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Research Letter

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First description of a new uncultured epsilon sulfur bacterium colonizing marine mangrove sediment in the Caribbean: *Thiovulum* sp. strain karukerense

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One sentence summary: The sulfide-producing sediment of the marine edge of mangrove in Caribbean is not covered only by cyanobacteria but also by large gamma proteobacteria of *Beggiatoaceae* and by epsilon-proteobacteria belonging to the genus *Thiovulum* forming veils on the top of the sediment.
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ABSTRACT

Here, the first description is reported of an epsilon sulfur-oxidizing bacterium from sulfide-rich sediments of marine mangrove in the Caribbean. By transition electron microscopy it was shown that this new strain contains intracytoplasmic large internal sulfur granules, which was confirmed by energy-dispersive X-ray spectroscopy analyses performed using an environmental scanning electron microscope. The sulfur distribution obtained for this sulfur-oxidizing bacterial strain allowed us to conclude that elemental sulfur is formed as an intermediate oxidation product and stored intracellularly. By conventional scanning electron microscopy it was shown that the bacterial cells are ovoid and extremely motile by lophotrichous flagella. Phylogenetic analyses based on partial sequence of the 16S rRNA gene confirmed that the bacterial strain belongs to the *Thiovulum* cluster and could be a representative of a new species in this poorly studied genus of sulfur-oxidizing free-living bacteria. Thus, reduced sediment of marine mangrove represents a sulfide-rich environment sustaining development of both gamma and epsilon sulfur-oxidizing Proteobacteria.

Keywords: phylogeny; ultrastructure; STEM analysis; EDX; chemoautotrophic bacteria

INTRODUCTION

In the Caribbean, the marine edge of the mangrove ecosystem is characterized by the presence of *Rhizophora mangle* trees, the roots of which are in 'normal' seawater, with a pH, temperature, salinity and dissolved oxygen concentration similar to those in other tropical marine environments. The periphyton (consisting mostly of cyanobacteria) lies close to the submerged roots of *R. mangle*. It covers the sediment and is the dominant primary producer in the marine mangrove ecosystem (Guidi-Rontani *et al.* 2014). The granularity of the sediment particles, their total inorganic content, atomic total organic carbon/total nitrogen ratio and $\delta^{13}C$ values seem to be the principal parameters influencing the occurrence of microbial mats at the sediment–water interface (Gontharet *et al.* 2017). Another important element is the large amount of sulfides emitted from the sediment due to the anaerobic degradation of organic matter by sulfate-reducing bacteria. Sulfides (HS^-) are used by various bacteria, including anoxygenic photolithotrophic bacteria (such as purple sulfur bacteria) within the sediment, and sulfur-oxidizing bacteria located at the oxic–anoxic interface at the top of the sediment. Some of these sulfur-oxidizing bacteria are free-living colorless filamentous bacteria from the Beggiatoaceae that form

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dense bacterial mats (Jean *et al.* 2015), whereas others have developed symbiotic relationships with diverse marine organisms, from unicellular protists (Ott, Bright and Bulgheresi 2004) to metazoans, such as meduzoans (Abouna *et al.* 2015), bivalves (Frenkiel, Gros and Mouëza 1996) and nematodes (Himmel *et al.* 2009). Among these sulfur-oxidizing bacteria, *Thiovulum majus* (Marshall *et al.* 2012) is the only large-celled (cell diameter >5 μm) colorless sulfur epsilon-proteobacterium described to date. The species of genus *Thiovulum* was first described as *Volvox punctum* (Müller (1786) in Marshall *et al.* 2012), but were subsequently reclassified as *Thiovulum muelleri* based on morphological similarities to two species described by Hinze (1913): *Thiovulum majus* and *Thiovulum minus*.

In a nutrient gradient, *T. majus* cells aggregate, forming a community in which cells are attached to each other via a matrix of mucus tethers on top of the sediment. This matrix, which is known as a veil, may extend for several centimeters over the sediment surface. It is beneficial to its constituent cells in two ways (Petroff *et al.* 2015). First, it provides a permeable surface to which cells attach, allowing them to avoid the screening effect of surfaces and increasing the flow of water. Second, it provides a substrate on which the cells can organize to generate a macroscopic flow. Thus, *T. majus* exploits collective hydrodynamic effects to change its natural environment, generating millimeter-scale convective flows that pull nutrient-rich water to the cells (Fenchel 1994; Petroff *et al.* 2015). In shallow marine environments, an ability to build conspicuous veils has also been reported for other epsilon-proteobacteria, such as those of the genus *Arcobacter* (Wirsen *et al.* 2002) and *Candidatus* 'Thioturbo danicus' (Muyzer *et al.* 2005). Moreover, colorless vibrioid-shaped bacteria have been observed in sulfide-containing sediments mixed with decaying seagrass and macroalgae in Denmark, and have been cultured (Thar and Kühl 2002). However, no phylogenetic data are currently available for these colorless vibrioid bacteria.

