6 The Family *Beggiatoaceae*

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Abstract

The family *Beggiatoaceae* contains a wide range of morphologically conspicuous, aerobic, or nitrate-dependent sulfide-oxidizing bacteria that span the range from obligate sulfur-based chemolithoautotrophy to heterotrophic growth supplemented by sulfur oxidation. The *Beggiatoaceae* are the model organisms for the concept of chemolithotrophy, developed by Sergei Winogradsky during his postgraduate studies using natural populations of filamentous freshwater *Beggiatoaceae* collected in sulfur springs. Since the metabolism of the *Beggiatoaceae* requires access to reduced sulfur species and oxidants such as oxygen or nitrate, these bacteria thrive in microbial mats, surficial sediments, and sediment–water interfaces where these electron donors and acceptors coexist and can be intercepted for microbial energy generation before gradual abiotic sulfide oxidation sets in. All *Beggiatoaceae* have the ability to oxidize sulfide to elemental sulfur that is stored as intracellular sulfur globules, which make the cells highly refractory and conspicuous with the unaided eye and under the microscope. This characteristic, together with the absence of photosynthetic pigments, has led to their traditional designation as members of the "colorless sulfur bacteria," in contrast to the photosynthetic purple and green sulfur bacteria or the cyanobacteria. The white, yellow, or occasionally orange color of the *Beggiatoaceae*, their frequently filamentous or chain-like morphology, their growth pattern in flocs and mats on sediment surfaces, and their large cell size and capacity for storing several different compounds intracellularly have made these organisms fascinating research targets. Extensive microscopic and morphological surveys have focused on these bacteria since the late nineteenth and early twentieth century. To a surprising extent, early microscopic and morphological observations on large, morphologically conspicuous sulfur bacteria can be reintegrated into the emerging molecular and phenotypic taxonomy of the *Beggiatoaceae* today.

Taxonomy, Historical and Current

The family *Beggiatoaceae* represents one of the major mutually exclusive phylogenetic lineages of the morphologically conspicuous sulfur bacteria (Bavendamm 1924) within the *Gammaproteobacteria*. The *Beggiatoaceae* have undergone major expansions and revisions in the recent past: The genera *Beggiatoa* and *Thioploca* were recognized as phylogenetically intertwined (Teske et al. 1999) and in need of taxonomic revision that better reflects their natural evolutionary relationships in relation to each other and to *Thiomargarita* (Jørgensen et al. 2005; Teske and Nelson 2006). To accommodate the emerging natural diversity among these bacteria after substantive revision based on 16S rRNA and its sequences, cell morphology, and physiology, the family *Beggiatoaceae* retains the currently recognized genera *Beggiatoa*, *Thioploca*, and *Thiomargarita* in revised form and also includes the recently proposed genus-level Candidatus groups Maribeggiatoa, Marithioploca, Marithrix, Isobeggiatoa, Parabeggiatoa, Allobeggiatoa, Halobeggiatoa, and Thiopilula, the revived candidate genus *Thiophysa*, and some distinct phylogenetic lineages that for now remain unnamed (Salman et al. 2011; Hinck et al. 2011; Grünke et al. 2012). The *Beggiatoaceae* do not include the filamentous, heterotrophic freshwater bacterium *Vitreoscilla*, a betaproteobacterium that does not form intracellular sulfur globules (Strohl et al. 1986).

Recently, the combined family name *Thiotrichaceae* was introduced to include the genera *Beggiatoa*, *Thioploca*, *Thiomargarita*, *Thiothrix*, *Leucothrix*, *Achromatium*, *Thiobacterium*, and *Thiospira* (Garrity et al. 2005). However, this polyphyletic assemblage comprises physiologically and phylogenetically divergent bacteria, including the type genera (*Beggiatoa*, *Leucothrix*, and *Achromatium*) of the validly published families *Beggiatoaceae*, *Leucotrichaceae*, and *Achromatiaceae*. The genera *Beggiatoa*, *Thioploca* and *Thiomargarita* form a monophyletic lineage within the *Gammaproteobacteria* (Ahmad et al. 2006; Jørgensen et al. 2005); the genera *Thiothrix* and *Leucothrix* form the second (Howarth et al. 1999); the genus *Achromatium* constitutes the third of these lineages (Head et al. 1996); *Thiobacterium* is not yet phylogenetically assigned (Grünke et al. 2010). This phylogenetic framework based on 16S rRNA sequences is remarkably consistent with the validly published families *Beggiatoaceae* (Leadbetter 1974; Strohl 1989), *Leucotrichaceae* (Brock 1974), and *Achromatiaceae* (Van Niel 1948) that were based on distinct cell morphology and physiological characteristics and hold up well in the light of modern molecular taxonomy. Since each family is monophyletic, they provide a robust, natural phylogenetic framework that can accommodate future updates and novel taxa and should be retained.

This chapter provides an overview of the family *Beggiatoaceae* that synthesizes published taxonomic descriptions, physiology based on pure cultures and field samples, morphology and size of filaments, cell clusters and single cells, and 16S rRNA gene sequences obtained from pure cultures and single cells (Fig. 6.1).

Beggiatoaceae Migula 1894.

Beg.gia.to.a'ce.ae. N.L. fem.n. *Beggiatoa* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Beggiatoaceae* the *Beggiatoa* family.

Type genus: *Beggiatoa* (Trevisan 1842).

Beg.gia.to'a. M.L. fem.n. *Beggiatoa* named for F.S. Beggiatoa, a physician of Vicenza.

Molecular Analyses

Molecular analyses have uncovered wide phylogenetic diversity within the family *Beggiatoaceae* and delineated the *Beggiatoaceae* from other families of morphologically conspicuous sulfur bacteria. Early on, *Beggiatoa alba* and *Thiothrix nivea* were recognized as distinct lineages of the Gammaproteobacteria, initially by reverse transcription sequencing of extracted 5S rRNA (Stahl et al. 1987) and 16S rRNA (Lane et al. 1992) and later by sequencing of PCR-amplified and cloned 16S rRNA genes (Teske et al. 1995). Since then, 16S rRNA gene sequencing of individual filaments or single cells has played a crucial role in defining mutually exclusive monophyletic

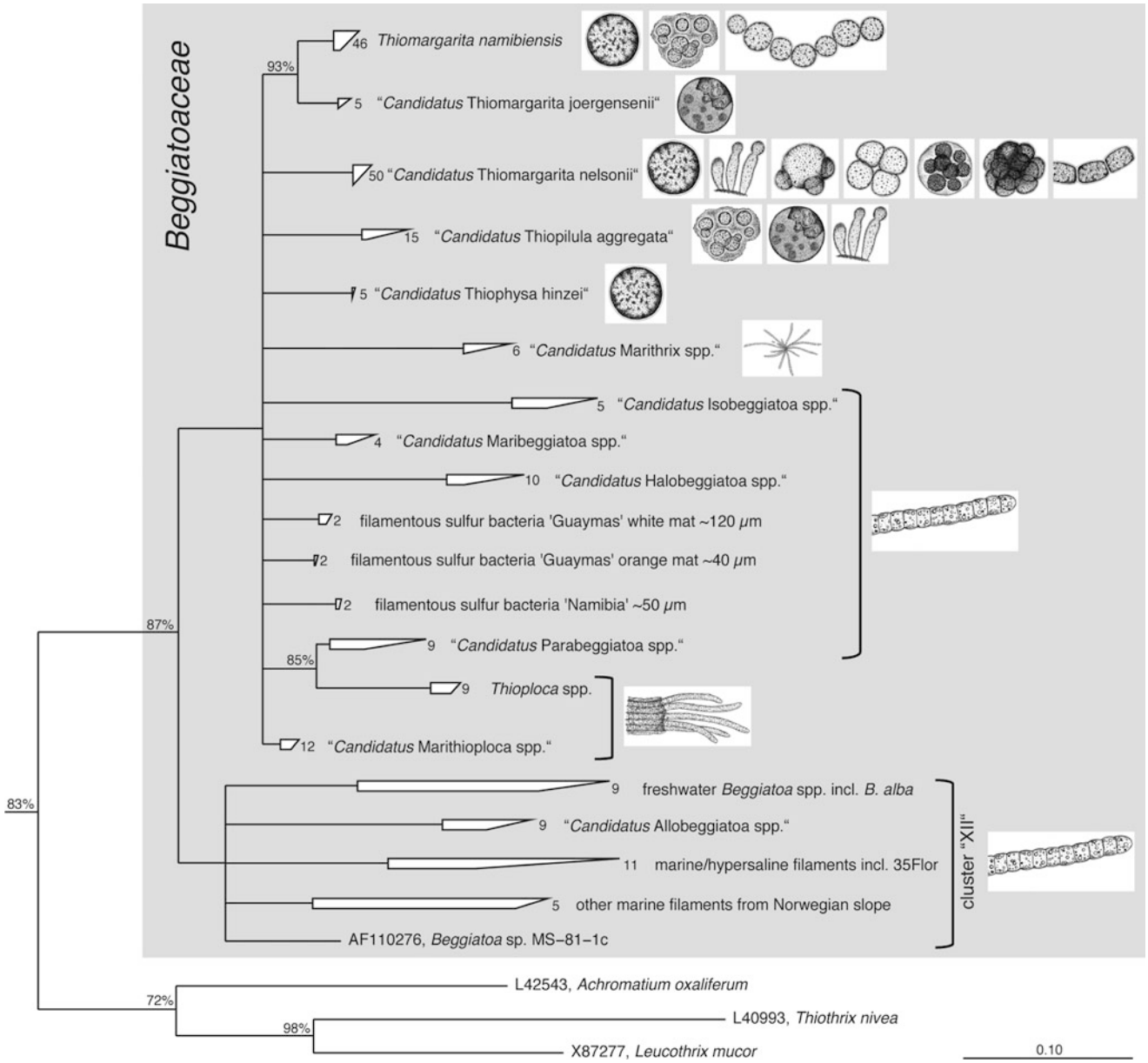

Fig. 6.1

Phylogenetic tree of Beggiatoaceae 16S rRNA gene sequences*. The phylogeny was inferred based on *E. coli* positions 279 to 1290, using maximum likelihood and 100 bootstrap runs. Nodes with less than 60 % bootstrap support were collapsed into polytomies. The family *Beggiatoaceae* separates into distinct phylogenetic groups: ''Cluster XII'' contains several lineages of filamentous sulfur bacteria, including the type species *Beggiatoa alba* (Salman et al. 2011). Subgroups within ''Cluster XII'' might need reclassification in the future. The top part of the tree with clusters I–XI contains filamentous and nonfilamentous large sulfur bacteria of various cell morphologies and arrangements. The 16S rRNA phylogeny shows that morphology is not a monophyletic feature within the *Beggiatoaceae

phylogenetic lineages that form the basis for several proposed candidate genera and species. Since these taxa are mostly uncultured and therefore incompletely described, no type strains can be given; listed instead are the currently known 16S rRNA gene sequences, morphological and physiological characteristics, and the environmental origin of natural samples and specimens.

A fundamental caveat for the study of natural *Beggiatoaceae* samples has to be kept in mind: Some key physiological characteristics (for example, intracellular nitrate accumulation) cannot be identified from the same filament or cell that is used for sequence identification; instead, morphologically indistinguishable filaments or cells from the same sampling site are used for parallel phylogenetic identification, as well as phenotypic and physiological characterization. Therefore, genotype/morphotype matches are to some extent inferred, especially for newly defined taxa with a small sample base. Repeated and consistent identification of natural populations in different settings and locations will solidify the evolving taxonomy of the *Beggiatoaceae*.

Genus *Beggiatoa*

In contrast to all other genera and candidate lineages of the *Beggiatoaceae*, the genus *Beggiatoa* has cultured representatives and a well-characterized type species, *Beggiatoa alba* strain B18LD, isolated from freshwater sediments near Baton Rouge, Louisiana, USA (Fig. 6.2c) (Mezzino et al. 1984). *Beggiatoa alba* is a deeply branching member of the *Beggiatoaceae* in 16S rRNA phylogenies (Ahmad et al. 2006); its phylogenetic position near the root of the *Beggiatoaceae*, among multiple lineages of freshwater and marine *Beggiatoaceae*, was recently confirmed in a comprehensive reanalysis of all known members of this group (Salman et al. 2011). *Beggiatoa alba* B18LD (AF110274; Strohl et al. 1981a) forms a monophyletic cluster with the closely related freshwater strains, *Beggiatoa alba* B15LD (L40944, Strohl and Larkin 1978a; Strohl et al. 1982), *Beggiatoa* sp. OH-75-2a (Fig. 6.2a) (AF110273; Nelson and Castenholz 1981a, b), and *Beggiatoa* sp. D-401 and D-402 (AY583995 and AY583996; Grabovich et al. 1998, 2001). The molecular mass of the *Beggiatoa alba* genome has been determined by CoT analysis as 2.02×10^9, which corresponds to 3.03×10^6 base pairs, similar to *E. coli* (Genthner et al. 1985). The G+C content for *Beggiatoa alba* strains B18LD, B15LB, and B25RD ranged from 40 to 42.7 mol%. The phenotypically

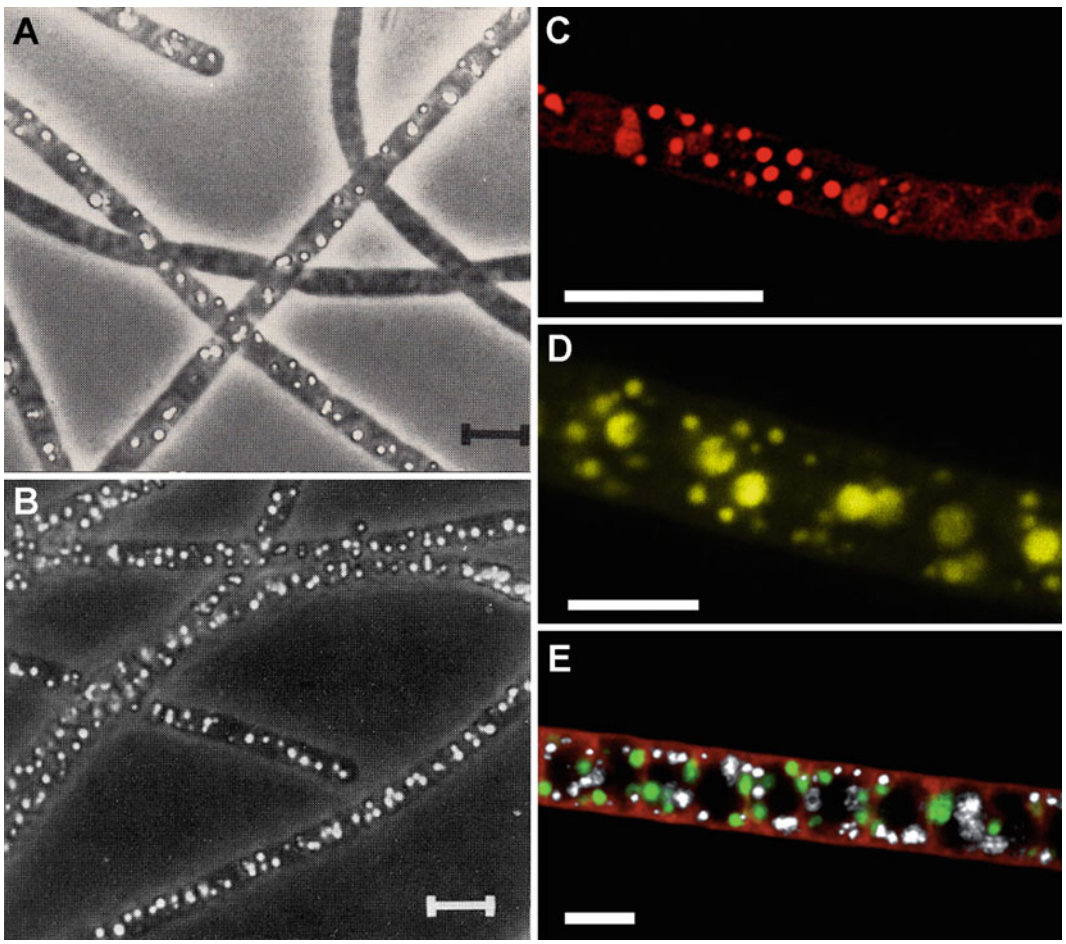

Fig. 6.2

Filaments affiliated with "Cluster XII." **(a)** ***Beggiatoa*** **sp. strain OH-75-2a, light micrograph showing bright white spots representing elemental sulfur and poly-β-hydroxyalkanoate inclusions. Scale bar 5 μm. (b)** ***Beggiatoa*** **sp. strain MS-81-1c, phase contrast micrograph likewise showing bright white inclusions bodies. Scale bar 5 μm. (c)** ***Beggiatoa alba*** **strain B18LD stained with Nile Red reveals membrane structures and inclusions of poly-β-hydroxyalkanoates. Scale bar 5 μm. (d)** ***Beggiatoa*** **sp. strain 35Flor stained with DAPI reveals polyphosphate inclusions at an emission wavelength of 525 nm (instead of 460 nm usually used for the specific detection of DNA). Scale bar 5 μm. (e) "*** ***Candidatus*** **Allobeggiatoa sp." filaments stained with Nile Red (*** ***red*** **) show location of membrane structures, and SYBR Green (*** ***green*** **) stains DNA nucleoids. White spots represent sulfur inclusions, and the void compartments in the center of each cell are the aqueous vacuoles, in which nitrate can be stored. Scale is 5 μm (Photos (a) and (b) Doug Nelson, University at California at Davis; Photo (c) Verena Salman, University of North Carolina at Chapel Hill; Photo (d) adapted from Brock et al. 2012; (e) adapted from Hinck et al. 2011)**

similar strain L1401-15 had a different G+C content of 51.7 mol % and appeared to be genetically distinct (Mezzino et al. 1984). The three former *Beggiatoa alba* strains contained plasmids with molecular masses of 12.3 to 12.8 × 10^6 or 18.9–19.7 kb with no described function (Minges et al. 1983). Independent analyses (Nelson, unpublished) determined the following mol G+C values: B18LD (37.1 %), B25RD (35.5 %), and OH-75-2a (38.5 %). *Beggiatoa alba* B18LD contains the genes for the linear C_1-oxidation pathway of alpha-, beta-, and gammaproteobacterial methylotrophic bacteria (Jewell et al. 2008). *Beggiatoa alba* B15LD (DSM 1416) also contains the soxB gene, shared with a wide range of sulfur-oxidizing *Proteobacteria* and *Chlorobia* (Genbank accession number EF618583) (Meyer et al. 2007), and the chaperonin-60 gene (Genbank accession number JF745935). Genome sequencing of type strain *Beggiatoa alba* B18LD has been completed at the Joint Genome Institute (JGI Project ID 16466). Pending additional analyses, only the *Beggiatoa alba* cluster may constitute the phylogenetically validated genus *Beggiatoa* in the strict sense.

Beggiatoa alba **(Vaucher 1803) Trevisan 1842**

Al'ba. L. adj. *albus*, white.

B. alba grows chemoorganotrophically and aerobically, with a preference for microaerobic conditions. When grown in the presence of reduced sulfur sources, sulfur is deposited in inclusions surrounded by the cytoplasmic membrane. Anaerobic cell maintenance with sulfur as electron acceptor is possible. Necridia and hormogonia can be formed. Colonies on agar may appear as circuitously curled filaments. The filaments of *B. alba* are about 1.5–4 µm in diameter and may vary with growth conditions. Cells are usually 3.0–9.0 µm long, with filament lengths averaging 60–120 µm.

The neotype strain, B18LD, was isolated from an enrichment obtained from a rice paddy in Lacassine, Louisiana, USA. This strain is described in detail by Mezzino et al. (1984). The well-characterized strains OH-75-2a and B15LD should be considered strains of *B. alba*.

Type strain: LSU B18LD, ATCC 33555.

Genbank accession numbers of 16S rRNA gene sequence: AF110274.

Other morphologically and physiologically similar isolates and enrichments of filamentous sulfur bacteria constitute sister lineages to *Beggiatoa alba* radiating near the base of the *Beggiatoaceae*; these form separate 16S rRNA branches and cannot be subsumed under the *Beggiatoa alba* lineage (Ahmad et al. 2006). These lineages were termed "cluster XII" (*Fig. 6.1*), understood as a temporary designation until more taxonomic work establishes several well-defined groups (Salman et al. 2011). Several of these "cluster XII" organisms appear in the literature under the genus name *Beggiatoa*, but they are overdue for updated formal description and taxonomic revision, as suggested previously based on 16S rRNA sequences (Salman et al. 2011) and also by heterogeneous G+C content of genomic DNA (Mezzino et al. 1984). This taxonomic revision has started with the recently proposed Candidatus genus-level group "Allobeggiatoa" (Hinck et al. 2011). Two additional groups of *Beggiatoaceae* within "cluster XII" require taxonomic revisions (*Fig. 6.1*):

1. Filamentous *Beggiatoa*-like bacteria from freshwater habitats include not only the *B. alba* strains B15LD and B18LD (L40994 and AF11024, Strohl and Larkin 1978a; Strohl et al. 1981, 1982), but also the pure culture strains *Beggiatoa* sp. OH-75-2a (AF110273, Ahmad et al. 2006), *Beggiatoa* sp. AA5A (Genbank No. AF110275, Ahmad et al. 2006), *Beggiatoa* sp. D-401 and D-402 (AY583995 and AY583996, Grabovich et al. 1998, 2001), *Beggiatoa* sp. 1401–13 (L40997; Pringsheim 1964), *Beggiatoa* sp. LPN from a sewage outlet (EU015402, Kamp et al. 2006), and thin (5–7 µm diameter) *Beggiatoa* filaments naturally enriched in a cave stream for which a 16S rRNA FISH probe has been designed (DQ133935; Macalady et al. 2006, 2008). The older literature contains several studies of *Beggiatoa* strains that grew preferentially under heterotrophic conditions or with organic carbon amendments to chemoautotrophic media (Faust and Wolfe 1961; Scotten and Stokes 1962; Morita and Stave 1963; Burton et al. 1966; Kowallik and Pringsheim 1966; Pringsheim 1967); these strains might have their taxonomic home in "Group XII" as well. A 16S rRNA gene sequencing survey and further characterization of those strains that might have survived in culture collections (for example, Schlösser 1982) are overdue. A sequence-based study should also reexamine the taxonomic borders between heterotrophic *Beggiatoa* spp. and morphologically similar, filamentous *Vitreoscilla* spp. that share the same freshwater benthic habitat; the genus *Vitreoscilla* differs from *Beggiatoa* by not forming sulfur globules (Strohl et al. 1986).
2. A second group consists of uncultured marine morphotypes from hypersaline lagoons (GU117706 and GU117707; de Albuquerque et al. 2010), several phylotypes from the Håkon Mosby mud volcano in the Arctic Ocean (FR847882 to FR847887; Grünke et al. 2012), the cultured autotrophic marine strain MS-81-6 (AF110277) from Sippewissett salt marsh near Woods Hole, MA (Nelson et al. 1982; no longer available in culture), brackish-water filaments enriched from sediments off southeast India (HM598303, JN588607, JN674459; Saravanakumar et al. 2012), and the cultured marine strain 35Flor (FR717278) originating from corals infected with black band disease. The members of this marine cluster have filament diameters of ca. 2–7 µm (compiled in Brock et al. 2012). Near the root of this cluster branches the autotrophic marine strain MS-81-1c, also isolated from Sippewissett salt marsh (AF110276; Nelson et al. 1982) but no longer available in culture (*Fig. 6.2b*). Currently, strain 35Flor is the only marine *Beggiatoa* strain that is available in culture (coculture with a *Pseudovibrio* sp.); it has been studied extensively for its polyphosphate

inclusions (Fig. 6.2d) (Brock and Schulz-Vogt 2011; Brock et al. 2012) and anaerobic sulfur respiration (Schwedt et al. 2012).

