GAYANA

Gayana (2025) vol. 89, No. 1, 66-72

SHORT COMMUNICATION

First record of multicellular filamentous benthic bacteria from freshwater lagoons in central Chile

Primer registro de bacterias bentónicas filamentosas multicelulares en lagunas de agua dulce en Chile central

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ABSTRACT

Observations on “macrobacteria” inhabiting the reduced bottoms of two freshwater lagoons in central Chile are here presented. The new finding of morphologically diverse, colorless multicellular filamentous bacteria expands their known presence into Chilean continental freshwater environments and confirms their association with reduced habitats. Their morphologies and ecology appear to connect them to similar benthic marine findings and Precambrian microfossils, encouraging their exploration in similar systems around the world and guiding the search for biosignatures in extraterrestrial paleoenvironments.

Keywords: exobiology, freshwater bacterial community, macrobacteria.

RESUMEN

Se reportan las observaciones sobre “macrobacterias”, que habitan los fondos reducidos de dos lagunas de agua dulce en Chile central. Estos nuevos registros de bacterias filamentosas morfológicamente diversas amplían el conocimiento sobre su presencia a ambientes dulceacuícolas en Chile y confirman su asociación con hábitats reducidos. Sus morfologías y ecología las vinculan con hallazgos bentónicos marinos y con microfósiles del Precámbrico, alentando su exploración en sistemas similares de todo el mundo y guiando la búsqueda de biofirmas en paleo-ambientes extraterrestres.

Palabras clave: comunidad bacteriana de agua dulce, exobiología, macrobacterias.

Large multicellular filamentous bacteria are key inhabitants of benthic communities in marine and freshwater systems (Schulz & Jørgensen 2001; Gallardo *et al.* 2013; Kjeldsen *et al.* 2019). They mediate biogeochemical processes at the sediment-water interface (Jørgensen 1982; Kjeldsen *et al.* 2019). Their morphology has been used to connect them with ancient Precambrian microfossils (Schopf *et al.* 2007) and to classify them into two ecomorphotypes: the vacuolated “megabacteria” with cell diameters larger than *ca.* 10 µm (Maier & Gallardo, 1984), and the smaller than *ca.* 10 µm in diameter, non-vacuolated “macrobacteria” (Gallardo & Espinoza 2007a, 2007b).

Subsequent findings have revealed distinct habitat patterns for each ecomorph. Megabacteria inhabit the low-oxygen, nitrate-rich benthic sediment-water interface (Huettel *et al.* 1996), whereas the macrobacteria are typically found fully immersed in anoxic sediment (Gallardo & Espinoza 2007a, 2007b; Gallardo *et al.* 2013). Moreover, the association between morphotypes and reduced sulfidic habitats suggests that microfossils found in evaporite

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sulfates may correspond to similar communities, indicating that these species have undergone relatively little change over hundreds of millions of years (Gallardo & Espinoza 2007b; Schopf *et al.* 2012; 2017). Therefore, the discovery of new systems where filamentous multicellular bacteria occur provides valuable insights into Precambrian life and, possibly, future extraterrestrial findings. Specifically, they could serve as modern analog systems and, at the same time, as easily accessible terrestrial study models for comparison with Martian environments.

Larger multicellular filamentous megabacteria have been identified in diverse aquatic systems (Schulz & Jørgensen 2001), consistently associated with the sulfide-rich bottom sediments. The Humboldt Sulfuretum is the most conspicuous benthic filamentous bacteria ecosystem where megabacteria and macrobacteria have been found (Gallardo 1977; Gallardo & Espinoza 2007a, 2007b; Gallardo *et al.* 2013). In the Benguela Upwelling System off Namibia, an analogous habitat, the presence of megabacteria has already been confirmed (Gallardo *et al.* 1998, Schultz *et al.* 1999). While macrobacteria have not yet been reported in the latter environment, the rich sublittoral *"slimy grass"* reported by von Bonde (1928) for the southeastern Atlantic, could well refer to a bacterial community like that of the South-eastern Pacific which harbours both types of filamentous life. Although not always classified as such, macrobacteria have been reported in sewage treatment plants (Eikelboom 1975), rice cultivation systems (Joshi & Hollis 1977), and mangroves (Gallardo & Espinoza 2007b). However, marine macrobacteria were largely overlooked until they were rediscovered and gained more attention due to their abundance and morphological diversity in highly reduced sublittoral marine sediments from central Chile (Gallardo & Espinoza 2007a, 2007b).

