Dense Populations of a Giant Sulfur Bacterium in Namibian Shelf Sediments
H. N. Schulz, et al.
Science 284, 493 (1999);
DOI: 10.1126/science.284.5413.493

The following resources related to this article are available online at www.sciencemag.org (this information is current as of February 4, 2008):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/cgi/content/full/284/5413/493

This article cites 18 articles, 6 of which can be accessed for free:
http://www.sciencemag.org/cgi/content/full/284/5413/493#otherarticles

This article has been cited by 74 article(s) on the ISI Web of Science.

This article has been cited by 20 articles hosted by HighWire Press; see:
http://www.sciencemag.org/cgi/content/full/284/5413/493#otherarticles

This article appears in the following subject collections:
Microbiology
http://www.sciencemag.org/cgi/collection/microbio

Information about obtaining reprints of this article or about obtaining permission to reproduce this article in whole or in part can be found at:
http://www.sciencemag.org/about/permissions.dtl
the time of implantation. The mechanical strength of the explanted grafts could not be assessed accurately because of postoperative fibroblastic migration and collagen deposition at the outer surface of the engineered vessel.

In contrast, the two nonpulsed autologous grafts remained open for 3 weeks and then developed thrombosis, which may have been caused by gradual shearing loss of the luminal polymer region and of the endothelial layer due to arterial flows (Fig. 5, C and D). Histologically, the walls of the autologous explanted vessels showed highly organized structure and minimal inflammation as compared to the xenograft. For all vessels, there was no evidence of bleeding at the anastomoses or mechanical breakdown at explantation.

Important areas of future work include the effects of culture conditions on graft longevity, the stimulation of elastin in the vessel wall, and the minimization of residual polymer fragments (28) in the engineered tissues. Clinically useful engineered vessels should approximate the patency rate of 90% at 30 days that is observed with autologous vein grafts (29). Although further studies are required to assess the biological function of these vessels during short-term and long-term implantation, the feasibility of culturing autologous implantable arteries and the important effects of pulsatile culture conditions have been demonstrated.

References and Notes

25. The wall stress [σ] was calculated from (13, 14) as follows:

\[
\sigma = 8 \cdot \rho \cdot r_s \cdot \left( r_s - r_e \right) \cdot \left( r_e - r_i \right)^2 \left( 1 - \frac{r_i}{r_e} \right)^2
\]

where \( r_s \) is the measured external radius, \( r_e \) is the internal radius calculated from the measured external radius and cross-sectional area, and \( \rho \) is the intraluminal pressure. Wall strain \( \varepsilon \) was calculated at the midwall radius as follows:

\[
\varepsilon = \frac{\left( r_e - r_i \right)^2}{2 \left( r_e - r_i \right)^2} - 1
\]

where \( r_i \) and \( r_e \) are the external and internal vessel radii under unstressed conditions (at \( P = 25 \) mm Hg) (13, 14). The formulation for \( E_{\text{finc}} \) is based on models previously reported to describe native vessels (elastin staining in engineered vessels was negative, so elastin is neglected) (13, 14):

\[
E_{\text{finc}} = E_{\text{finc}} W_f c_f + E_{\text{finc}} W_m
\]

where \( E_{\text{finc}} \) is the incremental modulus, calculated from the slope of the stress-strain curves (Fig. 3B). 

Filamentous, nitrate-accumulating sulfur bacteria of the genus Thioploca form extensive populations of up to 120 g wet weight/m² along the coast of Chile and Peru (1–3). Similar to the South American continental shelf, the shelf off Namibia has high upwelling with high plankton productivity and oxygen-depleted bottom water (4). In a search for Thioploca along the Namibian coast, we obtained sediment samples from water depths of ~100 m during a cruise in April 1997 aboard the R/V Petr Kottsov. Thioploca and its close relative Beggiatoa were present, but only in low numbers. Instead, we discovered large populations of a previously undescribed sulfur bacterium that occurred at biomasses of up to 47 g m⁻². These giant bacteria grow as a string of pearls, which shine white because of refractive sulfur globules and are large enough to be visible to the naked eye (Fig. 1A). We suggest the genus and species name Thiomargarita namibiensis, “Sulfur pearl of Namibia,” for this organism.

