New communities of large filamentous sulfur bacteria in the eastern South Pacific

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Summary. New complex communities of morphologically diverse and sometimes abundant large, multicellular, filamentous bacteria were discovered in the oxygen-deficient, organically laden, shelf sediments under the oxygen minimum zone off the coast of the eastern Pacific, i.e., off the coasts of central and northern Chile; central and northern Perú; Roca Redonda, Galápagos Archipielago, Ecuador; and off the Pacific coasts of Panamá and Costa Rica. Similar microbial communities were also observed in the reduced layer of a muddy-sand beach adjacent to a mangrove swamp on Coiba Island, Pacific Panamá, and in the organically laden bottom underneath a salmon culture pen in southern Chile (region X). Of varying morphology, the diameters of the bacteria range from <1 to <10 μm, and their lengths from around 10 μm to usually several hundreds but at times several thousands of micrometers. The new filamentous bacterial component is at least one order of magnitude smaller than the also multicellular "megabacteria" *Thioploca* spp. and *Beggiatoa* spp., and is collectively referred to as "macrobacteria". A recent review only mentioned a few of these free-living filamentous bacteria, remarking on their scarcity despite the obvious advantages of a large size. This prokaryote size-window has been rarely investigated optically by researchers; thus, assemblages that appear to have had world-wide distribution probably since pre-Cambrian times have been overlooked. [**Int Microbiol** 2007; 10(2):97-102]

Key words: *Thioploca* spp. · *Beggiatoa* spp. · *Thiomargarita* · filamentous bacteria · large sulfur bacteria · eastern South Pacific · anoxic environments · ENSO cycle

Introduction

Despite their size, the finding of large multicellular filamentous bacteria in the sediments of the present oceans has been a relatively recent development [2,3,8,9]. Since the discovery, in 1962, of massive mats of the giant *Thioploca* spp. in highly reduced shelf bottoms under the oxygen minimum zone (OMZ) of the productive waters of northern Chile [32], much of the research effort has focused on these "megabac-

teria" [8]. Conversely, little attention has been paid to the accompanying, very diverse, and at least one order of magnitude smaller filamentous forms making up the "macrobacteria" component [3]. The latter have been found in remarkable abundance in highly reduced sediments from central Chile—both at the COPAS Time Series Station 18 (TSS-18; 88 m depth) and at other shallower as well as deeper localities—during the exceptionally long inter-El Niño period that has followed the last event (1997–1998). This rediscovery of the "macrobacteria" prompted us to undertake a detailed optical study and to survey other localities along the eastern Pacific. The goal of the latter effort was to test the hypothesis that these "macrobacteria" occur wherever overlying oxygen-deficient waters [6] and reduced (sulfidic) sediments are present. Here we present the results of this preliminary biogeographic study, which consisted mostly of samples from sublittoral settings but also samples from intertidal and a human-impacted site.

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Materials and methods

Data describing the sites where "macrobacteria" were sampled are summarized in Table 1. Sublittoral reduced sediment samples were obtained with either a mono- or a multicorer except off Ancón (Perú) and Iquique (Chile), where 0.05-m^2 and a 0.02-m^2 van Veen bottom grabs were used, respectively. Sediment samples from off the coasts of Perú and Chile were collected from under the OMZ (<0.5 ml/l). In Golfo Dulce (Costa Rica), oxygen concentrations were >1 ml/l. In the Gulf of Panamá, sediments were green and slightly sulfidic. Sediment cores from the multi- and monocorer were sub-sampled into smaller tubes and kept at appropriate temperatures in thermally insulated boxes until their live examination in the laboratory. From the grab samples, sub-samples were obtained from the upper surface. However, this method was not as effective in collecting the "macrobacteria" as it was in collecting deeply buried filamentous "megabacteria". Finally, samples were obtained by hand by divers from a warm sulfide seep off Roca Redonda, Galápagos Archipielago, Ecuador, and underneath a salmon culture pen in southern Chile and by hand-coring at low tide in an organically

rich beach off a mangrove swamp at Coiba Island (Pacific coast of Panamá). Figure 1 shows the geographical location of the different sampling sites along the coast in the eastern South Pacific, both in Central and South America.