Several studies have used *T. majus* as a model organism, to investigate the fastest known motility in the prokaryote kingdom (Garcia-Pichel 1989; Fenchel 1994; Petroff and Libchaber 2014; Petroff *et al.* 2015) or the physical advantages of veil formation for the physiology of sulfur-oxidizing bacteria in their environment (Jørgensen and Revsbech 1983; Thar and Fenchel 2001). By contrast, very few molecular data are available for this epsilon-proteobacterial genus. The only molecular analysis of *Thiovulum* species published to date is that of Marshall *et al.* (2012), who performed single-cell genome analyses on four individual cells collected from phototrophic mats in California. These analyses led to the determination of a partial 16S rRNA gene sequence that is now available for conventional phylogenetic analysis.

In sulfide-rich aquatic environments, *Beggiatoa* and *Thiovulum* species are typical of the gradient-type colorless sulfur bacteria occupying a transition niche between O_2 and H_2S (Thar and Fenchel 2001). They are often observed as isolated patches on sediments rich in organic matter located along the protected shores of lakes and the sea. However, *Thiovulum* species have never before been described in the Caribbean.

In this study, we explored the contribution of epsilon sulfur-oxidizing bacteria to marine mangrove microbial communities, through a combination of phylogenetic analysis (16S rRNA gene) and ultrastructural investigations (environmental scanning electron microscopy, scanning transmission electron microscopy, energy-dispersive X-ray (EDX) spectroscopy). We provide the first description of a sulfur-oxidizing epsilon-bacterial community from this typical tropical marine environment.

MATERIALS AND METHODS

Sampling site

Samples were collected manually, with a syringe, from the sediment of a tropical marine mangrove under a *Rhizophora mangle* canopy in Guadeloupe (French West Indies). Samples were collected at two sites (N16°16′560″/W61°33′303″ and N16°16′826″/W61°33′220″).

Scanning electron microscopy

Samples for scanning electron microscopy were fixed by incubation for 2 h at 4°C in 2.5% glutaraldehyde in cacodylate buffer (900 mOsM, pH 7.2). They were then dehydrated in a series of acetone solutions of increasing concentration, dried to critical point in CO_2 and sputter-coated with gold before observation with a FEI Quanta 250 electron microscope at 20 kV.

Energy-dispersive X-ray spectroscopy analysis

The chemical elements (such as sulfur) present in the individuals sampled were detected by observing freshly fixed samples (as described above for scanning electron microscopy preparation) in an environmental scanning electron microscope (FEI Quanta 250) operating at 15 kV, under an environmental pressure of 130 Pa (low-vacuum mode). EDX measurements were made with an M-max 50 mm^2 Oxford detector. The samples were briefly rinsed in deionized water immediately before observation, to remove salts. They were then placed rapidly in the electron microscope. Elemental mappings were obtained after an acquisition time of 180 seconds.

Scanning transmission electron microscopy

Freshly collected samples were prefixed by incubation for 1 h at 4°C in 2.5% glutaraldehyde in 0.1 M pH 7.2 cacodylate buffer adjusted to 900 mOsM with NaCl and $CaCl_2$ to improve membrane preservation. The bacterial cells were briefly rinsed in the same buffer and fixed by incubation for 45 min at room temperature in 1% osmium tetroxide in the same buffer. The cells were then rinsed in distilled water and post-fixed by incubation with 2% aqueous uranyl acetate for 1 h at room temperature before embedding in epoxy resin. Ultrathin sections (60 nm thick) were cut, and four to five such sections were observed in an FEI Quanta 250 electron microscope at 20 kV. The scanning transmission electron microscopy micrographs presented in this study are representative of all of the cells examined.

