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## CYANOBACTERIAL MATS FROM THE CARRIZAL, A GEOTHERMAL SPRING POOL IN MEXICO

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### HIGHLIGHTS

- Cyanobacterial mats
- Geothermal spring in Mexico

### ABSTRACT

Cyanobacterial microflora is very diverse in different habitats over the world and closely connected with the ecology of the habitat. In particular, extreme habitats are colonised by numerous specialised morpho- and ecotypes which are unique to these ecologically specialised environments. A variety of methods are needed to characterize cyanobacterial assemblages in thermal water environments. We report here on the community structure of the cyanobacterial mats at the hot spring of the alkaline and low sulphide artificial pool of “Carrizal” (Villa Emiliano Zapata, Mexico). The most common organisms were *Anabaena* sp., *Phormidium* sp. and *Pseudoanabaena* sp. Some filamentous cyanobacteria showed a 98%-95% 16S rDNA gene similarity with *Oscillatoria* sp. and cluster together with other filamentous cyanobacteria from the thermal environments.

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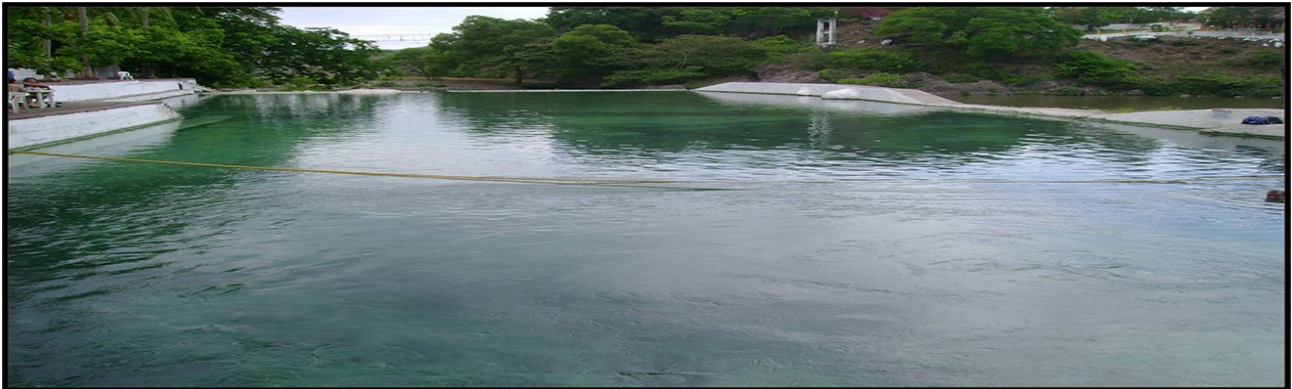
Microorganisms communities

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## 1. INTRODUCTION

The study on the biogeography of species provide to understand the mechanisms which are at the base of the biodiversity, like speciation, extinction, dispersion and interactions among and into the species. The geographic distribution of plants has been studied by the biologists since 1900 while research on biogeography of microorganisms is more recent. The application of more advanced molecular technologies has offered the possibility to analyses the biodiversity of a microbial community (Cennamo & Ciniglia 2017, Ciniglia et. al 2004, Ciniglia et al. 2005). Considering extremophilic microbial communities, it to note these communities require extreme habitats, which are often characterized by high temperature and different pH values (Brock 1986; Cennamo & Ciniglia, 2017). The extreme environments are widespread in nature and are host to organisms whose ecophysiological features are very useful, to provide models of interpretation of many biological phenomena (Castenholz, 1978; Ciniglia et al 2004; Ciniglia et al. 2005). Geothermal environments (e.g. hot springs) host a diversity of biota formed by thermophilic organisms. Scientific exploration on the life in geothermal environments has accelerated in recent decades, and dramatically new forms of life have been discovered in recent years. (Castenholz, 1978, HindàK, 2001, Ward et al. 1998). Microbial mats composition in geothermal springs are featured by different temperature and pH. Studies on microorganisms from these environments utilized 16S and 18S rRNA gene data from environmental samples and showed the occurrence of different lineages of bacteria and cyanobacteria, in particular in the thermal sites *Synechococcus* and *Oscillatoria* species are abundant.



