where the pH ranged from 2 to 4. These bacteria were assigned to the iron-sulphur oxidizer *Acidithiobacillus ferroxidans*. A broader microbiological survey of the same system reported that the lake's pH ranges from 0.2 to 1.1 (Chiacchiarini et al. 2010); these authors used diverse media and incubation conditions and cultivated bacteria that use inorganic reduced sulphur compounds as energy sources, and CO_2 (chemolithoautotrophs) or organic compounds (chemolithoheterotrophs) as carbon sources. Among these were *Bacteria* (e.g., *Leptospirillum ferrooxidans*, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*) and *Archaea* (*Acidianus*, *Sulfolobus*), plus yeasts and filamentous fungi from sites that extended from a 70 °C, pH ~1.0 hot spring (el Vertedero) emerging from the mountain, just below the lake, and along the Upper and Lower Rio Agrio, and from Caviahue Lake. Temperature along this water course drops to ~8 °C and pH increases to 4.2. An unidentified extremely acidotolerant (pH < 2) filamentous fungus was cultivated, as were over a dozen yeasts from at least three genera (*Cryptococcus*, *Rhodotorula*, *Sporidiobolus*). That no microbes were cultivated from the Laguna del Volcán by both Lavalle et al. (2005) and Chiacchiarini et al. (2010), although water samples were inoculated to media, is consistent with observations elsewhere that bacterial growth does not proceed in crater lakes when the pH is <1 (Satake and Saijo 1974; Takano et al. 1994a).

A discussion of microbes in the environment should not overlook the potential for viral infections. Just as the role of viruses in marine biogeochemical cycling has been reassessed in the last couple of decades, so too might we find that viruses are instrumental in controlling populations of thermophiles and acidophiles in volcanic settings (Short and Suttle 2002; Rice et al. 2001; Snyder et al. 2003). Almost 30 years ago, Janekovic et al. (1983) described a family of viruses that infect *Thermoproteus tenax*, a thermophilic *Archaea* found in hot springs. Multiple viruses in *Sulfolobus*, which inhabit high temperature hydrothermal environments, are now known (Zillig et al. 1996; Arnold et al. 2000). Geslin et al. (2003) reported the first virus in an hyperthermophilic archaeon, *Pyrococcus abyssi* from marine hydrothermal vents. An attempt about 10 years ago to isolate phage from water in Ruapehu crater (pH ~2, 45–55 °C) found neither phage nor DNA that could be amplified (Hugh Morgan, pers. comm.).

3.2 Microbial Diversity in Volcanic Systems by Molecular Methods

Molecular 'fingerprinting' methods are routinely applied in microbial ecology to compare phylogenetic diversity in microbial communities. Such diversity in volcanic environments has to date been largely investigated by denaturing gradient gel electrophoresis (DGGE) of ribosomal (rRNA) gene fragments (Muyzer et al. 1993; Löhr et al. 2006a, b; Wendt-Potthoff and Koschorreck 2002). Gene fragments with different nucleotide sequences in different species are visualized in DGGE as different 'bands' (Fig. 3). The DGGE profile, or community fingerprint, of different samples can be statistically analyzed for comparative purposes. Determining the nucleotide sequence of the rRNA gene fragment in each band permits tentative assignment of the original bacterium to a taxonomic group. More recently, 'chip-based' techniques have been developed, such that specific RNA probes on a microarray can detect their complementary genes, allowing detection of potentially thousands of species in a single experiment (De Santis et al. 2003). In this respect, an acidophilic bacterial microarray was developed and tested in habitats such as Spain's Rio Tinto, where the extremely acidic conditions are similar to those in some volcanic lakes (Garrido et al. 2008).