To evaluate the hypothesis regarding the association of filamentous multicellular macrobacteria with reduced bottoms of natural freshwater lagoons, in 2014 the exploration started with a survey of two urban freshwater systems in Chile: Laguna Chica and Laguna Grande, located in San Pedro de la Paz, Biobío region, Chile (Fig. 1). These freshwater systems have experienced an increasing process of eutrophication (Parra 1989; Scasso & Campos 2000; Cruces *et al.* 2001; Parra *et al.* 2003) with their bottoms experiencing more severe hypoxic conditions during the summer months (Parra *et al.* 2003). These conditions create a favorable environment for the primeval life here described, which had previously gone undetected.

We here present results from a sampling at three stations per lake (see Table 1). Sediment core samples were obtained using a homemade gravity corer (the "Oticorer"). The cores were extracted undisturbed and redox measurements were immediately performed using a Waterproof Handheld Meter Kit Oakton PD 650. At the shallowest station in Laguna Chica, sediment with a strong hydrogen sulfide odor was recovered with the benthic corer at the same time that bubbles reached the surface. The microscopic observations were performed on the day of collection at the Benthos and Exobiology Lab of the Universidad de Concepción. Microphotographs were captured using a Canon DS126291 camera which was mounted on a Zeiss Axio microscope. Filamentous diameter measurements were made with the EOS Utility and AxioVision SE64 Rel 4.9.1 software. To search for giant filamentous macrobacteria, an inspection of reduced sediment was conducted on the first ten centimeters.

The microscopic inspection revealed a morphologically diverse community of filamentous bacteria (Fig. 2). As shown in Fig. 3, all filaments belonged to the so-called "macrobacteria" (*sensu* Gallardo & Espinoza 2007b), showing a morphological diversity that previously reported from marine samples (Gallardo & Espinoza 2007b). Macrobacteria were found in all sampling stations, although there was an evident decrease in abundance at greater depths. In both lakes, the sediment redox measurements indicated a sudden change in the oxidation-reduction potential after the first centimeter at all sampling stations (see Fig. 4), consistent with previous findings (Gallardo & Espinoza 2007a, 2007b). No filamentous bacteria were found beyond the first centimeter of surface sediment.

Table 1. Geographic information and sampling station depths. / Información geográfica y profundidad de las estaciones de muestreo.

	Laguna Chica			Laguna Grande		
Sta.	**Lat. (°S)**	**Long. (°W)**	**Depth (m)**	**Lat. (°S)**	**Long. (°W)**	**Depth (m)**
1	36°50'59"	73°05'09"	16	36°51'14"	73°06'25,5"	10
2	36°50'50"	73°04'56"	12	36°50'57,7"	73°06'28,5"	6
3	36°50"41"	73°05'28"	2.5	36°50'48"	73°06'40"	2

Figure 1. Position of sampling stations within the study lagoons. / Ubicación de las estaciones de muestreo en las lagunas bajo estudio.

The first sampling expedition to Laguna Chica and the only survey at Laguna Grande have documented the first occurrence of macrobacteria communities in a Chilean freshwater system. Notably, in all four Laguna Chica samples, the macrobacteria community was consistently present. Unlike the marine prokaryotic communities documented from the eastern South Pacific, here the vacuolate megabacteria were absent.

Measurements from filamentous samples collected from both lagoons revealed that cell diameters ranged from 0.76 µm to 8.47 µm (Fig. 3). In general, all morphotypes exhibited some form of characteristic movement. For instance, the morphotypes in Figs. 2c, 2i, were observed moving laterally. Others, such as those shown in Figs. 2a, 2d, and 2j, displayed gliding movements, and what appeared to be sensitive, ocurring at the tips of the multicellular filament suggesting active chemotaxis. These behaviors have been interpreted as adaptations to varying chemical gradients (Jørgensen & Revsbech 1983). Such movements have also been reported in the very thin (0.4 – 2.2 µm) multicellular filamentous macrobacteria known as "cable bacteria", which belong to the family Desulfobulbaceae (Bjerg *et al.* 2016) whose diameters largely overlap with those reported here (Fig. 3). Cable bacteria inhabit both marine and freshwater environments (Risgaard-Petersen *et al.* 2015) and can connect sulfide oxidation with oxygen or nitrate reduction through long-distance electron transport between vertical phases (Kjeldsen *et al.* 2019). Similarly, some of our morphotypes exhibit globules inside their cells (Fig. 2a, 2b, 2d, and 2j). In colorless sulfur bacteria *i.e.*, Beggiatoaceae, this characteristic has been identified as stored elemental sulfur (Teske & Nelson 2006), suggesting similar metabolic pathways are present in the morphotypes reported here.

