

Cyanobacteria and photosynthetic species as part of the microbial community structure of biofilms in Copahue geothermal springs (Neuquén, Argentina)

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Abstract. Copahue is a geothermal field located in the Northwest corner of Neuquén province in Argentina. It is dominated by the still active Copahue volcano. In the area there are many acidic pools, hot springs and solfataras with different temperature and pH conditions that influence their microbial diversity. On the surrounding rocks and the borders of the pools, where water movements and thermal activity are less intense, many biofilms can be found. They have different aspects and structure, and they present less extreme temperature and pH conditions than the ponds and hot springs. Biofilms are a different ecological niche and they have different microbial community structure. In this study carried out by molecular ecology techniques, mainly 16S and 18S rRNA gene sequencing, we report a strong presence of cyanobacteria, chloroflexi and eukaryotes, not detected in previous biodiversity studies done on water samples. Almost no acidophilic bacteria were found, with the exception of members of genus *Thiomonas*, also found in the acidic pools. Archaea were detected only in one of the biofilms and the structure of that community seems to be similar to those found in water samples, with many uncultured species mainly related to order *Sulfolobales*. The aim of this study was to assess microbial community diversity in the biofilms present in this acidic geothermal area, with particular emphasis on detection of cyanobacteria and eukaryotes with potential biotechnological applications like production of alternative energy sources, synthesis and accumulation of biomolecules with antiviral or antibiotic activities or potential ability to bioremediate contaminated areas.

Introduction

Copahue Geothermal region is located in the Cordillera Norpatagónica in the north west of Neuquén province, Argentina. The area is crowned by Copahue volcano (2965 m above sea level). Approximately 100 m below the crater there are two hydrothermal springs that are the source of acidic Río Agrio. The river flows down Copahue-Caviahue region. Its biodiversity has been studied by Urbietta et al. [1]. Associated with Copahue volcano geological origin and activity, the area is characterised by many fumaroles, pools, ponds, and hot springs. The prokaryotic biodiversity of those pools water is determined by temperature; high temperature ponds are dominated by

thermoacidophilic archaea, mainly from the order *Sulfolobales*, whereas moderate temperature ponds practically do not show archaea and the bacteria detected there are related to acid mine drainage environments, like *Thiomonas*, *Acidithiobacillus* and *Acidiphilium* (Urbieta, unpublished). Cyanobacteria are unique prokaryotes, as they are autotrophs able to do photosynthesis. Their morphological and physiological structures allow them to adapt to different environmental conditions. The ability of cyanobacteria to develop in acidic environments has been questioned by several authors [2, 3]. However in this work we describe the biodiversity of biofilms in Copahue acidic geothermal area, making particular emphasis on cyanobacteria and photosynthetic species presence, as they have many interesting and versatile biotechnological applications.

Methods

Biofilms from four different sampling stations of Copahue geothermal area were collected in December 2009. Samples were kept in sterile plastic jars at room temperature until further processing. Temperature and pH were measured *in situ* with properly calibrated instruments. DNA extraction was done using the Fast DNA Spin kit for soil (Bio 101, Carlsbad, CA, USA) according to the manufacturer's instructions. Clone libraries of complete 16S rRNA genes for *Bacteria* and *Archaea* domains were generated from environmental DNA templates. 16S rRNA genes were amplified by PCR using forward primers 8F for bacteria and 25F for archaea; reverse primer for both was 1492r [4, 5]. 18S rRNA eukaryotes genes were amplified using the primers: Euk 20F and Euk Br [6]. Amplified 16S and 18S rRNA genes products (>1,400 bp) were cloned using the Topo Ta Cloning Kit (Invitrogen, CA, USA) and sequenced using M13F and M13R primers and the Big-Dye sequencing kit (Applied Biosystem) following the manufacturer's instructions. Sequences from the three clones libraries constructed were checked for potential chimeras using Bellerophon Chimera Check program (http://greengenes.lbl.gov/cgi-bin/nph-bel3_interface.cgi) and Maillard software. Sequences detected as chimeras were retrieved from further analysis. OTUs were defined at 97% sequence similarity using ARB software package (<http://www.arb-home.de>). Further analysis of one representative of each OTU was done using the Classifier and Taxomatic on line tools of Ribosomal Database Project (RDP) (<http://rdp.cme.msu.edu>).