Genus *Thioploca*. Lauterborn 1907

Thi.o.plo'ca Gr. neut. n. *thein* (latin transliteration thium) sulfur; Gr. fem.n. *ploke* a braid, a twist; M.L. fem. n. *Thioploca* sulfur braid. The genus *Thioploca* includes thin filaments occurring in sheathed bundles that inhabit freshwater and brackish-water surficial sediments and decaying plant material (Fig. 6.3g). The type species of the genus *Thioploca*, *T. schmidlei* from Lake Constance, Germany (Lauterborn 1907), has been observed recently in Lake Baikal, Russia (Zemskaya et al. 2009), but it is not represented by 16S rRNA gene sequences. The second described species *T. ingrica* is morphologically similar to *T. schmidlei*, but has a smaller filament diameter (Wislouch 1912; Maier 1984). *T. ingrica* is represented by a tight cluster of mutually similar 16S rRNA gene sequences (AF452892; AY115530; AB263619; FR690997; FR690998; EU718069-71; L40998; AB699673 to AB699684) that were obtained from filaments in temperate freshwater lakes of Japan and Germany (Kojima et al. 2003, 2006), from Lake Baikal (DQ338566; Zemskaya et al. 2009), from brackish fjords in Denmark (Høgslund et al. 2010; Salman et al. 2011), and from a shallow tropical lake in Cambodia (Nemoto et al. 2012). A specific 16S rRNA FISH probe for this cluster has validated the 16S rRNA sequencing results for environmental filaments (Kojima et al. 2003). The microbial epibionts inhabiting the sheaths produced by *T. ingrica* have been analyzed by 16S rRNA gene sequencing and FISH, yielding predominantly *Chloroflexi* phylotypes (Kojima et al. 2006; Nemoto et al. 2011). Intergenic spacer region and partial 23S rRNA gene sequences (AB699673 to AB699684) allow for a fine-scale resolution of the genus *Thioploca*; the tropical *Thioploca* phylotypes diverge from their temperate lake counterparts (Nemoto et al. 2012).

Thioploca schmidlei. Lauterborn 1907

schmid'le.i. M.L. gen.n., *schmidlei* of Schmidle.

Identified from sediments of freshwater and brackish-water localities in Europe and from Lake Baikal, Russia. Originally found in Lake Constance, southern Germany. Multicellular filaments, diameter 5–9 µm, constant width over the entire length of the filament, forming bundles, gliding motility.

Type strain: none isolated.

Thioploca ingrica. Maier 1984

In'gri.ca. M.L. adj. ingrica pertaining to Ingria, ancient district of St. Petersburg, Russia.

Identified from sediments of freshwater and brackish-water localities in central Europe, from Lake Erie, USA; from Lake Biwa, Japan; and Lake Tonle Sap, Cambodia. Multicellular filaments, constant width over the entire length of the filament, diameter 2–4.5 µm, forming bundles; gliding motility.

Type strain: none isolated.

Genbank accession number of 16S rRNA gene sequence: L40998.

Taxonomic note: The genus *Thioploca* is not represented by pure cultures; its type species *T. schmidlei* is only rarely found, and its sole described species, *T. ingrica*, remains uncultured and incompletely characterized. Such a combination is usually characteristic of a Candidatus group; at present, *Thioploca* retains its status as a validly described genus due to historical precedent.

"*Candidatus* Marithioploca". Salman et al. 2011

This group of uncultured, filamentous, sheath-forming sulfide-oxidizing bacteria (Fig. 6.3a–f) was originally included in the genus *Thioploca* and contained the large marine *Thioploca* species *T. araucae and T. chileae* (Maier and Gallardo 1984b). Since it constitutes a monophyletic 16S rRNA gene lineage distinct from freshwater *Thioploca* (Teske et al. 1995, 1999) and also shows substantial physiological differences, the marine and freshwater *Thioploca* sp. were separated into two taxonomic groups. The smaller brackish and freshwater representatives are retained as the genus *Thioploca sensu stricto*, and the large marine strains constitute the *Candidatus* taxon "Marithioploca" (Salman et al. 2011). FISH hybridization experiments with group-specific 16S rRNA probes have validated the 16S rRNA sequences obtained from size-sorted and cleaned filaments (Teske et al. 1995, 1999). The "Marithioploca" group forms two separate, yet mutually closely related subclusters. One subcluster contains the original published partial sequence of *Thioploca araucae* (L41043; Teske et al. 1995), the near-complete sequence of a large, single, marine *Beggiatoa*-like filament from the Bay of Concepción (AF035956; Teske et al. 1999), and several sequences from bundled and single filaments collected offshore Concepción, Chile (FR690987 to FR690993; Salman et al. 2011). The filament diameter range within this group is largely congruent with the range given in the original description of *T. araucae* (30–43 µm; Maier and Gallardo 1984b). The second subcluster contains the original partial sequences for *T. chileae* (L40999; Teske et al. 1999) and three other sequences originating again from bundled and single filaments (FR690994 to FR690996; Salman et al. 2011) that are congruent with the published size range for *T. chileae* (12–20 µm; Maier and Gallardo 1984b). 16S rRNA gene sequence identities among the two clusters are as high as 98.3–99.5 %, which could argue against a taxonomic separation. On the other hand, the two species show not only nonoverlapping filament diameter distributions but also distinct environmental distributions and habitat preferences (Schulz et al. 1996, 2000). Based on mutually consistent ecophysiological, morphological, and molecular differences, the two species are retained.

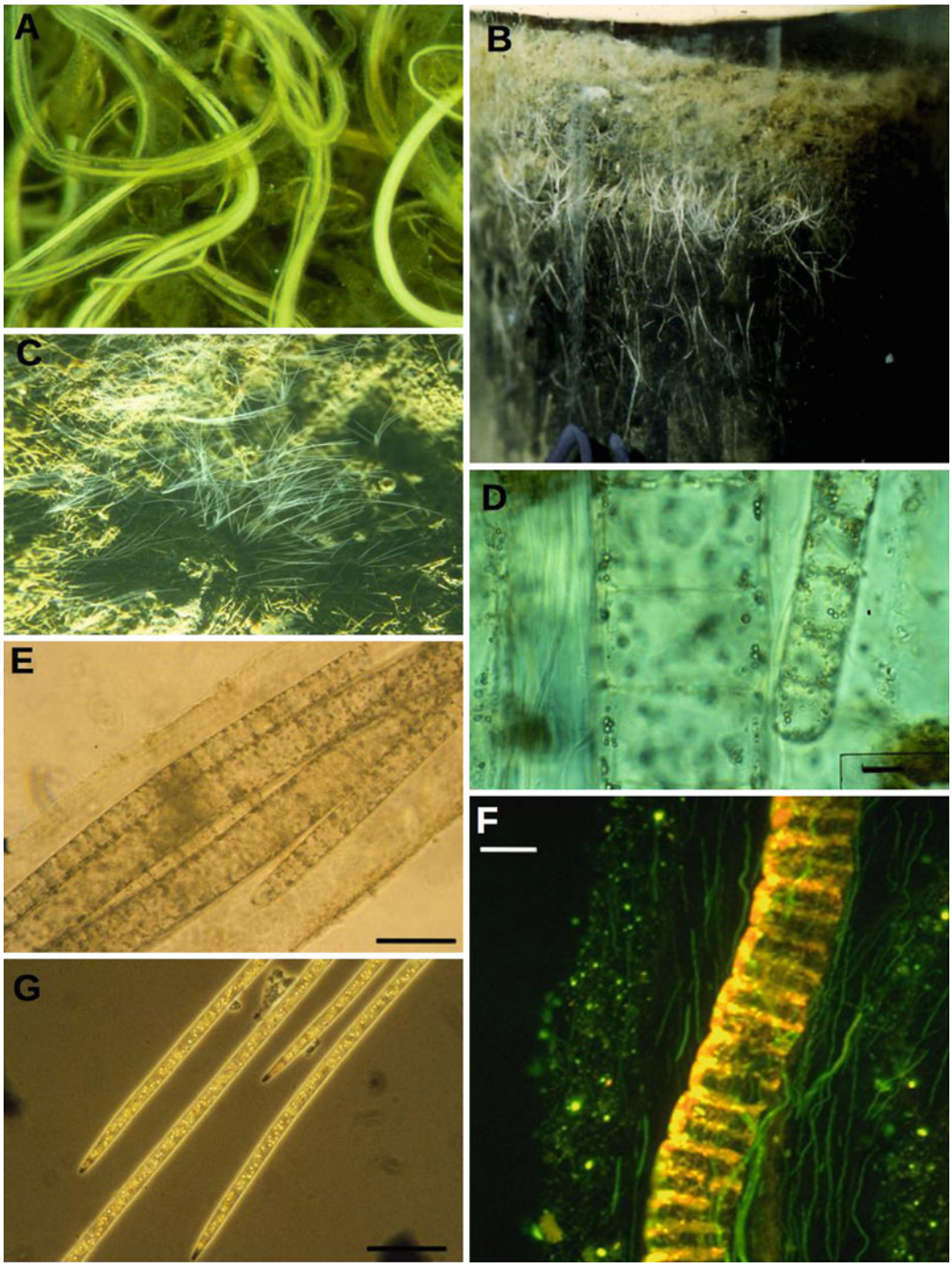

Fig. 6.3
''*Candidatus* Marithioploca'' *and Thioploca*. (a) Washed bundles of ''*Candidatus* Marithioploca'' from the continental shelf of Chile. The filaments appear white due to their internal sulfur content; bundles of filaments are surrounded by transparent sheaths. (b) Sediment core with reducing marine sediment, embedded vertically oriented ''Marithioploca'' filament bundles, and olive-green phytoplankton debris on top. (c) Individual filaments of ''Marithioploca'' emerging from their buried sheaths to take up nitrate from the overlying seawater (Hüttel et al. 1996). (d) Adjacent filaments of ''Marithioploca araucae'' (*left*) and ''Marithioploca chileae'' (*right*). Note the vacuole space taking up the cell interior and the sulfur globules within the peripheral cytoplasm. Scale bar = 10 μm. (e) Filaments of ''Marithioploca araucae'' (*left*) and ''Marithioploca chileae'' in a shared sheath. Scale bar = 50 μm. (f) Filament of ''Marithioploca araucae'' surrounded with filamentous bacterial epibionts (members of the sulfate-reducing genus *Desulfonema*; Fukui et al. 1999). Scale bar = 25 μm. (g) Filament tips of *Thioploca ingrica* from a brackish fjord (Randersfjord, Denmark). Scale bar = 20 μm (Photos (a–c) Markus Hűttel, Florida State University; (d) Jan Kűver, Institute for Material testing, Bremen; (e, g) Andreas Teske, University of North Carolina at Chapel Hill; (f) Manabu Fukui, Hokkaido University, Sapporo, Japan)

"*Marithioploca araucae*". Salman et al. 2011

Ma.ri.thi.o.plo'ca. L. gen. n. *maris* of the sea; N.L. fem. n. *Thioploca* a genus name; N.L. fem. n. *Marithioploca* the *Thioploca* of the sea, the truly marine *Thioploca*; T. araucae (Maier and Gallardo 1984b), Approved Lists 1980; a.rau'ca.e. N.L. fem. adj. *araucae* of Arauco in Central Chile.

Identified from oxygen-poor upwelling area offshore Concepción, Chile. Multicellular filaments either free-living filaments or bundled by a common mucous sheath; filament diameter 25–43 μm; constant width over the entire length of the filament; gliding motility; vacuolated; ability to store nitrate; sulfur inclusions, marine.

Genbank accession number of 16S rRNA gene sequence: L41043; FR690987 to FR690993.

"*Marithioploca chileae*". This Publication

Ma.ri.thi.o.plo'ca. L. gen. n. *maris* of the sea; N.L. fem. n. *Thioploca* a genus name; N.L. fem. n. *Marithioploca* the *Thioploca* of the sea, the truly marine *Thioploca*; T. chileae (Maier and Gallardo 1984b), Approved Lists 1980; chi'le.ae. N.L. gen.n. *chileae* of Chile.

Identified from oxygen-poor upwelling area offshore Concepción, Chile. Multicellular filaments either free-living filaments or bundled by a common mucous sheath; filament diameter 12–20 μm; constant width over the entire length of the filament; gliding motility; vacuolated; ability to store nitrate; sulfur inclusions, marine.

Genbank accession number of 16S rRNA gene sequence: L40999, FR690994, FR690995, FR690996.

Taxonomic note: The near-identical 16S rRNA gene sequences of filaments growing as sheathed filament bundles and those thriving as single, free-living filaments affiliating with this taxon show that the morphological (sheath-based) distinction of the genera *Beggiatoa* and *Thioploca* was phylogenetically shallow (Teske et al. 1999; Salman et al. 2011).

Genus *Thiomargarita*. Schulz et al. 1999

The first discovery of nonfilamentous, very large, vacuolated, nitrate-accumulating, and spherical cells in highly reducing marine sediments offshore Namibia led to the description of the new genus *Thiomargarita* based on a distinct morphology (◉ *Fig. 6.4a*) and 16S rRNA gene sequence (AF129012) (Schulz et al. 1999). *Thiomargarita* is among the largest known bacteria by volume; observed cell diameters diverge widely, and current observations indicate a range of 16–750 μm (Salman et al. 2011). Large *Thiomargarita* cells are discussed as an alternate explanation for late proterozoic microfossils that are commonly regarded as eukaryotic blastocytes (Bailey et al. 2007).

The chain-forming *Thiomargarita* specimens of the original description have been supplemented by 16S rRNA gene sequencing of numerous unicellular (◉ *Fig. 6.4b*) or aggregate-forming (◉ *Fig. 6.4e*) *Thiomargarita* cells from Namibia, Chile, and Costa Rica (FN811663; FR690879 to FR690921); these phylotypes cluster together and form the species *Thiomargarita namibiensis* (Salman et al. 2011). Two additional *Thiomargarita* species are proposed as species-level Candidate taxa. *Candidatus* "Thiomargarita joergensenii" forms a distinct clade of 16S rRNA gene sequences (FR690922 to FR690925) and shows a homogenous morphology: multiple spherical cells are inhabiting an intact centric diatom frustule that has no openings or passages for fully grown cells, suggesting an initial colonization of the frustule by substantially smaller daughter cells (Salman et al. 2011).

This morphology/lifestyle is also found in the candidatus taxon "Thiopilula." Cells belonging to the candidate species "Thiomargarita nelsonii" occur in a wide range of morphologies (◉ *Fig. 6.4c, d*), i.e., spherical unicells; cylindrical cells in chains; symmetrically arranged, fourfold-divided aggregates; aggregates of several tens of cells; attached budding cells (◉ *Fig. 6.4f*); and nonattached, extremely large spherical cells with budding spherical caps. This species is also represented by a distinct 16S rRNA gene lineage (FR690926 to FR690967, FN811658, FN811659, FN811661, FN811662, HF954103, HF954105, HF954106, HF954108-110, HF954113) (Salman et al. 2011; Bailey et al. 2011; Salman et al. 2013). Specific PCR primers for the genus *Thiomargarita* were developed for specific amplification of 16S rRNA genes from contamination-prone single cells (Bailey et al. 2011; Salman et al. 2011).

Members of the genus *Thiomargarita* contain up to four self-splicing introns within their 16S rRNA genes; they enlarge the 16S rRNA genes considerably (up to app. 3.5 kb) and interfere with PCR amplification of 16S rRNA genes (Salman et al. 2012). One or two introns are also found within the 16S rRNA genes of the candidate genera "Marithioploca," "Thiopilula," and "Thiophysa." The introns occur in specific, conserved positions within the 16S rRNA gene (*E. coli* positions 795, 1078, 1396, and 1495) and often encode genes for intron-encoded homing endonuclease proteins. Intron persistence within the large, vacuolated sulfide-oxidizing bacteria might be connected to their suggested degree of polyploidy (Salman et al. 2012); an unusually high amount of nucleoids is documented for cells of *Thiomargarita namibiensis* (Schulz 2006). A few intron-encoded endonucleases would be sufficient to jump-start endonuclease-catalyzed spread of intron sequences throughout the polyploid genome (Salman et al. 2012).

Thiomargarita namibiensis Schulz et al. 1999

Thi'o.mar.ga.ri'ta Gr. neut. n. *theion* (Latin transliteration *thium*), sulfur; L. n. *margarita* pearl; N.L. fem. n. *Thiomargarita* sulfur pearl; na.mi.bi.en'sis. M.L. gen. n. *namibiensis* of Namibia.

Spherical cells, occurring unicellular, in chains or in aggregates; single cells occasionally motile by slow jerky rolling; vacuolated; can store nitrate in vacuole (up to 800 mM); sulfur

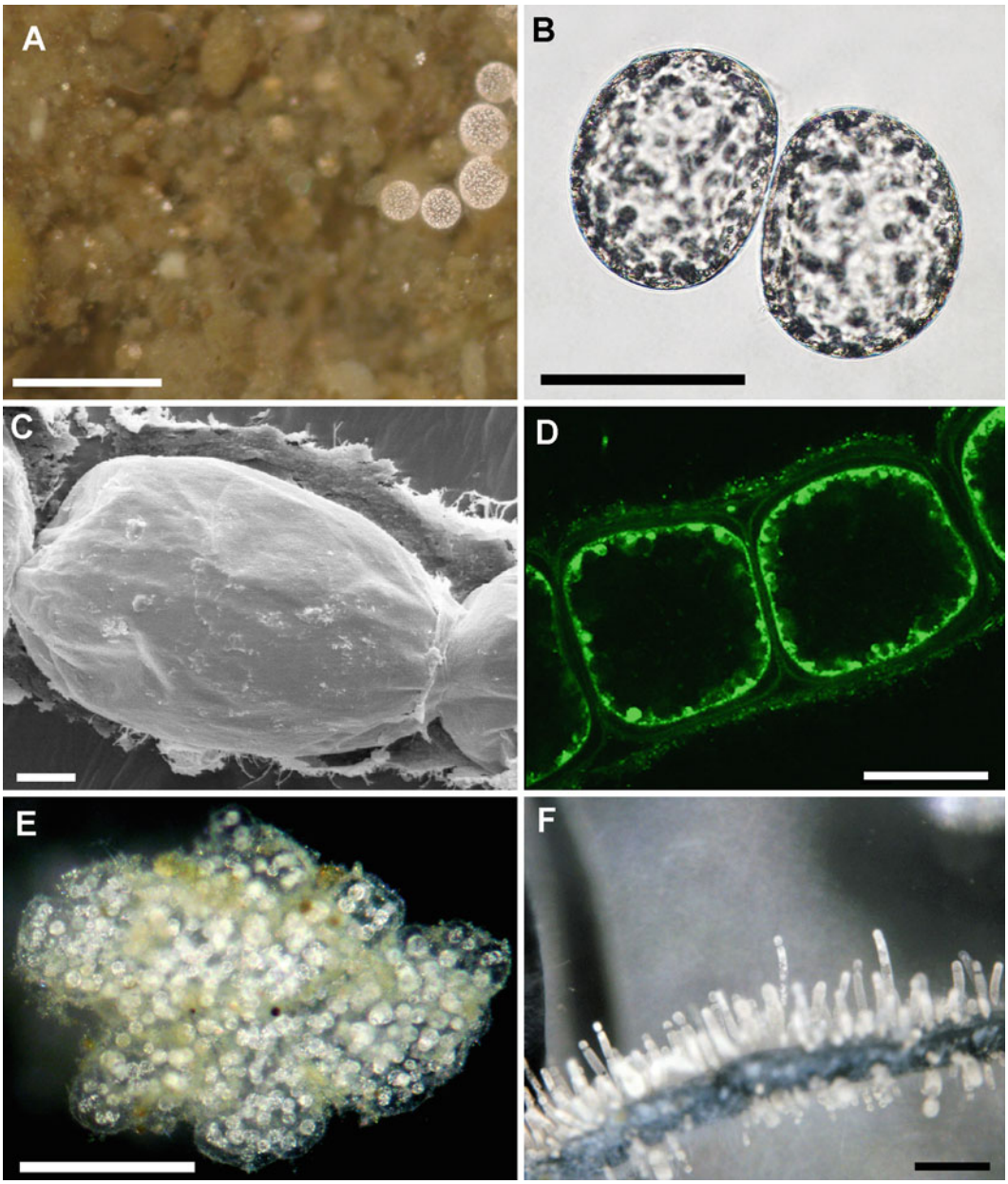

Fig. 6.4

***Marine nonfilamentous sulfur bacteria*.** (**a**) Namibian sediment is a liquid decaying diatomaceous ooze that contains predominantly nonfilamentous, chain-forming giant sulfur bacteria of the genus *Thiomargarita*. Scale bar 0.5 mm. (**b**) Microscopic image of two cells showing their elemental sulfur inclusions as black drop-like spots surrounding a large void internal vacuole—"in-focus" are the inclusions of the outermost rim of the cells, and they appear "out-of-focus" in the center as they are actually located above and below the focal plane. Scale bar 100 μm. (**c**) After removing the outer sheath of a "*Candidatus* Thiomargarita nelsonii" cells, the outer cell envelope can be observed with scanning electron microscopy. Scale 20 μm. (**d**) FITC staining of a "*Candidatus* Thiomargarita nelsonii" chain reveals the thin cytoplasmic rim at the periphery of each cell and leaves the inside (vacuole) unstained. The mucus and epibionts living thereon are stained as well. Scale is 50 μm. (**e**) Nonfilamentous sulfur bacteria like these collected off Namibia produce a mucous sheath that holds numerous spherical cells in a large aggregate. Scale is 0.5 mm. (**f**) Some unicellular sulfur bacteria are capable to produce a holdfast structure to attach themselves to solid surface and proliferate by forming small spherical buds at the apical ends. These cells were collected at the Costa Rica margin. Scale is 1 mm (Photos (**a**–**c**) Verena Salman, UNC Chapel Hill; (**d**) adapted from Salman et al. 2011; (**e**) Verena Salman University of North Carolina at Chapel Hill; (**f**) Jake Bailey, University of Minnesota)

inclusions; in sediments off Namibia, Chile, and Costa Rica and at mud volcano off Egypt; marine.