PCR amplification and DNA sequencing

Bacteria were collected and centrifuged for 3 min at 6000 *g*. The pellet obtained was suspended in 0.22μm sterile seawater and washed three times. The final pellet was used for DNA extraction with the DNEasy extracting kit (Qiagen), according to the manufacturer's protocol. Genes encoding the bacterial 16S rRNA were amplified with universal primer sets (8F-1492R and 533F-1492R), as previously described (Gros *et al.* 1996). The PCR products were directly sequenced by GATC Biotech (Germany) (http://www.gatc-biotech.com).

Phylogenetic analysis

A consensus sequence (1320 bp) was identified from the sequences obtained and its best hits according to BLAST (Altschul

et al. 1990) was aligned, with Clustal X software (Thompson *et al.* 1997), and refined manually. Phylogenetic analyses were performed with MEGA version 7 software (Kumar, Stecher and Tamura 2016). Phylogenetic trees were inferred by the maximum-likelihood method (Felsenstein 1981). Node robustness was assessed by performing 1000 bootstrap replicates (Felsenstein 1985). *Spirochaeta halophila* strain RS1 (M88722) was used as an outgroup.

Fluorescence *in situ* hybridization experiments

Bacteria were fixed by incubation for 2–3 h at 4°C in 4% paraformaldehyde in seawater. Specimens were then washed three times, for 10 min each, at 4°C, in seawater. They were then stored in 70% ethanol at 4°C until analysis. Two oligonucleotide probes (Cy3-labeled) were used: Eub338 (5′-GCTGCCTCCCGTAGGAGT-3′), targeting most members of the Eubacteria (Amann *et al.* 1990), and a more specific probe, EPS914 (5′-GGTCCCCGTCTATTCCTT-3′), targeting most members of the epsilon-proteobacteria (Grote *et al.* 2007).

Hybridization experiments were performed as previously described (Gros *et al.* 2012). The *Thiovulum* strain investigated here displayed no autofluorescence with the Cy3 filter used.

RESULTS

Ultrastructural and elemental analyses of the colorless spherical bacterium collected from marine mangrove sediment

Large veils of colorless microorganisms have been detected on the sediment close to *Rhizophora* roots in marine mangrove environments (Fig. 1A and B). These veils are invariably located at the interface between the anaerobic sediments and oxygenated seawater environments. We found that these white veils consisted almost exclusively of a dense population (Fig. 1D and E) of highly motile, colorless, spherical cells. Bright-field microscopy revealed the presence of refractive granules (Fig. 1E) randomly distributed in spherical cells with a mean diameter of 13.75 ± 1.92 μm (Fig. 1D and E).

Scanning electron microscopy observations showed that these microorganisms were present as single ovoid cells with large numbers of flagella at one of their poles (Fig. 2A and B). Scanning transmission electron microscopy observations showed that these bacterial cells had a double membrane typical of that in Gram-negative bacteria (Fig. 2C and D). No gas vesicles were detected. The 'large empty vesicles' observed within the cytoplasm probably correspond to sulfur inclusions lost during the dehydration and embedding processes. For characterization of the chemical content of these cells, we analyzed freshly collected samples with an environmental scanning electron microscope coupled to an EDX detector. The EDX spectra obtained from several individuals clearly have a major peak corresponding to sulfur (Fig. 1C), suggestive of the presence of elemental sulfur granules. Sulfur was strongly detected in all the cells, as shown by the sulfur distribution and mixmap picture obtained for this element (Fig. 1F). These results suggest that elemental sulfur is formed as an intermediate oxidation product and stored as refractive globules readily detected by light microscopy (Fig. 1F). Thus, on the basis of its phenotypic properties, we classified this species as a colorless sulfur-oxidizing bacterium.

Molecular identification of the colorless spherical bacterium collected from marine mangrove sediment