Results

Biodiversity and community structure were analysed in biofilms from four different sampling stations of Copahue geothermal area. Their temperature and pH values are presented in Table 1. Several metals (Na, K, Mg, Ca, Mn, Ni, Cu, Pb, Cr, Zn, Cd) were measured by inductively coupled plasma mass spectrometry (ICP-MS) but they were not detected in significant concentrations.

Amplifications with bacterial primers were positive for the four biofilms studied allowing clone libraries construction, whilst with archaeal primers, only LMi sample gave positive results.

Table 1. Sampling sites names (abbreviations used in the text) and physicochemical characteristics

	T (°C)	pH		T (°C)	pH
Las Máquinas (LMa)	36.0	4.80	Las Maquinitas (LMi)	35.0	3.50
Baño 9 (B9)	30.0	2.73	Laguna Verde (LVE)	30.0	4.80

When analyzing bacteria from the four biofilms together species across the whole phylogenetic tree were found. According to RDP classifier tool, the 16S rRNA sequences from Copahue's biofilms were affiliated with the taxonomical groups *Acidobacteria*, *Firmicutes*, *Chloroplast*, *Cyanobacteria*, *Deinococci*, *Delta*, *Gamma*, *Beta* and *Alphaproteobacterias*, *Bacterioidetes*, *Nitrospira*, *Caldilineaea* and *Chloroflei*. However, as can be seen in Figure 1, OTUs composition was different in each biofilm.