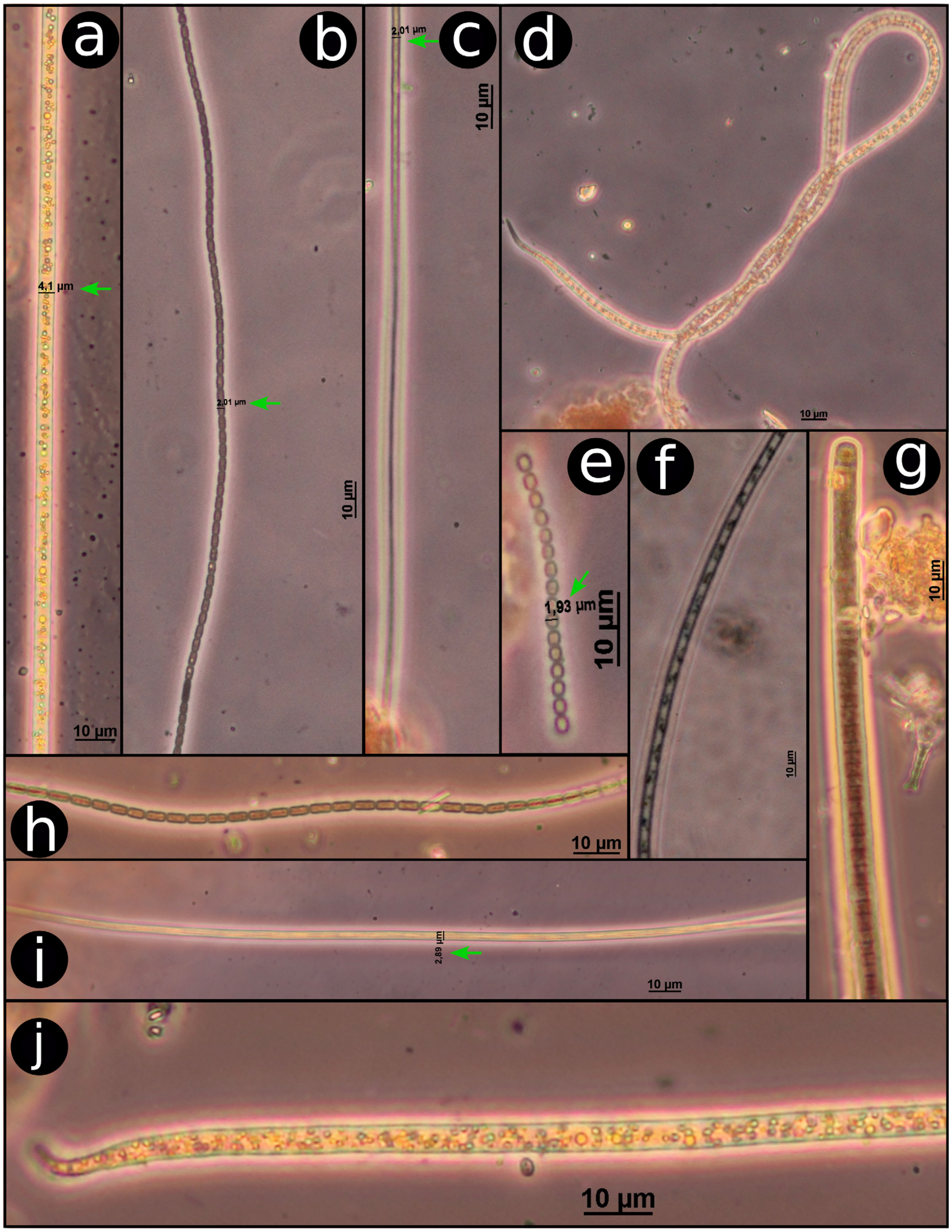

Figure 2. Colorless morphotypes of benthic filamentous macrobacteria collected in 2014 from central Chile freshwater lagoons: a-e. Benthic filamentous macrobacteria from Laguna Chica. f-j. Benthic filamentous macrobacteria from Laguna Grande. Arrows indicate diameter: b-c. 2.01 µm; i. 2.89 µm. Photographs by F.D.-G. / Morfotipos de macrobacterias incoloras recolectadas en lagunas de agua dulce de Chile central en 2014. a-e. Macrobacterias filamentosas bentónicas de la Laguna Chica. F-j. Ejemplos de bacterias filamentosas encontradas en Laguna Grande. Las flechas indican el diámetro. Fotografías por F.D.-G.

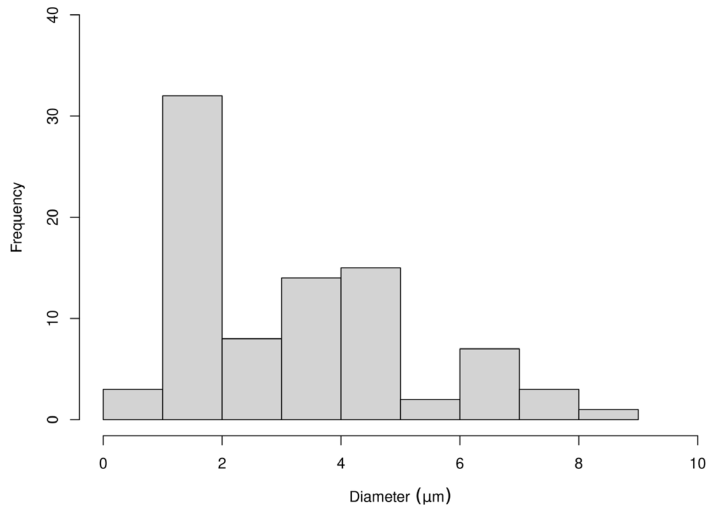

Figure 3. Cell diameter distribution of 89 filamentous multicellular macrobacteria randomly chosen from both surveyed lagoons. / Distribución del diámetro celular de 89 macrobacterias filamentosas elegidas al azar desde ambas lagunas.

