Thiomargarita namibiensis: Giant Microbe Holding Its Breath

The largest known bacterium survives in a highly sulfidic environment with only occasional access to electron acceptors

Heide N. Schulz

Thiomargarita namibiensis, the largest known bacterium, was discovered in sediments off the west coast of Africa in April 1997. Single spherical cells typically are 100–300 μm in diameter, but may be as large as 750 μm, exceeding the volume of all other known prokaryotes by several orders of magnitude. In terms of size, a *T. namibiensis* cell is to an *Escherichia coli* cell what blue whale is compared to a newly born mouse.

The single spherical cells, held together in a chain by a slime sheath, shine white due to intracellular sulfur inclusions (Fig. 1). This particular morphology inspired the selection of the genus and species names, which mean "Namibian sulfur pearl." Although these bacteria were discovered recently, in their natural environment they are by no means rare and may occur at biomass densities of up to 180 g per square meter, accounting for up to 0.8% of the sediment volume and making *Thiomargarita namibiensis* the dominant benthic organism in Namibian shelf sediments.

Thiomargarita namibiensis shares many traits with members of the marine sulfide-oxidizing genera *Beggiatoa* and *Thioploca*

A New Genus within the Old Group of Colorless Sulfur Bacteria

Most members of the group of colorless sulfur bacteria were described from the middle of the 19th until the beginning of the 20th century, when numerous genera where defined based on morphology. For these sulfur bacteria this approach to nomenclature has survived remarkably well, since the numerous diverse morphologies are often directly linked to their physiology. All members of the group are unpigmented and carry internal sulfur inclusions—reflecting their characteristic physiology, namely the lithotrophic, nonphotosynthetic oxidation of sulfide. Almost everywhere in nature where free sulfide occurs, either in fresh or salt water, some member of the colorless sulfur bacteria can be found.

The best-known, and possibly also the most widespread, colorless sulfur bacteria belong to the genus *Beggiatoa*, whose members form fast-gliding filaments that accumulate in a thin zone where sulfide diffusing from the sediment contacts oxygen diffusing into the sediment. In 1887, the Russian microbiologist Sergei Winogradsky reasoned, based on his observations on accumulation and disappearance of sulfur inclusions, that *Beggiatoa* filaments gain energy by the oxidation of sulfide. This was the first proposal of a lithotrophic physiology. Despite many efforts by Winogradsky and other microbiologists, a lithoautotrophic *Beggiatoa* strain was not isolated into pure culture until 1980. This difficulty of isolating strains into pure cultures remains a common feature for many members belonging to the group of colorless sulfur bacteria.

A Giant in a Family of Large Bacteria

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Seeing Bacteria from Sea to Shining Sea

Heide Schulz was completing her doctoral degree work in 1999 when her report on the discovery of gargantuan *Thiomargarita namibiensis*, the largest bacterial species yet known, appeared in *Science*. The publication coincided with her thesis defense— confronting her not only with nerve-wracking technical questions from professors but also with a flood of calls and very different kinds of questions about *T. namibiensis* from reporters at major newspapers, trade publications, and broadcasters, including CNN. "It was difficult to manage all of that at the same time," she says, with studied understatement.

In retrospect, Schulz says that the publicity proved well worth the short-term scramble because her findings helped to remind the general public that bacteria are more than mere disease agents. "I thought it was very nice that people realize that bacteria do things that are important for the ecological system," she says. "I think that's very nice to get this idea more into the public attention."

Schulz did not embark on her scientific career planning to do microbiology, but began by pursuing oceanography at the Max Planck Institute for Marine Microbiology in Bremen, Germany. "I'm not really a microbiologist," she says. "I'm an oceanographer who got interested in microbial ecology." She attributes her love of the sea and early interest in oceanography to having grown up in Kiel, along Germany's Baltic coast. The prospect of research expeditions aboard oceanographic vessels also appealed to her nomadic spirit. "I like travelling very much, which is one reason why I think I chose this career," says the young scientist who has been working at the University of California, Davis (UCD) on a two-year fellowship since April 2001. "But I like coming home also."