We determined the phylogenetic position of this colorless marine bacterium, by amplifying and partially sequencing the 16S rRNA gene (1320 bp fragment). The phylotype obtained was most similar (93% identity) to *Thiovulum* sp. ES (M92323), for which a full genome sequence is available (AKKQ00000000, Marshall *et al.* 2012) and to various sequences recovered from epsilon-proteobacteria (Campbell *et al.* 2006) of the genera *Sulfurimonas* (90% identity), *Sulfuricurvum, Sulfurovum, Sulfurospirillum* and *Helicobacter* (between 89 and 87%). The phylogenetic reconstruction confirmed these affiliations (Fig. 3). However, a comparison of three 16S rRNA gene sequences available for *Thiovulum* (*Thiovulum* sp. ES, *Thiovulum* sp. [AH003133], and *Thiovulum* sp. strain karukerense), revealed that these sequences were 88–96% identical with the sequence of the isolated bacterium, for the overlapping region. According to Yarza *et al.* (2014), these distances suggest that these strains, defined as '*Thiovulum*', may actually belong to at least two different genera. However, a larger number of longer 16S rRNA gene sequences from this as yet uncultured group will be required to determine its taxonomy accurately. The phylogenetic data were checked by fluorescence *in situ* hybridization with the probe designed for epsilon-proteobacteria (Grote *et al.* 2007). This experiment confirmed that almost all the bacterial cells forming the white veils collected from the mangrove were epsilon-proteobacteria (Fig. 2E).

DISCUSSION

The terrestrial parts of mangroves have been well investigated, but little is known about the microbes of the marine part of this ecosystem. Microbial mats in mangrove tidal channels are often covered by an outer layer of cyanobacteria (Guidi-Rontani *et al.* 2014), whereas their inner layers are composed of anoxygenic phototrophic bacteria (Lopez-Cortes 1990; Sheridan 1992). In areas characterized by weak hydrodynamic conditions, white patches consisting of an aggregation of colorless filamentous sulfur-oxidizing bacteria from the Beggiatoaceae may be seen on the surface of the marine sediment (Jørgensen 1977; Jean *et al.* 2015). Mangrove sediments are a sulfide-rich environment that (i) sustains the development of various free-living bacteria and (ii) facilitates the colonization of this particular ecological niche by various marine invertebrates harboring sulfur-oxidizing bacterial symbionts (Dubilier, Bergin and Lott 2008; Abouna *et al.* 2015). All of these bacteria are gamma-proteobacteria.

In the Caribbean mangrove, white veils of colorless rounded bacteria can be observed on the sediment, covering surfaces of several square meters. Our results suggest that these bacteria are epsilon-proteobacteria belonging to the genus *Thiovulum*. Several species of *Thiovulum* have been described, including *T. majus, T. minus* and *T. muelleri*, which differ in terms of cell size, motility and habitat (Hinze 1913; Kuever, Wawer and Lillebaek 1996; Garrity, Bell and Lilburn 2005; Marshall *et al.* 2012). Hinze (1913) described *T. majus* as being 11 × 9 to 18 × 17 μm in size. *Thiovulum minus* is smaller, in the range 9.6 × 7.2 to 11 × 9 μm, and both species display essentially unidirectional motility (Marshall *et al.* 2012). The intermediate-sized *T. muelleri*, which measures 6.3 × 4.9 to 12.8 × 10.2 μm (Petroff and Libchaber 2014; Petroff *et al.* 2015), displays a largely undirected zigzag pattern of movement. The size and motility of the *Thiovulum* isolated from Guadeloupe, i.e. *Thiovulum* sp. strain karukerense (mean

