



Ecology

Effect of seasonality and physicochemical parameters on bacterial communities in two hot spring microbial mats from Araró, Mexico

Efecto de la estacionalidad y de los parámetros fisicoquímicos sobre las comunidades bacterianas de tapetes microbianos de manantiales termales de Araró, México

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Abstract

In this study, we explored the diversity of culturable bacterial communities residing in hot springs from Araró, México, and analysed the effect of seasonality and related changes in physicochemical parameters of spring water. Two hot springs with unique features, Tina and Bonita, were analysed. Seventy-nine unique 16S rRNA gene phylotypes were detected, belonging to the bacterial phyla Firmicutes, Proteobacteria, and Actinobacteria. A group of dominant phylotypes of the genus *Bacillus* was recovered in 3 out of 4 of the sampling seasons. Another group of phylotypes was recovered in 2 samplings, while the remaining groups were detected in only 1 season. Ecological indexes for species richness and evenness showed moderate to low diversity in both hot springs, and a Sørensen analysis revealed that the 2 communities shared 64% of their bacterial phylotypes. Physicochemical parameters measured every season showed slight variations, except for temperature and arsenic content. Fluctuations in bacterial composition in the Tina hot spring were correlated mainly with salt content, while diversity in the Bonita hot spring was significantly correlated with temperature, pH, and arsenic content.

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Keywords: Microbial mats; Hot springs; Bacterial diversity; Environmental factors; Arsenic; Thermophilic communities

Resumen

En este estudio se explora el efecto de la estacionalidad y los cambios fisicoquímicos sobre la diversidad cultivable de la comunidad bacteriana de dos manantiales termales de Araró, México. Se encontraron 79 filotipos de 16S rRNA de las divisiones bacterianas Firmicutes, Proteobacteria y Actinobacteria. Un grupo de filotipos del género *Bacillus* fue el más dominante, aislado en 3 de las 4 estaciones muestreadas, otro grupo de filotipos se recuperó en 2 muestreos, mientras que los grupos restantes se detectaron en sólo 1 temporada. Los índices ecológicos para riqueza de especies y equitabilidad muestran una diversidad de moderada a baja en ambos manantiales, mientras que el índice de Sørensen resulta en que ambas comunidades de comparten hasta un 64% del total de los filotipos. Los parámetros fisicoquímicos medidos muestran ligeras variaciones estacionales, excepto la temperatura y el contenido de arsénico. Las fluctuaciones en la composición bacteriana en Tina estuvieron correlacionadas principalmente al contenido salino mientras que en Bonita las variaciones se asociaron con la temperatura, pH y contenido de arsénico.

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Palabras clave: Tapetes microbianos; Manantiales termales; Diversidad bacteriana; Factores ambientales; Arsénico; Comunidades termófilas

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Introduction

Hot springs can be found in many regions of the world. Each hot spring has unique geophysical and biological characteristics, making them interesting subjects of research with potential value for either biotechnological or ecological purposes (Briggs et al., 2014; Brock, 1997; Klatt et al., 2011; Lau, Aitchison, & Pointing, 2009; Tirawongsoj et al., 2008). Because of these differences, bacterial diversity in microbial mats is expected to be different among hot springs. Analyses of microbial diversity have been reported for hot springs located in many countries, including the USA (Ward, Ferris, Nold, & Bateson, 1998), Russia (Perevalova et al., 2008), Australia (Kimura et al., 2005), Thailand (Portillo, Sirin, Kanoksilapatham, & González, 2009), Bulgaria (Tomova et al., 2010), Colombia (Bohorquez et al., 2012), Philippines (Huang et al., 2013), Chile (Mackenzie, Pedrós-Alió, & Díez, 2013) and China (Briggs et al., 2014).

The physicochemical parameters of source waters in some hot springs can vary by season while in others, these parameters can be very stable. These parameters can influence the microbial diversity in springs. A few studies have assessed these parameters in association with microbial community composition in hot springs (Briggs et al., 2014; Ferris & Ward, 1997; Mackenzie et al., 2013). For example, microbial diversity was surveyed in 3 hot springs located in La Patagonia, Chile, over 2 seasons. The authors found differences in the microbial communities between the seasons, probably due to temperature variations (Mackenzie et al., 2013). Other factors, such as pH, levels of hydrogen sulphide, and temperature, have been associated with the presence of certain microbial species (Briggs et al., 2014; Purcell et al., 2007; Ward & Castenholz, 2000).