Genbank accession numbers of 16S rRNA gene sequence: FR690879-FR690921, FN811663, HF954102, HF954104.

"*Candidatus* Thiomargarita joergensenii" Salman et al. 2011

Thi.o.mar.ga.ri'ta. Gr. neut. n. *theion* (Latin transliteration *thium*), sulfur; L. n. *margarita* pearl; N.L. fem. n. *Thiomargarita* sulfur pearl; joer.gen.se'ni.i. N.L. gen. n., *joergensenii* of Jørgensen, named in honor of Bo Barker Jørgensen, a Danish microbiologist.

Single, spherical cells; occurring in empty diatom frustules; sporadic slow jerky rolling movement; vacuolated; sulfur inclusions; in sediments off Namibia; marine.

Genbank accession numbers of 16S rRNA gene sequence: FR690922–FR690925, HF954107.

"*Candidatus* Thiomargarita nelsonii" Salman et al. 2011

Thi.o.mar.ga.ri'ta. Gr. neut. n. *theion* (Latin transliteration *thium*), sulfur; L. n. *margarita* pearl; N.L. fem. n. *Thiomargarita* sulfur pearl; nel.so'ni.i. N.L. gen. n. *nelsonii* of Nelson, named in honor of Douglas C. Nelson, an American microbiologist.

Cells of highly diverse morphology and life modes; ability to divide in multiple planes, to attach, or to form gonidia; single cells or those in envelopes sporadic slow jerky rolling movement; vacuolated; sulfur inclusions; in sediments off Namibia, Chile, and Costa Rica and around cold seeps at Hydrate Ridge and Costa Rica; marine.

Genbank accession numbers of 16S rRNA gene sequence: FR690926–FR690967, FN811658–FN811659, FN811661, FN811662, HF954103, HF954105, HF954106, HF954108-110, HF954113.

Taxonomic comment: The partial 16S rRNA sequence of the original publication (AF129012) of *T. namibiensis* matches the "T. nelsonii" phylotype and not *T. namibiensis*. Obviously, the chains of cylindrical cells of "T. nelsonii" can be mistaken for the large, spherical cells in *T. namibiensis* chains.

"*Candidatus* Maribeggiatoa" Salman et al. 2011

Beggiatoa-like, large, vacuolated, nitrate-accumulating filaments from reducing marine sediments form this monophyletic lineage based on 16S rRNA gene sequences from individual filaments (Salman et al. 2011). The group contains phylotypes from the central Californian coast, Monterey Canyon (AF064543, Ahmad et al. 1999), Carmel Canyon (AY580013, Kalanetra et al. 2004), and Monterey Bay (FJ814745, FJ814753). Large individual filament diameters are found in this group: 20–76 µm for Carmel Canyon and 65–85 µm for Monterey Canyon filaments (*Fig. 6.5a–b*). With the exception of clone FJ814753, the sequences cluster tightly together and constitute the species-level candidate taxon "Maribeggiatoa vulgaris" (Salman et al. 2011). Phylotypes related to "Maribeggiatoa" were also obtained by sequencing of reverse-transcribed 16S rRNA from microbial mats in the Gulf of Mexico (partial sequences with Genbank numbers AY324499, AY324511) (Mills et al. 2004). A FISH probe for "Maribeggatoa" has been developed to distinguish "Maribeggiatoa" from "Marithioploca" (Ahmad et al. 1999).

"*Candidatus* Maribeggiatoa vulgaris" Salman et al. 2011

Ma.ri.beg.gi.a.to'a. L. gen. n. *maris* of the sea; N.L. fem. n. *Beggiatoa* a genus name; N.L. fem. n. *Maribeggiatoa* the *Beggiatoa* of the sea, the truly marine *Beggiatoa*; vul.ga'ris. L. fem. adj. *vulgaris* usual, common.

Disc-shaped cells forming multicellular filaments; constant width over the entire length of the filament, rounded terminal cells; gliding motility; vacuolated; ability to store nitrate; sulfur inclusions; marine; at seep sites and hydrothermal vents.

Genbank accession numbers of 16S rRNA gene sequence: FJ814745, AY580013, AF064543.

Taxonomic note: Two clusters of large, vacuolated, nitrate-accumulating marine *Beggiatoa*-like filaments from Guaymas Basin hydrothermal sediments consist of orange filaments with ca. 25–35 µm diameter (JN793553, JN793555, JN793556) and of very large colorless filaments of ca. 120 µm diameter (JN793554, JN793557) and form a multilineage cluster with the Candidatus taxa "Maribeggiatoa" and "Marithioploca" (McKay et al. 2012). The near-complete genome of a single orange filament has been obtained and analyzed after whole genome amplification (MacGregor et al. 2013a, b, c). Although published as "Maribeggiatoa," it became apparent that the orange Guaymas filaments share only weak bootstrap support (between 50 % and 60 %) with "Maribeggiatoa" (Salman et al. 2013). The white Guaymas filaments do not fit into currently described candidatus taxa (Salman et al. 2013). Therefore, the orange and the white Guaymas filaments are included here as separate lineages of *Beggiatoaceae* (*Fig. 6.1*).

"*Candidatus* Marithrix" Salman et al. 2011

Large, vacuolated filaments growing attached to hydrothermal vent chimneys and surrounding methane and mud seeps are exposed alternately to sulfidic and oxygenated seawater (*Fig. 6.5e*) and form this monophyletic lineage among the *Beggiatoaceae* (Kalanetra et al. 2004; Heijs et al. 2005; Kalanetra and Nelson 2010; Grünke et al. 2011, 2012). The 16S rRNA gene

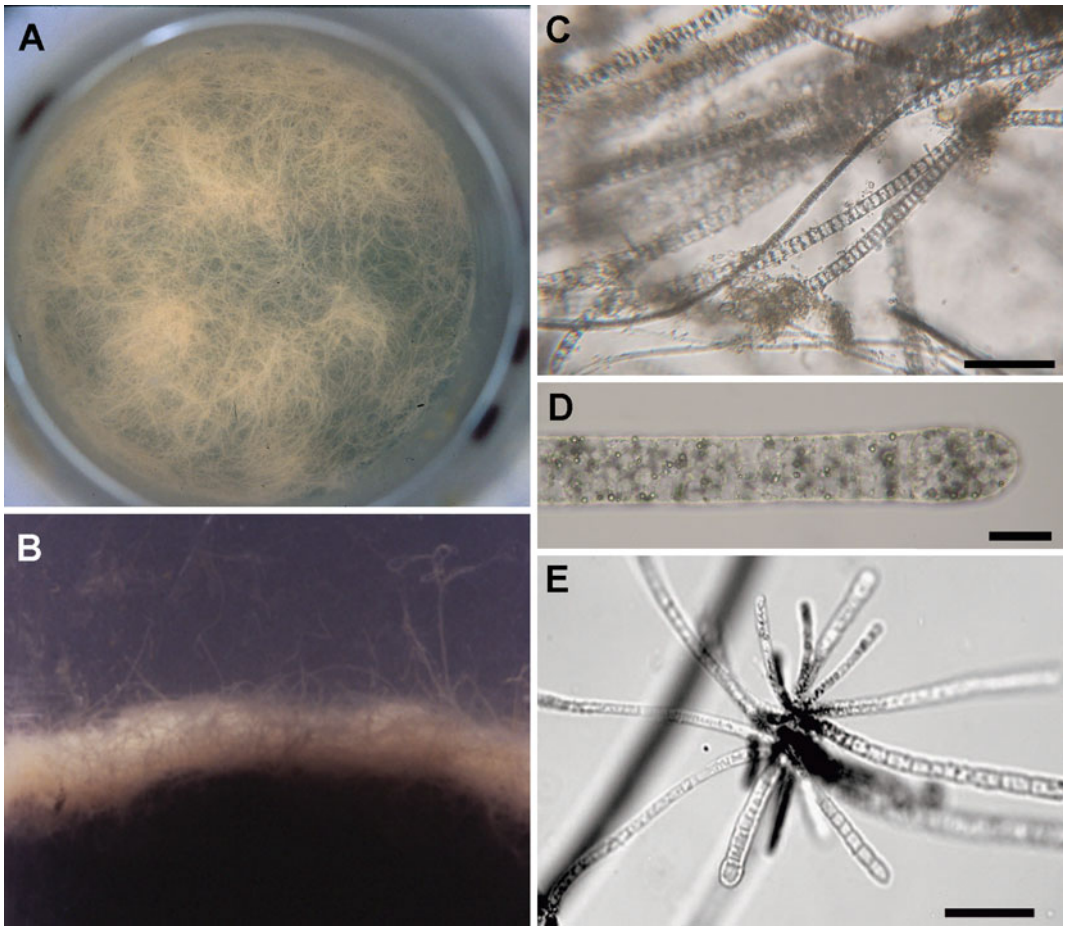

Fig. 6.5

***Marine filamentous sulfur bacteria*. (a) Mat-covered surface of a sediment core from Monterey Canyon, dominated by large, nitrate-accumulating filamentous sulfur oxidizers "*Candidatus* Maribeggiatoa." (b) Viewing the same mat from the side reveals individual filaments reaching out from the mat and into the supernatant water. (c) Microscopic image of filaments collected from a microbial mat at the Håkon Mosby mud volcano off Norway. The community consists of filaments of various diameters. Scale bar 50 μm. (d) Close-up view of a vacuolated marine filament sampled in Eckernförde Bay, Germany. The filament is in the correct size range for "Parabeggiatoa" but requires molecular identification for a definitive attribution. Scale bar 25 μm. (e) Attached filamentous sulfur bacteria ("Marithrix") sampled at White Point off Oregon forming a rosette. Scale bar 40 μm (Photos (a, b) Douglas Nelson, University of California at Davis; (c) Stefanie Meyer, Max Planck Institute for Marine Microbiology, Bremen; (d) Marc Mussmann, Max Planck Institute for Marine Microbiology, Bremen; (e) modified from Kalanetra et al. 2004)**

sequences of "Marithrix" filaments have been determined by multiple PCR amplifications with general and specifically developed group-specific primers and were validated by FISH hybridization of fresh filaments, using the rRNA equivalent of the group-specific PCR primer site as probe target (Kalanetra et al. 2004). The filament diameters of the target organism are variable: positive FISH hybridizations were obtained with filaments in the range of 10–38 μm, plus a few larger filaments (Kalanetra et al. 2004). Attached filaments from the Juan de Fuca vents ranged in diameter mostly from 9–30 μm; a minority of larger filaments reached up to 96 μm (Kalanetra and Nelson 2010). So far, filaments from two deep-sea hydrothermal areas (Juan de Fuca, Escanaba Trough) and a coastal hydrothermal vent (White Point, California) have identical 16S rRNA sequences (AY883933; AY883934; AY496953); very similar 16S rRNA transcripts were recently obtained from the Menez Gwen hydrothermal vent site (FR827864; Grünke et al. 2012) and Lucky Strike hydrothermal field (FR670384; Crépeau et al. 2011) on the Mid-Atlantic Ridge, from the Amon (FR666859, Grünke et al. 2011) and Milano (AY592917, Heijs et al. 2005) mud volcanoes in the Mediterranean Sea, and from a Storegga gas chimney off Norway (FR847874, Grünke et al. 2012). Overall, the members of this group share 16S rRNA gene sequence similarities of at least 98 %. The name of this candidate genus and species, *Candidatus* "Marithrix sessilis," reflects their distinctive surface-attached and rosette-forming growth mode that is otherwise seen in the genus *Thiothrix* (Salman et al. 2011).

"*Candidatus* Marithrix sessilis" Salman et al. 2011

Ma'ri.thrix. L. gen. n. *maris* of the sea; Gr. n. *thrix* hair; N.L. fem. n. *Marithrix* hair of the sea; ses'si.lis. L. adj. sessilis sitting, adhering to a surface.

Attached, multicellular filaments, constant width over the entire length of the filament; diameter of most filaments in the range of 10–38 µm (outliers up to 112 µm have been observed), rounded ends, sometimes forming rosettes; ability to produce gonidia; nonmotile; sulfur inclusions; vacuolated or non-vacuolated; marine; at cold seeps and hydrothermal vents.

Genbank accession numbers of 16S rRNA gene sequences: AY883933–AY883934, AY496953, FR827864.

"*Candidatus* Isobeggiatoa" Salman et al. 2011

Beggiatoa-like, vacuolated, nitrate-accumulating filamentous bacteria from a wide range of marine sediments constitute the genus-level candidate taxon "Isobeggiatoa," defined as a monophyletic lineage by 16S rRNA analysis (Salman et al. 2011). At present, this group contains representatives from Arctic fjords of Svalbard, Norway (FN561862; Jørgensen et al. 2010); Tokyo Bay, Japan (AB108786; Kojima and Fukui 2003); the Chilean coast (FJ875195; Aranda et al. 2010); and a cluster of similar sequences from Limfjorden in Denmark (AF532775) and Jadebusen in Germany (AF532769; Mussmann et al. 2003) that have been proposed as the candidate species-level taxon "Isobeggiatoa divolgata" (Salman et al. 2011). Filament diameters of geographically separated populations with distinct 16S rRNA sequences show a wide range from approximately 10–30 µm (Jørgensen et al. 2010; Aranda et al. 2010). A single filament from Eckernförde Bay in Germany (Filament PS; near 30 µm diameter) was used for whole genome amplification and subsequent pyrosequencing, yielding a partial genome of 6.769 contigs with 17x coverage and a total sequencing length of 7.6 Mb (Mussmann et al. 2007).

"*Candidatus* Isobeggiatoa divolgata" Salman et al. 2011

I.so.beg.gi.a.to'a. Gr. adj. *isos* equal, similar; N.L. fem. n. *Beggiatoa* a genus name; N.L. fem. n. *Isobeggiatoa* the bacterium similar to *Beggiatoa*; di.vol.ga'ta. L. fem. adj. *divolgata* widespread, common.

Disc-shaped cells forming multicellular filaments; constant width over the entire length of the filament, rounded terminal cells; gliding motility; vacuolated; ability to store nitrate; sulfur inclusions; brackish or marine, also arctic latitudes.

Genbank accession numbers of 16S rRNA gene sequence: AF532769, AF532775, FJ875195, AB108786, FN561862.

"*Candidatus* Parabeggiatoa" Salman et al. 2011

Morphologically similar to medium-sized, filamentous marine "Isobeggiatoa," this monophyletic group of uncultured, sulfide-oxidizing large filamentous bacteria is defined by 16S rRNA gene sequencing of single filaments (Salman et al. 2011). These bacteria occur in two distinct phylogenetic clusters: one cluster represented by filaments from brackish sediments of Limfjorden in Denmark (AF532770; AF532772-774; Mussmann et al. 2003) contains the candidatus taxon "Parabeggiatoa communis" (Salman et al 2011). The 16S rRNA sequences of this cluster are validated by FISH with a group-specific 16S rRNA probe; the FISH-stained filaments of this cluster range in diameter from approximately 33–40 µm (Mussmann et al. 2003). The second cluster consists of filaments with diameters in the range of 20–30 µm that were collected from reducing marine sediment underneath the cages of a salmon farm in southern Chile (FJ875196 to FJ875199) (Aranda et al. 2010). A single filament from Eckernförde Bay in Germany (Filament SS, ca. 30 µm diameter) was used for whole genome amplification and subsequent Sanger sequencing, yielding a low-coverage (3x) partial genome assembly of 1,091 contigs with a total sequencing length of 1.3 Mb (Mussmann et al. 2007). Recently, "Parabeggiatoa" was also found in extensive sulfide-oxidizing mats on hydrothermal sediments of Guaymas Basin in the Gulf of California, Mexico (JN793555; McKay et al. 2012).

"*Candidatus* Parabeggiatoa communis" Salman et al. 2011

Pa.ra.beg.gi.a.to'a. Gr. prep. *para* beside, like; N.L. fem. n. *Beggiatoa* a genus name; N.L. fem. n. *Parabeggiatoa* resembling the genus *Beggiatoa*; com.mu'nis. L. fem. adj. *communis* common, widespread.

Disc-shaped cells forming multicellular filaments; diameter 33–40 µm, constant width over the entire length of the filament, rounded terminal cells; gliding motility; vacuolated; ability to store nitrate; sulfur inclusions; brackish or marine.

Genbank accession numbers of 16S rRNA gene sequence: AF532770, AF532772–AF532774, FJ875196–FJ875199.

"*Candidatus* Allobeggiatoa" Hinck et al. 2011

Strains of the genus-level Candidatus group "Allobeggiatoa" (*Fig. 6.2e*) were enriched from solar salterns in Spain and hypersaline cyanobacterial mats in Spain and Mexico (Hinck et al. 2007, 2011) and represent a monophyletic lineage in 16S rRNA phylogenies (EF428583 and EU919200; Hinck et al. 2007; FR687024 to FR687036; Hinck et al. 2011). The phylogenetically clustered filaments from Spain constitute the species-level candidate group "Allobeggiatoa salina"

(Hinck et al. 2011). The 16S rRNA gene sequences have been validated by group-specific FISH probes and hybridization experiments (Hinck et al. 2011). The "Allobeggiatoa" group constitutes a sister lineage to *Beggiatoa alba* and related deeply branching *Beggiatoa*-like filamentous bacteria (Hinck et al. 2011). It differs not only by 16S rRNA phylogeny but also morphologically. The filaments of "Allobeggiatoa" are vacuolated (*Fig. 6.2e*) and have a diameter of predominantly 6–14 µm, which is distinct from the non-vacuolated, thinner (2–3 µm) filaments for *Beggiatoa alba* and its freshwater relatives (Hinck et al. 2011).

"*Candidatus* Allobeggiatoa salina" Hinck et al. 2011

Al.lo.beg.gi.a.to′a. L. gen. n. *allos* the other; *Beggiatoa* genus name; M.L. fem. n. *Allobeggiatoa*, the other *Beggiatoa*; sa.li′na. L. fem. adj. *salina* salted, saline.

Disc-shaped cells, forming filaments of 6–14 µm in diameter, constant width over the entire length of the filament, rounded terminal cells; gliding motility; intracellular storage of nitrate (up to 650 mM); each cell contains a large central vacuole; the vacuole accounts for about 80 % of cellular biovolume and is surrounded by a cytoplasmic layer; intracytoplasmic sulfur storage (up to 250 mM); facultative anaerobic and presumably performing reduction of intracellular nitrate; microaerophilic; chemolithoautotrophic sulfide oxidizer; halotolerant (tested range 3–15 % salinity); filaments do not form macroscopically visible mats, but are distributed within certain cyanobacterial mat layers that are exposed to sulfide gradients; habitat: sulfide-rich microbial mats at shallow permanently hypersaline lakes and ponds of solar saltern systems with salinities reaching up to 15 %.

Genbank accession numbers of 16S rRNA gene sequence: EF428583, EU919200, FR687024 to FR687033.

Taxonomic note: The detection of filamentous sulfur bacteria in hypersaline environments implied that the organisms were halophilic. Yet, culture studies showed identical growth at salinities ranging from 3–15 %, a characteristic that should be called halotolerant. In order to refer to the highly saline habitat where filaments were encountered, instead to an understudied physiological capability, the species name "halophila" was changed to "salina" before publication in Hinck et al. (2011). Care should be taken because the published phylogenetic tree (Hinck et al. 2011) contains the erroneous name "Allobeggiatoa halophila," which has been corrected by the erratum in Environmental Microbiology Vol. 14, Issue 12, p. 3287.

"*Candidatus* Halobeggiatoa" Grünke et al. 2012

The genus-level *Candidatus* group "Halobeggiatoa" represents a monophyletic group (95.9–100 % 16S rRNA gene sequence identity) of nitrate-accumulating marine single filaments of up to 10 µm diameter (*Fig. 6.5c*). The sequences of this phylogenetic group were obtained from filaments collected from white mats at the Håkon Mosby mud volcano offshore northern Norway (FR847864 to FR847873; Grünke et al. 2012) and from nearshore sediments in Tokyo Bay (AB106784, AB106785; Kojima and Fukui 2003).

"*Candidatus* Halobeggiatoa borealis" Grünke et al. 2012

Ha.lo.beg.gi.a.to'a. Gr. n, *hals* salt; N.L. fem. n. *Beggiatoa* a genus name; N.L. fem. n. *Halobeggiatoa* the salt *Beggiatoa*; bo.re.al.is. L. fem. adj. *borealis* northern

This species-level candidate group is based on seven identical 16S rRNA gene sequences from filaments collected at the Håkon Mosby mud volcano (HMMV) offshore northern Norway (Lichtschlag et al. 2010; Grünke et al. 2012).

Disc-shaped cells, forming filaments of 8–10 µm in diameter, constant width over the entire length of the filament; intracellular nitrate accumulation; gliding motility; filaments occur in conspicuous white mats on HMMV methane seep sediments. Genbank numbers are FR847864 to FR847870.

