the time of explantation. The mechanical strength of the explanted grafts could not be assessed accurately because of postsurgical fibroblast 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 (*26*, *27*), 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.

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

$$\sigma = 8 \cdot P \cdot (r_e \cdot r_i)^2 / [(r_e^2 - r_i^2) \cdot (r_e + r_i)^2] \quad (1)$$

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

$$\varepsilon = [(r_e + r_i)/2] / [(R_e + R_i)/2] - 1 \quad (2)$$

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

$$E_{inc} = E_C \cdot W_C \cdot f_C + E_{SM} \cdot W_{SM} \quad (3)$$

where E_{inc} is the incremental modulus, calculated from the slope of the stress-strain curves (Fig. 3B): $E_{inc} = 0.75(d\sigma/d\varepsilon)$; E_C and E_{SM} are elastic moduli for collagen and SMCs, respectively; W_C and W_{SM} are amounts of collagen and SMCs in the vessel wall; and f_C is the recruitment function for collagen. The recruitment function describes the fraction of total collagen fibers that support wall stress at a given strain. Previously reported values of maximal $E_C \cdot W_C$ fall in the range of $1.3 \pm 0.6 \times 10^8$ Pa. From Fig. 3A, we calculate the maximal SMC-supported wall stress for any vessel to be approximately 6×10^3 Pa. This negligible SMC contribution effectively reduces the model to a function of the collagen content alone.
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Dense Populations of a Giant Sulfur Bacterium in Namibian Shelf Sediments

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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 16*S* ribosomal DNA sequence data, these bacteria are closely related to the marine filamentous sulfur bacteria *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.

Filamentous, nitrate-accumulating sulfur bacteria of the genus *Thioploca* form extensive populations of up to 120 g wet weight/m^2 along the coast of Chile and Peru (*1*–*3*). Similar to the South American continental shelf, the shelf off Namibia has strong 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 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 Palgrave 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 $^{35}SO_4^{2-}$ 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

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3 cm of the sediment. The biomass of *Thiomargarita* declined exponentially with sediment depth down to 10 to 14 cm (Fig. 2A).

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 *Beggiatoa*, 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 3×10^6 μm^3, whereas the largest observed cells had a biovolume of 200×10^6 μm^3. In comparison, the largest known sulfur bacteria, *Beggiatoa* 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 1×10^6 μm^3 per cell. The largest described bacteria, *Epulopiscum 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 0.3×10^6 to 3×10^6 μm^3 per cell.

The phylogenetic position of *Thiomargarita* was determined by fluorescent in situ hybridization and 16*S* 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 *Beggiatoa* and *Thioploca* (*11*). 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 16*S* 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-

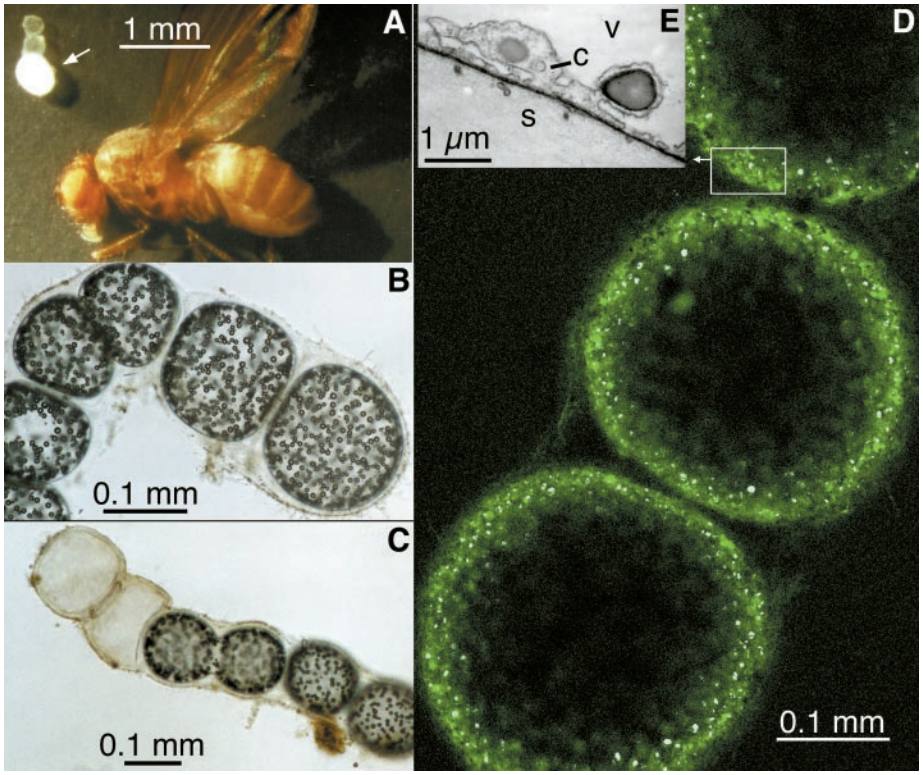

Fig. 1. *Thiomargarita namibiensis*. (**A**) The white arrow points to a single cell of *Thiomargarita*, 0.5 mm wide, which shines white because of internal sulfur inclusions. Above there is an empty part of the sheath, where the two neighboring cells have died. The cell was photographed next to a fruit 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).