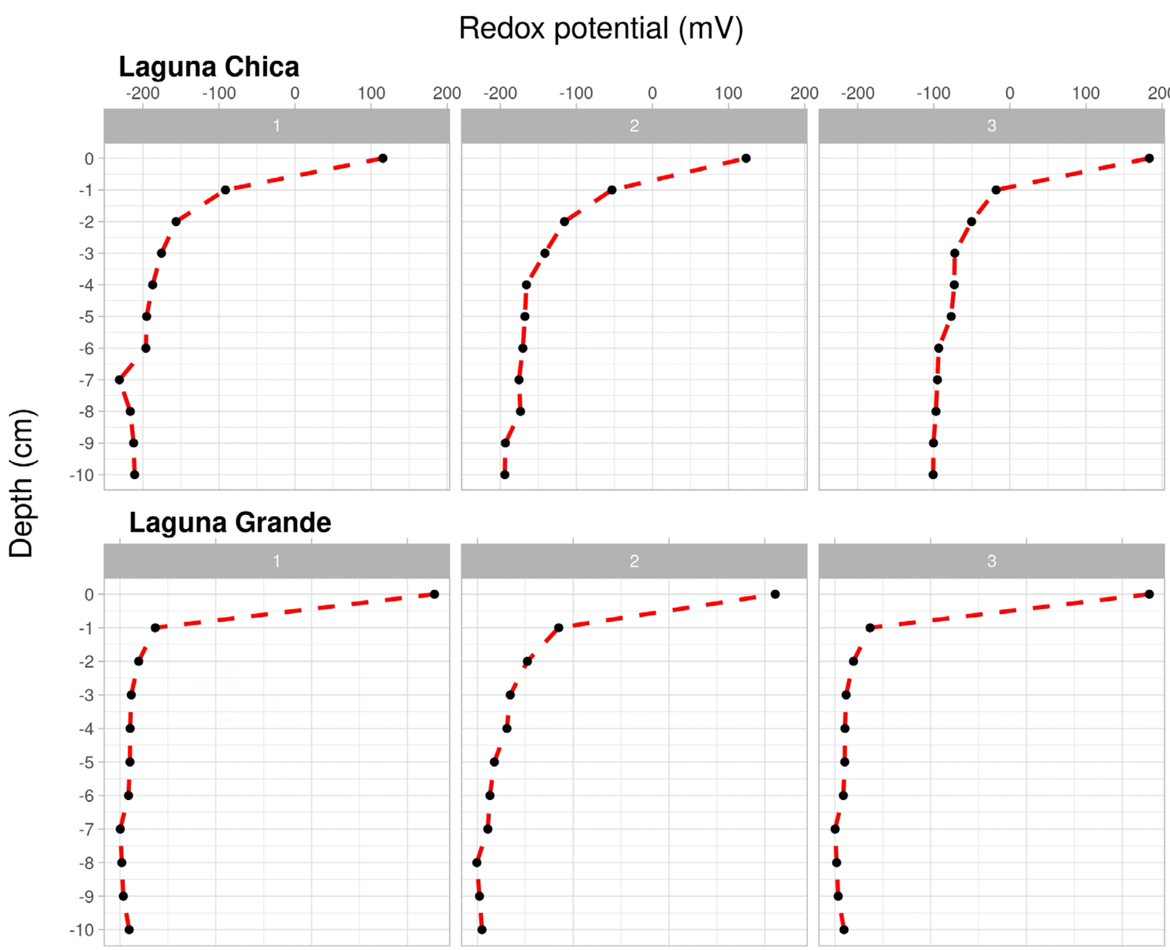

Figure 4. Redox potential measured in the first 10 cm of depth of the sediment core. / Potencial redox medido en los primeros 10 cm de profundidad del testigo de sedimento.

We emphasize the diversity of these filamentous bacteria, which need to be described and assigned to their corresponding taxa, while also elucidating their role within the community. Although some morphological features described above (internal globules, diameter range and movements) suggest their belonging to macrobacterial families, we concur with Salman *et al.* (2011, 2013) that morphological characteristics alone are insufficient for the taxonomic assignment of the larger sulfur bacteria, requiring also a molecular systematic approach. Additionally, some morphotypes may correspond to filamentous cyanobacteria, such as *Oscillatoria*-like species (Hauerová *et al.* 2021), which have occasionally been recognized as sharing the marine environment with megabacteria and macrobacteria (Gallardo 1977, Jørgensen 1982). It is also necessary to explore the physiological diversity within these communities to comprehend their roles in this ancient hypoxic, sulfide-rich habitat, and to extrapolate to past and potential extraterrestrial environments (Gallardo & Espinoza 2007a, 2007b; Schopf *et al.