Taxonomic note: Given the diversity of filamentous *Beggiatoa*-like organisms in the HMMV mats, the identification of "*Candidatus* Halobeggiatoa borealis" should be regarded as preliminary and requires future validation by FISH hybridization and filament-specific physiological characterization.

"*Candidatus* Thiopilula" Salman et al. 2011

The candidate genus-level group "Thiopilula" includes large, nonfilamentous, vacuolated cells that resemble *Thiomargarita* in spherical cell morphology but occur attached to surfaces (Bailey et al. 2011), in colony-like aggregates or within diatom frustules, and form a distinct 16S rRNA lineage (Salman et al. 2011). Specimens collected from benthic marine sediments off Namibia and attached in the vicinity of seeps off Costa Rica have been sequenced (FR690968 to FR690981; FN811660 and FN811664) and are proposed as members of the species-level candidate taxon "Thiopilula aggregata" (Salman et al. 2011).

"*Candidatus* Thiopilula aggregata" Salman et al. 2011

Thi.o.pi'lu.la. Gr. neut. n. *theion* (Latin transliteration *thium*), sulfur; L. fem. n. *pilula* little ball, little globule; N.L. fem. n. *Thiopilula* little sulfur ball; ag.gre.ga'ta. L. fem. adj. aggregata joined together.

Spherical cells aggregated in variable arrangements; recorded diameters 15–65 µm; ability to attach and form gonidia; sporadic slow jerky rolling movement; vacuolated; sulfur inclusions; marine.

Genbank accession numbers of 16S rRNA gene sequence: FR690968–FR690980, FN811660, FN811664.

"*Candidatus* Thiophysa" Salman et al. 2011

The candidate genus-level group "Thiophysa" includes large, nonfilamentous, motile single spherical cells with sulfur inclusions that by 16S rRNA gene sequence (FR690982 to FR690986) form a distinct monophyletic group (Salman et al. 2011). Comparable cells have been described originally as *Thiophysa volutans* (Hinze 1903) and were later reclassified as members of the genus *Achromatium* (*A. volutans*, Van Niel 1948). However, 16S rRNA gene analysis places these bacteria clearly into the *Beggiatoaceae*, not into the *Achromatiaceae*. Therefore, the genus name *Thiophysa* has been revived and the species-level candidatus taxon "Thiophysa hinzei" proposed (Salman et al. 2011).

"*Candidatus* Thiophysa hinzei" Salman et al. 2011

Thi.o.phy'sa. Gr. neut. n. *theion* (Latin transliteration *thium*), sulfur; Gr. fem. n. *physa* bubble, breath; N.L. fem n. T*hiophysa* sulfur bubble; hin'zei. N.L. gen. n. *hinzei* of Hinze; named in remembrance of G. Hinze, a German microbiologist, who first described marine, large, spherical sulfur bacteria.

Single, spherical cells; recorded diameters from 56 to 90 µm; vacuolated; sporadic slow jerky rolling movement; sulfur inclusions; marine.

Genbank accession numbers of 16S rRNA gene sequence: FR690982–FR690986.

Phenotypic Analyses

Phenotypic Characteristics of the *Beggiatoaceae*

One of the basic, defining features of the *Beggiatoaceae* is the formation of intracellular sulfur globules by oxidation of reduced sulfur sources (e.g., *Figs. 6.2a* and *6.4b*). The physiological roles of sulfide oxidation and sulfur accumulation are complex and diverge between different physiological types of the *Beggiatoaceae*. Sulfide is a source of energy and electrons for autotrophic carbon fixation and growth (Dworkin 2012; Winogradsky 1887); it can be oxidized with oxygen or nitrate as terminal electron acceptors and can be supplemented or replaced by organic carbon compounds as energy source for heterotrophic growth; in the latter case, elemental sulfur from sulfide oxidation is stored as an alternate electron acceptor. For overview purposes, the diverse genera and candidatus groups within the *Beggiatoaceae* can be divided into several groups with shared phenotypic characteristics.

(A) The heterotrophic non-vacuolate freshwater strains with thin filament diameter are represented by several well-studied strains: the type strain of the genus *Beggiatoa*, *B. alba* B18LD (*Fig. 6.2c*, Mezzino et al. 1984); the *B. alba* strains B15LB (Strohl and Larkin 1978a, b; Strohl et al. 1982); OH-75-2a (*Fig. 6.2a*, Nelson and Castenholz 1981a, b); and the distantly related strain L1401-13 (Pringsheim 1964; Kowallik and Pringsheim 1966). (B) The autotrophic non-vacuolate marine strains with thin filament diameters are represented by the facultatively autotrophic strain MS-81-6, by the obligately autotrophic strains MS-81-1c (*Fig. 6.2b*, Nelson et al. 1982; Nelson and Jannasch 1983; Nelson et al. 1986b; Hagen and Nelson 1996, 1997), and by the marine strain 35Flor (*Fig. 6.2d*, Kamp et al. 2008; Brock et al. 2012; Schwedt et al. 2012). These strains exhibit strong chemotactic behavior and orient themselves as *Beggiatoa* "plates" in the steep oxygen/sulfide gradients which they maintain by fast sulfide oxidation under microoxic conditions (*Fig. 6.6*). (C) The small freshwater and brackish-water genus *Thioploca* is characterized by sheathed bundles of thin filaments embedded in surface sediment or decaying plant material (*Fig. 6.3g*, Høgslund et al. 2010). (D) The large, vacuolated, nitrate-accumulating autotrophic marine Candidatus groups "Maribeggiatoa," "Isobeggiatoa," "Parabeggiatoa," (*Fig. 6.5a, b, d*) and related *Beggiatoaceae* remain uncultured so far. The cells of these large, marine Beggiatoaceae filaments are hollow, i.e., composed of a thin cylinder of cytoplasm surrounding a large central vacuole. This extensive vacuolation is usually linked to high intracellular nitrate concentration (Hinze 1901; Jannasch et al. 1989; Nelson et al. 1989; Larkin and Henk 1996; McHatton et al. 1996), with the possible exception of "*Candidatus* Marithrix," in which nitrate could not yet be detected in the vacuoles (Kalanetra et al. 2004) (*Fig. 6.5e*). (E) Large size, vacuolation, and the ability to accumulate nitrate also apply to *Thiomargarita* and "*Candidatus* Marithioploca," although these organisms are set apart by their conspicuous morphology and lifestyle: the filaments of "Marithioploca" occur predominantly in bundles within sheaths embedded in surface sediment and move within their sheaths to bridge spatially separated pools of the electron donor sulfide in the sediment and the electron acceptor nitrate in the overlying seawater (Hüttel et al. 1996) (Figs. *6.3a–f* and *6.7*). (F) In contrast, *Thiomargarita*, "Thiopilula," and "Thiophysa" have very limited mobility or grow even attached to surfaces (*Fig. 6.4*); they rely primarily on their large cell size and high intracellular storage capacity to survive fluctuating redox regimes and temporary electron donor and acceptor shortages (Schulz and Jørgensen 2001).

Genus *Beggiatoa*

A physiological characterization of the genus *Beggiatoa* depends on how its taxonomic borders are drawn. If the genus is not reduced to its only recognized species, the heterotrophic

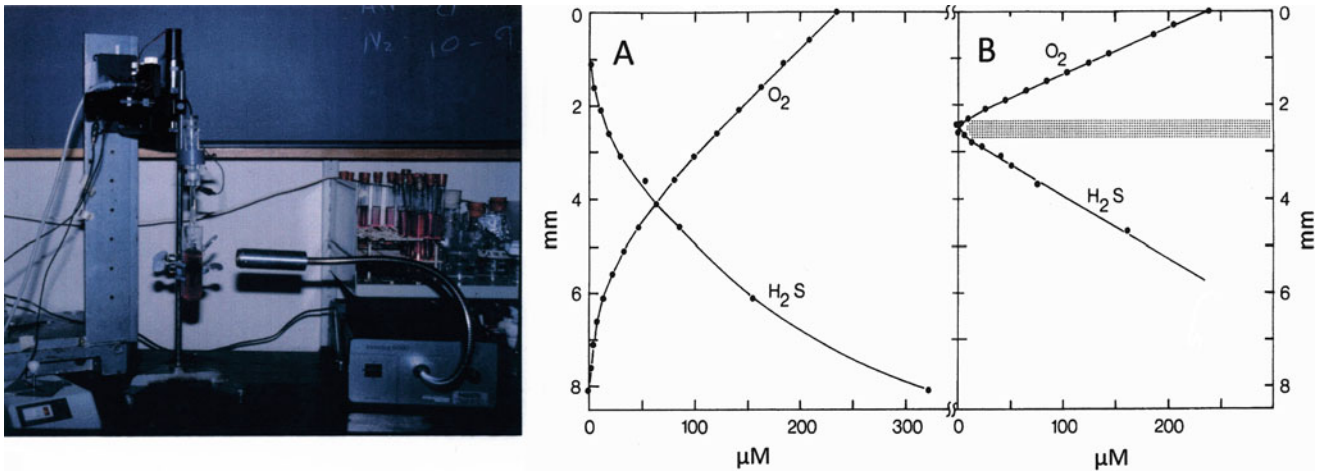

Fig. 6.6

Beggiatoa as gradient organism at the sulfide/*oxygen interface*. *Left*, photo of gradient culture of aerobic, autotrophic sulfide-oxidizing marine *Beggiatoa* spp. The oxygen profile in a gradient culture of *Beggiatoa* spp. is determined with an oxygen microelectrode mounted to a micromanipulator (Nelson et al. 1986a). The whitish *Beggiatoa* plate is visible near the surface of the gradient culture; its position is indicated by the point light source (Photo by Douglas Nelson, UC Davis). *Right*, overlap between H_2S and O_2 profiles in control medium without *Beggiatoa* spp. (a) or inoculated with a *Beggiatoa* culture after 3 days (b). Zero depth indicates the air/agar interface. The shaded area in (b) indicates the *Beggiatoa* plate (Nelson et al. 1986b)

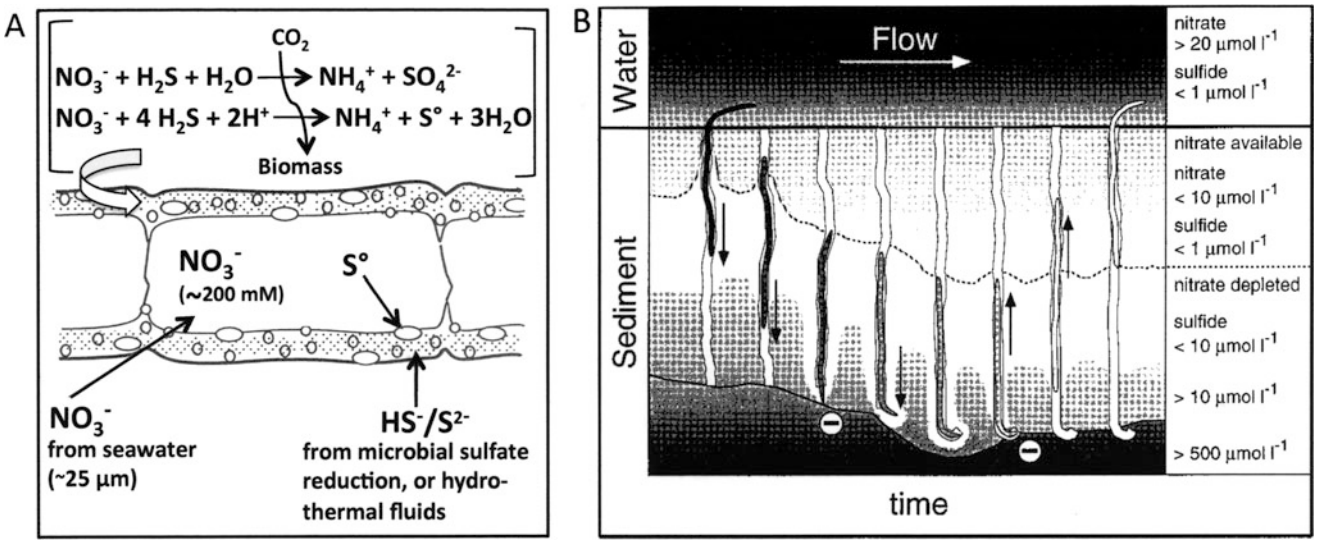

Fig. 6.7

***Physiology of nitrate-accumulating Beggiatoaceae*: *the case of "Marithioploca."* *Left*, schematic links between nitrogen, carbon, and sulfur metabolism in large, marine *Beggiatoaceae*. Nitrate is taken up from seawater, concentrated by four orders of magnitude, and stored in the central vacuole; it serves as electron acceptor for sulfide oxidation to elemental sulfur and sulfate. The resulting energy is used for autotrophic carbon fixation. The process is supposed to be localized in the cytoplasm (see *arrow*). This scenario is based on studies with "Marithioploca" (Otte et al. 1999) and "Maribeggiatoa" (McHatton et al. 1996). *Right*, diagram showing how chemotactic responses and the concentration of an internal trigger may control vertical shuttling in "Marithioploca" spp. The *shading* of the trichomes reflects the concentrations of the trigger (e.g., nitrate) in the filaments. The *arrows* indicate the chemotactic attraction of and the movement towards sulfide and nitrate, respectively. The *minus* signs indicate phobic responses to high concentrations of oxygen or sulfide (Hüttel et al. 1996) (Diagram by Markus Hüttel, Florida State University)**

freshwater filamentous bacterium *Beggiatoa alba* B15LD and B18LD (Strohl and Larkin 1978a; Strohl et al. 1981, 1982) and its close relative OH-75-2a (Nelson and Castenholz 1981a, b), it should also include a wide variety of freshwater and marine strains with small filament diameters and a metabolic spectrum that reaches from aerobic heterotrophy coupled with auxiliary sulfur metabolism (sulfur respiration under anoxia) in freshwater strains to microaerophilic, sulfur-based autotrophy in marine strains (Nelson et al. 1982; Nelson and Jannasch 1983). *Beggiatoa* strain 35Flor is the only marine strain currently available in culture; it is capable of autotrophic growth by aerobic sulfide oxidation, and it can survive anoxic episodes by changing to anaerobic sulfur respiration (Schwedt et al. 2012).

Beggiatoa alba

Beggiatoa alba requires organic carbon substrates for aerobic, heterotrophic growth and resembles in this regard numerous freshwater strains (Faust and Wolfe 1961; Burton et al. 1966; Pringsheim 1964; Kowallik and Pringsheim 1966; Scotten and Stokes 1962; Strohl and Larkin 1978a, b; Nelson and Castenholz 1981a, b). Most *Beggiatoa* strains examined can grow with acetate as a sole source of carbon and energy. All strains of the type species *Beggiatoa alba* (B18LD, B15LD, B25RD) grow well in the presence of sulfide and additions of 0.001–0.05 % acetate (Mezzino et al. 1984), very similar to previous results on other freshwater *Beggiatoa* strains that, when grown with H_2S as energy source, required acetate additions in the range of 0.01–0.0001 % (w/v) (Kowallik and Pringsheim 1966). *Beggiatoa alba* B18LD can use acetate as an energy source and oxidize both acetate C atoms to CO_2; it can also incorporate ^{14}C-labeled acetate into a wide range of cellular and storage compounds, e.g., poly-β-hydroxyalkanoates (*Fig. 6.2c*). Also, acetate significantly increases the capability of this strain to assimilate CO_2, probably through anaplerotic reactions of the tricarboxylic acid (TCA) cycle (Strohl et al. 1981a). Recently, it was shown that *Beggiatoa alba* B18LD and its close relative OH-75-2a can grow on methanol as the sole carbon substrate, when cultured in sulfide gradient tubes (Jewell et al. 2008); the strains are therefore facultative methylotrophs. Other methylated substrates or methane did not support growth (Jewell et al. 2008).

A functional TCA cycle with a glyoxylate bypass has been demonstrated in detailed enzymological studies of *Beggiatoa* strain OH-75-2a (Nelson and Castenholz 1981b), a close relative of *Beggiatoa alba* B18DL and most likely a strain of the same species (Mezzino et al. 1984; Ahmad et al. 2006), and in the chemoheterotrophic *Beggiatoa* strain D-405 (Grabovich et al. 1993). *Beggiatoa* strain OH-75-2a can grow on acetate, ethanol, lactate, pyruvate with a small addition of yeast extract, and TCA cycle intermediates in combination with acetate.

The heterotrophic *Beggiatoa* strain OH-75-2a was quantitatively studied for autotrophic and mixotrophic growth by sulfide and thiosulfate oxidation (Nelson and Castenholz 1981a) to test whether these inorganic electron donors reduce the need for carbon oxidation, lead to increased carbon assimilation and biomass yield, and increase the ecophysiological flexibility of *Beggiatoa* in nature (Pringsheim 1967; Strohl and Schmidt 1984). Sulfide oxidation does not result in additional biomass yield for *Beggiatoa* strain OH-75-2a beyond that obtained from the oxidation of organic carbon sources; mixotrophic growth enhancement by sulfide oxidation was not found (Nelson and Castenholz 1981a). Other attempts to demonstrate mixotrophy for this strain showed experimental shortcomings and need to be revisited (Güde et al. 1981; Nelson and Jannasch 1983). Sulfur globules serve as an electron acceptor reserve that allows a rudimentary anaerobic respiration with sulfur. In *Beggiatoa* strain OH-75-2a, sulfur globules that had accumulated during aerobic thiosulfate oxidation subsequently sustained anaerobic metabolism and growth during several days of anoxia (Nelson and Castenholz 1981a). Reduction of sulfur globules to sulfide, coupled to de novo synthesis of cell material, was also found in *Beggiatoa alba* B18LD during anoxic incubation (Schmidt et al. 1987). This mechanism helps the filaments to survive periods of anoxia in their natural interface habitat (Schmidt et al. 1987).

These results for strain OH-75-2a indicate that *Beggiatoa alba* B18LD does not use sulfur as a chemolithoautotrophic or mixotrophic source of energy. There is circumstantial evidence that acetate and sulfide oxidation compete for oxygen; the addition of acetate and other carbon sources inhibited sulfide oxidation and accumulation of intracellular sulfur globules in *Beggiatoa alba* B18LD considerably (Schmidt et al. 1987). The obligately aerobic oxidation of reduced sulfur compounds in *Beggiatoa alba* B18LD, consistent with the presence of c-type cytochromes (Cannon et al. 1979), stops essentially at the stage of the elemental sulfur globules. Under a wide range of test conditions, *Beggiatoa alba* B18LD filaments harboring sulfur globules did not release significant amounts of soluble sulfur oxidation products into the surrounding medium (Schmidt et al. 1987). During anoxic incubation, *Beggiatoa alba* B18LD reduces sulfur globules to sulfide, coupled to de novo synthesis of cell material (Schmidt et al. 1987). Thus, a major physiological role for sulfide oxidation in *Beggiatoa alba* is the formation of internal sulfur globules as an alternate electron acceptor reservoir.

Beggiatoa alba strains can use nitrate, nitrite, ammonia, and casamino acids as sole nitrogen source (Mezzino et al. 1984), and the list also includes urea, aspartate, asparagine, alanine, and thiourea that tested positive for strain B18LD (Vargas and Strohl 1985a). *Beggiatoa alba* B18LD assimilates ammonia by the glutamine synthetase–glutamate synthase pathway (Vargas and Strohl 1985a). Nitrate cannot be used as electron acceptor for growth with sulfide oxidation; it allows a limited degree of acetate oxidation, but does not sustain growth as the sole electron acceptor. The enzyme activity is associated with the soluble fraction, not with the cell membranes, and generates ammonia as the waste product. Based on its cellular localization and biochemical properties, the nitrate reductase of *Beggiatoa alba* appears to be an assimilatory nitrate reductase (Vargas and Strohl 1985b). In contrast to *Beggiatoa alba*, other freshwater

Beggiatoa strains can use nitrate as terminal electron acceptor (Sweerts et al. 1990; Kamp et al. 2006).

Beggiatoa alba tests positive for nitrogen fixation and thus contributes to total nitrogen fixation in its natural habitats. Nitrogenase activity in *Beggiatoa alba* is strongly regulated by nitrogen bioavailability: nitrate and nitrite additions to the growth medium prevent induction of nitrogenase; in vivo nitrogenase activity is inhibited by ammonia and urea (Polman and Larkin 1988). Similar nitrogenase repression was found in the heterotrophic freshwater strain OH-75-2a and several other strains isolated from a warm freshwater spring (Nelson and Castenholz 1981a, b). Tightly regulated nitrogenase activity is also shared with marine autotrophic strains MS-81-6 and MS-81-1c (Nelson et al. 1982).

Beggiatoa sp. MS-81-6 and MS-81-1c

These two marine strains, although phylogenetically distinct from *Beggiatoa alba*, remain the best-studied examples for autotrophic carbon fixation and chemolithotrophic sulfur oxidation among the *Beggiatoaceae* (*Fig. 6.2b*). Carbon assimilation processes and pathways of *Beggiatoa* came under investigation shortly after Winogradsky began to develop the concept of microbial chemolithoautotrophy based on his initial investigations with this organism (Winogradsky 1887). Almost a century later, the first clearly autotrophic *Beggiatoa* strains MS-81-6 and MS-81-1c were isolated in pure culture (Nelson et al. 1982; Nelson and Jannasch 1983). In autotrophic *Beggiatoa* strains, carbon fixation occurs via the Calvin cycle, as judged by the activity level and regulation of RuBPC/O (Ribulose-1,5-bisphosphate carboxylase/oxygenase). In the obligately autotrophic strain MS-81-1c, RuBPC/O cannot be repressed by acetate additions and is always active at similar levels. The facultatively autotrophic strain MS-81-6 tightly regulates autotrophic vs. heterotrophic growth and is almost certainly mixotrophic with regard to both carbon and energy metabolism (Hagen and Nelson 1996). Acetate additions reduce the activity of RuBPC/O to a small fraction of its activity in organic-free medium; increase the activity of 2-oxoglutarate dehydrogenase (Hagen and Nelson 1996), a key enzyme of the citric acid cycle; and open the way to respiratory oxidation of C_2 substrates. Under sulfide-induced autotrophic growth conditions, 2-oxoglutarate dehydrogenase is not expressed thus "interrupting" the citric acid cycle at the stage of 2-oxoketoglutarate. As a result, autotrophically fixed carbon is not oxidized, but used for synthesis of cellular compounds.