Schulz's first research expedition was a three-week expedition off the coast of Chile to study *Thioploca*, a colorless sulfur bacteria commonly found in those waters. Her *Thioploca* studies eventually expanded into a 15-month sojourn in the South American nation and subsequently led to further expeditions along Africa's coast, where Schulz studied other forms of colorless sulfur bacteria. "You could say that I got dragged into this group of bacteria pretty much immediately as I started to do research on my own," she says. "I did not get away from them again because they're so interesting. They fascinate me because they are beautiful . . . and they are not so well investigated, even though the first one was described in the middle of the 19th century. There are so many things you can still learn about them."

The size of these colorless sulfur bacteria makes them relatively easy and fun to work with, Schulz says. Even without microscopes, "you can actually see what they are doing; you can see whether they are doing well or not. It's almost as if you are doing zoology instead of microbiology."

On such ocean-going expeditions, Schulz typically spends as many as three weeks without a break in cramped quarters. Despite some discomforts, she enjoys the camaraderie that develops among team members working and living together in such limited spaces. Also, she adds, doing research at sea hones one's organizational and creative skills because, if any analytic tools or equipment is unavailable, one must improvise. There's also the matter of doing research while earning one's "sea legs." Hardly a landlubber, she admits that she occasionally feels twinges of seasickness aboard smaller boats.

Though her research on the colorless sulfur bacteria has led Schulz to publish several papers, she expects eventually to broaden her focus beyond this fascinating group of bacteria. For now, however, her studies on these bacteria are expanding, and they continue to hold her main interest. "It's a very small niche," she says, noting that only a handful of scientists worldwide specialize in this bacterial group. "But the niche has gotten bigger and bigger since I got into it. You keep finding new and interesting things, and you feel like you don't want to abandon it. So I hope I can carry on with the colorless sulfur bacteria while finding a bigger research area."

When Schulz finishes her fellowship at UCD, she plans to return to Germany and hopes to secure a position at a research institution there. "I would like at some point to settle down a bit more and maybe have a family," she muses, but then adds, "it's a long way from finishing your Ph.D. to getting a permanent position in Germany." The opportunities for the equivalent of tenure-track positions in American institutions are fewer and harder to land in her home country, though recent initiatives may improve the situation. She notes that many young German scientists who take on fellowships or post-doctoral studies at U.S. institutions end up staying on instead of returning home. But asked if she would consider that option, she replies, "as I said, I like travelling, but I like coming home as well. I would like to work in the German system if that is possible at all."

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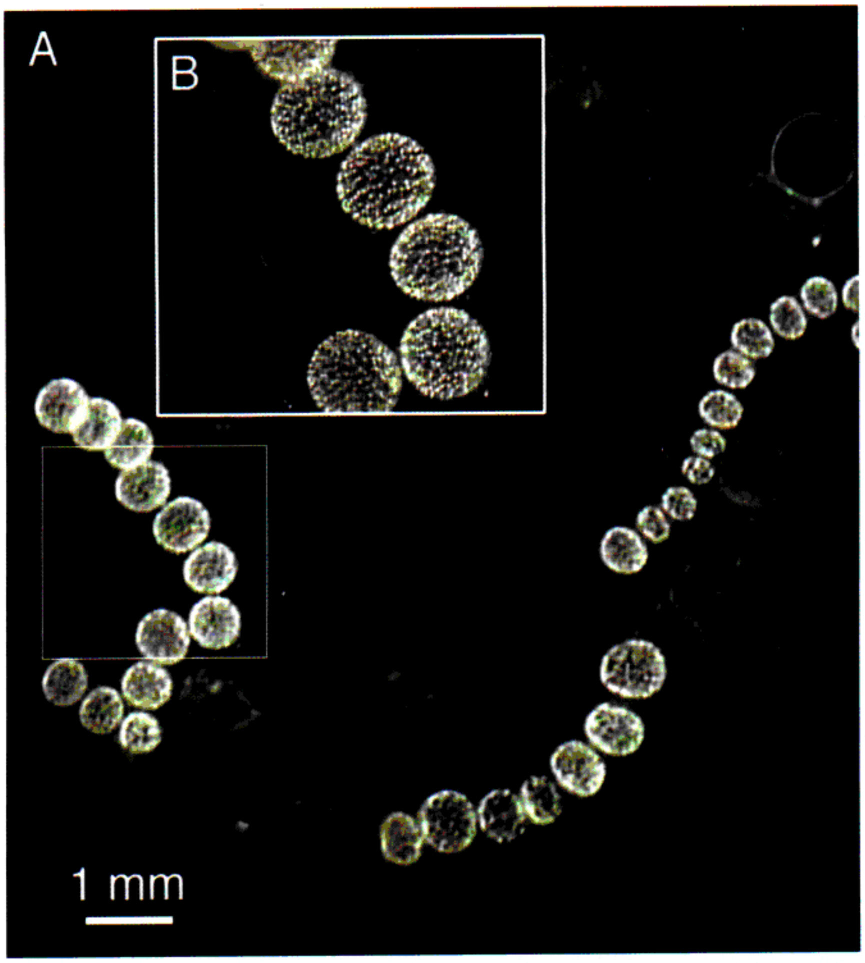
FIGURE 1