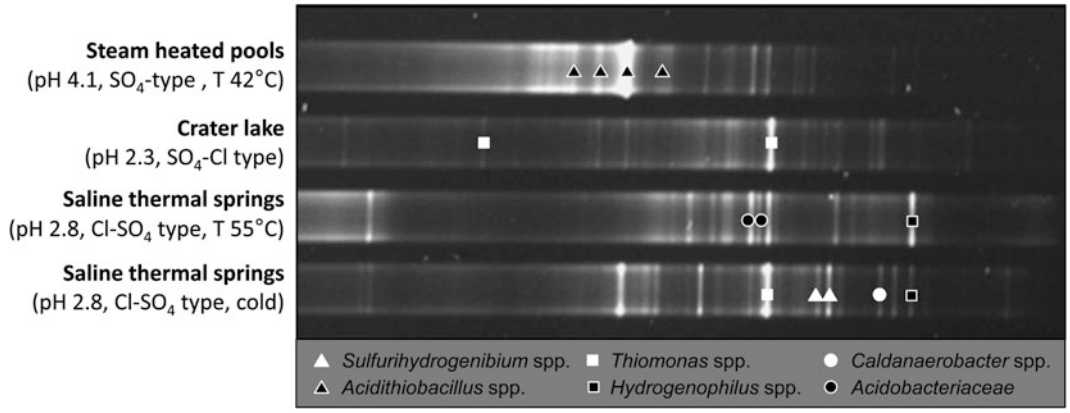

Fig. 3 DGGE gel of amplified 16S rRNA genes from El Chichón crater lake and associated thermal springs. Bands in each lane represent a different bacterial species. Symbols show which band corresponds to a particular *Bacteria* genus or family

In addition to the cultivation-based data described above for White Island, cloned 16S rRNA gene fragments from the acid stream affiliated with *Pandoraea* and *Ralstonia* (*Burkholderiaceae*, *Betaproteobacteria*), *Rhodovulum* (*Rhodobacteraceae*, *Alphaproteobacteria*), *Acidosphaera* (*Acetobacteraceae*, *Alphaproteobacteria*), and the photoautotrophic *Chlorobium vibrioforme* (Chlorobi) (Donachie et al. 2002). No DNA was detected in water collected from the pH ~0, 58 °C lake (Donachie et al. unpubl.).

The largest known acidic crater lake is Kawah Item, Indonesia, whose pH 0.3 water is clearly extreme compared to that of more typical lakes and rivers (Geller and Schultze 2009). As the river Banyupahit-Banyuputih flows from Kawah Ijen, its pH increases from 0.39 to 7.62. Microbial diversity in the lake and river investigated by DGGE detected no *Bacteria* or *Eucarya* in the lake, although they were detected along the effluent rivers (Löhr et al. 2006a). No attempts were made to cultivate microbes, nor were other sequencing strategies with larger sample sizes conducted. *Bacteria* sequences detected by DGGE in the highest pH river water affiliated with *Betaproteobacteria*, *Gammaproteobacteria* and Flavobacteria; in the most acidic sample, only a sequence affiliated with the *Pseudomonadaceae* family was detected. None of the *Bacteria* detected were known acidophiles, and *Eucarya* were absent from low pH waters in both the Kawah Ijen lake and the upper reaches of the river Banyupahit-Banyuputih. However, *Eucarya* diversity was high in water at pH ≥2.66, and included both algae and diatoms (Löhr et al. 2006b). *Archaea* sequences were detected at all sites, but taxonomic diversity was generally low and reached minima in the most acidic sites. *Archaea* typical of acidic environments were not detected (Löhr et al. 2006b).

Microbial communities in the Copahue-Caviahue system of Argentina have also been studied (Urbieta et al. 2012; Löhr et al. 2006a). Here, extremely acidic water (pH 0.2–1.1) flows from L. Copahue in the Copahue volcano into the Río Agrio. The river can be divided into the Upper Río Agrio (URA) that flows into L. Caviahue (pH 2.1–3.7), and its effluent Lower Río Agrio (LRA). Bacterial abundance in water from the Upper Río Agrio and in L. Caviahue determined by microscopy was $\sim 2 \times 10^5$ cells ml^{-1}, of the order of that reported in acid mine lakes, the anthropogenic homologue of volcanic lakes (Wendt-Potthoff and Koschorreck 2002). A more recent study by CARD-FISH detected 10× more cells in the Upper Rio Agrio (Urbieta et al. 2012). Measurements of microbial activity such as oxygen consumption, and iron oxidation and reduction at three points along the URA, one of which was immediately before the inflow to L.