Phase-contrast observations and microphotography were done on living material with either a Standard Zeiss microscope supplied with a Sony digital camera or, more recently, with an AxioZeiss microscope supplied with AxioVision Software and a Canon digital camera. Slides for microscopic observations were prepared by extracting small quantities of the interstitial water from the first few millimeters of the cores, either with an Eppendorf or a simple Pasteur pipette. Microdilution of the sample on the slide with water from the overlying layer was usually necessary to obtain cleaner images. The amount of water under the cover slip was just enough to yield a tight preparation, in order to allow for immersion-oil observation when needed. A 4× objective was used to quickly scan the preparation for quality, a 20× objective for quick systematic scanning of the preparation, and a 40× objective for most of the microphotography. Smaller forms or details were recorded under immersion oil with either a 63× or a 100× objective. 10× oculars were always used. Since the aim of our study in this phase was to confirm the presence of "macrobacteria" in the different settings and to record as many morphs as possible, no systematic attempt was made to

Table 1. Sample location and associated data of the communities of the large filamentous sulfur bacteria in the eastern South Pacific

Sample location	Locality (Country)	Date	Depth (m)	Location	Oxygen (ml/l)	Gear	Observations
Warm seep	Off Roca Redonda Galápagos Islands (Ecuador)	Feb. 2004	19	0° 16′ 20″ N 91° 38′ 0″ W	ND	Diving	Sulfide warm seep
Time Series Station 18, shelf	Off the Bay of Concepción (Chile)	2004-2007	88	36° 30.8′ S 73° 07.7′ W	<0.5	Multicorer and monocorer	Sulfidic
Enclosed sea area (Station 7)	Bay of Concepción (Chile)	2005-2007	35	36° 36.5′ S 73° 00.6′ W	<0.5	Monocorer	Highly sulfidic
Enclosed sea area (Station 4)	Bay of Concepción (Chile)	2005-2007	28	36° 38.7′ S 73° 02.2′ W	<0.5	Monocorer	Highly sulfidic
Inshore area	Hauyquique, Iquique (Chile)	Aug. 2004	35	20° 16′ S 70° 08′ W	<0.5	van Veen grab	Sulfidic
Shelf, open ocean	Antofagasta (Chile)	Oct. 2005	106	22° 58′ 58″ S 70° 23′ 44″ W	<0.5	Multicorer	Sulfidic
Below salmonc ulture pen	Región X (Chile)	2005	Diving depth	ca. 42° S	ND	Hand coring	Sulfidic
Inshore area	Ancón (Perú)	Aug. 2004	15, 34, 50	11° 45′ S 72° 12′ W	<0.5	van Veen grab	Sulfidic
Inshore area	Off Callao (Perú)	Oct. 2004	94	12° 02′ 82″ S 77° 29′ 13″ W	<0.19	Monocorer	Sulfidic
Coiba Island, mangrove beach (low tide)	Gulf of Chiriqui (Panamá)	Apr. 2007	Beach	7° 37.4′ N 81° 37′ W	ND	Hand coring	Sandy mud sulfidic below surface
Enclosed shelf area, off SW Oroque Island	Gulf of Panamá (Panamá)	Apr. 2007	23	8° 31.8′ N 79° 40′ W	ND	Monocorer	Slightly sulfidic green mud
Enclosed sea area	Golfo Dulce (Costa Rica)	Sep. 2005	195	8° 35′ 47″ N 83° 13′ 64″ W	>1	Monocorer	Slightly sulfidic

ND, not determined.
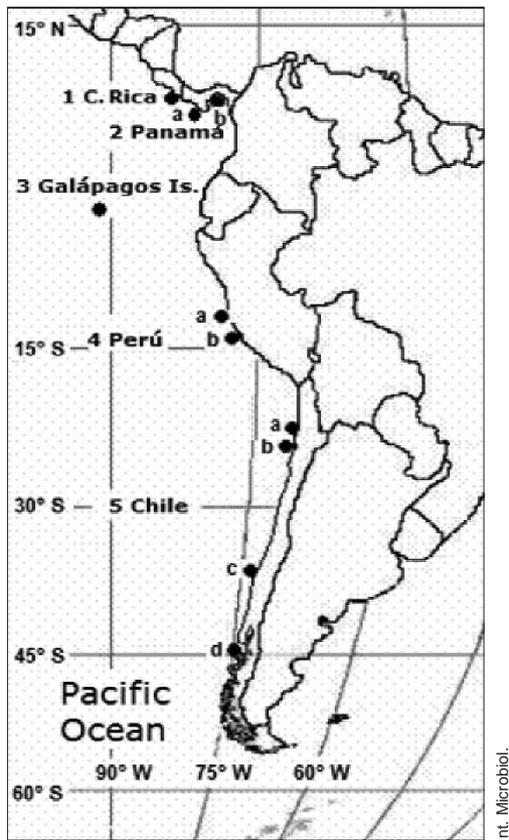
Fig. 1. Map with the sampling localities in the eastern South Pacific. (1) Costa Rica (Golfo Dulce). (2) Panamá: (2a) Coiba I., Gulf of Chiriquí; (2b) off SW Oroque I., Gulf of Panamá. (3) Ecuador (Roca Redonda, Galápagos Is.). (4) Perú: (4a) Ancón; (4b) Callao. (5) Chile: (5a) Huayquique, Iquique; (5b) Mejillones; (5c) Concepción (COPAS shelf Time-Series Station 18, and Stations 4 and 7 in Bay of Concepción); (5d) Región X.