Even the obligately autotrophic *Beggiatoa* strain MS-81-1c increases its growth yield by ca. 20 % after addition of acetate, indicating that acetate can be used as an auxiliary carbon source for the synthesis of cell material in a manner analogous to other chemolithoautotrophic sulfide-oxidizing bacteria (Hagen and Nelson 1996).

Autotrophic growth of *Beggiatoa* strain MS-81-6 was achieved in sulfide gradient cultures, where *Beggiatoa* filaments grew as a defined band in a slush soft agar column at the sulfide–oxygen interface (Nelson and Jannasch 1983). The *Beggiatoa* filaments in the gradient culture migrated over time and kept themselves positioned at the sulfide–oxygen interface. Their growth depends on the availability of both compounds, oxygen and sulfide, in opposed overlapping gradients (*Fig. 6.6*, Nelson and Jannasch 1983; Nelson et al. 1986b). Depending on growth stage, *Beggiatoa* strain MS-81-6 adjusts the oxidation pathways of sulfide. When sulfide is abundant, it is oxidized to the stage of elemental sulfur; when the supply of sulfide is limited and has to be used more effectively, sulfide oxidation proceeds to sulfate (Nelson et al. 1986b). Sulfide is biologically oxidized at a rate that is roughly three orders of magnitude faster than the competing chemical oxidation, with half-life times of a few seconds in the oxygen–sulfide transition zone (Nelson et al. 1986b). In comparison to other autotrophic sulfide-oxidizing bacteria, both marine autotrophic *Beggiatoa* strains tested have high molar growth yields (8 g/mol for *Beggiatoa* str. MS-81-6 and 16 g/mol for MS-81-1c) on sulfide in gradient cultures (Nelson et al. 1986b; Hagen and Nelson 1997).

Physiological differences between the obligately autotrophic strain MS-81-1c and the facultatively autotrophic strain MS-81-6 are apparent in different enzyme systems for sulfur oxidation (Hagen and Nelson 1997). *Beggiatoa* strain MS-81-1c uses APS reductase (adenosine 5'-phosphosulfate reductase, located in the cytosol) in the AMP-dependent oxidation of sulfite to APS. In a second step catalyzed by the enzyme ATP sulfurylase, the pyrophosphate-dependent substrate-level phosphorylation of APS produces ATP and sulfate:

1. $SO_3^{2-} + AMP + acceptor_{oxidized} \rightarrow APS + acceptor_{reduced}$
2. $APS + PP_i \rightarrow SO_4^{2-} + ATP$

Both enzymes are highly active regardless of the sulfur source (H_2S gradient, thiosulfate, or thiosulfate with acetate). Substrate-level phosphorylation during sulfur oxidation opens a new source of energy for this *Beggiatoa* strain, in contrast to other *Beggiatoa* strains that appear to lack this pathway and depend on respiratory sulfur oxidation instead. *Beggiatoa* strain MS-81-6 completely lacks APS reductase activity. The activity of the ATP sulfurylase is two orders of magnitudes lower than in strain MS-81-1c and in the typical range for assimilatory ATP sulfurylases. An assimilatory role for the ATP sulfurylase is supported by the ability of strain MS-81-6 to grow with acetate on sulfate as the only sulfur source (Nelson and Jannasch 1983).

An AMP-independent, apparently membrane-associated, sulfite:acceptor oxidoreductase systems represent a second sulfur oxidation pathway, which is found in *Beggiatoa* strains MS-81-1c, MS-81-6, and also in the heterotrophic strain OH-75-2a. Since these sulfite oxidases are localized in the cell membrane, they are most likely integrated with the respiratory chain and use cytochrome c as electron acceptor. In strain MS-81-1c, sulfite: acceptor oxidoreductase is upregulated in the presence of H_2S and is at least 3 times higher than in strain MS-81-6. The different rates of respiratory sulfur oxidation and additional substrate phosphorylation coupled to sulfite oxidation by the APS reductase system in strain MS-81-1c probably contribute to

the differences in molar growth yield between strains MS-81-1c and MS-81-6 (Hagen and Nelson 1997).

DNA hybridizations were used to check the presence of dissimilatory ATP sulfurylase genes in different *Beggiatoa* strains. The gene probe was a fragment of the ATP sulfurylase gene of the autotrophic, sulfur-oxidizing endosymbiont of the hydrothermal vent tube worm *Riftia pachyptila*; the endosymbiont utilizes ATP sulfurylase and APS reductase in dissimilatory sulfur metabolism. DNA of the autotrophic strain MS-81-1c hybridized positively, whereas DNA of the facultatively heterotrophic strain MS-81-6 and of the heterotrophic strain OH-75-2a hybridized negatively, indicating that the latter two strains harbor assimilatory, not dissimilatory ATP sulfurylases (Laue and Nelson 1994).

Strain 35Flor

The marine *Beggiatoa* strain 35Flor was isolated in 2001 from a microbial community associated with scleractinian corals suffering from black band disease off the coast of Florida. This *Beggiatoa* strain grows under chemolithoautotrophic conditions in an agar-stabilized oxygen–sulfide gradient medium gaining energy from the aerobic oxidation of sulfide (Kamp et al. 2008; Brock and Schulz-Vogt 2011), and it grows in obligate coculture with a *Pseudovibrio* strain (Brock and Schulz-Vogt 2011; Schwedt et al. 2012). Currently, strain 35Flor is the only marine, autotrophic strain within the *Beggiatoaceae* that is continuously maintained in monospecific culture. Strain 35Flor is a member of the same monophyletic lineage as strain MS-81-6 and can be considered its closest cultured relative. This strain was the first marine isolate capable of sulfur respiration with concurrent PHA dissolution under anoxic conditions (Schwedt et al. 2012). Most likely, the stored carbohydrates are oxidized and excreted, while S globules are reduced to sulfide. In this way, strain 35Flor removes excess sulfur globules that accumulate intracellularly during microoxic sulfide oxidation (Schwedt et al. 2012).

Comparative Aspects

Interestingly, the heterotrophic freshwater *Beggiatoa* strain OH-75-2a showed a similar spectrum of sulfide- and sulfur-oxidizing enzymes as the facultative autotrophs. AMP-independent sulfite:acceptor oxidoreductase was present and active in a similar range as in strain MS-81-6, allowing in principle the energy-gaining oxidation of sulfur compounds. However, the activities of ATP sulfurylase were an order of magnitude lower than in strain MS-81-6 and three orders of magnitude lower than the ATP sulfurylase in strain MS-81-1c; thus, the ATP sulfurylase appears to be assimilatory rather than dissimilatory (Hagen and Nelson 1997).

The sulfur-oxidizing enzyme system of the facultatively autotrophic freshwater *Beggiatoa* strain D-402 shared important features with strains MS-81-6 and OH-75-2a. AMP-dependent APS reductase was absent; sulfur-oxidizing enzymes that are not involved in substrate-level phosphorylation (sulfite:cytochrome C oxidoreductase and thiosulfate:ferricyanide oxidoreductase) were active and were upregulated under autotrophic cultivation conditions with thiosulfate as sulfur source (Grabovich et al. 1998, 2001; Patritskaya et al. 2001). The unusually high activities of RubisCo and of sulfur-oxidizing enzymes in strain D-402 could be connected to its high growth yield (12.2 g/mol oxidized thiosulfate). Direct comparisons of *Beggiatoa* strains with identical culture conditions, sulfur sources, and enzyme assays are necessary to determine the physiological diversity and activity of sulfur-oxidizing enzymes in marine and freshwater *Beggiatoa*.

Photoresponses of *Beggiatoa* spp.

The migrations of *Beggiatoa* filaments in microbial mats and sediments are not only regulated by the combined effects of oxygen and sulfide, but are in parallel controlled by light. Experiments with *Beggiatoa* cultures from a warm freshwater spring (Hunter Spring) have shown a statistically significant photophobic response of individual *Beggiatoa* filaments that was still detectable at low ambient light levels as low as ca. 2 % of full summer sunlight intensity. Filaments were most sensitive in the blue to blue-green (400–500 nm) spectrum that in nature is blocked out by cyanobacterial carotenoids. *Beggiatoa* filaments in field material maintained in the laboratory enrichments retreated into the sediment after short periods of illumination and returned to the sediment surface after several hours of darkness (Nelson and Castenholz 1982). Such distinct responses to blue light invite closer examination. In the genome of an undescribed *Beggiatoa* sp., a DNA sequence encodes an adenylyl cyclase directly linked to a BLUF (blue light receptor using FAD) type light sensor domain, which led to the working hypothesis of a light-triggered cyclic AMP signaling mechanism (Stierl et al. 2011).

Genus *Thioploca*

The genus *Thioploca* is widespread in freshwater and brackish-water habitats (*Fig. 6.3g*), for example, in sediments of Lake Constance and other lakes in Germany (Lauterborn 1907; Koppe 1924; Maier and Preissner 1979), in the Neva river at St. Petersburg (Wislouch 1912), in the Rhine and in Baltic coastal lagoons (Kolkwitz 1912), in Lake Erie (Maier 1980), in Lake Ontario (Dermott and Legner 2002), in Lake Baikal in Siberia (Namsaraev et al. 1994; Zemskaya et al. 2001), and in Lake Biwa, Japan (Nishino et al. 1998; Kojima et al. 2003). Studies on field samples of freshwater and brackish-water *Thioploca* spp. (*T. ingrica*) from Japan (Kojima et al. 2007) and Denmark (Høgslund et al. 2010) have provided an initial outline of their physiology. Autoradiography experiments have shown that *T. ingrica* assimilates acetate and bicarbonate, although bicarbonate incorporation could not be detected in the specimens from Japan (Høgslund et al. 2010; Kojima et al. 2006). It is

assumed that *T. ingrica* links inorganic carbon assimilation to sulfur oxidation; however, only indirect support for sulfur oxidation is available. A correlation of elevated sulfate concentrations and *T. ingrica* biomass was shown (Kojima et al. 2007), but enzymatic or genetic studies of carbon assimilation are currently lacking. Oxygen and nitrate are required for carbon assimilation (Høgslund et al. 2010). Nitrate is accumulated intracellularly at concentrations of 2–3 mM; given environmental nitrate concentrations of ca. 10–20 µM (Lake Biwa) to ca. 100 µM (Danish fjords), *T. ingrica* concentrates nitrate by one to two orders of magnitude (Høgslund et al. 2010; Kojima et al. 2007). The large, nitrate-accumulating vacuoles that are typical of many large members of the *Beggiatoaceae* (e.g., ❯ *Fig. 6.4d*) are not found in *T. ingrica*; therefore, nitrate must be accumulated and stored in some other way, such as within smaller cytoplasmatic vacuoles, in the cytoplasm itself, or in the periplasm that can feature cytoplasmic membrane invaginations extending into the cytoplasm (Maier and Murray 1965). However, elemental sulfur, and not nitrate, is the electron acceptor of choice during prolonged anoxia. Anoxic incubation experiments in closed bottles have shown that storage globules of elemental sulfur serve as the electron acceptor during prolonged oxygen and nitrate depletion; *T. ingrica* can survive as a sulfur-reducing heterotroph for over 2–3 months (Høgslund et al. 2010). Therefore, elemental sulfur has the same role in *T. ingrica* as in *Beggiatoa alba* and other thin marine and freshwater *Beggiatoa* that rely on their intracellular sulfur reservoir under anaerobic conditions.

Genus *Thiomargarita*

Cells of the genus *Thiomargarita* show spherical, barrel-like, or bulbous shapes and either are unicellular (❯ *Fig. 6.4b*), are organized in chains surrounded by a mucous sheath (e.g., ❯ *Fig. 6.4a* and *d*), form clusters or aggregates (❯ *Fig. 6.4e*), or grow attached to surfaces (❯ *Fig. 6.4f*, Schulz et al. 1999; Kalanetra et al. 2005; Schulz 2006; Salman et al. 2011). They are among the contenders for the largest prokaryotic cells on earth; the initial discovery of *Thiomargarita* in organic-rich and sulfidic sediments on the continental shelf of Namibia reported individual cells of up to 750 µm in diameter, although sizes of 100–300 µm were more common (Schulz et al. 1999). Their nitrate-storing vacuoles fill the cell volume and restrict the cytoplasm to a thin layer sandwiched between cell membrane and vacuolar membrane; the cytoplasm presumably also contains the intracellular sulfur globules (Schulz 2006). Although some rolling motility is now reported for some morphotypes in this genus, *Thiomargarita* cannot move over long distances vertically within the surficial sediments, such as *Marithioploca*, to efficiently bridge spatially separated pools of oxidant and reductant. Instead, *Thiomargarita* relies on its extreme storage capacity for sulfur and nitrate, an essential capability in benthic habitats that experience long periods of sulfidic conditions and oxygen or nitrate depletion (Schulz et al. 1999). *Thiomargarita* is currently the only sulfur bacterium that combines four types of intracellular inclusions that ensure prolonged survival under energy limitation: nitrate-storing vacuoles and sulfur inclusions provide intracellular reserves of electron acceptor and donor, polyphosphate inclusions serve as an additional energy buffer, and glycogen (or another polyglucose) represents a carbon reserve (Schulz and Schulz 2005).

Based on analogies with previously characterized "Marithioploca" and "Maribeggiatoa" spp., *Thiomargarita* is most likely a facultative chemolithoautotrophic sulfur oxidizer. *Thiomargarita namibiensis* cells can take up and utilize acetate as a carbon source, although not as an electron donor (Schulz and de Beer 2002), and can store this organic carbon intracellularly as glycogen (Schulz and Schulz 2005). Sulfide oxidation and sulfide flux towards *Thiomargarita* cells are stimulated by the presence of oxygen; even highly oxygenated conditions (ca. 200 µM O_2 in solution) were tolerated, showing that *Thiomargarita* is not a microaerophile such as "Marithioploca" and "Maribeggiatoa" (Schulz and de Beer 2002). Oxygen additions to anoxic medium increased the sulfide flux into *Thiomargarita* cell chains by ca. 1/3 (from approximately 5–7.5 pmol sulfide per cm^2 s), indicating that while oxygen stimulated the sulfide flux, most of it was still sustained by intracellularly stored nitrate. This flexibility in usage of electron acceptor and tolerance to different electron donor and acceptor concentrations may explain the long survival of *Thiomargarita* cells in natural sediment samples in the lab.

The large cell size of *Thiomargarita*, and the spatial extent of its cytoplasm and membranes, requires a mechanism to overcome diffusional limitations to intracellular transport, signaling pathways, and coordinated gene expression across the cell volume. Assuming the absence of directional transportation via bacterial tubulins or other analogs to mitotic spindles, how does messenger RNA reach the portions of the cytoplasm that are separated by hundreds of micrometers from the location of the genome? Nucleic acid-stained condensed genomic equivalents or nucleoids are distributed throughout the cytoplasm (Schulz 2006) and suggest a polyploid genome (Angert 2012) similar to the large bacterial endosymbiont *Epulopiscium fishelsoni* (Robinow and Angert 1998; Mendell et al. 2008; Angert 2012).

"*Candidatus* Marithioploca"

Members of "*Candidatus* Marithioploca" are large, vacuolated, sulfide-oxidizing, and nitrate-reducing filamentous bacteria (❯ *Fig. 6.3 a–f*) capable of assimilating inorganic carbon as well as organic substrates for biomass production (Jørgensen and Gallardo 1999). Multiple filaments are usually surrounded by a sheath that is embedded in surficial sediment. The unique combination of high storage capacity for electron donor (sulfur) and acceptor (nitrate) and vertical migration capability between bottom seawater and sediment enables "Marithioploca" to thrive at sediment–water interfaces where electron donor and acceptor undergo major fluctuations in concentration, availability, and spatial separation (❯ *Fig. 6.7*).

All physiological studies on "Marithioploca" have been performed on freshly collected, sheathed filament bundles from the continental shelf of Chile (Maier and Gallardo 1984a, b; Fossing et al. 1995; Otte et al. 1999; Høgslund et al. 2009). The two dominant members of "Marithioploca" from the Chilean continental shelf, previously named *Thioploca chileae* and *T. araucae* and distinguished by filament diameter (12–20 µm and 30–43 µm) (Maier and Gallardo 1984b), were both able to incorporate radiolabeled bicarbonate as well as acetate and amino acids (Maier and Gallardo 1984a). In freshly collected "Marithioploca" samples, CO_2 fixation occurred at rates of 0.4–1.5 nmol carbon per minute and mg protein, similar to those of large hydrothermal vent *Beggiatoaceae* (Otte et al. 1999). Acetate was incorporated at a rate roughly equal to CO_2, with no apparent terminal oxidation, suggesting that "Marithioploca" spp. are mixotrophic with respect to carbon source (Otte et al. 1999). Mixotrophic potential was also supported by quantitative microautoradiography experiments that showed increased inorganic carbon fixation after the addition of acetate and propionate (Høgslund et al. 2009).

Oxidation of reduced sulfur compounds is linked to nitrate reduction. Incubation experiments of freshly collected "Marithioploca" with ^{15}N-labeled nitrate have shown that nitrate is taken up and subsequently reduced to ammonia at rates of 5–6.5 and 1–4 nmol nitrate uptake and reduction per minute and mg protein, respectively (Høgslund et al. 2009). "Marithioploca" filaments respond with positive chemotaxis to nitrate additions (20–30 µm) and emerge, while remaining anchored in their sediment-embedded sheaths, up to several centimeters into nitrate-amended, flowing seawater (Hüttel et al. 1996). Subsequently, the filaments can retreat (at a speed of 3–5 mm h^{-1}) into the sediment and use their stored nitrate for sulfide oxidation (Hüttel et al. 1996). Nitrate can be stored within large vacuoles at concentrations between 10 and 500 mM (Otte et al. 1999; Fossing et al. 1995; Zopfi et al. 2001); however, nitrate can be reduced to ammonia directly without prior storage and dilution of the ^{15}N-signal in the vacuole (Otte et al. 1999). Small amounts of dinitrogen are also produced (Otte et al. 1999), but these denitrification activities result from bacterial epibionts and contaminants on the "Marithioploca" sheaths (Høgslund et al. 2009). Due to nitrate ammonification, ammonia accumulates in "Marithioploca"-harboring sediment surface layers (Thamdrup and Canfield 1996; Hüttel et al. 1996) and can be microbially reassimilated; such a trend would counteract nitrogen loss through denitrification (Farias et al. 1996; Farias 1998). On the other hand, ammonia can also be reoxidized in situ by anammox bacteria (*Candidatus* Scalindua) that grow on "Marithioploca" sheaths; this microbial consortium then amplifies denitrification and enhances nitrogen efflux towards parity of ammonia and N_2 loss from the sediment (Prokopenko et al. 2006, 2013).

Oxygen can be consumed at rates comparable to nitrate, but aerobic respiration during sulfide oxidation can be sustained only at low external oxygen concentrations (ca. 30 µM, 10 % oxygen saturation) that leave the filaments within the "Marithioploca" bundles anoxic. Higher oxygen concentrations (ca. 100 µM, 30 % oxygen saturation) penetrated into the bundles and killed the filaments within 8 h of oxygen exposure (Høgslund et al. 2009). In closed-flume system experiments, dissolved oxygen concentrations of 100–150 µM in seawater triggered a retreat of seawater-exposed filaments into the sediment (Hüttel et al. 1996). Thus, "Marithioploca" can oxidize sulfur as a microaerophile, but does not survive at higher oxygen concentrations, and avoids them by retreating into the sediment.

Sulfide oxidation and ammonia production rates in "Marithioploca" samples showed a stoichiometric ratio of ca. 2, indicating that nitrate reduction to ammonia is accompanied by concomitant sulfide oxidation to elemental sulfur (stoichiometric ratio 1:4) and sulfate (ratio 1:1); no sulfur intermediates were detected. Apparently, sulfide is oxidized first to elemental sulfur, which acts as an intracellular electron donor reservoir, and then in a second step to sulfate. In the absence of sulfide, the ratio of ammonia production from nitrate (requiring 8 electrons per ammonia) and elemental sulfur oxidation to sulfate (yielding 6 electrons per sulfate) is close to the predicted stoichiometric ratio of 4/3 (Otte et al. 1999). Elemental sulfur is an essential electron donor that can be accumulated and stored until needed. The high intracellular concentrations of sulfur and nitrate are correlated to the position and activity of "Marithioploca" filaments in the gradient. Deeply located "Marithioploca" filaments tend to consume their nitrate content for sulfide oxidation and build up sulfur globules and replenish their nitrate stocks at the surface where sulfur is consumed (Zopfi et al. 2001). Given the fluctuations in sulfide supply, intracellular sulfur storage provides an essential electron donor reservoir. "Marithioploca" is capable of faster sulfide uptake than sulfide oxidation (0.5–15 compared to 2–3 nmol per minute and mg protein), indicating that sulfide can be just as limiting as nitrate and has to be taken up rapidly when available (Otte et al. 1999; Høgslund et al. 2009).

"*Candidatus* Maribeggiatoa"

Members of the genus-level candidate taxon "Maribeggiatoa" are large, vacuolated, nitrate-accumulating sulfide-oxidizing filamentous bacteria (*Fig. 6.5 a, b*) with autotrophic capabilities; they resemble "*Candidatus* Marithioploca" spp. in vacuolation and nitrate storage capacity (McHatton et al. 1996). Uncultured "Maribeggiatoa" from cold sulfide seeps in the Monterey Canyon (McHatton et al. 1996) and "Maribeggiatoa"-related filaments from the Guaymas Basin hydrothermal sediments (Nelson et al. 1989) showed high RuBisCO activity in the range of 7.5–15 and 5–6 nmol C fixed per minute and mg protein, respectively. Carbon assimilation by the Calvin cycle was consistent with the ^{13}C-isotopic signature of large "*Maribeggiatoa*"-like filaments from cold seep sediments in the Gulf of Mexico (Larkin et al. 1994).