Caviahue, showed a gradient of microbial abundance and activity. Iron- and sulphur-oxidizing bacteria and iron-reducing bacteria were previously reported, as one might expect given the abundances of iron and sulphur in this type of ecosystem (Wendt-Potthoff and Koschorreck 2002). FISH detected acidophilic bacteria with a potential for iron and sulphur metabolism, such as *Sulfobacillus*, *Leptospirillum*, *Acidithiobacillus* and *Acidimicrobium*, in the hot spring of Copahue village (Giaveno et al. 2009a). *Acidithiobacillus ferroxidans*, *Acidithiobacillus thiooxidans* and members of the genus *Leptospirillum* and the *Archaea* genus *Sulfolobus* were cultivated from different parts of the Copahue-Caviahue system in a study to isolate microbes that may bioleach ores and recover precious metals (Chiacchiarini et al. 2010). Bacterial diversity in four water samples from the Upper Río Agrio was also recently reported on the basis of 16S rRNA gene clone libraries and CARD-FISH (Urbieta et al. 2012). Sampling sites were characterized by increasing pH (1–2) and temperature (6.7–59 °C). CARD-FISH revealed *Archaea* were the most abundant members of the community, albeit not diverse since most sequences affiliated with just one species, the chemolithoautotrophic, iron-oxidizing, *Ferroplasma acidiphilum*. Members of the *Ferroplasma* genus are key players in the sulphur cycle through oxidation of sulphides and regeneration of Fe^{3+}, the primary oxidant of pyrite at low pH (Urbieta et al. 2012). In contrast to what one might expect in an 'ecological dogma' context, that more extreme conditions exert a stronger selective pressure that results in a less complex community, a *Bacteria* 16S rRNA gene clone library from the sample with the lowest pH (1) and highest temperature (59 °C) contained the most phylogenetically diverse community. Moreover, a 'less extreme' habitat, that characterized by pH 2 and 15.9 °C, showed the lowest diversity indices. *Bacteria* clones in URA waters affiliated with *Alpha-, Beta-* and *Gammaproteobacteria*, and the phyla *Actinobacteria*, *Firmicutes* and *Nitrospirae*, the latter represented only by the genus *Leptospirillum*, typically found in acid environments with high concentrations of reduced iron and sulphur. The distribution of *Acidithiobacillus ferrooxidans*, a known iron-sulfur oxidizing bacterium, along the URA correlated with the iron content of the water and pH value, confirming previous cultivation-dependent experiments (Chiacchiarini et al. 2010). Clone libraries also contained an *Acidiphilium* sp., an important observation given they may scavenge organic molecules that would otherwise be toxic to chemolithoautotrophs such as *Acidithiobacillus ferrooxidans* (Johnson 1995; Urbieta et al. 2012). Sulphur-oxidizing species affiliating with *Acidithiobacillus thiooxidans* and *Acidithiobacillus albertensis* were also detected in the URA; these species, along with *Sulfobacillus* spp., are typically found in natural or man-made acidic habitats, e.g., the Río Tinto in the Iberian Pyritic Belt, or in acid mine drainage.