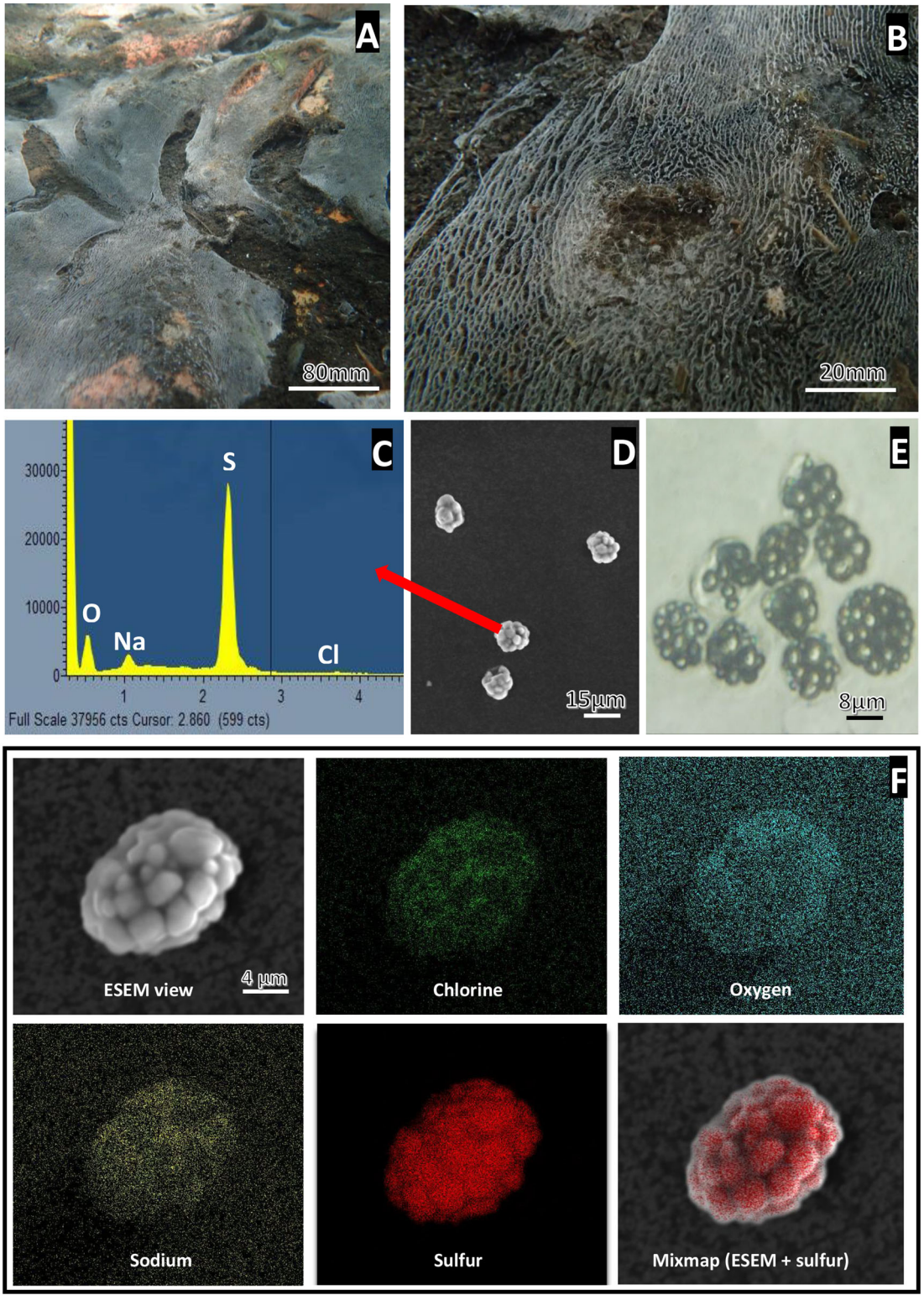

Figure 1. Description of white veil from marine mangrove in the Caribbean. (**A**, **B**) *In situ* light micrographs showing the veils of *Thiovulum* sp. strain karukerense covering the reduced sediment of marine mangrove around Guadeloupe. (**C**) EDX spectrum obtained from one single organism (arrow) clearly indicates that the main element present is elemental sulfur (S°). (**D**) Low vacuum image collected with secondary electron detector under 650 mPa water vapor atmosphere with 15 kV accelerating voltage. The image is dominated by backscattered electrons due to the high penetration power of the incident electrons in the specimen. (**E**) Light micrograph (phase contrast) showing single ovoid cells containing several refractive internal granules. The cytoplasm of this epsilon-proteobacterium is usually filled with these highly refractive granules. (**F**) Elemental mapping of the main elements detected by EDX spectroscopy. Sodium, oxygen and sulfur are only located within the bacterial cell. The sulfur map clearly demonstrates that sulfur is located inside the bacteria, more specifically within the intracellular granules, as confirmed by the mixmap image. ESEM, environmental scanning electron microscopy.