**Figure 1:** El Carrizal's pool. Source: photo by P. De Luca

In this study we report information on the community structure of the microbial mats in hot springs of the alkaline artificial pool of Carrizal (Villa Emiliano Zapata, Mexico, Fig. 1) 250m on sea level, with a mean annual temperature was of 29°C annual and a vegetation mainly represented by *Ficus tecolutensis*, *F. glabrata*, *Bursera simaruba*, *Salix humboldtiana*, *Plumeria alba* and many other species of caducifolia. Carrizal, a few miles off Highway 140 coming up from the Gulf Coast to Xalapa, is a hot spring next to a river "Los Pescados", with a huge thermal pool, a variety of pleasantly warm swimming pools. The water of the hot spring discharged into an artificial pool (50X12 m), where the temperature was 40°C. This artificial pool is an example of urban settlement at the edge of the city, something very common in many European cities (Sgobbo, 2011; Mangoni & Sgobbo, 2013; Moccia et al., 2016). The pool was built with volcanic rocks, and the main elements present in the water are Na, Mg, Cl, Li and S. All mats possessed highly ordered layers of airspaces at both the macroscopic and

microscopic levels, as a result of an adaptation to a pre-floating growth habit. The diversity was evaluated in parallel employing both morphological and molecular techniques (among the latter, 16S rDNA sequencing and Denaturing Gradient Gel Electrophoresis - DGGE).

## 2. METHODS

### 2.1 Environmental samples.

El Carrizal (Formative Period 1500 B.C.-A.D. 100) is a site in south-central Veracruz, in Mexico; north of Los Pescados River, it is in the Dos Rios Municipal District and is easily accessed from Xalapa and Veracruz city. The area is famous for the thermal pool resorts, which are a tourist attraction.

Mats samples in the pool (Fig. 1) were collected during two seasons (spring and autumn 2009). Twenty biofilm samples were collected with a sterile scalpel, placed into sterile plastic vials and transferred to laboratory, where they were grown on different media (for bacteria, cyanobacteria and algae). Mats generally occurred in shallow (4-5 cm depth) water. Temperature and pH in the pool where mat growth was recorded; the thermometer probes were also inserted into holes left from biomass sampling (Fig. 2).

### 2.2 Microscopic observations.

Optical microscopy was used to detect microorganisms (Nikon Eclipse E800). SEM was also employed to study biofilms characteristics. For SEM observations, after critical point drying, samples were coated with gold in an Emitech k550 Sputter Coater and photographed by a Philips EM 208S in order to examine the morphology of the different microbial populations and the relationships between microbial growth and mineral substrate (Fig. 3).

### 2.3 DNA extraction and PCR amplification.

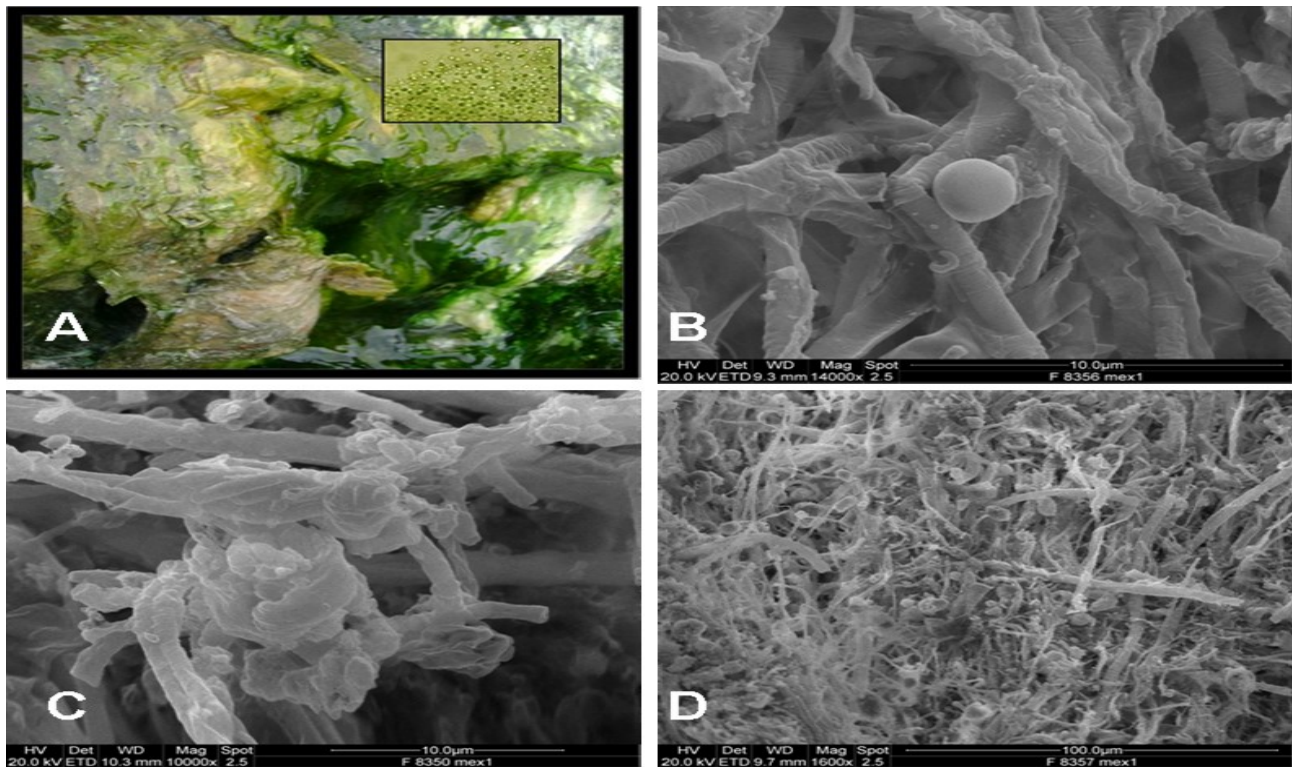
DNA was extracted from liquid cultures following the procedure described in Doyle & Doyle (1990). PCR amplification was performed with about 10 ng of extracted DNA using the primer set Euk 1A and 516-GC (Ferris et al. 1996).