As with studies of the Upper Río Agrio, a DGGE survey of the El Chichón crater lake (Mexico) revealed the presence of chemolithoautotrophic bacteria able to synthesize organic carbon from carbon dioxide (Fig. 3). *Alpha-, Beta-* and *Gammaproteobacteria* along with *Aquificae*, *Firmicutes* and *Acidobacteria* were detected in acidic, sulphate-rich waters of the lake (Peiffer et al. 2011; Mapelli, pers. comm.). The presence of *Sulfurihydrogenibium*, *Acidithiobacillus*, *Thiomonas* and *Hydrogenophilus,* members of which are involved in sulphur and iron cycling, and hydrogen oxidation, reflects the chemical composition of El Chichón's lake and thermal springs.

Volcanic lakes present unique challenges for both microbes and microbiologists. After a few decades of cultivation- and microscopy-based studies, plus a decade or so of molecular methods, it seems we might conclude the microbial flora in the few lakes studied is limited to a handful of microbial eukaryotes, and some *Archaea* and *Bacteria* (Schleper et al. 1995; Donachie et al. 2002). On the other hand, these lakes have been so rarely investigated in microbiological terms that we should reserve judgment until more extensive data are available.

4 Potential Applications of Microbiological Studies of Volcanic Lakes

Interest in microbial diversity in crater lakes is both scientific and economic in nature. For example, knowledge of ecological processes in natural and artificial acidic systems provides leads for established and novel biotechnological applications (Antranikian et al. 2005; Liu and Zhang 2008). Such studies may allow improvement of or development of new applications, such as in biomining, and the development of strategies to restore waters and ecosystems affected by acidification. Applications of biological resources from volcanic systems extend to reclaiming or scavenging metals from contaminated substrates or toxic mixtures. For example, Chiacchiarini et al. (2010) showed a putative *Acidothiobacillus ferrooxidans* from the Upper Rio Agrio could reduce chromium and zinc concentrations in municipal sludge to below permitted levels; consortia of bacteria from Copahue–Caviahue could also extract more gold from a sulphide concentrate than could pure cultures of *Acidothiobacillus ferrooxidans* DSM 11477 *and Leptospirillum ferrooxidans* ATCC 29047^{T} (Giaveno et al. 2009b). These type strains and related species have long been the focus of bioleaching studies (Porro et al. 1989; Donati et al. 1996).

An innovative application of microbiological research in volcanic systems is that of Plant Growth Promoting (PGP) bacteria, those that help sustain plant growth in extreme geochemical settings. Associations with microbes are an acknowledged evolutionary strategy in plants that may improve the bioavailability of nutrients and protect against pathogens. Plant-microbe associations can also enhance a plant's survival against abiotic stresses such as extreme pH or presence of toxic compounds. Only certain pioneer plants can tolerate the severe environmental conditions that typify soils affected by volcanic activity, such as high temperature, anomalous gas fluxes through soils and sediments, low pH, and presence of potentially toxic elements in aerosols and water. A PGP bacterial activity that may be widespread and important to plants colonizing volcanic systems is the detoxification of phytotoxic compounds, such as heavy metals. PGP bacteria isolated from pioneer plants in volcanic areas have potential in the formulation of 'biofertilizers' able to sustain plant growth in altered and degraded soils. Potential PGP bacteria associated with a pioneer plant growing in a hot, acidic stream emanating from the crater lake of El Chichón volcano (Mexico) have been isolated and characterized (Fig. 4). PGP bacteria can be isolated from the root interior or the adhering soil, the so-called "rhizosphere", of pioneer plants and screened in vitro for known PGP activities and resistance to abiotic stressors. To develop an effective 'biofertilizer' it is essential to determine if the bacteria are rhizocompetent, i.e., able to colonize the plant root. Rhizocompetence can be evaluated by engineering the cells to express a 'green fluorescent protein'. Such bacteria can be used in root colonization experiments on model plants and observed in root sections by epifluorescence microscopy. The most efficient or rhizocompetent bacteria strains in vitro and in vivo are tested in field trials on plants growing in suboptimal conditions. Microbial inocula such as these, with detoxification or growth stimulation activity, can enhance a plant's tolerance of stress, and promote rhizoremediation, phytoremediation and phytostabilization techniques (Ma et al. 2011; Regvar et al. 2006). The cultivation of microbes from volcanic systems, or the application of genetic information from these and uncultivated taxa, may well contribute to socially acceptable, environmentally friendly practices in land restoration.