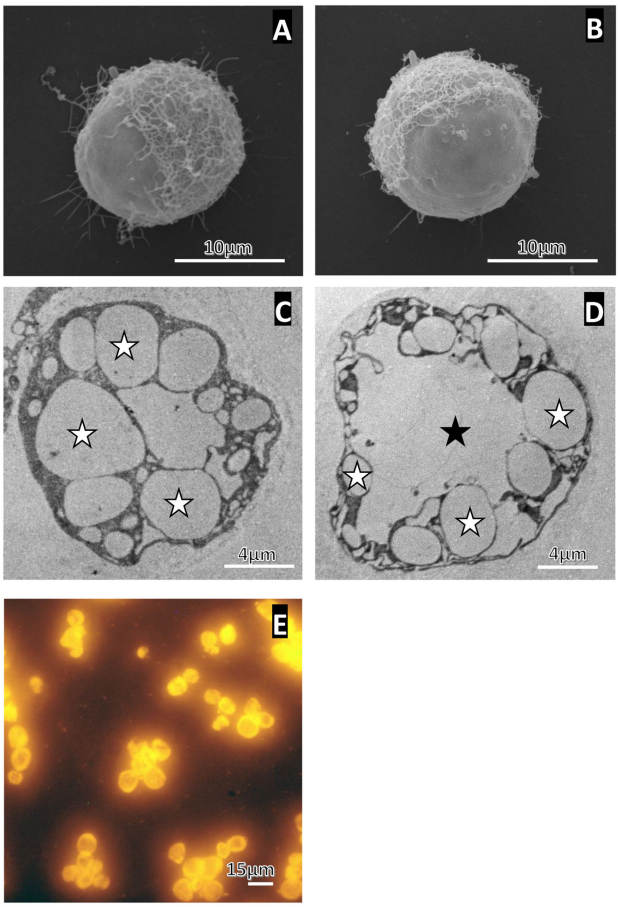

Figure 2. Ultrastructural analysis of freshly collected individuals from marine mangrove. (**A**, **B**) Low magnification scanning electron micrographs of three cells showing the ovoid shape of this bacterial strain. Two important features are obvious here, the presence of numerous flagella covering only one pole of the cell showing that these bacteria are extremely motile by means of lophotrichous flagella. (**C**, **D**) Scanning transmission electron micrographs of transverse sections of a single bacterial cell. The cytoplasmic volume appears filled with large vesicles corresponding to internal former sites of sulfur granules (white stars). In some sections, the center of the bacterial cell is occupied by an internal vacuole (black star). (**E**) Fluorescence *in situ* hybridization experiments showing a strong hybridization with the epsilon-proteobacteria probe (probe EPS914, Cy3 dye labelled) from the bacterial cells constituting the veils.

diameter of 13.75 ± 1.92 μm), suggest that this strain is actually phenotypically close to *T. majus*. *Thiovulum* sp. strain karukerense cells are 60% larger than those of the strain studied by Petroff *et al.* (2015), and their cytoplasm contains many large sulfur granules. By contrast, in the strain studied by Petroff *et al.* (2015), sulfur granules were present only at the posterior pole of the cell. The sulfur granules in the cytoplasm of *Thiovulum majus* are sometimes gathered at the posterior pole, but they may also be spread throughout the cell, as described by Hinze (1913). These structural data could support the fact that these two strains are probably different, as strongly suggested by the phylogenetic analysis based on 16S rRNA gene sequences. Several morphotypes have been described, but only a few sequences are available from the database for phylogenetic analysis. Thus, the strain collected from the marine mangrove environment may correspond to a new strain or a new species within this poorly studied genus of epsilon-proteobacteria.

The scanning transmission electron microscopy sections obtained here resemble published images of *Thiovulum* cells (De Boer, La Rivière and Houwink 1961; Wirsen and Jannasch 1978; Garrity, Bell and Lilburn 2005). The cytoplasm is filled with internal sulfur granules and a central vacuole can be seen on some sections (Fig. 2D). The presence of such a vacuole is unusual in this bacterial genus. Other sulfur-oxidizing bacteria have been reported to have large central vacuoles that may be nitrate vacuoles, as described in large marine Beggiatoaceae (Mußmann *et al.* 2003), whereas in *Thiotrix* species, such vacuoles appear to be nitrate-free (Kalanetra, Huston and Nelson 2004). Bacteria with nitrate vacuoles can survive anaerobically, by oxidizing sulfides through nitrate reduction to gaseous nitrogen and ammonia (Schulz and Jørgensen 2001; Sayama *et al.* 2005). *Thiovulum* species have been reported to be microaerophilic, their cells unable to survive in anaerobic or O_2-saturated conditions, but it is unclear whether they can oxidize nitrate. However, anaerobic growth using nitrate as an electron acceptor is a common trait in colorless sulfur bacteria, particularly epsilon-proteobacteria (Kodama and Watanabe 2004; Grote *et al.* 2012). A single-cell genome analysis of *Thiovulum* sp. ES by Marshall *et al.* (2012) showed that this species of *Thiovulum* contained genes encoding the α- and β-subunits of periplasmic nitrate reductase, suggesting that *Thiovulum* may be capable of anaerobic growth with sulfide oxidation coupled to nitrate reduction. Further investigations of this dense population of *Thiovulum* sp. strain karukerense would improve our understanding of the real capacity for nitrate reduction in genus *Thiovulum* under anaerobic conditions.