### 2.4 DGGE (Denaturing Gradient Gel Electrophoresis).

DGGE analyses were conducted following the protocol of Ferris et al. (1996). DGGE bands were sequenced after excision from gel and reamplification. Briefly, bands were excised, resuspended in 20 ml of water DNase free, and stored at 4°C over night. The majority of DGGE bands were cloned, sequenced and matched with the GenBank database; percentage similarities ranged from good (99%) to poor (88%). The sequences obtained from DGGE bands were aligned to the already available sequences in the database (Gene Bank) of the Bio Edit software (Hall, 1999).

## 3. COMMUNITY STRUCTURE OF THE CYANOBACTERIAL MATS STRUCTURE

Resulted morphological, biochemical and physiological characters did not allow to place the strain of bacteria into any know genus.



**Figure 2:** Optical (A) and SEM (B-D) observation of microbial mats. Source: photo by P. Cennamo

For this reason the morphological data on the isolated strains were supplemented with phylogenetic analyses based on 16S rRNA gene sequencing. This allowed us to sequence over 1,400 bp of the gene. The sequences were aligned and plotted as a tree using the neighbor joining algorithm. Among the bacteria, the maximum similarity of the 16S rRNA gene sequences was with *Rhodospirillum rubrum* sp. clustering in the members of the class of Gammaproteobacteria, typical of the warm water (Alfredsson et al. 1988, Alfredsson et al. 1994). The sequences indicated the presence of another bacteria group forming by the *Leptothrix* sp. group. The results indicated that these bacteria belong a thermophilic genus in the *Rubrivivax* subgroup of the Proteobacteria, described also by Elbanna et al. (2003) in a thermal site in Egypt. In all sampling sites during two collecting period we found *Acanthamoeba* species. *Acanthamoeba* is a free-living opportunistic protozoan parasite which is frequently in aquatic environments and hot springs (Bing-Mu, 2009). In many cases, *Acanthamoeba* species are associated with bacteria of *Legionella* which can survive and grow in *Acanthamoeba*. In fact, in many samples we observed the presence of *Legionella*. These data indicated that the coexistence of *Acanthamoeba* with *Legionella* should be considered a potential public health threat. It is important to remember that *Legionella* is pathogenic to humans, but the risk is reduced in widely airy open environments. Cyanobacteria are the dominant components of the mats present in the pool of the Carrizal. The recognized filamentous cyanobacteria resulted belonging to two lineages, formed by *Oscillatoria* and *Synechococcus* sp.; showing 95%- 98% 16S RNA gene similarity and clustering together with some other filamentous cyanobacteria from thermal environments (data not shown). Strain *Pseudoanabaena* sp. and *Leptolyngbya* sp. were found in each sampling sites. The comparison with other sequence available in GenBank showed that the ours *Leptolyngbya* strains found by us fall in the clade of other *Leptolyngbya* isolated in geothermal environments, probably the condition in thermal environments condition conserved the genotype of this species. We obtained the same result for the *Pseudoanabaena* species. A specific interesting finding is the present of *Ochromonas* sp. that that on the

basis of the molecular analyses resulted forming a different group in the clade of *Ochromonas* species. *Ochromonas* is a single-celled, motile, golden-brown alga frequently found in thermal and acidophilic environments. Occurrence of this species in the Carrizal pool shows that this organism is able to live in alkaline thermal environments, as also reported for geothermal sites in Italy (Ciniglia et al. 2004). The characterization of the microbial community of the mats present in the Carrizal pool allowed to identify species commonly living in thermal environments, like to *Rhodobacters* sp., *Leptothrix* sp., *Acanthamoeba*, *Legionella*, *Pseudoanabaena*, *Leptolyngbya* and is important to evidence the presence of a new lineage of *Ochromonas* in alkaline environments. In conclusion, we have characterized community by morphological and molecular analysis of the thermophilic mats. The 16S/18S rRNA gene-defined diversity of all mats was only in part confirmed by the microscopic observations, whereas genotypes resolved at all the identification of these microorganisms.

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