Volcanic activity has always been part of Earth's history, so investigating microbial life in volcanic crater lakes and their effluent rivers may provide clues to the origin of life on Earth, and how life has adapted over time to extreme conditions. Astrobiology is a multidisciplinary field focusing on the origin, evolution and pattern of life on Earth and extraterrestrial bodies. The only incidence in the solar system of life as we know it is on Earth. We thus define conditions required

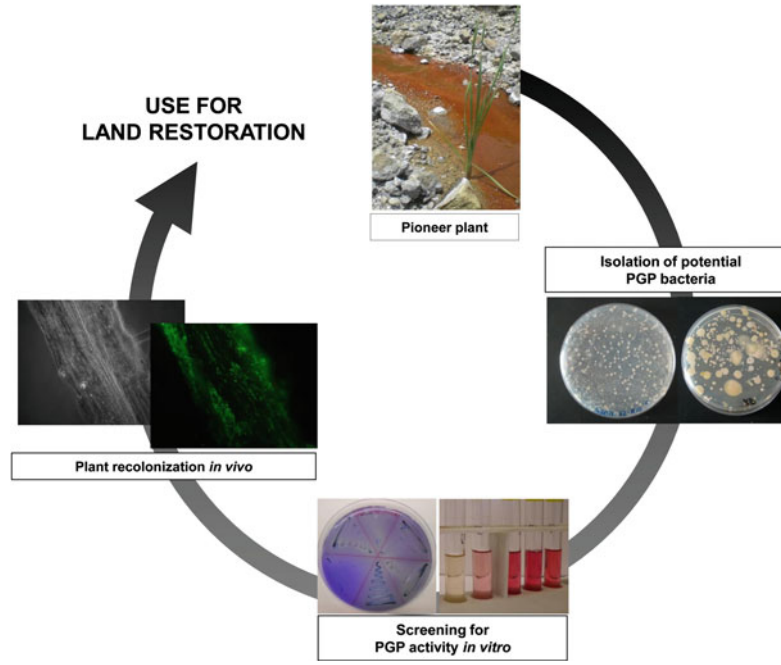

Fig. 4 Extremophilic bacteria can be isolated from pioneer plants, studied under laboratory and in vivo conditions, and may be used in environmental biotechnology, e.g., for land clean-up and restoration.

for the development of life according to observations here, and especially in volcanic systems where extreme pH, temperature and chemistry are considered analogs of both early Earth and extraterrestrial habitats. Geochemical features such as pH, and sulphur and iron species in crater lakes, appear to be the principal forces shaping the composition of autochthonous microbial communities. Iron in particular plays a fundamental role in the biogeochemistry of many volcanic ecosystems, and was probably important on the early Earth (Weber et al. 2006). Signatures of acidic aqueous systems with iron- and sulfur-based redox cycles have been found on the Martian surface (Bibring et al. 2007). For example, jarosite, an iron oxide mineral found on the surface of Mars by the 'Opportunity' rover (Klingelhofer et al. 2004) can be produced from pyrite by *Acidithiobacillus ferrivorans* cultivated from iron bioweathered soil (Borin et al. 2009), and from El Chichón crater lake (Mapelli, pers. obs.). *Acidithiobacillus* spp. and other extremophiles have been proposed as biomarkers for life detection strategies on planetary bodies (Gómez and Parro 2012). Moreover, some of the most extreme crater lakes, such as those in the Chilean Altiplano, are eminently suited to investigations of microbial physiology and community dynamics in rapidly changing and extreme environments (Demergasso et al. 2010). Considerable technical challenges await those who choose to work on the microbiology of volcanic crater lakes, but the field will surely yield both exciting methodological developments and scientific discoveries.

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