quantify the morphs. Nevertheless, a single quantitative exercise was carried out on "macrobacteria" samples collected on March 2004 from off the coast of Concepción, central Chile. This attempt yielded ca. 3.6×10^5 cells/ml and about 280 g wet weight/m^2. The latter amount is of similar magnitude to that reported in the same area for the macrofauna [4]. A database of microphotographs of 209 morphs, mostly differing in diameter, is offered as supporting information (SI) online. A collection of video recordings of bacterial movements is also being built but is not presented.

Results and Discussion

The presence of morphologically diverse, multicellular, and motile filamentous "macrobacteria" was particularly striking in the hypoxic/anoxic shelf sediments under the OMZ off central Chile during the late austral summer (March) of 2004 at the COPAS TSS-18, located at 36° 30.8′ S, 73° 07.7′ W, 88 m depth, and 28.4 km from the coast. Although sporadic sampling started at TSS-18 in 1994 (the "Thioploca-Chile Expedition of the Max Planck Institute for Marine Microbiology, Bremen, Germany" [2]), the March 2004 sampling was the first time that the "macrobacteria" were optically targeted, following a simple protocol developed for sediment samples taken at a warm sulfide seep off Roca Redonda, Ecuador, during the Galápagos Leg of the Sorcerer II Expedition [11].

Since the last major El Niño episode (1997–1998), the sediments of the central Chile locality, over and beyond seasonal variations, have experienced a long period of normal, cold, productive ENSO (El Niño southern oscillation) conditions. The result of this remote oceanographic forcing is increasingly anoxic bottoms, i.e., black, and sulfide-