Three strings of *Thiomargarita namibiensis* viewed under a dissecting microscope (A). The cells are illuminated from the side to show the numerous, light-reflecting sulfur inclusions, which become visible at higher magnification (B). The larger cells are about 0.5 mm in diameter. In the lower of the three strings at both ends of the string a few cells died, leaving behind empty pockets of mucus. On the upper right of the picture there are empty diatom shells which make up a large fraction of the fluid sediments populated by *Thiomargarita namibiensis*. The cells shown in this picture have been sampled in August 2000 and are since kept alive in their original sediment.

genera include bacteria that are unusually large, with diameters ranging from 10 to more than 100 μm. These giant cells contain a liquid vacuole that accounts for much of the internal space, while the cytoplasm is confined to a thin 1- to 2-μm layer surrounding the vacuole. Despite their overall large size, the active cytoplasm of such cells is not much thicker than that of an ordinary-sized bacterium. Hence, like other prokaryotes, these giant cells can take up nutrients by means of diffusion without needing special transport systems.

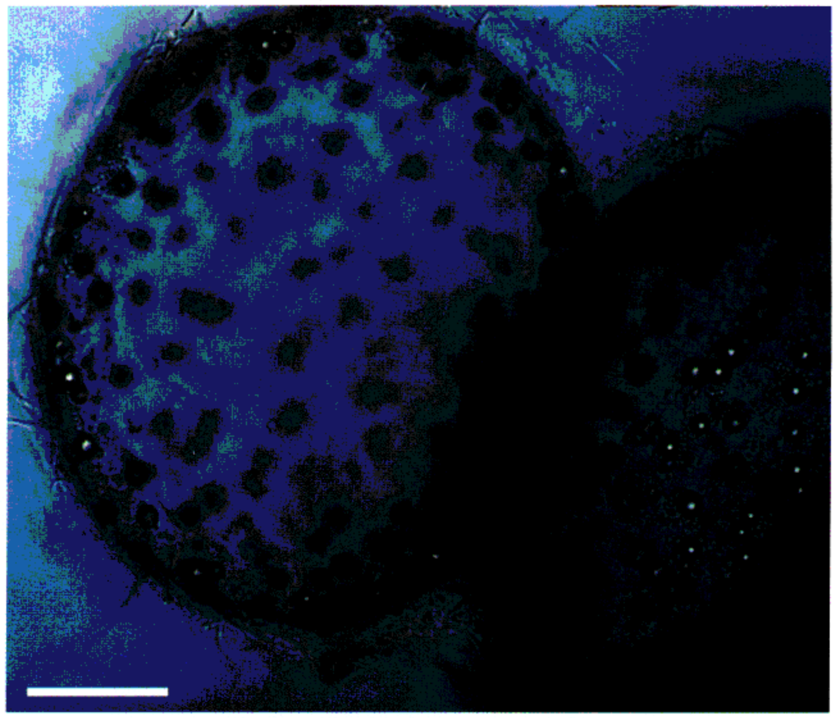
Like all colorless sulfur bacteria, *Thiomargarita* as well as *Beggiatoa* and *Thioploca* cells contain numerous sulfur inclusions (Fig. 2). The sulfur, which is stored in the periplasm, apparently represents conversion of the otherwise toxic electron donor sulfide into a nontoxic, highly condensed form that can be safely stored. Under sulfide starvation conditions, the sulfur inclusions slowly disappear.