To determine the bacterial diversity in microbial mats, both cultivation-dependent and -independent approaches have been employed, depending on the research goals. A cultivation-independent methodology was used in a recent PhyloChip microarray analysis of the microbial communities inhabiting hot springs of the Tengchong region in China (Briggs et al., 2014). However, this technology is not appropriate for the isolation of thermophilic microorganisms, their genes, or their proteins to determine their potential importance for biotechnology applications (Brock & Freeze, 1969). The 2 approaches can be complementary; however, an initial microbial diversity analysis can be a first step in searching for bacteria with enzymatic activities for industrial application. A good example of this approach is a report by Kanokratana, Chanapan, Pootanakit, and Eurwilaichitr (2004), which first described the biodiversity of bacteria and archaea from hot springs in Thailand. Years after this work, they reported on a functional screening of a metagenomic library derived from sediments of the hot springs, which resulted in the isolation of 2 novel genes encoding an esterase and a phospholipase (Tirawongsoj et al., 2008).

In the present study, using a culture-dependent approach, we investigated the seasonal variation (Spring, Summer, Fall, and Winter) in bacterial diversity of 2 hot springs located in the geothermal system of Araró, Michoacán, México, which is a system independent from Los Azufres geothermal zone (Brito et al., 2014; Viggiano-Guerra & Gutiérrez-Negrín, 2005). The

physicochemical parameters of both hot springs were assessed and associated with fluctuations in bacterial biodiversity.

Materials and methods

The geothermal system of the Araró region is located in the central part of Mexico, inside the Trans Mexican Volcanic Belt located in Michoacán State. The region is approximately 20 km west of the well-known Los Azufres geothermal field (Fig. 1). The zone known as Zimirao (19°53'54" N, 100°49'50" W) is where most of the hot springs are located. There are about 50 hot springs in the Araró region, and many of them are used for recreational activities; however, the selected hot springs, Tina and Bonita, have relatively little disturbance and are far from the recreational area. Another interesting feature of these hot springs is that Bonita has low water emission and forms colourful microbial mats, while Tina has a constant water effluent and a little running stream, with microbial mats formed along the stream (Fig. 2). Compared to Bonita, Tina presents only a very thin bacterial mat. Therefore, the systems exhibit different features that might influence the communities present in microbial mats.

Four samplings were conducted on February 2nd (Winter), June 5th (Spring), October 6th (Summer), and December 5th (Fall) of 2013. Three samples were directly collected from microbial mats in each of the 2 hot springs at a depth of 30–50 cm from the surface. A criterion for selection of these 2 hot springs was whether the system was closed or open. One spring selected was a “closed system” with low water emission (Bonita), while the other (Tina) was an “open system” with constant water emission (Fig. 2). Microbial mat samples were immediately transported on wet ice to the lab. For water sampling, 500-mL sterile Kinex flasks were used to take water from each hot spring. Water samples were stored in darkness and transported to lab on dry ice.

Physicochemical parameters of water in the hot springs were measured during sampling of biological material. The parameters, including temperature (°C), electrical conductivity, pH, and dissolved oxygen, were measured *in situ* with a Corning® Checkmate™ II modular meter system.

Physicochemical analyses, including faecal coliforms analysis, were performed in collaboration with the National Water Commission (Conagua-México) (Table 1). Arsenic concentrations in the water samples of the Bonita and Tina hot springs were measured by absorption spectroscopy using an atomic absorption spectrometer (Perkin-Elmer Analyst 200) with a hydride generation system. The measurement of fluoride was carried out with a conventional fluorimeter.

A small sample (0.1 g) of the microbial mat from each spring was placed into Eppendorf tubes (1.5 mL) with 990 µl of sterile water, vortexed, and plated onto 3 different 10-fold-diluted culture media. These included salted, rich, and poor media (Luria-Bertani, Nutrient Agar (NA), and Minimal Medium (MM), respectively, purchased from Sigma-Aldrich). The LB medium contained (/L) yeast extract (5 g), tryptone (10 g), NaCl (10 g). NA medium contained (/L) peptone (5 g) and beef extract

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