Fig. 2. Key to the different morphologies found in the assemblages of multicellular "macrobacteria". These examples are all from sediment samples collected off Concepción, central Chile (~36°S lat.) in the period 2004–2007. For completeness, slots are included for the spherical, unicellular, *Thiomargarita*-like forms (**A**), and the cylindrical, vacuolated, multicellular, *Thioploca* and *Thioploca*-like forms (**B.II.iii.5**).
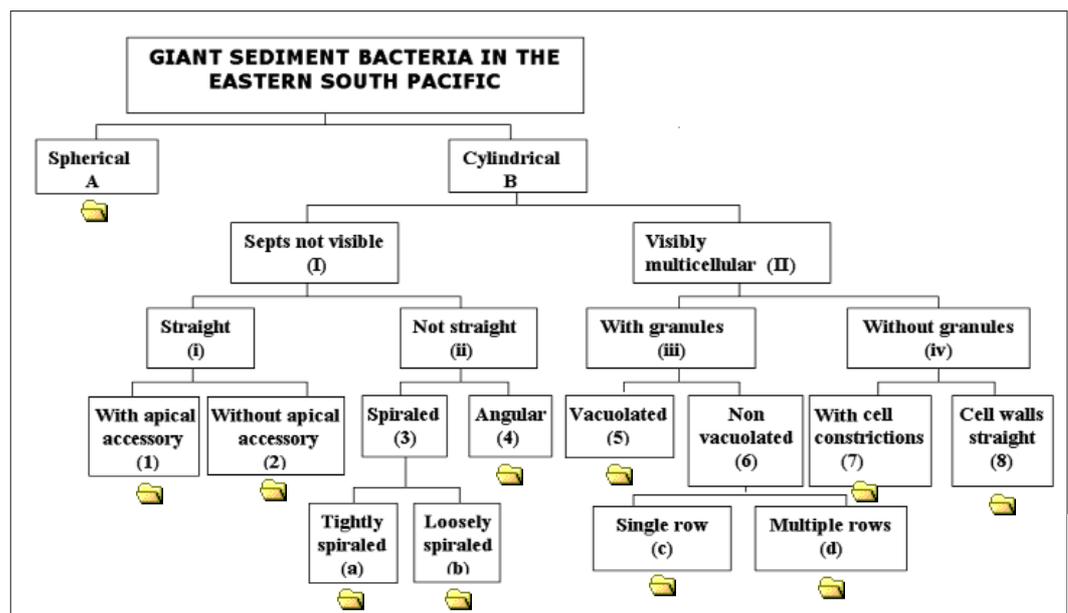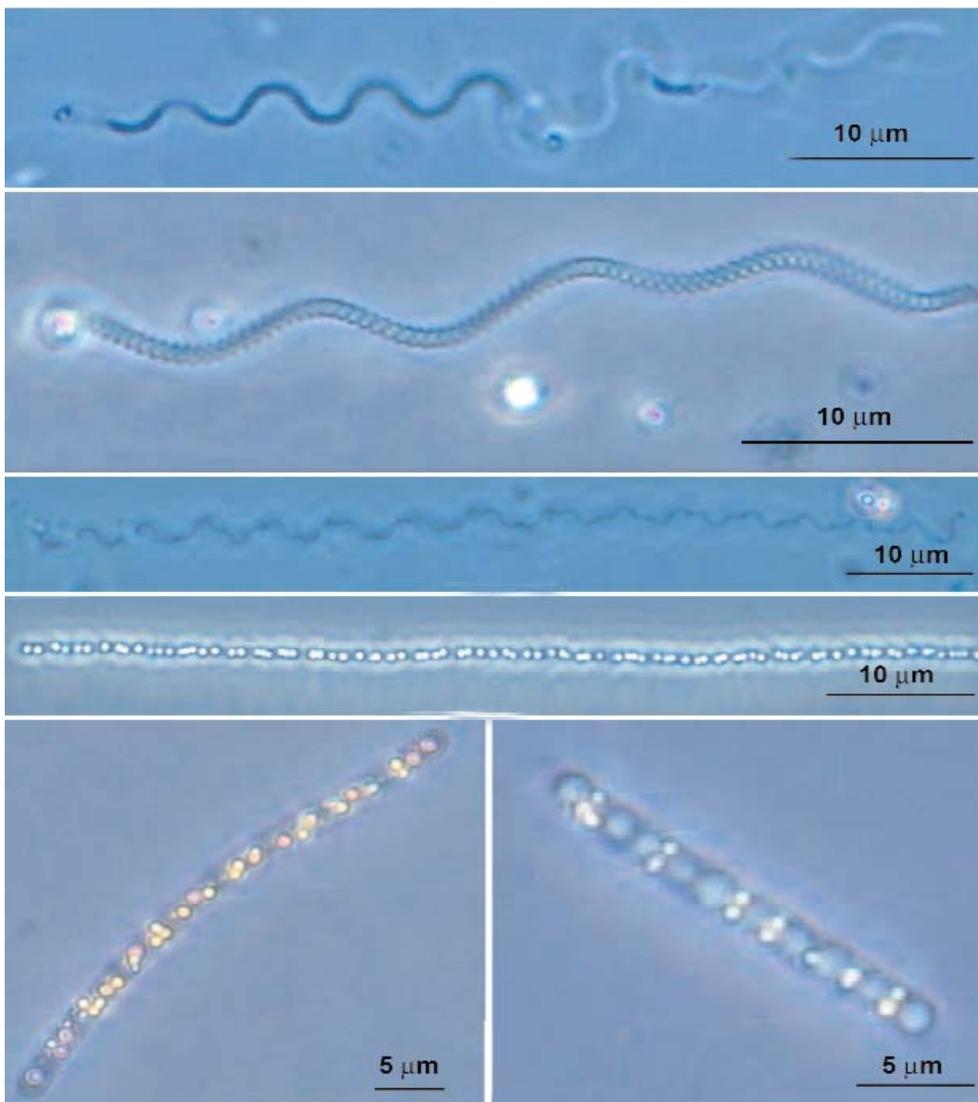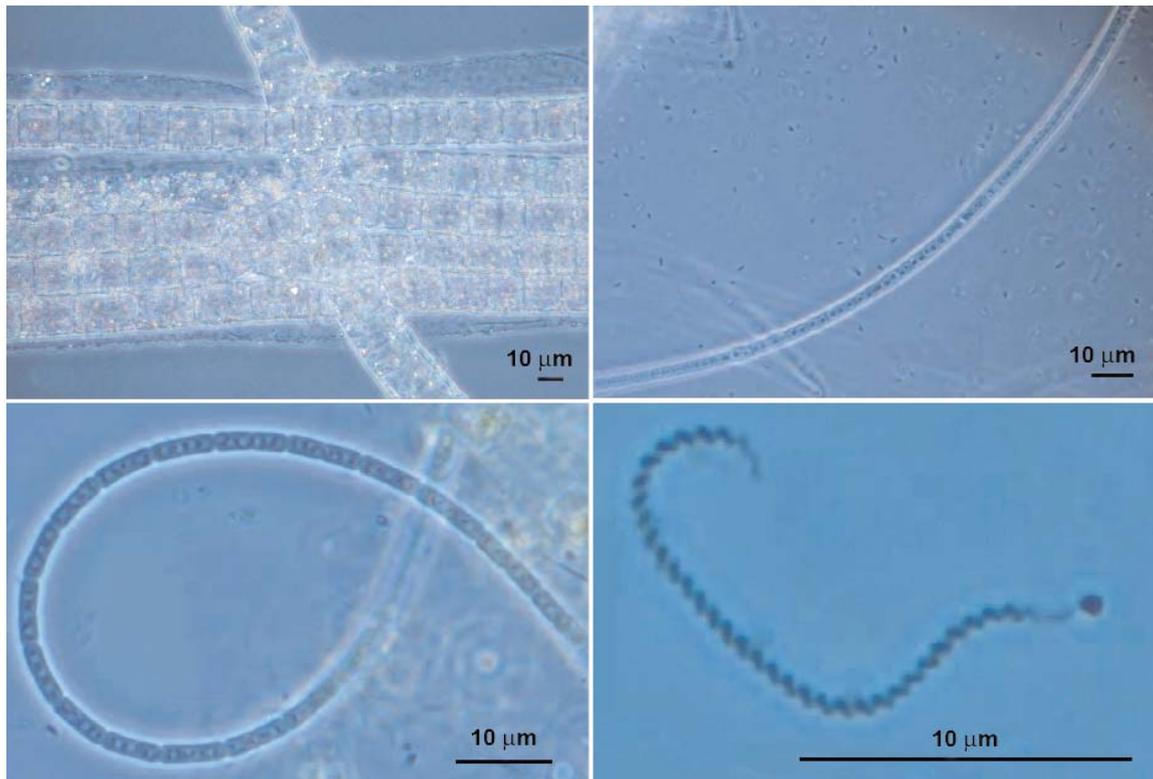
Fig. 3. A sample of "macrobacteria" representing different morphologies distinguished in the dichotomic key of Fig. 2 and the on line gallery [SI]. For this photo gallery, only shorter forms were selected.