In 1995, Osvaldo Ulloa and collaborators at the University of Concepcion in Chile found that the vacuole stores nitrate in very high concentrations (>0.1 M), maintaining this internal concentration against a steep gradient. Nitrate in the ambient bottom water typically occurs in concentrations below 50 μM, and often falls below detection limits within the sediment. The mechanism by which *Thiomargarita* and the larger *Beggiatoa* and *Thioploca* cells concentrate nitrate and maintain such high internal concentrations has not been studied. It appears that the ability to accumulate nitrate has only evolved once, since, according to the few 16S rDNA sequences that have been obtained so far, nitrate-storing species of *Beggiatoa*, *Thioploca*, and *Thiomargarita* seem to form a monophyletic group.

The large nitrate-storing sulfur bacteria typically occur in sediments characterized by high sulfide fluxes, due to either high sulfate reduction rates or to transport of sulfide from deeper layers to the sediment surface as is found at thermal vents or hydrocarbon seeps. In spite of the close phylogenetic relationship of these bacteria and their many similarities, each of these sulfide oxidizers seems to occupy a slightly different ecological niche.

Apart from relative differences in size, the most notable morphological difference between *Thiomargarita* on the one side and *Beggiatoa* and *Thioploca* on the other is that the latter two form filaments, whereas *Thiomargarita* cells do not. Each individual *Thiomargarita* cell is surrounded by a slime layer that attaches neighboring cells to one another after cell division (Fig. 1). Motility has not been observed and seems unlikely within this viscous sheath enclosing each cell.

FIGURE 2



Light micrograph of the terminal *Thiomargarita namibiensis* cell in a chain surrounded by mucus. The larger inclusions are sulfur globules. The smaller inclusions have not yet been identified. Both types of inclusions are restricted to the outer layer of the cell whereas the inner part, which is filled by the vacuole, appears hollow. On the sheaths there are numerous filamentous epibionts, possibly of the genus *Desulfonema*.

Internal Stored Nitrate Used for Oxidation of Sulfide

Ulloa and his collaborators, studying two dominant marine species of *Thioploca*, were the first to recognize this vacuole-based capacity to store nitrate in high concentrations. These marine species dominate sediments underlying the highly productive upwelling areas off the coast of Peru and Chile.