Filaments of "*Candidatus* Maribeggiatoa" accumulate nitrate intracellularly in vacuoles and—as suggested by analogy

to "*Candidatus* Marithioploca"—use nitrate as a respiratory electron acceptor for sulfur oxidation. The "Maribeggiatoa" population at Monterey Canyon showed an intracellular nitrate concentration of ca. 160 mM (McHatton et al. 1996); the Guaymas Basin population of "Maribeggiatoa"-like mat-forming filaments accumulated intracellular nitrate in the range of 50–100 mM (McKay et al. 2012). These concentrations would not be possible with dissolved oxygen; its saturation concentration in fully aerated seawater at 1 atm pressure and 7 °C is limited to ca. 300 μM. In comparison to other Beggiatoaceae, the "Maribeggiatoa" population at Monterey Canyon showed the highest level of nitrate reductase activity. Nitrate reductase activity was predominantly found in the particulate fraction, indicating a membrane-bound location within the respiratory chain (McHatton et al. 1996). The Monterey Canyon "Maribeggiatoa" also consume oxygen at a rate considerably greater than the average rate of nitrate consumption (8–25 nmol O_2 compared ca. 1–4 nmol NO_3^- per minute and mg protein) (Kalanetra and Nelson 2010).

Nitrate transformations in mats of "Maribeggiatoa" and related *Beggiatoaceae* provide new avenues for biochemical research. The nearly complete genome sequence of a single orange filament (related to "*Candidatus* Maribeggiatoa," pending more detailed classification) from a hydrothermal microbial mat in Guaymas Basin (Gulf of California, Mexico) harbored the gene encoding an abundant soluble orange-pigmented protein in Guaymas Basin mat samples (MacGregor et al. 2013a). The predicted protein sequence grouped with octaheme cytochromes whose few characterized representatives are hydroxylamine or hydrazine oxidases. The protein was partially purified and shown by in vitro assays to have hydroxylamine oxidase, hydrazine oxidase, and nitrite reductase activities. In the context of *Beggiatoaceae* physiology, nitrite reduction was inferred as the most likely in vivo role of the octaheme protein (MacGregor et al. 2013a). The surficial sediments associated with the *Beggiatoaceae* mats in Guaymas Basin showed high denitrification activities that were inhibited by sulfide accumulation; the working hypothesis can be inferred that sulfide removal by *Beggiatoaceae* may catalyze denitrification in the Guaymas Basin sediments (Bowles et al. 2012).

Detailed studies of sulfide and sulfur oxidation pathways and activities are currently lacking for "Maribeggiatoa." However, their conspicuous content of sulfur globules, as in "Marithioploca," suggests that sulfide serves as energy source and elemental sulfur as a storage compound and electron donor when sulfide is not available. In "Maribeggiatoa"-related large, orange-colored *Beggiatoaceae* from the Guaymas Basin (30 μm filament diameter, sample 1615), diverse c-type cytochromes were found, whose hemes have appropriate oxidation–reduction midpoint potentials for respiratory sulfide oxidation (Prince et al. 1988). The near-complete genome of an orange-colored filament in this size class from Guaymas Basin showed a wide repertoire of sulfur oxidation and assimilation pathways (MacGregor et al. 2013c).

"*Candidatus* Marithrix"

The physiology of "*Candidatus* Marithrix" presents a conundrum: the large filaments contain sulfur globules and large vacuoles, but so far nitrate could not be detected in the filaments (Kalanetra et al. 2004; Kalanetra and Nelson 2010). Given that "Marithrix" grows on surfaces that are alternately exposed to mixed sulfidic vent fluids and oxygenated seawater, oxygen is the most likely electron acceptor (Kalanetra et al. 2004). Since the vacuoles are not used for nitrate storage, they could serve either as oxygen reservoirs that provide storage capacity for a few minutes of oxic respiration (Kalanetra and Nelson 2010) or as structural element contributing to filament strength. "Marithrix" has autotrophic capability (average 2.5 nmol CO_2 fixed per min and mg protein), similar to "Maribeggiatoa" and "Marithioploca" (Kalanetra and Nelson 2010).

"*Candidatus* Isobeggiatoa"

Filaments of the genus-level candidate taxon "Isobeggiatoa" are vacuolated and accumulate nitrate as well as sulfur intracellularly (Mussmann et al. 2003; Jørgensen et al. 2010). Instead of forming conspicuous microbial mats on the sediment surface, "*Candidatus* Isobeggiatoa" strains occur within surficial sediment layers where oxygen and sulfide are excluded or occur only at low concentrations. Although these sediment layers often show high sulfate reduction rates, porewater sulfide is rapidly consumed by sulfide-oxidizing bacteria or by reoxidation with metals and does not build up. In this habitat, "Isobeggiatoa" filaments do not dominate in terms of sulfide-oxidizing activity or cell number, although they constitute a larger proportion of sedimentary biomass due to large cell size (Jørgensen et al. 2010). A partial genome for an "Isobeggiatoa" filament matches the sulfide-oxidizing, nitrate-reducing physiology that can be inferred from the habitat characteristics (Mussmann et al. 2007). The partial genome contains a sulfide quinone oxidoreductase and flavocytochrome c-sulfide dehydrogenase for sulfide oxidation, the reverse dissimilatory sulfate reductase pathway for sulfur oxidation, and a partial sox pathway for thiosulfate oxidation (Mussmann et al. 2007). Sulfur respiration is also supported by the presence of genes for the respiration of dimethyl sulfoxide and the reduction of thiosulfate (Mussmann et al. 2007). The genome has a partial nitrate reduction pathway, but the preferred end product of nitrate reduction (N_2 or NH_3) cannot be inferred. Two cytochrome c oxidases that most likely differ by oxygen affinity indicate the capability for aerobic respiration (Mussmann et al. 2007). The "Isobeggiatoa" genome combines a nearly complete tricarbonic acid cycle with some key genes of the Calvin cycle, including form I RubisCO. "*Candidatus* Isobeggiatoa" has genes for glycolate oxidation, for the synthesis of the storage compound poly-beta-hydroxybutyric acid, for glycogen synthesis, and for ATP synthesis through substrate-level phosphorylation by fermenting

pyruvate to lactate (Mussmann et al. 2007). The genome indicates a major role for phosphate uptake and storage; it encodes a phytase for accessing inorganic phosphates, selective porins and ABC phosphate transporters for phosphate uptake, and a polyphosphate kinase for intracellular polyphosphate synthesis (Mussmann et al. 2007). Non-ribosomal peptide synthetases and polyketide synthetases indicate the potential for secondary metabolite synthesis. Numerous glycoproteins in "*Candidatus* Isobeggiatoa" are most likely involved in cell adhesion and aggregation. These two gene categories have often cyanobacterial affinities, suggesting horizontal gene transfer between *Cyanobacteria* and *Beggiatoaceae* in shared sedimentary and microbial mat habitats during long periods of coexistence in the earth's past (Mussmann et al. 2007).

"*Candidatus* Parabeggiatoa"

Members of "*Candidatus* Parabeggiatoa" were originally found in surficial sediments of a brackish fjord, Limfjorden, in Denmark, and formed a monophyletic cluster of large (33–40 µm filament diameter), vacuolated, presumably nitrate-accumulating filaments (Mussmann et al. 2003). "Parabeggiatoa" and "Isobeggiatoa" were identified during a reexamination of previously discovered *Beggiatoa*-like filaments within surficial sediments of Limfjorden (Jørgensen 1977). These filaments did not occur in the narrow zone of overlapping oxygen and sulfide gradients at the sediment surface, but in the surficial sediment interval where oxygen was no longer available but sulfide did not visibly accumulate. In retrospect, this was the first indication of the nitrate-reducing and potentially sulfur-reducing mode of metabolism that sustains these bacteria in their anoxic habitat. The filaments of "Parabeggiatoa" were larger than those of their sister group "Isobeggiatoa" (33–40 µm vs. 9–17 µm filament diameter, respectively) and occurred preferentially deeper in the sediment in close proximity to the sulfidic zone (Mussmann et al. 2003), possibly due to a greater reservoir of intracellular electron acceptors, nitrate, and elemental sulfur. At present, nitrate and sulfur content of the Limfjorden "Parabeggiatoa" are incompletely reported; the smaller "Isobeggiatoa" filaments from Limfjorden contain 156 ± 71 mM nitrate, but the nitrate content of the larger "Parabeggiatoa" filaments is unspecified. The elemental internal sulfur concentration of all measured Limfjorden filaments was 369 ± 176 mM, indicating large sulfur storage capacity in a similar range as nitrate concentrations (Mussmann et al. 2003). A single filament from Eckernförde Bay in Germany (Filament SS) was used for whole genome amplification and subsequent Sanger sequencing, yielding a low-coverage (3x) partial genome assembly of 1,091 contigs with a total sequencing length of 1.3 Mb (Mussmann et al. 2007). The"Parabeggiatoa" partial genome is more incomplete and fragmented than the "Isobeggiatoa" partial genome that was reported in the same study. Both partial genomes contain genes for the reverse dissimilatory sulfate reductase pathway, for putative nitrate reductases, and for genes that channel acetate into general metabolism (acetate/cation symporters, acetate kinase, and acetyl-CoA (coenzyme A) synthase) (Mussmann et al. 2007).

"*Candidatus* Allobeggiatoa"

Thin, sulfur- and nitrate-accumulating vacuolated filaments with diameters of 6–14 µm from hypersaline cyanobacterial mats (*Fig. 6.2e*) constitute the candidate genus "*Candidatus* Allobeggiatoa." So far, "Allobeggiatoa" has been observed and identified by 16S rRNA sequencing and FISH in hypersaline cyanobacterial mats in saline lagoons and salterns in Spain and Mexico (Hinck et al. 2007, 2011). Given the preference of this group for hypersaline conditions, previous observations of *Beggiatoa*-like filaments in the hypersaline cyanobacterial mat of Guerrero Negro, Mexico, most likely include populations of "Allobeggiatoa" (Garcia-Pichel et al. 1994; Jørgensen and DesMarais 1986). Within hypersaline cyanobacterial mats, "Allobeggiatoa" occur predominantly at the upper sulfide horizon where oxygen and sulfide meet during daytime photosynthetic activity; they remain almost stationary at night and do not follow the retreating oxygen gradients towards the mat surface (Hinck et al. 2007). Intracellular sulfur and nitrate concentrations are highly variable. In sulfide-gradient enrichment culture, where they could be sustained for several weeks, "Allobeggiatoa" grow at the sulfide–oxygen interface where they most likely respire with oxygen; under these conditions they accumulate nitrate to high concentrations (430–650 mM) and deplete their intracellular sulfur store (6–25 mM). In their cyanobacterial mat habitat, intracellular nitrate concentrations are depleted (4 mM), and elemental sulfur accumulates (250 mM); thus, "Allobeggiatoa" appears to be nitrate-limited in its natural habitat (Hinck et al. 2011).

"*Candidatus* Halobeggiatoa"

The preliminary phenotypic characterization of this filamentous group is based on a habitat study of "Halobeggiatoa" from white mat patches at the Håkon Mosby mud volcano at 1,260 m depth in the Barents Sea (*Fig. 6.5c*, Lichtschlag et al. 2010; Grünke et al. 2012). These filamentous sulfur oxidizers grow in an intermediate zone of the mud volcano where the sulfide supply is high enough to support growth, but not to exceed the available oxygen and nitrate supply. Sulfide is sequentially oxidized with elemental sulfur as intermediate. The filaments have a diameter of approx. 8–10 µm and accumulate nitrate intracellularly (average 110 mM, range 73–149 mM) in the same range as intracellular S° (average 120 mM, range 45–289 mM) (Lichtschlag et al. 2010). The cells of this population disintegrate at temperatures higher than 8 °C and therefore represent genuine psychrophiles that are adapted to the permanently cold (−0.7 °C) in situ temperature in their habitat (Grünke et al. 2012).

"*Candidatus Thiopilula*"

The candidate genus "*Thiopilula*" was described from free-living specimens in Namibian sediments and from specimens attached to solid substrates in sediments of the Costa Rica margin (Salman et al. 2011). These large, spherical cells (25–67 µm diameter) occur mostly in aggregates within a thick mucus envelope; smaller individual cells (11–24 µm diameter) of the same phylogenetic lineage reside in diatom frustules (Salman et al. 2011). Similar-sized cells in mucus-ensheathed clusters were also documented earlier from Namibian sediment samples (Schulz 2006). The cells contain sulfur inclusions. Most cells are vacuolated and could store nitrate, but intracellular nitrate concentrations have not been tested yet. The attached cells are most likely dividing by budding from mother cells attached to solid substrates (Bailey et al. 2011). Cells within aggregates and diatom frustules show binary division stages and occasional motility by jerking, rolling movement (Salman et al. 2011).

"*Candidatus* Thiophysa"

The candidate genus "*Candidatus* Thiophysa" is physiologically almost entirely uncharacterized. These large, single, spherical cells (56–90 µm diameter) from Namibian sediments contain sulfur inclusions and also vacuoles that could store nitrate, but intracellular nitrate concentrations remain to be tested (Salman et al. 2011). They are motile by slow, rolling, and jerking motions (Salman et al. 2011). The genus was originally described from sandy sediments of shallow marine sulfur springs in the Gulf of Naples, strongly smelling of hydrogen sulfide (Hinze 1903). These specimens lost their sulfur inclusions during 1 or 2 days of incubation in oxygenated seawater, consistent with a sulfur-oxidizing metabolism (Hinze 1903). The Namibian and Neapolitan specimens resemble each other in morphology; the original, beautifully detailed microscopic drawings (Hinze 1903) are close equivalents of the modern microphotographs (Salman et al. 2011). Yet, the cell diameter of the Neapolitan "Thiophysa" is given as 7–18 µm (Hinze 1903), approx. 1/5 to 1/8 of the Namibian specimens. This difference suggests unexplored morphotype and species diversity either within the candidate genus "*Thiophysa*" or other taxa of the family *Beggiatoaceae*.

Cell Structure

Vacuolation

The cells of many large members of the *Beggiatoaceae* appear hollow in microscopic examination; they are composed of a thin cylinder of cytoplasm surrounding a large central vacuole, or several large vacuoles, a characteristic that has been initially observed and described more than a century ago (Hinze 1901, ❯ *Figs. 6.4d* and ❯ *6.8a*, *b*). This extensive vacuolation is characteristic for most large *Beggiatoaceae* (Jannasch et al. 1989; Nelson et al. 1989) above a size threshold of approx. 10 µm (Larkin and Henk 1996). Since vacuolation commonly coincides with high intracellular nitrate concentration (McHatton et al. 1996), the vacuoles are presumed to be the locations where nitrate is accumulated for respiration, either by denitrification or by dissimilatory reduction to ammonia. However, important caveats apply: the small, freshwater species *Thioploca ingrica* lacks the extensive vacuolation of large, marine *Beggiatoaceae* (Maier and Murray 1965), but is still capable of nitrate accumulation (Høgslund et al. 2009). Analyses of "Candidatus Marithrix" demonstrate that large vacuoles cannot be equated with nitrate accumulation (Kalanetra et al. 2004).

The large intracellular vacuoles have been studied in greater physiological detail in "Allobeggiatoa," using a combination of vacuolar pH measurements (Beutler et al. 2009), immunostaining, and selective inhibition of membrane proteins (Beutler et al. 2012). Nitrate addition increased the vacuolar proton motive force and acidified the vacuole; the resulting proton gradient from vacuole interior to cytoplasm can be used for ATP and pyrophosphate generation in the cytoplasm. Nitrate addition also led to the production of nitric oxide (NO) from its precursor nitrite in the vacuole (Beutler et al. 2012); subsequently, NO could be reduced to the denitrification intermediate nitrous oxide (N_2O).

Cell Envelope

Ultrastructural analyses of cell envelope and cell inclusions have focused on freshwater *Beggiatoa alba* (Strohl et al. 1982; Strohl and Larkin 1978b), on *Thioploca ingrica* (Maier and Murray 1965), and on marine filamentous *Beggiatoaceae* (de Albuquerque et al. 2010; Larkin and Henk 1996). Thin sectioning and freeze-etching techniques have shown that filaments of the freshwater species *Beggiatoa alba* (strain B15LD) have a cell envelope consisting of five distinct layers that is continuous over the entire filament (❯ *Fig. 6.8c*); the individual cells within each filament are separated only by their cytoplasmic membranes plus a septum that is contiguous with the inner layer of the envelope (Strohl et al. 1982). In other freshwater *Beggiatoa* strains, similar multilayer cell envelops and single-layer cell septa have been observed, in lower resolution due to technical limitations (Morita and Stave 1963; Drawert and Metzner-Küstner 1958). Recently, marine *Beggiatoa* filaments (non-vacuolated, average diameter 4.4 µm, related to the marine autotrophic strain MS-81-6) were shown to have a five-layer envelope and a single-layer cell septum (presumably murein) that is contiguous with the inner layer of the envelope (de Albuquerque et al. 2010). Large, vacuolated marine *Beggiatoaceae* (size not explicitly specified, but most likely 10–30 µm filament diameter) showed a similarly complex cell envelope composed of four distinct layers (de Albuquerque et al. 2010) (❯ *Fig. 6.8d*, *e*).

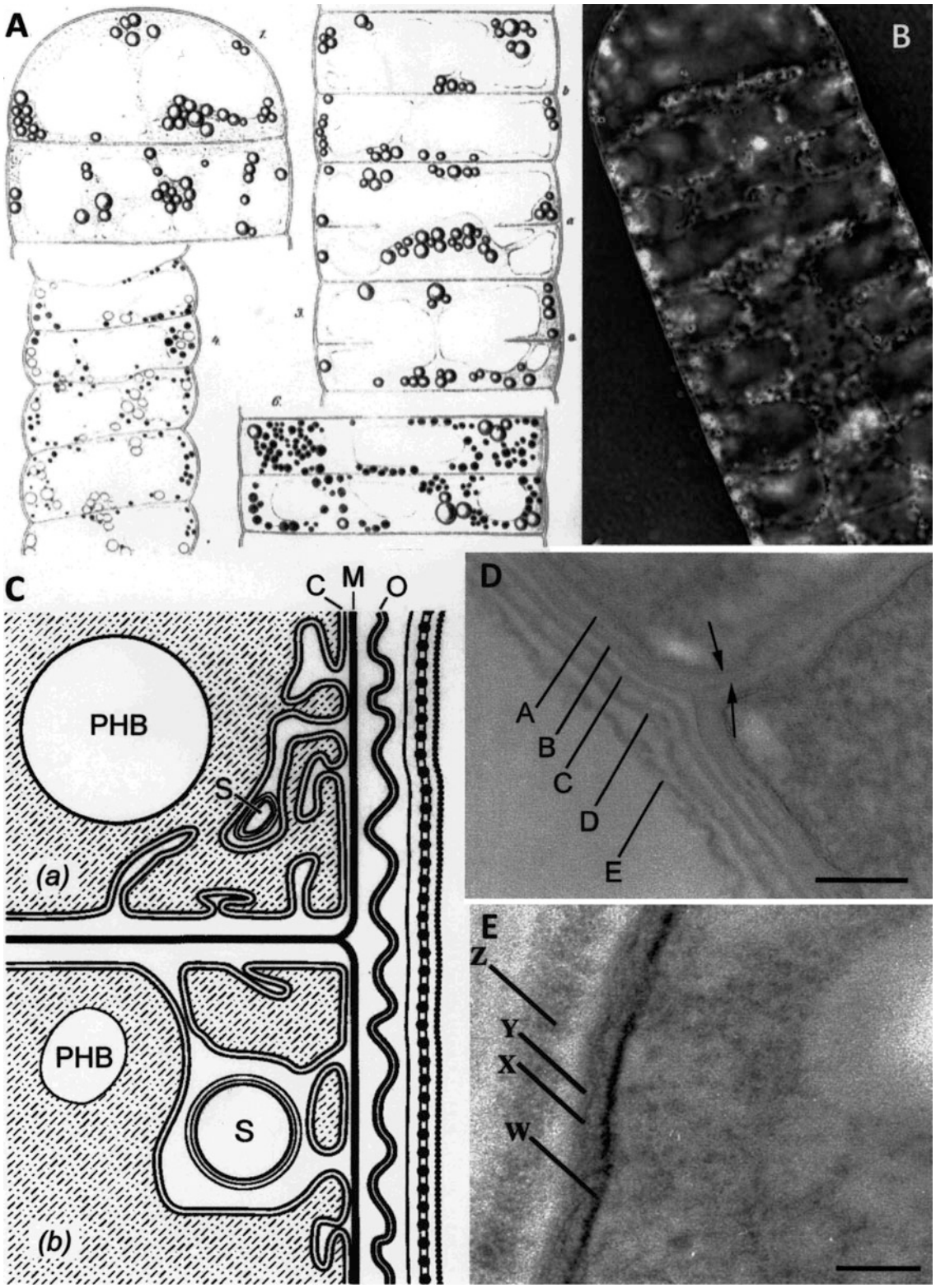

Fig. 6.8

***Cell structure of Beggiatoaceae*.** (**a**) Drawings from the original publication on the structure of large *Beggiatoaceae* filaments (Hinze 1901) show the extensive vacuoles that take up most of the cell volume, the sulfur globules embedded into the cytoplasm (drawn as three-dimensionally shaded globules), empty membranes after dissolution of sulfur globules, and small carbohydrate inclusions (in *black*), all based on microscopic observation of large *Beggiatoaceae* filaments (ca. 45 μm diameter) collected in Kiel Harbor. (**b**) Microphotograph of a large vacuolated *Beggiatoaceae* filament collected from the Chilean continental shelf shows the close

Surrounding the cell envelope and embedding the thin marine filaments, a sheath-like outer layer of fibrillar or striated material was observed (de Albuquerque et al. 2010). Large marine filaments show small round pores, ca. 15 nm in diameter, arranged in rows on the outer surface of a filament; a possible role in mucus secretion was suggested (Larkin and Henk 1996). Linearly arranged longitudinal fibrils have been observed on the cell envelope surface layer of *Beggiatoa alba*; most likely, they expel mucus forming a trail of mucilage around the filaments, inside which they can glide (Strohl et al. 1982). In *Beggiatoa alba*, the mucilage trail is composed of neutral polysaccharides (mannose and glucose) (Larkin and Strohl 1983). Earlier electron microscopic observations of conspicuously striated sheath material in freshwater *Beggiatoa* spp. (Drawert and Metzner-Küstner 1958) could correspond to such an outer cell envelope layer of longitudinal fibrils, or to the mucilaginous coating itself. Mucilaginous coats surrounding individual *Beggiatoa* are significant for filament movement (Møller et al. 1985); they could be a direct homolog for the larger sheaths surrounding *Thioploca* and "Marithioploca" filament bundles. Consistent with such a derivation, these sheaths appear to have a striated texture that runs parallel to the filaments; epibiotic filamentous bacteria on and within the sheath matrix tend to be aligned parallel to the sheath striation and the "Marithioploca" filaments (Fukui et al. 1999; Muyzer and Ramsing 1995) (*Fig. 6.3f*).

