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How flexible are the prokaryote consortia in the extreme habitat of the Copahue Geothermal system?

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Abstract. Microbial consortia taken from an extreme environment were grown at different temperatures and enrichment media. The consortia response to environmental changes was evaluated in order to investigate their metabolic flexibility. The molecular technique, DGGE (denaturing gel gradient electrophoresis) was carried out to evaluate the biodiversity. The results show that each consortium was able to grow according to the available resources, demonstrating their flexibility. A selective development was detected when growing conditions were similar to those found in the natural environment even though some species were able to grown even in conditions far away from those present in the sampling sites.

Introduction

Copahue-Caviahue geothermal system (Neuquén, Argentina) is an extreme natural environment characterised by many ponds and geothermal springs with wide ranges of temperatures, pH values and high concentration of heavy metals. The microorganisms that colonise this geothermal spring are mainly acidophilic, mesophilic, moderate and extreme thermophilic, both bacteria and archaea [1]. Due to the particular environmental conditions found in the area, the microorganisms that inhabited Copahue might have potential applications in different bioprocesses, such as bioleaching of metal sulphides [1,2].

The goal of this study is to analyse the flexibility of Copahue microbial communities by growing samples taken from ponds with different physicochemical conditions, using different temperatures and enrichment media.

Material and Methods

The water and sediment samples used to inoculate the different enrichment cultures, were taken from four ponds of Copahue-Caviahue geothermal system. Table 1 shows the physicochemical characteristics of those sampling sites. They were selected in order to have representation of the different physicochemical conditions of the area.

Original samples were incubated in 140 mL flasks, agitated at 125 rpm, at three different temperatures (30ºC, 50ºC, and 70ºC) and using three enrichment media with different energy sources: elemental sulphur, iron(II) and/or yeast extract.

Table 1. Sampling sites and the corresponding measurements of temperature and pH at field.

Sampling Site	Label	Temperature [ºC]	pH
Laguna Slfurosa (LS3)	1	67	4.00
Las Maquinitas (LMi2)	2	87	2.50
Baño 9 (B91)	3	87	6.50
Las Maquinitas (LMi1)	4	34	3.00

Table 2 shows the factorial design scheme used to assess growth flexibility of Copahue communities. Iron free 9K medium [3], named 0K was used, and initial pH was adjusted with H_2SO_4. Sterile controls were done in every case.

Table 2. Factorial design scheme used for the enrichment of cultures.

Enrichment media	Temperature		
	T1 = 30ºC	T2 = 50ºC	T3 = 70ºC
M1 = 0K + Sº (10 $g.L^{-1}$) pH=3	M1-T1	M1-T2	M1-T3
M2 = 0K + Fe^{2+} (1.4 $g.L^{-1}$) pH=2	M2-T1	M2-T2	M2-T3
M3 = M1+ Fe^{2+} (1.4 $g.L^{-1}$) + yeast extract (1 $g.L^{-1}$) pH=2	M3-T1	M3-T2	M3-T3

Iron(II) oxidation was determined by permanganometry and changes of redox potential, while sulphur oxidation was monitored by protons titration with sodium hydroxide solution and pH measurements.

Diversity of microbial cultures was analysed by DGGE (denaturing gel gradient electrophoresis). The DGGE banding patterns of 16S rRNA genes PCR products, from the natural and enriched samples, was performed using specific *Eubacteria* and *Archaea* primers [4].

Visual comparison of band profiles, cluster analysis, and diversity indexes [5,6] were used to analyse DGGE data. Visual comparison of band profiles analyse presence/absence of bands and changes in bands intensity. In the cluster analysis, similarities between banding patterns generated by DGGE were analysed using the Pearson correlation coefficient. The clustering algorithm used to perform the analysis, was an unweighted pair group method with arithmetic averages (UPGMA) [5]. The cluster analysis and dendrogram generation were obtained by GelCompar II software. Additionally, the species richness (S) and evenness (E) factors, and also the Simpson (D and D-1) indexes were evaluated [7].

Results and discussion

Microorganisms from all natural samples were able to grow at the three selected temperatures, using at least one of the energy sources present in the different culture media. In some cases, they grew at pH values far from the ones measured in their natural habitats. In general most samples were able to grow in all media, under all the conditions assayed. The results presented in this paper were selected as they represent the general behaviour of the cultures.

Figure 1 shows iron(II) oxidation kinetics in cultures with M3 media. Iron(II) was oxidised in all samples at 30ºC and 50ºC. As temperature increased, iron(II) oxidation rate was slower. In particular, sample 3 at 70ºC presented almost no iron(II) oxidation. However, sample 3 at lower temperature presents iron consumption, probably due to the presence of dormant state species which developed under lower temperatures conditions [8]. Similar iron(II) oxidation kinetics were observed for all samples grown on M2 media (data not shown).