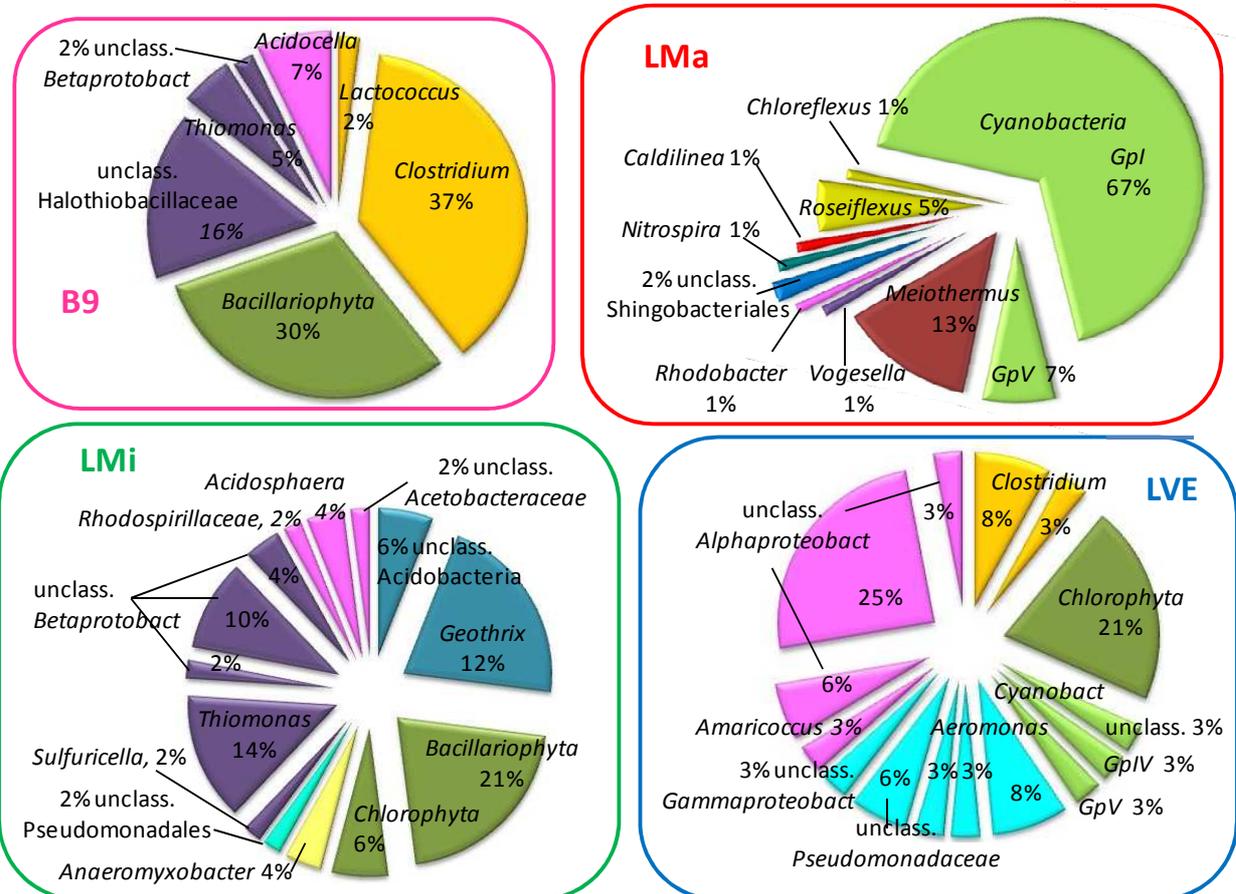


Figure 1. Bacterial clone libraries of the four biofilms studied. Different colours correspond to different classes. Taxonomic classifications were done using RDP.

Photosynthetic species were detected in the four samples, with higher prevalence and diversity in LMa. *Cyanobacteria* were detected in LMa and LVE. LMa was dominated by Group I species, 100% similar to *Mastigocladus laminosus*, thermophilic cyanobacteria typical of hot springs. In LVE three OTUs of cyanobacteria were found, one of them 98% similar to *Leptolyngbya* (GpIV). In preliminary results from a pyrosequencing study of these biofilms we detected *Cyanobacteria* species in the four of them. B9, LVE and LMi showed between a 21 and a 30% of the bacteria clones related to chloroplasts from photosynthetic autotrophic eukaryotes (*Bacillariophyta* and *Chlorophyta*). Among *Alphaproteobacteria* photosynthetic species were also detected. In LMi 2% of the clones were affiliated with the family *Rhodospirillaceae* and in LMa 1 % of the clones were affiliated with the genus *Rhodobacter*, both taxonomical groups composed of photosynthetic purple non sulphur bacteria. Two OTUs from *Chloroflexi* class were detected in LMa library. One was related to genus *Roseiflexus* known as thermophilic photosynthetic bacteria that form red mats in

natural environments. The other was 98% similar to *Chloroflexus aurantiacus*, a photoheterotrophic green non sulphur bacteria.

As regards LMi archaea library the sequences were related to sequences detected in geothermal or acidic environments, some of them in Copahue's ponds water or in Rio Agrio [1]. In contrast to what was found in the ponds water, dominated by *Crenarchaeota* mainly from *Sulfolobales* order, archaea community in biofilms showed a stronger presence of *Euryarchaeota* (unclassified *Thermoplasmatales*, and genera *Ferroplasma* and *Thermigomonas*). The sequences affiliated to *Crenarchaeota* domain were related to thermophilic species like *Sulfolobus*, *Pirobaculum* and *Vulcaniasaeta*. Autochthonous *candidatus "Acidianus copahuensis"*, isolated in Copahue's ponds [7], was not detected in this study.

18S rRNA gene clone libraries were of poor quality and the study of eukaryotes biodiversity was repeated using pyrosequencing technique (unpublished). However, we could detect sequences of *Scenedesmus*, *Chaetophora*, *Pinnularia*, *Semispithidium* and *Platyreta* genera.

Conclusion

Biofilms community structure in Copahue geothermal springs presents many photosynthetic species from various genera of bacteria and eukaryotes. Considering the species detected, biofilms presumably have wide versatility of photosynthetic metabolisms. Although cyanobacteria are generally rare to find in acidic environments, we have detected them in the Copahue's acidic biofilms. As we have pointed out in previous studies, Copahue is a niche of many potential new species with possible uses in different bioprocesses, from biofuel and bioplastic production, to biomining and bioremediation of contaminated environments.

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