A cell envelope similar to *Beggiatoa* spp. has been observed in an electron microscopic ultrastructure survey of *Thioploca ingrica* filaments (Maier and Murray 1965). The envelope surrounds the entire filament, including the filament tips, and consists of multiple distinct layers. Adjacent to the cytoplasmic membrane, an electron-dense inner layer is contiguous with the cell septa and then follows a complex, multilayered "quadruple profile" and on the outside two distinct outer envelope layers (Maier and Murray 1965). A similar cell envelope is found in the Chilean "Marithioploca" filaments (Maier et al. 1990). The inner layer, which is continuous with cell septa, is followed by interstitial material and the adjacent undulating "triple layer"; after a gap, two outer layers are completing the cell envelope. On the inside of the cell envelope, periplasmic spaces appear in some locations, followed by the cytoplasmic membrane. Incomplete cell septa branch off from the cell envelope inner layer and extend into the cytoplasm and the ventral vacuole. However, they do not close off a cell, but they remain surrounded by the cytoplasmic membrane and the vacuolar membrane (Maier et al. 1990). The incomplete septa seen in various lengths suggest a mode of cell division where growing septa bisect a cell and its central vacuole. Interestingly, the early study by Hinze (1901) records a similar mechanism of cell division in microscopic drawings of large *Beggiatoaceae*.

Cell Inclusions

Four types of inclusions have been reported for members of the *Beggiatoaceae*: polyhydroxyalkanoates (PHA) (Pringsheim 1964; Pringsheim and Wiessner 1963; Strohl and Larkin 1978a; Strohl et al. 1982; Schwedt et al. 2012), glycogen or a similar polyglucose (Schulz and Schulz 2005), polyphosphate (Maier and Murray 1965; Schulz and Schulz 2005; Brock et al. 2012), and sulfur (Strohl et al. 1981b, 1982; Winogradsky 1887).

PHA Inclusions

Production of PHA appears to be a universal feature of heterotrophic freshwater strains (*Fig. 6.2c*). Interestingly, in heterotrophic *Beggiatoa* spp. the deposition of the alkanoate polyhydroxybutyrate seems to correlate primarily with high aeration (Pringsheim 1964), and it can account for up to 50 % of total dry weight under aerated conditions in the absence of sulfide (Güde et al. 1981). Numerous cell inclusions that resemble PHA were also found by TEM in the freshwater and brackish-water species *Thioploca ingrica* (Kojima et al. 2003). In the marine *Beggiatoaceae* strain 35Flor, PHA is used up under prolonged anoxia, most likely by oxidation using intracellular sulfur as electron acceptor (Schwedt et al. 2012).

Sulfur Inclusions

The sulfur inclusions of *Beggiatoa* are periplasmic in location, being enclosed in invaginations of the cell membrane. The sulfur globules are surrounded by their own electron-dense monolayered envelope, followed by the cytoplasmic membrane (de Albuquerque 2010). In large, vacuolated filaments, the sulfur inclusions are located in the narrow cytoplasmic space between

Fig. 6.8 (continued) correspondence between the drawings by Hinze and modern observations (Photo by Jan Küver, Institute for Materials Testing, Bremen). The positive photo slide was scanned as a negative slide for improved contrast. (c) Ultrastructure drawing of *Beggiatoa alba* strain B15LD (ATCC#33554). Symbols: C, cell membrane; M, presumed murein layer; O, presumed outer membrane layer; S, globule of S°; PHB, poly-β-hydroxybutyrate granule. (1) Note large PHB inclusion and rudimentary S° globule typical of cells grown in acetate-supplemented mineral medium. (2) Note small PHB inclusion and large S° globule typical of cell grown in the presence of sulfide or thiosulfate and a low concentration of acetate (Figure adapted from Strohl et al. 1982). (d) Transmission electron micrograph of cell surface envelope with five layers from a small non-vacuolated filament of marine *Beggiatoaceae*; the two small arrows indicate the septum that separates two cells within the same filament. (e) Transmission electron micrograph of cell envelope with four layers from a large marine vacuolated filament. Both filaments were collected from coastal lagoons in Brazil (Figure adapted from de Albuquerque et al. 2010)

the cell membrane and the large central vacuole (Jannasch et al. 1989; Larkin and Henk 1996; de Albuquerque et al. 2010). Very similar observations were made for the Chilean "Marithioploca" species; a thin cytoplasmic layer containing membrane-enclosed sulfur globules surrounds the central vacuole (Maier et al. 1990). The vacuole is surrounded by its own vacuolar membrane in addition to the cytoplasmic membrane.

In *Beggiatoa alba* strain B15LD, the sulfur globules are enclosed within a multilayered sulfur inclusion envelope of 12–14 nm thickness (Strohl et al. 1982), while in other strains the S° globule envelope appears to be composed of a single protein layer 4–5 nm thick (Strohl et al. 1981b). The extraction of S° globules with solvents such as pyridine and their refractile appearance when intact cells are viewed with phase contrast microscopy have proven very useful in confirming their presence (Skerman et al. 1957). *Beggiatoa* cells grown in the absence of reduced sulfur compounds apparently contained small, "rudimentary" S° inclusion envelopes (Strohl et al. 1982). Dehydration solvents (e.g., ethanol) that are necessary for preparation of electron microscopy dissolve the S° and complicate the determination whether the rudimentary inclusions completely lack elemental sulfur. The sulfur globules consist of fine-grained, microcrystalline elemental sulfur in the common, stable S_8 ring configuration (Pasteris et al. 2001; Prange et al. 2002) without significant additions of other elements (Lawry et al. 1981); they are surrounded by extensions of the cytoplasmic membrane plus an inner sulfur inclusion envelope (Lawry et al. 1981; Strohl et al. 1981b). The cyclooctasulfur globules of *Beggiatoa alba* (strain DMSZ 1416) and of *Thiomargarita namibiensis* differ from sulfur globules produced by other sulfur oxidizers, such as polythionate sulfur globules of *Acidithiobacillus thiooxidans* and sulfur chain-dominated sulfur globules of anoxygenic phototrophs (Prange et al. 2002); the differences in sulfur speciation probably reflect different sulfur deposition pathways.

Polyphosphate Inclusions

Cells of *Thiomargarita namibiensis* accumulate phosphate intracellularly and store it as polyphosphate granules (Schulz and Schulz 2005). Polyphosphate accumulation and phosphate release by *Thiomargarita* lead to the precipitation and accumulation of phosphate-rich minerals (Schulz and Schulz 2005). These observations link microbially catalyzed precipitation of authigenic phosphate minerals in marine sediments to the in situ activities of sulfur-oxidizing bacteria and sulfate-reducing bacteria (Arning et al. 2009; Williams and Reimers 1983) and realize earlier suggestions made after the discovery of the Chilean Marithioploca mats (Gallardo 1977b). Although it is very likely that these granules provide an energy buffer for *Thiomargarita* cells, the environmental or biogeochemical triggers for polyphosphate accumulation and degradation in *Thiomargarita* remain to be identified.

In addition to *Thiomargarita*, polyphosphate inclusions have been documented for different filamentous *Beggiatoaceae* (Maier and Murray 1965; de Albuquerque et al. 2010; Brock and Schulz-Vogt 2011); they appear to be absent from "Marithioploca," at least at the time of sampling (Holmkvist et al. 2010). Experiments with *Beggiatoa* strain 35Flor—a strain that accumulates phosphate intracellularly as large inclusions surrounded by lipids, most likely a membrane (Brock et al. 2012)—have shown that phosphate is released in response to increasing sulfide concentrations; acetate additions have a similar effect on *Thiomargarita* but most likely act by stimulation of sulfate reduction and concomitant sulfide production in the sediment (Brock and Schulz-Vogt 2011).

Isolation, Enrichment, and Maintenance Procedures

Enrichments from Natural Environments

Natural enrichments of *Beggiatoaceae* can be transplanted into the laboratory for observation and community succession studies; a highly reducing *Beggiatoa*-rich sediment can be maintained in an aquarium for continued observation. For example, the development of a *Beggiatoa* mat in the laboratory unfolded over 10 days and revealed a succession of different *Beggiatoa* size classes (Bernard and Fenchel 1995).

Similar aquarium setups can be used for selective enrichments (Nelson 1992). The bottom of a shallow pan or aquarium (approximately 30 × 30 × 12 cm) is covered with a few centimeters of sand; seaweed or shredded paper is added as a source of complex organic polymers, plus approx. 20 g of $CaSO_4$ and a few grams of K_2HPO_4, followed by several centimeters of sulfide-rich marine mud and sufficient seawater to overlay the entire enrichment by about 1–2 cm. Subsequent dark incubation minimizes competition with phototrophic bacteria. The enrichment is certain to contain the proper sulfide–oxygen interface somewhere in the vessel if air is introduced near the sediment surface using an airstone. Water lost by evaporation should be replaced by distilled water. Alternatively, a slow steady flow of freshly aerated seawater, with a drain maintaining a constant level, will provide the necessary O_2. A similar freshwater enrichment inoculated with mud from a sulfur spring and maintained on a light–dark cycle (10 h:14 h) provided viable tufts of *Beggiatoa* spp. for almost 1 year (Nelson and Castenholz 1982). Sewage treatment plants are also an excellent source of enrichment material (Burton and Lee 1978; Williams and Unz 1985).

Enrichment in extracted hay medium (Cataldi 1940) provides a useful strategy for enriching *Beggiatoaceae*, and several modifications of this method have been employed successfully for enriching freshwater *Beggiatoa* strains from decaying plant material and aquatic sediment (Faust and Wolfe 1961; Joshi and Hollis 1976; Saravanakumar et al. 2012). These materials provide good inocula even when conspicuous mats are absent; as filaments break at necridia or "sacrifical cells," trichomes as short as 3–10 μm are produced and widely dispersed (Pringsheim 1964; Strohl and Larkin 1978b; Kamp et al. 2008). Enriched tufts of *Beggiatoa* filaments then provide start material

for single filament isolation by excising individual filaments after transfer of tufts on agar plates (Faust and Wolfe 1961).

Liquid Media

Liquid media can be used for enrichment, MPN enumeration, and bulk cultivation of *Beggiatoa*. Early attempts to use liquid media for bulk cultivation (Kowallik and Pringsheim 1966) had already demonstrated the importance of small amounts of carbon substrates, either soil or hay extracts or small amounts of acetate, for successful cultivation of heterotrophic or mixotrophic freshwater *Beggiatoa*. The type species and strain, *Beggiatoa alba* B18LD, and related strains, are generally grown in liquid media that include a salt base, acetate as carbon source, and variable yeast extract and sulfide additions (Mezzino et al. 1984; Schmidt et al. 1986).

In an extensive study, Strohl and Larkin (1978a) have tested several liquid media formulations for isolation and MPN enumeration of heterotrophic *Beggiatoa* filaments from organic-rich freshwater ditches and lakes. A soil extract amended with 0.05 % (w/v) acetate, 15–35 U catalase per mL, and 1 % (w/v) hay extract yielded the best results. Following a recently published version of this protocol (Saravanakumar et al. 2012), hay is extracted by boiling in tap water for about 30 min and the water is decanted. Repeated boiling and decanting is carried out at least five times with cold tap water rinses between each boiling/decanting step. The extracted hay is left in water overnight and then decanted and dried at room temperature for 2 days. Approximately 1 g of dried hay is added to 100 mL of artificial seawater in a 250-mL Erlenmeyer flask and autoclaved. Filter-sterilized catalase is added to the medium at a final concentration of 35 U/mL. The medium is subsequently inoculated with 1–2 g of sediment sample. After 1–2 weeks incubation at 28 °C in the dark, the enrichments are checked for the presence of whitish threadlike mats and tufts, and these are examined microscopically for the presence of typical *Beggiatoa* filaments. The tufts from enrichment cultures are washed twice with sterile 0.01 % sodium azide solution (Strohl and Larkin 1978a) prepared in artificial seawater, followed by two washes and a 5-min soak in filtered artificial seawater containing catalase (35 U/mL). Washed filaments are then used for pure culture isolation procedures using heterotrophic media (Burton and Lee 1978; Strohl and Larkin 1978a) or sulfide gradient cultures (Nelson and Jannasch 1983).

Isolations on Agar Plates

Agar plate enrichments have commonly led to the isolation of heterotrophic *Beggiatoa* strains, but can be adjusted for autotrophic enrichments by minimizing or removing the organic carbon source in the agar medium. Tufts of *Beggiatoa* filaments are collected from the environment or an enrichment, washed in a sterile washing solution, and placed on an agar plate that contains dilute organic substrates, such as small amounts (1–0.25 ‰ w/v) of peptone or yeast extract or 0.5 mM acetate. Growing filaments that move away from the central inoculum are cut out on agar blocks and are used as inoculum for new agar plates (Pringsheim 1967); individual filaments can also be pulled away from the inoculum with a suitable micromanipulation needle or finely pointed watchmaker's forceps (Nelson 1992). The surface of the agar plates should be dry and free of condensation water droplets.

To enrich and isolate heterotrophic freshwater *Beggiatoa*, the representative DTA medium (Nelson 1992) for agar plates is prepared as follows. The pH is adjusted to 7.0 prior to autoclaving.

ND stock solution	50 mL
$(NH_4)_2SO_4$	0.13 g
Sodium acetate	0.68 g (may be reduced)
K_2HPO_4	0.027 g
$Na_2S_2O_3 \times 5H_2O$	0.50 g
$CaCl_2$	0.10 g
Distilled water	950 mL
Agar	12 g
ND stock solution (Castenholz 1988)	
Distilled water	1000 mL
NTA (nitrilotriacetic acid)	2.0 g
Micronutrient solution	10 mL
$FeCl_3$ solution	(0.29 g/L) 20 mL
$CaSO_4 \times 2H_2O$	1.2 g
$MgSO_4 \times 7H_2O$	2.0 g
NaCl	0.16 g
Na_2HPO_4	1.4 g
KH_2PO_4	0.72 g
Micronutrient solution	
Distilled water	1,000 mL
H_2SO_4 (concentrated)	0.5 mL
$MnSO_4 \times H_2O$	2.28 g
$ZnSO_4 \times 7H_2O$	0.50 g
H_3BO_3	0.50 g
$CuSO_4 \times 5H_2O$	0.025 g
$Na_2MoO_4 \times 2H_2O$	0.025 g
$CoCl_2 \times 6H_2O$	0.045 g

By using defined mineral media and reducing the organic carbon content of the agar medium (Nelson and Castenholz 1981b), heterotrophic contaminants are selected against, and the enrichment and isolation of autotrophic *Beggiatoa* is favored. Agar plates made with filtered seawater, trace elements, and vitamin mix and supplemented with sodium sulfide, ammonium sulfate, sodium thiosulfate, and sodium acetate were used for the isolation of marine *Beggiatoa* strains that in subsequent tests showed autotrophic growth (Nelson et al. 1982). The marine basal medium (J3) without carbon substrate amendments can be

used for agar plates selecting for autotrophic *Beggiatoa* spp. (Nelson 1992) and is prepared starting with three solutions that are separately autoclaved in Erlenmeyer flasks.

Solution 1:
Aged natural seawater (salinity 3.2–3.5 %), 500 mL
Prefiltered (Whatman #1 or Gelman GF/F) and filtered (0.45 µm)

Solution 2 (in larger flask):	
Distilled water	200 mL
Agar	9.0 g
Solution 3:	
NH_4NO_3	0.06 g
Trace element solution SL8	0.75 mL
Mineral stock	50 mL

The aged natural seawater can be replaced by artificial seawater, containing per liter NaCl, 27.5 g; $MgCl_2 \times 6H_2O$; $MgSO_4 \times 7H_2O$, 4.1 g; $CaCl_2 \times 2H_2O$, 0.66 g; and KCl, 1.02 g (Kamp et al. 2008).

The trace element solution SL8 (Pfennig and Biebl 1981) contains per liter Na_2EDTA, 5.2 g; $FeCl_2 \times 4H_2O$, 1.5 g; $ZnCl_2$, 0.070 g; $MnCl_2 \times 4H_2O$, 0.100 g; H_3BO_3, 0.062 g; $CoCl_2 \times 6H_2O$, 0.19 g; $CuCl_2 \times 2H_20$, 0.017 g; $NiCl_2 \times 6H_2O$, 0.024 g; and $Na_2MoO_4 \times 2H_2O$, 0.036 g.

The mineral stock contains per liter K_2HPO_4, 0.52 g; Na_2MoO_4, 0.05 g; $FeCl_3 \times 6H_2O$, 0.29 g; $Na_2S_2O_5$ (sodium pyrosulfite), 0.75 g; and phenol red, 10 mL of a sterile 0.5 % solution (Gibco) (Nelson 1992).

After cooling to 50 °C, the autoclaved solutions are aseptically combined in the solution 2 flask (volume > 750 mL) and supplemented with 0.2 mL of Va vitamin solution, which contains (in mg per liter) B_{12}, 1; thiamine, 200; biotin, 1; folic acid, 1; para-aminobenzoic acid, 10; nicotinic acid, 100; inositol, 1; and calcium pantothenate, 100.

J3 basal medium is amended to produce an isolation medium (J-TS) by adding the following three sterile stocks, with final concentrations in parentheses (Nelson 1992): (1) 7.5 mL of 200 mM $Na_2S_2O_3$ (2 mM) and (2) 3.75 mL of freshly neutralized 200 mM Na_2S (1 mM). The Na_2S stock is autoclaved as a basic solution and then neutralized with an equimolar quantity of sterile HCl just prior to use. The Na_2S stock solution is kept for approx. a month during oxic storage, unless it is stored under N_2 gas. (3) 15 mL of 1 M $NaHCO_3$ (20 mM). To make this stock, autoclave 8.4 g of $NaHCO_3$ (dry), and add 100 mL sterile water when cool. The medium is buffered by the bicarbonate in conjunction with the atmospheric CO_2.

Immediately after solidification, plates are incubated in a bell jar for 24 h or more under anoxic conditions (99.5 % N_2, 0.5 % CO_2), with desiccant present to absorb water evaporating from the surface of the plates. After inoculation with a tuft of *Beggiatoa* spp., plates are placed in a microoxic atmosphere (0.5 % CO_2; 0.2 % O_2; balance N_2). The medium and the bacteria tolerate temporary air exposure during inoculation or single-filament isolation (Nelson 1992).

Methods for isolating marine *Beggiatoa* strains on agar under microoxic conditions should in principle work for freshwater *Beggiatoa* as well. While freshwater strains were typically isolated on a variety of media equilibrated with full air (Nelson and Castenholz 1981b; Strohl and Larkin 1978a; Williams and Unz 1985), microoxic conditions may be required on occasion: the cultivation of a freshwater *Beggiatoa* strain in liquid mineral medium on thiosulfate and HCO_3^- required dissolved oxygen concentrations in the range of 3–16 µM (0.1–0.5 mg O_2/L) (Patritskaya et al. 2001).

Isolations and Cultivation in Gradient Media

Motile *Beggiatoa* spp. display strong chemotactic movement and adjust their position in gradients of oxygen and sulfide; they form platelike aggregates near the microoxic surface of semisolid agar tubes or—in liquid culture—reticulate networks of filaments surrounding an FeS pellet as sulfide source (Faust and Wolfe 1961). These observations were extended into a cultivation approach based on gradient media, where two layers of agar (a sulfide-rich agar plug at the bottom overlaid with sulfide-free soft agar) containing opposed sulfide and oxygen gradients provide a suitable gradient habitat for maintaining and propagating marine, autotrophic, non-vacuolate *Beggiatoa* spp. (*Fig. 6.6*) (Nelson and Jannasch 1983).

Marine gradient medium JG8 (Nelson 1992) is constructed as follows: first a 4 mL quantity of J3 agar (pH 8.4; the $NaHCO_3$ concentration is lowered to 2.0 mM; thiosulfate may be omitted) supplemented with freshly neutralized Na_2S is solidified in the bottom of a screw-capped tube (Hungate tube, 16 × 150 mm or similar). The bottom agar plug contained initially a sulfide concentration of 8 mM (Nelson and Jannasch 1983), but 3–4 mM sulfide is sufficient (Kamp et al. 2008; Jewell et al. 2008); agar strength can be reduced from 1.5 % to 0.75 % (Kamp et al. 2008). This bottom agar is then overlaid with 8.0 mL of semisolid J3 agar (0.25 % agar; $NaHCO_3$ concentration lowered to 2.0 mM; no sulfide or thiosulfate, but may contain nitrate). The resulting two layers of agar contain opposed sulfide and oxygen gradients that allow the growth of a well-defined *Beggiatoa* layer at the sulfide–oxygen interface (Nelson et al. 1986a, b). The overlying air headspace reservoir in the tube constitutes an oxygen reservoir. Tubes are loosely capped to permit exchange of headspace gasses with the atmosphere.

Aging new gradient media for 2–3 days prior to inoculation establishes a sulfide–oxygen interface that is quite stable in both position and rates of nutrient fluxes; however, molecular diffusion and nonbiological reactions between sulfide and oxygen gradually alter the gradient (Nelson et al. 1986a, b). The sulfide–oxygen interface near the top of the agar column spreads out in the absence of a *Beggiatoa* inoculum and contracts after inoculation. For example, sulfide and oxygen overlapped for 6–7 mm in uninoculated medium during slow, nonbiological sulfide

oxidation (Nelson et al. 1986b), in marked contrast to an overlap of 0.2 mm or less in active *Beggiatoa* cultures where sulfide oxidation proceeded quickly (Nelson 1992). Whether inoculated at the surface of this medium or stabbed throughout the upper few centimeters, the filaments rapidly proliferate at the sulfide–oxygen interface, forming a marked layer or "plate" of variable thickness. Gliding motility and negative chemotactic responses allow these bacteria to track this interface as it slowly descends due to the gradual depletion of the sulfide reservoir.