The use of environmental scanning electron microscopy to observe samples that have not been dehydrated in solvents (such as ethanol or acetone) coupled with elemental sulfur detection by EDX spectroscopy analysis is not common in microbiological investigations. These two techniques were used to assess the ability of this new sulfur-oxidizing strain of *Thiovulum* to store elemental sulfur within its cytoplasm. EDX spectroscopy analysis has also been used to characterize the metabolic pathways of unculturable bacteria, including those capable of oxidizing iron (Zbinden *et al.* 2004), and many sulfur-oxidizing bacteria, including photosynthetic sulfur bacteria (Sun *et al.* 2017), to demonstrate that the sulfur stored in the cytoplasm of these organisms is the solid S_8 form (Pickering *et al.* 2001). We showed that *Thiovulum* sp. strain karukerense cells stored elemental sulfur in their cytoplasm when exposed to sulfides from their marine environment.

In this study, the internal component of the central space was not identified, but TEM images showed that the empty area had no intracytoplasmic membrane. This finding is consistent with the observations of De Albuquerque, Keim and Lins (2010), who reported an absence of internal membranes in the vacuoles of microbes forming marine and hypersaline mats.

The veils of *Thiovulum* cells were generally observed in areas of weak hydrodynamics, as favored by the presence of *Rhizophora mangle* roots, in which large amounts of fine sediment were deposited. Gontharet *et al.* (2017) recently showed that the granularity of sediment particles, their total carbonate content, and $\delta^{13}C$ values were the principal parameters correlated with the occurrence of sulfur-oxidizing microbial mats in marine mangrove environments. These microbial mats may be formed from gamma-proteobacteria, such as large filamentous Beggiatoaceae (Jean *et al.* 2015), or epsilon-proteobacteria (as in this study). The sediments associated with microbial mats contain larger amounts of clay and have higher total organic carbon and total nitrogen levels, and higher total organic carbon/total nitrogen ratios, with lower total carbonate contents and $\delta^{13}C$ levels. These findings are consistent with the presence of *Thiovulum*