* 2012). This could provide evidence to evaluate predictions about the formation of Archean consortia, hypothesized to have been established during the Paleoarchean anoxic atmosphere (Schopf *et al.* 2017). Moreover, the potential biotechnological applications derived from specific taxa of macrobacteria, such as the so-called "cable bacteria", which have potential applications in bioelectronics and bioremediation (Meysman 2018), need to be explored.

Considering: (1) that sulfur cycling assemblages of filamentous bacteria presently regarded as "living microbial fossils" could have been a major component of the Paleoarchean near-surface biosphere (Schopf *et al.* 2017); and, (2) that stratigraphic studies on the Martian Jezero paleo-lake have begun in search for biosignatures within a variety of sulfate phases (Benison *et al.* 2024), our lacustrine findings of modern filamentous multicellular macrobacteria serve as a valuable guide for studying Archean and Proterozoic community analogues, linking micropaleontology and exobiology (Gallardo & Espinoza 2007; Schopf *et al.* 2012, 2015, 2017).

ACKNOWLEDGEMENTS

We thank D. Barrera, A. Cáceres, C. Contreras, E. Ehrenfeld, M. González, L. Rivera, J. Toro, Á. Zúñiga B. Ernst, E. Tarifeño, and Mr. Monné for fieldwork and logistics help.

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Received: 02.10.2024
Accepted: 21.03.2025

Editor: Fulgencio Lisón