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smelling, with growing but modest biomasses of "megabacteria" and decreasing abundances and biomasses of macrofauna (Gallardo and Espinoza, in prep.). Furthermore, in recent years, large filamentous bacteria have been observed off the coasts of northern Chile (Iquique and Mejillones), Perú (Ancón and Callao), Costa Rica (Golfo Dulce), and off the Pacific coast of Panamá (Gulf of Panamá). Sediment samples from a mangrove-influenced beach (Coiba Island, Gulf of Chiriquí, Panamá) and from under salmon culture pens in the austral region X in Chile also showed elements of this kind of bacteria. In most sites, the newly observed smaller forms were accompanied by either isolated, unsheathed, filaments (trichomes) or a mat of sheathed *Thioploca*-like filaments. Monthly biological and abiotic observations from COPAS TSS-18 indicated that the habitat there has become more favorable for an almost exclusively diversified anaerobic prokaryotic system than for the usual mixed, prokaryote/eukaryote—*Thioploca*-dominated—system previously described for the area [3,4]. In central Chile, the community of new large bacteria was found to be associated with both an increasing biomass of the "megabaterium" *Thioploca* and dwindling biomasses of macrofauna. The new filamentous bacterial component is at least one order of magnitude smaller than the "megabacteria" and is here collectively referred to as "macrobacteria". Of varying morphology (Figs. 2, 3, 4 and [SI]), the diameters of the "macrobacteria" range from <1 μm to nearly 10 μm, and their lengths from around 10 μm to mostly several hundreds or even several thousands of micrometers. Communities also containing spherical *Thiomargarita*-like forms [12] were found off the coasts of Chile, Perú, and Costa Rica, as were protista—foraminifera [10], ciliates, flagellates—and small metazoans (mainly nematoda).