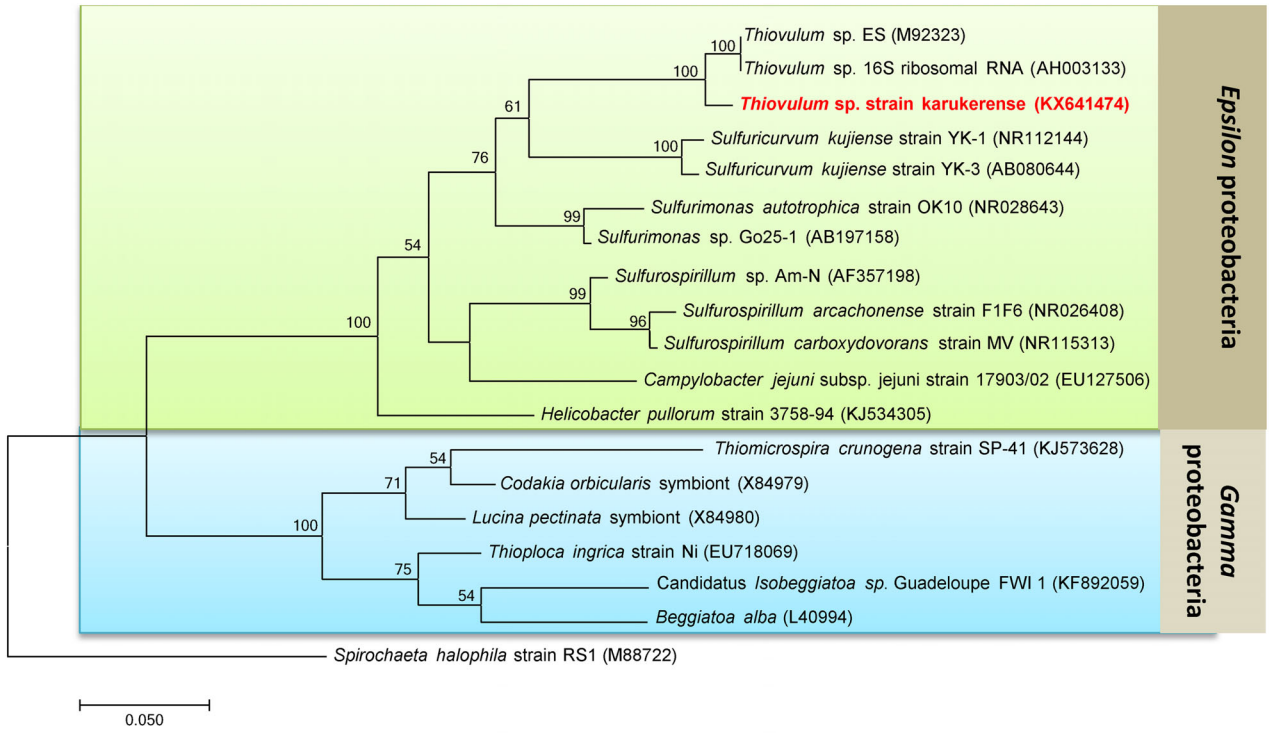

Figure 3. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences (773 bp) within related species of the family Helicobacteraceae. The phylogenetic tree was constructed using the Clustal X (Thompson *et al.* 1997) and MEGA version 7 (Kumar, Stecher and Tamura 2016) programs. Gaps in sequence alignments were omitted in the calculation. The sequence accession numbers are shown in parentheses. The bootstrap values obtained by 1000 replications (Felsenstein 1985) over 50% are beside nodes. The outgroup was specified as *Spirochaeta halophila* strain RS1 (M88722). Bar, 5 nucleotide substitutions per 100 nucleotides.

microbial mats close to the roots of mangrove trees, or on the surface of decaying organic matter from dead animals (known as the 'whale fall' ecosystem) and plants (known as the 'wood fall' ecosystem), which produce large amounts of sulfides (HS^-; Laurent *et al.* 2013).

In situ sulfide measurements have already been performed on mangrove sediment. The mean sulfide concentration obtained for mangroves is 1187 ± 728 μM (Jean *et al.* 2015), confirming the ability of marine mangrove sediment to sustain the development of free-living sulfur-oxidizing bacteria from the epsilon and gamma groups of Proteobacteria.

Pascal *et al.* (2014) assessed the importance of filamentous sulfur-oxidizing bacteria as an infaunal food source, by studying natural isotopic compositions and performing a ^{13}C enrichment study. They showed that the ingestion of sulfur-oxidizing bacterial mats by the associated meiofauna, dominated by rotifers and, to a lesser extent, small polychaetes and nematodes, could occur even if the proportion of organic matter represented by sulfur bacteria is much smaller than that for the total bacterial fraction in this environment. The availability of this chemosynthetic food resource thus has a limited local effect. This conclusion was recently confirmed by Pascal, Gros and Boschker (2016), who performed a survey of the stable isotopic compositions of four grazers and four food sources in a marine mangrove ecosystem. None of the grazers analyzed had an isotopic composition correlated with that of colorless filamentous *Beggiatoa*, suggesting that sulfur bacteria were not a predominant part of the diet of any grazer. Epsilon-proteobacteria such as *Thiovulum* species are, therefore, probably also a poor food source.

Freshwater environments generally have low sulfide levels. By contrast, in seawater, sulfate-reducing bacteria often rely on the activity of sulfur-oxidizing phototrophs or colorless *Beggiatoa* to generate electron donors (Ghosh and Dam 2009). Sulfur-oxidizing bacteria play an important role in the biogeochemical cycling of carbon and sulfur in stratified ecosystems (van Gemerden 1993; Grote *et al.* 2008). They are therefore likely to play a key role in carbon and sulfur turnover in mangrove sediment. The contribution of epsilon-protebacteria to marine mangrove C and N cycles and their role in sustaining local food webs are underestimated. The real contribution of *Thiovulum* communities to total primary production in marine mangroves is complex and requires further study. The results presented here provide a first insight into the contribution of *Thiovulum*-dominated microbial mats to the biochemical cycles and food web of marine mangroves. They should serve as a basis for further studies of marine mangrove microbial mats.

Nucleotide sequence accession number

Thiovulum sp. strain karukerense 16S rRNA gene partial sequence obtained in this study was deposited in the GenBank database under accession number KX641474.

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Conflict of interest. None declared.

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