Thiomargarita was found at stations between Pelagric Point and Lüderitz Bay. The highest biomasses were between Cape Cross and Conception Bay. The surface sediment in this area is a fluid, green diatom ooze (5). Oxygen concentrations were low, 0 to 3 µM, in the overlying water at all stations, whereas nitrate was present at 5 to 28 µM. Sulfate reduction rates measured by the \( ^{35} \text{SO}_4^{2-} \) tracer technique were high, 14 to 76 mmol m⁻² day⁻¹ in the upper 19 cm, and gave rise to high sulfide concentrations of 100 to 800 µM in the upper 3 cm of the sediment. Frequently, the water directly overlying the sediment smelled of sulfide. Most of the bacteria were found in the top

Dense Populations of a Giant Sulfur Bacterium in Namibian Shelf Sediments

H. N. Schulz,¹ T. Brinkhoff,² T. G. Ferdelman,¹ M. Hernández Marín,³ A. Teske,⁴ B. B. Jørgensen¹

A previously unknown giant sulfur bacterium is abundant in sediments underlying the oxygen minimum zone of the Benguela Current upwelling system. The bacterium has a spherical cell that exceeds by up to 100-fold the biovolume of the largest known prokaryotes. On the basis of 16S ribosomal DNA sequence data, these bacteria are closely related to the marine filamentous sulfur bacterium Thioploca, abundant in the upwelling area off Chile and Peru. Similar to Thioploca, the giant bacteria oxidize sulfide with nitrate that is accumulated to ≥800 millimolar in a central vacuole.

¹Max Planck Institute for Marine Microbiology, Cell-ssiustrasse, D-28359 Bremen, Germany. ²Institute for the Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg, Post Office Box 2503, D-26111 Oldenburg, Germany. ³Facultad de Farmacia, Universitat de Barcelona, Av. Joan XXIII, s/n, 08028 Barcelona, Spain. ⁴Department of Biology, Woods Hole Oceanographic Institution (WHOI), Woods Hole, MA 02543, USA.

*To whom correspondence should be addressed. E-mail: hschulz@mpi-bremen.de

11 January 1999; accepted 18 March 1999
The giant cells of *Thiomargarita* have many similarities to those of the gliding, filamentous relatives, *Thioploca* (2, 3, 6, 7). *Thiomargarita* also occurred in an oxygen-poor environment with high sulfate reduction rates. Each cell had a large central vacuole (Fig. 1) in which nitrate was accumulated to a concentration of 0.1 to 0.8 M. Electron micrographs showed that the cytoplasm was restricted to a thin outer layer of 0.5- to 2-μm thickness (Fig. 1, D and E). The remaining 98% of the biovolume consisted of a liquid vacuole. The bacteria contained sulfur stored in the form of globules, which were situated in the thin outer layer of cytoplasm at a concentration per total biovolume equivalent to 0.4 to 1.7 M. The depth distribution of biomass in the sediment observed for *Thiomargarita* (Fig. 2A) was similar to that of *Thioploca* off the Chilean coast (3). In contrast to the multicellular *Thioploca* and *Beggiatooa*, the cells of *Thiomargarita* were not attached to each other but were evenly separated by a mucus sheath (Fig. 1). Motility was not observed. Most of the chains were linear and contained on average 12 cells, but sometimes they branched or coiled together in a ball. Long chains of, for example, 40 to 50 cells tended to break easily when manipulated.

Most cells had diameters of 100 to 300 μm (Fig. 2B). Most cells in a chain were of a similar diameter (Fig. 2C), but in some chains a single cell occurred with a much larger diameter of up to 750 μm. These extremely large forms also occurred as single cells (Fig. 1A). The average *Thiomargarita* with a diameter of 180 μm had a volume of $3 \times 10^7 \mu m^3$, whereas the largest observed cells had a biovolume of $200 \times 10^6 \mu m^3$. In comparison, the largest known sulfur bacteria, *Beggiatooa* spp., found at hydrothermal vents in the Guaymas Basin, Gulf of California, can reach diameters of 160 μm (8). The height of their disc-shaped cells is ~50 μm and their volume is $1 \times 10^6 \mu m^3$ per cell. The largest described bacteria, *Epulopiscium fishelsoni*, a symbiont of the surgeonfish (9), is typically 250 μm by 40 μm large, but individual cells can reach 600 μm by 80 μm. This corresponds to a volume of $0.3 \times 10^6$ to $3 \times 10^7 \mu m^3$ per cell.

The phylogenetic position of *Thiomargarita* was determined by fluorescent in situ hybridization and 16S ribosomal RNA (rRNA) sequencing. A hybridization analysis with competitive beta- and gamma-proteobacterial probes (10) identified *Thiomargarita* as a gamma proteobacterium, a bacterial phylum that also harbors *Beggiatooa* and *Thioploca*. We then tested *Thiomargarita* with the *Thioploca araucae*– and *Thioploca chileae*–targeted probe 829 (11) and found a positive hybridization. This probe was subsequently used as a specific primer to amplify positions 24 to 828 of the 16S rRNA gene of *Thiomargarita* (12). *Thiomargarita* was found to be the closest relative to the marine, vacuolated, nitrate-accumulating *Thioploca* species, *T. araucae* and *T. chileae*, thus separating them from the smaller freshwater species, which do not have large vacuoles (13) (Fig. 3). The possession of a large vacuole in connection with intracellular nitrate accumulation appears to be congruent with this phylogeny.