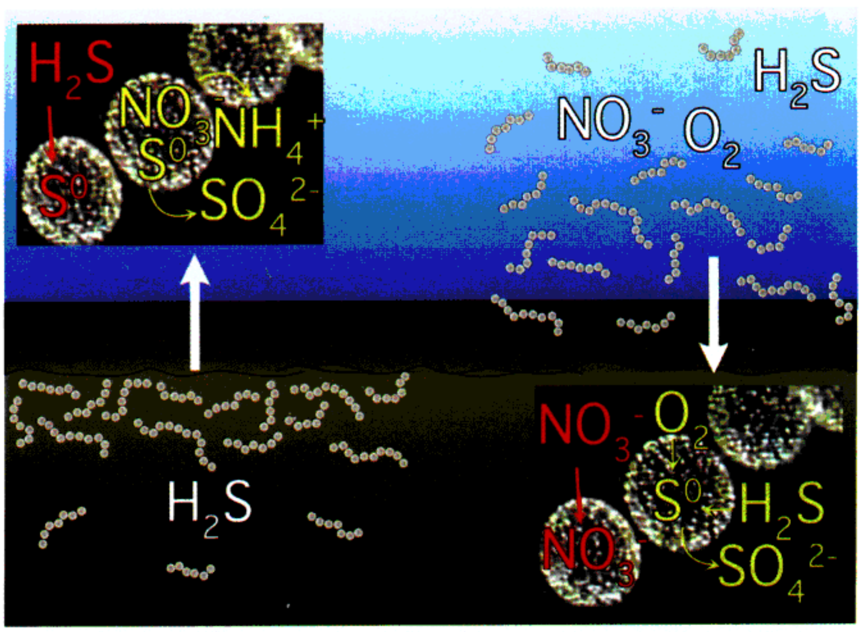
Thioploca filaments are specialists in bringing together nitrate, which is available only in the bottom water overlying the sediment, with sulfide, which is found several centimeters below the surface of the sediment. These filaments occur in bundles held together by mucus sheaths that penetrate many centimeters into the sediment. The sheaths connect the deeper sulfidic zones with the bottom water that contains nitrate. By shuttling up and down in these conduits, *Thioploca* filaments oxidize sulfide at a sediment depth where no other electron acceptor, apart from their internally stored nitrate, is available. The end product of the nitrate reduction seems to be ammonia, whereas nitrite may be formed under environmental stress. So far, there is no indication that *Thioploca* filaments can use oxygen as an electron acceptor. The dense populations of *Thioploca* off the west coast of South America seem to be very sensitive to either oxygen or sulfide in higher concentrations.

Beggiatoa of the narrow, nonvacuolate variety occur as individual filaments at the surface of sediments where sulfide, diffusing from below, creates a steep sulfide gradient that overlaps with oxygen diffusing into the sediment from the water above. The filaments avoid high concentrations of sulfide and oxygen, accumulating in a small band directly in the narrow zone where their electron donor and acceptor overlap. Larger, vacuolated *Beggiatoa* filaments also can store nitrate. Nevertheless, the larger forms of *Beggiatoa* filaments tend to populate distinct horizontal zones in the sediment rather than spreading deep into the sediments, as is typical for *Thioploca* filaments. Because *Beggiatoa* spp. are usually not found in environments that are completely anoxic, it seems that the larger, nitrate-storing *Beggiatoa* spp. might use nitrate as an alternative electron acceptor only during anoxic periods.

Namibian Shelf Sediments: a Highly Changeable Environment

Beggiatoa and *Thioploca* spp. both require motility to oxidize sulfide. Thus, the discovery of a third genus of nitrate-storing sulfur bacteria apparently lacking motility points to a previously unrecognized strategy for using internally stored nitrate. *Thiomargarita namibiensis* occurs off

FIGURE 3



Life mode of *Thiomargarita namibiensis*: Most of the time the cells are buried in the sediment with very high sulfide concentration and no access to nitrate. Under these conditions they can take up and store sulfide as elemental sulfur which they may oxidize further to sulfate for gaining energy. For both oxidation steps internally stored nitrate is reduced to ammonia. Occasionally the very loose sediments may get resuspended and the cells gain access to nitrate which they take up and concentrate in the vacuole. When the sediment is suspended in the oxic water column cells, *Thiomargarita* cells may gain energy by oxidizing either internal sulfur or sulfide if present.

the Namibian coast in sediments that are unusually fluid, consisting predominantly of empty diatom shells.

These high levels of organic material in Namibian coastal sediments lead to very high sulfide concentrations of up to 10 mM. Oxygen is usually absent in the bottom water covering these sediments, while nitrate may be found in low concentrations. Because they are not motile, *Thiomargarita* cells are trapped within the sediment without access to nitrate and are subject to sulfide concentrations that would be toxic for their relatives.