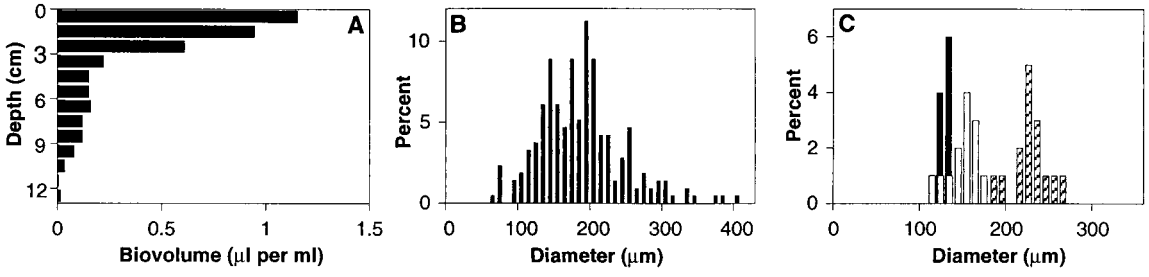

Fig. 2. Distribution of biomass and diameters. (**A**) Depth distribution of biovolume of *Thiomargarita* (in microliters per milliliter). Bars represent the mean values of three measurements. (**B**) Frequency of diameters of 214 randomly chosen cells. (**C**) Cell diameter distributions in three different chains.

bling of the cell number within 1 to 2 weeks. Addition of organic substrates such as acetate or glucose had no immediately detectable effect on growth. Although *Thiomargarita* appear to thrive best under low oxygen or anoxic conditions, exposure to atmospheric oxygen levels were not toxic as has been observed for *Beggiatoa* (*14*) and *Thioploca* (*15*). *Thiomargarita* showed an unusual ability to survive without growing. Small samples of 15-cm^3 fluffy surface sediment collected during an earlier research cruise, that were kept in 80 ml of air-saturated sea water and stored at 5°C without addition of nitrate or sulfide, contained intact cells after more than 2 years. The surviving cells were comparatively small, with diameters of 50 to 110 μm and occurring singly or in pairs.

The thickness of the cytoplasm corresponds to the usual small width of bacteria, and its peripheral distribution counteracts a potential diffusion limitation within the cell (*16*). Because the thickness of cytoplasm is independent of cell size, the ratio of vacuole- to cytoplasm-volume increases with the diameter. By doubling the diameter, the volume of vacuole storage capacity relative to cytoplasm also doubles. The observed potential of *Thiomargarita* to survive nitrate starvation for long periods might, accordingly, be explained by the following calculation: The mean protein content of *Thiomargarita* was 4.5 mg cm^{-3} volume (including the vacuole), less than half of what has been measured for the large, vacuolated *Beggiatoa*, which also accumulate nitrate (*17*). For a nitrate reduction rate of 1 nmol NO_3^- min^{-1} mg^{-1} protein as observed for *Thioploca* (*18*), a *Thiomargarita* cell with a diameter of 180 μm and 0.3 M nitrate stored could survive for at least 40 to 50 days without taking up nitrate. Because the intensity of the upwelling off the Namibian coast frequently changes (*4*), *Thiomargarita* could survive until sulfide or nitrate appear in higher concentrations and can be stored again for later use.

In most marine sediments, the zones of nitrate and hydrogen sulfide do not overlap. *Thioploca* has developed a strategy to overcome the problem that their electron acceptor and energy source do not coexist. They live in sheaths that allow the filaments to glide up and down and thereby commute between nitrate uptake from the overlying sea water and sulfide uptake within the sulfate reduction zone of the sediment (*2*, *3*). The high fluidity and instability of sediments at Walvis Bay (*5*), however, seem to prevent *Thioploca* from forming vertical sheaths and establishing dense populations. Instead, balloon-shaped sulfur bacteria thrive here. The discovery of *Thiomargarita* expands the range of known adaptations of prokaryotic organisms to a life in sulfide gradients. Whereas motility is a fundamental prerequisite for the filamentous *Thioploca* and *Beggiatoa* (*14*, *19*), *Thiomargarita* appear unable to move actively to an environment where its energy source and electron acceptor are optimally supplied. Instead, they may rely on passive transport by external processes such as periodic resuspension of the loose sediment or on temporal variations in the chemical environment. In accordance with this, it is more resistant to high levels of oxygen and sulfide than are the filamentous relatives, which show a phobic chemotactic response to oxygen (*19*). Both *Thiomargarita* and *Thioploca* face the same ecological challenge: to oxidize sulfide with nitrate, although their two substrates do not coexist. By their solution, to store both nitrate and sulfur, they may successfully compete with faster growing anaerobic sulfide oxidizers, such as *Thiobacillus denitrificans* and *Thiomicrospira denitrificans*. With *Thioploca*, sulfide and nitrate are 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, nitrate 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 *Beggiatoa*, 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.

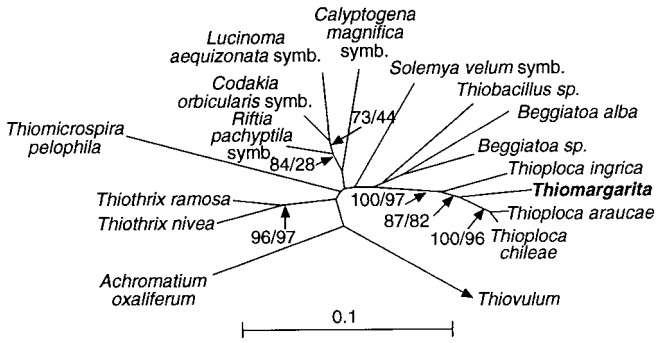

Fig. 3. Distance tree of *Thiomargarita namibiensis* and related sulfur-oxidizing bacteria of the gamma-proteobacterial subdivision. The distance tree is based on 16*S* rRNA position 358 to 802, which is the overlap of the partial 16*S* rDNA sequence of *Thioploca araucae*, *T. chileae*, and *Thiomargarita*. The tree was rooted with *Thiovulum 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).

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