Our attempts to isolate *Thiomargarita* into pure culture have not been successful. The bacteria may survive and grow in the laboratory in samples of their natural sediment for at least a year. Nitrate and sulfide addition led to a dou-

---

**Fig. 1. Thiomargarita namibiensis.** (A) The white arrow points to a single cell of *Thiomargarita*, 0.5 mm wide, which shines white because of internal sulfur inclusions. Above there is an empty part of the sheath, where the two neighboring cells have died. The cell was photographed next to a fruit fly (*Drosophila viriles*) of 3 mm length to give a sense of its size. (B) A typical chain of *Thiomargarita* as it appears under light microscopy. (C) At the left end of the chain there are two empty mucus sheaths, while in the middle a *Thiomargarita* cell is dividing. (D) Confocal laser scanning micrograph showing cytoplasm stained green with fluorescein isothiocyanate and the scattered light of sulfur globules (white). Most of the cells appear hollow because of the large central vacuole. (E) Transmission electron micrograph of the cell wall [enlarged area in (D)] showing the thin layer of cytoplasm (C), the vacuole (V), and the sheath (S).

**Fig. 2. Distribution of biomass and diameters.** (A) Depth distribution of biovolume of *Thiomargarita* (in microliters per milliliter). Bars represent the mean values of three measurements. (B) Frequency of diameters of 214 randomly chosen cells. (C) Cell diameter distributions in three different chains.
The thickness of the cytoplasm corresponds to the usual small width of bacteria, and its peripheral distribution counteracts a potential problem that their electron acceptor and energy metabolism is spatially separated, and Thioploca commute between these two sources. In contrast, Thiomargarita only obtain nitrate during occasional sediment resuspension events. Meanwhile they can effectively endure high sulfide concentrations until the next resuspension event occurs.

Sulfide production rates are high in coastal sediments around the world, wherever the sediment is rich in organic matter, particularly in upwelling regions (6). The bottom water in these areas is often depleted of oxygen because of intense heterotrophic respiration. As the second-most favorable electron acceptor, nitrogen may be used for the oxidation of sulfide. This results in a close coupling of the sulfur and the nitrogen cycles through these specialized sulfur bacteria. Thioploca predominates along the Pacific coast of South America, whereas Thiomargarita is abundant along the Namibian coast. In both upwelling areas, sediments with extremely high organic content and sulfate reduction rates harbor dense and conspicuous populations of giant sulfur bacteria. However, even the well-known Beggiaota, frequently encountered along the coast, have recently been shown in Baltic Sea sediments to accumulate nitrate (20). These findings indicate that a chemolithotrophic coupling of nitrate and sulfide through nitrate-storing sulfur bacteria may be a widespread feature of coastal sediments.

References and Notes
4. T. J. Hart and R. I. Currie, Discovery Rep. 31, 123 (1960);
5. G. Schuette and H. Schrader, in Coastal Upwelling, J. E. Richards, Ed. (American Geo-
12. To avoid contamination with other sulfur bacteria, the sheaths of Thiomargarita were dissolved with a com-
18. S. Otte et al., in preparation.
21. We thank the crew of the Petri Kottsov and the participants of the BENEFIT expedition, especially L. Pastel and C. Eichner for their cooperation. Special thanks are due to C. Suppes, J. Zoři, F. García Pichel, F. Widdel, and A. Friedrich for assistance with practical problems and for fruitful discussions and to H. G. Trüper for help in finding an appropriate name. The study was supported by the Max Planck Society. This is WHOI contribution number 9751.

5 October 1998; accepted 17 February 1999

www.sciencemag.org SCIENCE VOL 284 16 APRIL 1999 495

R E P O R T S

Fig. 3. Distance tree of Thiomargarita namibiensis and related sulfur-oxidizing bacteria of the gamma-proteobacterial subdivision. The distance tree is based on 16S rRNA sequence of Thioploca araucae, T. chileae, and Thiomargarita. The tree was rooted with Thio-

lum majus of the epsilon-proteobacterial subdivision as outgroup. Bootstrap values (200 runs) are given for nodes that have at least 70% support by distance (first) or parsimony bootstrap (second value). The bar corresponds to 0.1 Jukes-Cantor substitutions per nucleotide. The sequence has been deposited with GenBank (accession number AF129012).