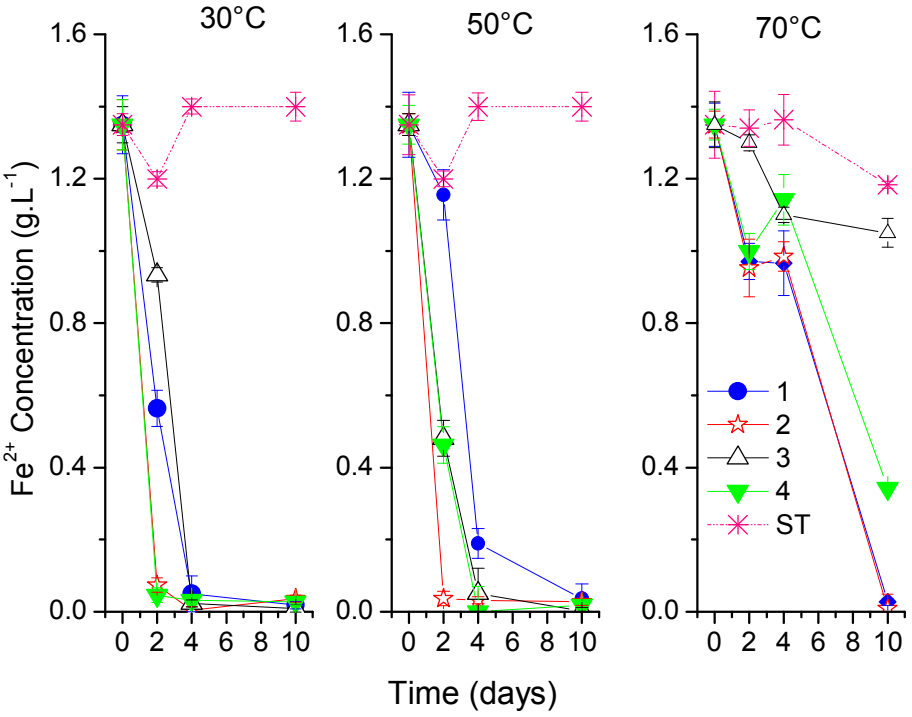

Figure 1. Iron(II) oxidation kinetics in cultures with M3 media at different temperatures.

All samples were able to oxidise sulphur at all temperatures assayed. Samples 1 and 4 grown on M1 medium (with sulphur) reached lower pH values at low temperatures; on the contrary, samples 2 and 3 reached lower pH values at high temperatures.

The diversity of natural samples and enrichment cultures was analyzed to evaluate the effect of changes in growth conditions and to assess their flexibility. Simpson's Diversity Index is a measure of diversity which takes into accounts both richness and evenness giving more weight to the more abundant species in a sample. It is often used the Simpson's Index of Diversity as (1 – D) which goes between 0 and 1; the greater the value, the greater the sample diversity. To calculate D and richness (S) number and intensity of bacterial DGGE bands were used. In general, the DGGE analysis showed that the original samples presented fewer bands, less intense than the ones observed in the enrichment cultures. DGGE profiles of *Eubacteria* showed more bands at lower temperatures. On the other hand, more bands were observed in the gels for *Archaea* at 70°C. Table 3 shows some of 1-D and S values for *Eubacteria* DGGE profiles.

Table 3. Richness and Simpson's diversity index calculated from *Eubacteria* DGGE analysis for samples grown in M3 under different conditions.

	Sample 1 (LS3) 67°C and pH=4.0				**Sample 3** (B91) 87°C and pH=6.5				**Sample 4** (LMi1) 34°C and pH=3.0			
	1	30°C	50°C	70°C	**3**	30°C	50°C	70°C	**4**	30°C	50°C	70°C
S	3	5	6	1	10	2	3	3	8	6	8	1
1-D	0.64	0.76	0.80	0.00	0.87	0.48	0.59	0.67	0.88	0.83	0.87	0.00

Finally, UPGMA dendrograms were obtained from DGGE banding patterns, using the Pearson correlation coefficient which takes into account not only the relative position of the bands, but also their intensity.

In general, the dendrograms showed that samples were grouped mostly by temperature, as it is shown in Figure 2 for sample 1.

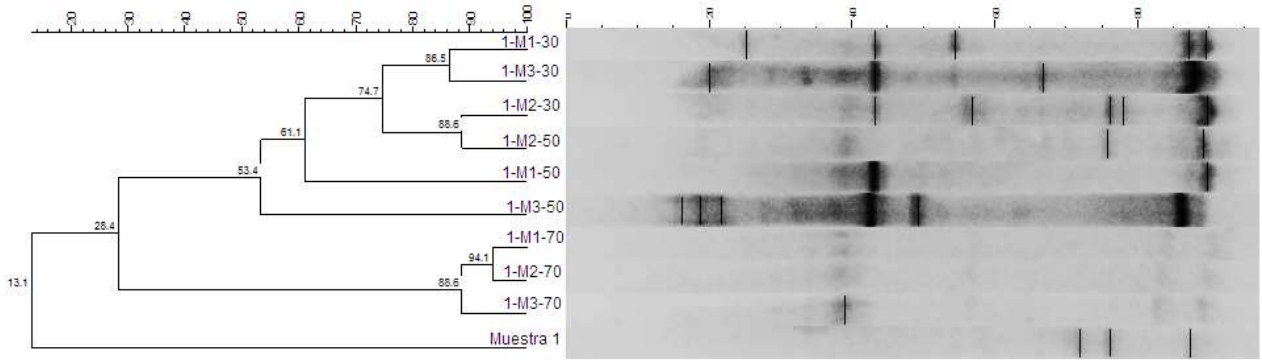

Figure 2. UPGMA dendrogram obtained from sample 1 DGGE banding patterns. Comparison made with Pearson correlation coefficient. Tolerance band position: 1%.

Each consortium developed according to available resources, demonstrating their flexibility. In general more significant microbial growth was detected whenever physicochemical conditions were similar to those found in their natural environment. However, some species were common in many of the enrichment cultures tested, even in conditions far away from those present in the sampling sites.

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