Occasionally, the loose sulfidic mud may be resuspended—for example, by methane eruptions that occur regularly in the area off Walvis Bay in Namibia and may be so dramatic that they raise "islands of mud" to the sea surface. Only such resuspension events would allow *Thiomargarita* cells to come into contact with nitrate for storage needed for later survival in the sulfidic sediment. During such events, the bacteria are also likely to encounter high oxygen concentrations that would be toxic for related microorganisms, including *Thioploca* filaments (Fig. 3).

Like the cytoplasm of the larger *Beggiatoa* and *Thioploca* cells, the active cytoplasm of *Thiomargarita* cells is typically 1–2 μm. Nevertheless, because such cells are spherical rather than cylindrical and because of their larger diameter, their vacuole-to-cytoplasm ratio is considerably greater than is that for either *Beggiatoa* or *Thioploca* cells. Thus, even though they have similarly high internal concentrations of nitrate, this nitrate storage can sustain them through a longer period than a *Thioploca* or *Beggiatoa* filament can withstand, assuming all of them have similar metabolic activity.

Therefore, the large size of *Thiomargarita* cells can be interpreted as an adaptation for surviving long periods without access to a suitable electron acceptor. In addition, *Thiomargarita* cells seem capable of surviving by reducing their metabolic activity under conditions of nitrate starvation. For example, Namibian sediments kept in the cold for more than four years without added nitrate still contained a few living cells of *T. namibiensis*.

In contrast to *Beggiatoa* and *Thioploca*, *Thiomargarita* cells can survive exposure to atmospheric oxygen and to sulfide in millimolar concentrations. This tolerance of a broad spectrum of environmental conditions from very reduced to very oxidized chemical milieus seems to be another adaptation to the fluid, sulfidic sediment off Walvis Bay. This, together with the capacity to store both nitrate and sulfide, and the ability to survive long intervals without access to nitrate, enables *Thiomargarita* cells to continue to oxidize sulfide, even though electron acceptors are seldom available in the sediment. Thus, unlike *Thioploca* filaments, whose electron acceptor and donor are spatially separated, the electron donors and acceptors used by *Thiomargarita* are separated in time.

Additional Use of Oxygen

Single cells of *T. namibiensis* are so large that their metabolic activity produces measurable gradients of substrates. Such measurements indicate that *Thiomargarita* cells do not simply survive exposure to atmospheric oxygen but also consume it. Sulfide enhances this uptake, and oxygen enhances sulfide uptake, suggesting that *Thiomargarita* cells use oxygen as an electron acceptor in addition to nitrate for oxidizing sulfide or internal sulfur. Thus, during periods of sediment suspension, when the sulfidic sediments mix with oxygen-containing seawater, *Thiomargarita* cells take up nitrate for later survival in the highly sulfidic mud and also may gain energy by rapidly oxidizing sulfide or stored sulfur globules with oxygen (Fig. 3).

In addition to passively enduring a highly reduced and occasionally very oxidized environment, *Thiomargarita* cells may actively switch between a slow metabolism during the long phases that the cells are buried in the sulfidic sediment and a very rapid metabolism during resuspension from that sediment when oxygen and sulfide are simultaneously available.

Anaerobic Oxidation of Sulfide

Nitrate-storing sulfur bacteria have been discovered in a broad range of marine sulfidic environments. Each of the genera of nitrate-storing sulfur bacteria can occur in enormously high biomass if they are sustained by a high flux of sulfide, as is often the case in coastal sediments. Thus, apart from showing exotic adaptations to sulfidic environments, these bacteria may play a more important role in the oxidative part of the sulfur cycle than was previously thought.

Although microbiologists long ago realized that bacteria may oxidize sulfide with nitrate, they considered anaerobic oxidation of sulfide to be of little importance, and it receives no mention in textbook illustrations of the sulfur cycle. Because the large marine *Beggiatoa* and *Thioploca* species use their vacuoles for storing nitrate, this view of the sulfur cycle now seems outdated. Furthermore, the discovery of *Thiomargarita*, a third genus of sulfur bacteria that can use nitrate as an electron acceptor for the oxidation of sulfide, stresses the underlying ecological importance of this process.

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