Fig. 4. Other examples of "macrobacteria" representing different morphologies distinguished in the dichotomic key of Fig. 2 and the on line gallery [SI].

Coupled with their varied multicellular morphology, some elements of this assemblage showed an interesting variety of movements that suggested advanced stimuli transmission along the train of cells and fine coordination mechanisms. These observations suggested that "macrobacteria", together with those of Abreu et al. [1], are true multicellular organisms, in which different cells along the chain might differ in function. Albeit quite slowly, most forms can slide forwards and backwards but progress in a U-shape disposition has also been observed. The presence of what appears to be sulfur globules inside the cells of some of the "macrobacteria" suggested sulfide-based chemosynthesis, but granules of other polymers might also be involved. The widespread occurrence of such large, free-living bacteria, (although at times some of them were seen associated with the sheath of *Thioploca*), beyond the models of the "megabacteria", i.e., *Beggiatoa* and *Thioploca*, had been anticipated. In fact, large size offers a competitive advantage in that it helps to overcome the thermodynamic limitations of the micro-scale [13]. These cylindrical forms overcome not only these limitations but also those imposed by large spherical shapes (e.g., *Thiomargarita* [12]), in that the surface area:volume ratio of a cylinder does not change very much with increasing length, allowing thin filamentous forms a larger exposure to environmental resources. The use of this strategy is well-exemplified in Fig. 5, where the distribution of cell diameters (in μm) for
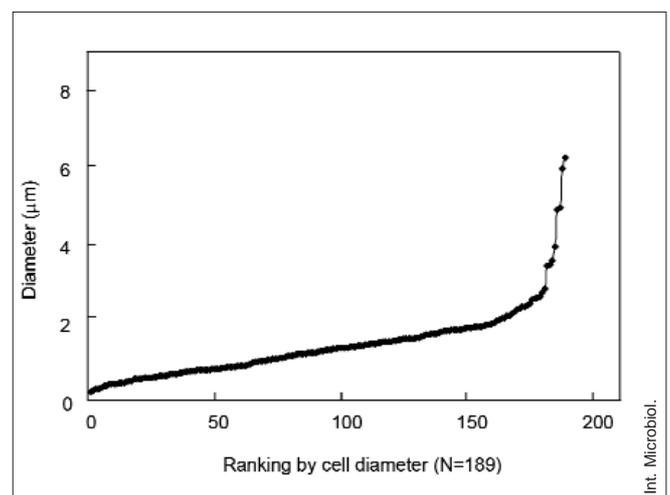
Fig. 5. Distribution of cell diameters (in μm) for 189 ranked morphs from off Concepción, central Chile. Since these bacteria reproduce by transversal sectioning, this diversity of cell diameters may represent different ecomorphs or even different species.

189 cell-diameter-ranked morphs, only from off the coast of Concepción, central Chile (COPAS TSS-18), is shown. It appears that the diameter of "macrobacteria" is maintained within strictly limited bounds and the exhibited variability might be associated with different ecological niches [5], thus potentially representing different ecomorphs (or, perhaps, "species").

Efforts have been initiated to elucidate the potentially varying phylogenies and physiologies among these novel large bacteria with respect to their spatial and temporal (seasonal and inter-annual) variations at the shelf of central Chile and the adjacent Bay of Concepción. The oxygen-deficient areas of the world's modern oceans are thought to provide analogs for the open-ocean benthic conditions that probably contained similar assemblages in the Archean and Proterozoic eons. The fact that large filamentous bacteria similar to the "macrobacteria" reported here are mostly known only as fossils [7,14] suggests that the study of these living representatives in present-day oxygen-deficient marine sediment settings can provide answers to paleobiological and paleoecological evolutionary questions and even insights into the discipline of astrobiology.

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