The gradient approach is not limited to marine *Beggiatoa*. For cultivation of estuarine strains, the soft agar medium is based on a 2/3-strength natural seawater medium that lacks reduced sulfur compounds but includes trace elements and vitamin mix and was supplemented with ammonium nitrate (J2 Medium) (Nelson and Jannasch 1983). For gradient culture of freshwater *Beggiatoaceae* (Kamp et al. 2006), the basal mineral medium is adjusted accordingly (per liter: EDTA, 0.010 g; $CaSO_4 \times 2\ H_2O$, 0.120 g; $MgSO_4 \times 7\ H_2O$, 0.200 g; NaCl, 0.016 g; Na_2HPO_4, 0.140 g; NaH_2PO_4, 0.138 g; $CaCl_2 \times 2\ H_2O$, 0.264 g; $FeCl_3$ solution [0.290 g/L]; 1 mL micronutrient solution). The micronutrient solution contains per liter 0.5 mL H_2SO_4 (>98 %); $MnSO_4 \times H_2O$, 2.28 g; $ZnSO_4 \times 7\ H_2O$, 0.5 g; H_3BO_3, 0.5 g; $CuSO_4 \times 5\ H_2O$, 0.025 g; $Na_2MoO_4 \times 2\ H_2O$, 0.025 g; and $CoCl_2 \times 6\ H_2O$, 0.045 g (Kamp et al. 2006); the vitamin solution remains the same.

Coculture and Obligate Associations

Some *Beggiatoaceae* could only be cultured in association with nonfilamentous bacteria (Kamp et al. 2006). One of these cocultures, an obligate association of the marine *Beggiatoa* strain 35Flor with a single, specific *Pseudovibrio* strain has been studied in more detail (Kamp et al. 2008; Brock and Schulz-Vogt 2011; Schwedt et al. 2012). Strain 35Flor grows only in the presence of an accompanying *Pseudovibrio* sp. strain that can be cultured without the *Beggiatoa* sp., but not vice versa. Since members of the genus *Pseudovibrio* are commonly isolated from marine invertebrates, the 35Flor-associated strain may have originated from the coral from which *Beggiatoa* sp. 35Flor had been isolated (Bondarev et al. 2013). Genome sequencing revealed that the *Pseudovibrio* symbiont has the genomic potential to attach to host cells, to produce secondary metabolites, and to provide the host organism with enzymatic cofactors (Bondarev et al. 2013).

Cultivation of *Thioploca*

Currently, no pure cultures or enrichments of *Thioploca* species or strains exist. All biochemical, physiological, and molecular work has been performed on *Thioploca* filaments collected from their natural environment, marine or freshwater sediments. Natural *Thioploca* populations can be kept alive in the laboratory for months or even years. Maier (1989) described the following procedures for freshwater *Thioploca*. Filaments may be maintained in jars overlaid with tap water at 8–20 °C in the dark; at approximately yearly intervals, a few stems of extracted grass (Scotten and Stokes 1962) may be stuck into the sediment, and *Thioploca* often colonizes these stems. Alternatively, 0.2–0.3 g of pulverized extracted hay is autoclaved in 60 mL of tap water in 125 mL Erlenmeyer flasks and inoculated with 4–10 mL of sediment (Maier 1980). After a month of undisturbed incubation at room temperature to avoid periods of maximum H_2S development, *Thioploca* bundles are added, and incubation continues for many weeks with intermittent inspection.

Attempts to enrich marine "Marithioploca" spp. have met little success. They may be maintained for months in undisturbed cores sampled from the natural populations and kept near the in situ temperature of 13 °C in a basin of anoxic seawater with nitrate added (H. Schulz-Vogt, pers. comm.). Physiological studies with harvested "Marithioploca" filaments required careful handling of the filaments, and avoidance of oxygen and air exposure, in order to prevent significant losses in enzymatic activities (Otte et al. 1999). Future cultivation approaches have to take into account the sensitivity of "Marithioploca" to high sulfide concentrations and to oxygen exposure and should maintain the delicate balance of sulfide, nitrate, and oxygen concentrations that characterizes its natural habitat (Hüttel et al. 1996; Schulz et al. 2000).

Strain Maintenance

Freshwater and marine strains of *Beggiatoa* spp. can be maintained in sulfide–oxygen gradient media. The smooth oxygen and sulfide gradients coupled with the chemotactic motility of the bacteria, which directs them to the proper microenvironment, make this approach especially attractive for obligately microaerophilic strains (Nelson et al. 1986b). A low concentration of acetate must be provided for the strains that do not show autotrophic capacity. For the typical medium geometry employed (Nelson 1992), transfers to new tubes of gradient medium should be made every 2–3 weeks. Gradient medium should be stored no more than 4–6 weeks; transfers into aged gradient media will not survive as long as those into freshly prepared media (Nelson 1992). Sustained propagation of heterotrophic freshwater strains on agar plates in the presence of full air is straightforward. By contrast, propagation of marine strains on agar plates under microoxic regimes in bell jars is best reserved for initial isolation and any required repurification steps. Cryopreservation of strains has proved problematic (Nelson and Schulz, pers. comm.).

Ecology

The *Beggiatoaceae* are gradient bacteria that occupy an ecological niche at the interface where fluxes of sulfide as electron donor and oxygen or nitrate as electron acceptor meet; different

genera have adapted to this niche in characteristic ways and prefer different types of interface habitats. These ecophysiological strategies of efficient sulfur oxidation are directly reflected in the structure of the mats and in the arrangement and the movements of the filaments and cells within the oxic/anoxic gradient. Thus, physicochemical habitat characteristics are directly linked to occurrence patterns, morphology, and physiology of *Beggiatoaceae* in nature.

Their ecophysiological flexibility allows the *Beggiatoaceae* to colonize a wide spectrum of freshwater and marine environments; *Beggiatoaceae* can be found in a wide range of habitats, including organic-rich, coastal marine sediments (Jørgensen 1977; Klas 1937; Mussmann et al. 2003; Rosenberg and Diaz 1993); benthic microbial mats (Teske and Stahl 2002) salt marshes (Nelson et al. 1982); eutrophic, oxygen-depleted bays (Graco et al. 2001; Vallius 2006); marine oxygen-minimum zones (Schmaljohann et al. 2001); oxygen-depleted marine basins (Williams and Reimers 1983); geothermally active submarine caves (Mattison et al. 1998); hydrothermal vents (Jannasch et al. 1989; Nelson et al. 1989); cold sulfide seeps (Sassen et al. 1994); and hydrocarbon seeps (Larkin et al. 1994). Freshwater habitats include sulfur springs (Uphof 1927; Caldwell et al. 1975; Nelson and Castenholz 1981b; Fukui et al. 1999); freshwater ditches, puddles, wetlands, and lake sediments (Koppe 1924; Pringsheim 1964; Scotten and Stokes 1962; Strohl and Larkin 1978a); terrestrial salt springs (Kolkwitz 1918); and sulfidic cave streams (Macalady et al. 2006, 2008). The cave stream study is of special interest for the ecology of the *Beggiatoaceae* since it outlines the environmental preferences of *Beggiatoa*-like filaments against single-celled sulfur-oxidizing epsilonproteobacteria and filamentous *Thiothrix* spp. that compete for different microhabitats within the same cave ecosystem. The Epsilonproteobacteria dominated extremely oxygen-depleted stagnant water with very little turbulent flow and oxygen in-mixing; the *Thiothrix*-like filaments preferred locations characterized by strong turbulent mixing, higher oxygen availability, and reduced sulfide concentrations, whereas the *Beggiatoa*-like filaments inhabited intermediate habitats over a wide range of oxygen and sulfide concentrations, as long as a sedimentary substrate allowing for mat formation was available (Macalady et al. 2008).

The Oxygen–Sulfide Interface at the Sediment Surface

The small freshwater and marine *Beggiatoa* spp. position themselves as a narrow layer at the oxygen–sulfide interface and separate the two compounds efficiently from each other. High sulfate reduction rates in surficial sediments maintain high fluxes of sulfide in the range of 10–100 mmol m^{-2} day^{-1} (see literature compilation in Schwedt et al. 2012); sulfide is then oxidized within *Beggiatoa* mats at the sediment–water interface. Oxygen from the overlying water is also consumed within *Beggiatoa* mats and does not penetrate the underlying sulfidic sediment (Jørgensen and Revsbech 1983; Møller et al. 1985; Nelson et al. 1986a; Fenchel and Bernard 1995). The microoxic growth zone of *Beggiatoa* is characterized by oxygen concentrations in the range of 1–2.5 μM (Nelson et al. 1986a). This microoxic niche of *Beggiatoa* is created by the highly dynamic sulfide-oxidizing metabolism of the *Beggiatoa* filaments themselves, but once established, it is remarkably stable (◉ *Fig. 6.6*). Laboratory gradient cultures remain active for several weeks and died only when the sulfide in the bottom agar was exhausted (Nelson et al. 1986a). A phobic response to high oxygen concentrations seems to be a driving force in establishing well-defined *Beggiatoa* mats. *Beggiatoa* filaments on a sediment surface adjust their position to short-term fluctuations in the sulfide and oxygen supply; they avoid high oxygen concentrations by contracting into the diffusive boundary layer directly at the sediment surface and expand after the oxygen stress has passed (Møller et al. 1985). Oxidative damage to essential enzymes by peroxide formation is one of the presumed reasons for the general oxygen sensitivity of *Beggiatoa* spp.; for example, H_2O_2 exposure inhibited fumarate hydratase, an essential TCA cycle enzyme, in the heterotrophically growing *Beggiatoa* freshwater strain D-405 (Grabovich et al. 1993).

The Anoxic, Non-sulfidic Surficial Sediment

In coastal, organic-rich marine sediments, *Beggiatoaceae* are often found in the intermediate sediment layer where porewater oxygen and nitrate are already depleted, but sulfide does not yet accumulate—a conspicuous departure from growth in overlapping oxygen–sulfide gradients at the sediment–water interface (Jørgensen 1977; Mussmann et al. 2003; Preisler et al. 2007; Jørgensen et al. 2010). These sediment populations do not form visually conspicuous mats at the sediment surface; their abundance becomes clear only after microscopic counts of the sediment-embedded filaments. Especially "Isobeggiatoa" and "Parabeggiatoa" occur in this habitat; their intracellular storage capacity for nitrate and sulfur is crucial in bridging the gap between the sedimentary porewater pools of electron acceptor and donor (Mussmann et al. 2003; Preisler et al. 2007). Even when they are abundant, these *Beggiatoa*-like filaments contribute only to a minor extent to overall anaerobic sulfide oxidation; precipitation with Fe^{2+} and oxidation with Fe^{3+} dominated sedimentary sulfide oxidation (Preisler et al. 2007). Filaments position themselves in the anoxic, sulfide-free zone by responding chemotactically to porewater concentrations of oxygen and sulfide; filaments in the favored zone reversed course frequently and were gliding shorter distances in randomized directions between reversals, whereas filaments in oxic or sulfidic sediments took more time between reversals and therefore glided longer distances (Dunker et al. 2010).

Hypersaline Cyanobacterial Mats

Beggiatoaceae, including members of the halotolerant Candidatus genus "*Allobeggiatoa*," occur in hypersaline benthic cyanobacterial mats (Hinck et al. 2007, 2011; Dillon et al. 2009). Cyanobacterial mats form a diurnally shifting gradient habitat (Jørgensen 1982). At daytime, the upper layers of cyanobacterial mats are photosynthetically active and become supersaturated with oxygen. At night, oxygen production ceases, and sulfide produced by sulfate reduction moves up towards the mat surface (Jørgensen et al. 1979; Jørgensen and Revsbech 1983). If this interface remains outside of the photic zone during the day, photosynthetic sulfide-oxidizing bacteria are excluded, and colorless sulfur bacteria grow along the oxic/anoxic interface (Jørgensen and DesMarais 1986). The response of motile filaments in the mat is modulated by their physiology. Migrating *Beggiatoa*-like filaments closely follow the diel up-and-down movement of the oxygen–sulfide interface, whereas a nonmigratory population in the same mat remains stationary at ca. 1 mm depth (Garcia-Pichel et al. 1994). Interestingly, nitrate-storing *Beggiatoaceae* do not migrate towards the mat surface to escape nighttime anoxia, but remain in the mat and stay several millimeters below the oxic surface layer (Hinck et al. 2007).

Close associations with cyanobacteria in microbial mat habitats may have left a genomic imprint in some *Beggiatoaceae* that is especially visible in genetic elements involved in cell differentiation (MacGregor et al. 2013b). The draft genome sequence of a single orange "Maribeggiatoa"-related filament from hydrothermal mats shows evidence of extensive genetic exchange with cyanobacteria, in particular for sensory and signal transduction genes. A putative homing endonuclease gene and Group I intron within the 23S rRNA gene; several Group II catalytic introns; GyrB and DnaE inteins, also encoding homing endonucleases; and multiple copies of sequences similar to the fdxN excision elements XisH and XisI (required for heterocyst differentiation in some cyanobacteria) all have close non-*Beggiatoaceae* matches with cyanobacterial sequences (MacGregor et al. 2013b). Sequences similar to the uncharacterized ORF and Xis elements are found in other *Beggiatoaceae* genomes, a variety of cyanobacteria, and a few phylogenetically dispersed pleiomorphic or filamentous bacteria (MacGregor et al. 2013b). Thus, gene transfer and evolutionary linkages between *Beggiatoaceae* and other filamentous bacteria (Reichenbach and Dworkin 1981) might be more significant than expected.

Hydrothermal Vents

Large filamentous sulfide oxidizers ("Maribeggiatoa" spp. and "Marithrix" spp.) occupy a distinct ecological niche characterized by fluctuating sulfide and oxidant levels. Conspicuous examples of this habitat have been studied in the hydrothermal sediments of Guaymas Basin in the Gulf of California (Jannasch et al. 1989; Nelson et al. 1989). Here, massive "Maribeggiatoa"-like mats of several cm thickness growing on hydrothermally active sediments are exposed to irregularly fluctuating pulses of oxygenated seawater and sulfidic sediment fluids (Gundersen et al. 1992) (*Fig. 6.9a*, *b*). The gradients of sulfide, DIC, and (most likely) low molecular weight organic compounds become steeper towards the center of a hydrothermal hot spot and appear to select for different morphotypes and genotypes in the center and in the periphery of the hydrothermally active sediment region (McKay et al. 2012). Orange filaments with a diameter of ca. 25–40 µm (*Fig. 6.9c*) dominate the center of the mat (*Fig. 6.6c*), whereas larger white filaments (>120 µm diameter, *Fig. 6.9c–e*) form the periphery of the mat (McKay et al. 2012). High temperature is unlikely to select for these different populations; in situ temperatures at the sediment–water interface across the multicolored mats remained cool (near 10 °C when measured with the Alvin temperature probe) and suitable for psychrophilic bacteria (McKay et al. 2012).

Filamentous mats do not only grow on hydrothermal sediments, but essentially on all solid substrates with a suitable regime where oxygenated seawater and reduced hydrothermal fluids mix, including the exterior of gradually seeping chimneys (*Fig. 6.9b*) and gaps and cracks in rocky debris that channel the flow of reduced fluids (*Fig. 6.9f*). Convective mixing of reduced vent fluids and oxygenated seawater also characterizes the habitat of "Marithrix" filaments; substrate-attached growth as rosettes allows "Marithrix" filaments to persist on exposed surfaces, such as hydrothermal chimneys (Kalanetra et al. 2004; Heijs et al. 2005; Kalanetra and Nelson 2010; Grünke et al. 2012). Most likely, these filaments have a higher oxygen tolerance than those in the Guaymas Basin mats.

Hydrocarbon Seeps

At hydrocarbon seeps, sediment areas characterized by active seepage of methane- and sulfide-rich fluids host conspicuous mats of *Beggiatoa*-like filaments (*Fig. 6.10*). Currently, most observations of this habitat type come from the continental slope of the northern Gulf of Mexico, an area exceptionally rich in hydrocarbon seeps (Larkin et al. 1994; Nikolaus et al. 2003). A cross section from the center to the margin of a mat-covered sediment area in the Gulf of Mexico (MC118) showed that the mat area coincided with high sulfate reduction and anaerobic methane oxidation rates in the surficial sediments; the microbial community underneath the mat was dominated by deltaproteobacterial sulfate-reducing bacteria and by methane-oxidizing archaea (Lloyd et al. 2010). The rates declined in bare sediments adjacent to the mat, and the microbial communities in the surficial sediments diversified considerably (Lloyd et al. 2010). The sulfidic seep sediments underneath the mats select for a specialized sulfur- and methane-cycling microbial community of reduced diversity (Lloyd et al. 2010).

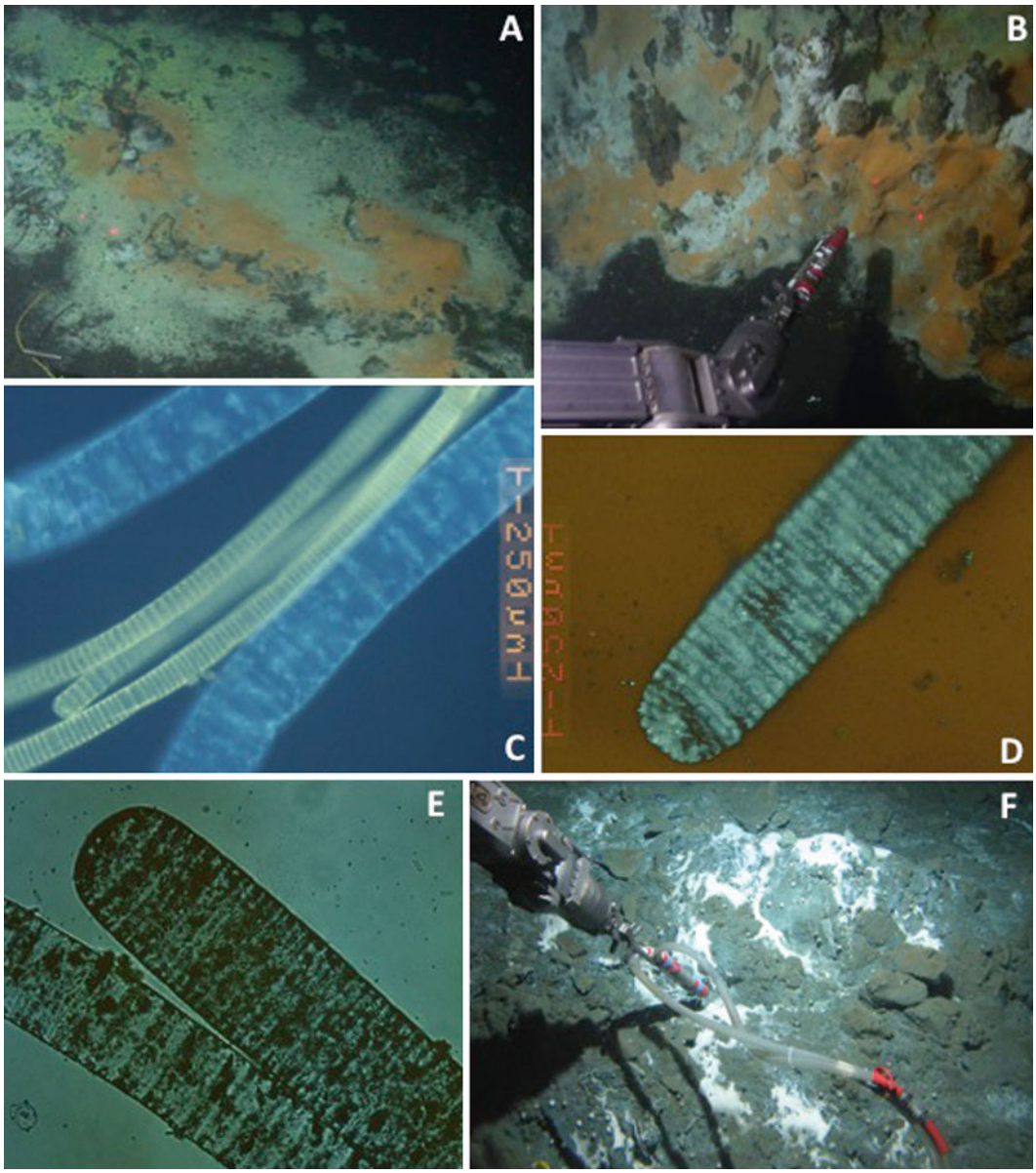

Fig. 6.9

***Hydrothermal vent Beggiatoaceae*. (a) Typical structure of Guaymas Basin mat of filamentous sulfur oxidizers: orange filaments at the center and white filaments at the periphery, surrounded by bare sediment (Alvin Dive 4569, N 27°00.47, W 111°24.431, 2,009 m depth). For this mat (M14), in situ temperature gradients, porewater geochemistry, filament types, and their 16S rRNA gene sequences are described in context (McKay et al. 2012). (b) Mats on chimney structure "Cathedral Hill" in Guaymas Basin (Alvin Dive 4573, near N 27°00.696, W 111°24.265, 2,013 m depth). The Alvin sampling device ("slurp gun") is visible in the foreground. (c) Epifluorescence microphotograph of the two dominant types of Guaymas Basin *Beggiatoaceae*, large white (ca. 120 μm diameter), and smaller orange (ca. 40 μm diameter) filaments under UV excitation light. Vertical scale bar, 250 μm. (d) Dark field microphotograph of large *Beggiatoaceae* filament, showing the sulfur globules and the salami-like arrangement of individual cells in the filament. Vertical scale bar, 250 μm. (e) Transmission light microphotograph of large *Beggiatoaceae* filament, same scale as photos (c) and (d). (f) Sampling of *Beggiatoaceae* mats at Costa Rica Jaco Scarp (Alvin Dive 4509, N 09°07.030, W 84°50.550, 1,866 m depth) (Photos (a, b) Woods Hole Oceanographic Institution; (c–e) Andreas Teske, University of North Carolina at Chapel Hill; (f) Jake Bailey, University of Minnesota)**

The large filamentous sulfur oxidizers from Gulf of Mexico hydrocarbon seeps (with filament diameters up to 200 μm; Larkin and Henk 1989, 1996) are taxonomically unidentified but resemble large "Maribeggiatoa" filaments (e.g., *Fig. 6.10e*). In several investigations of cold hydrocarbon seeps in the Gulf of Mexico, colorful *Beggiatoaceae* mats showed a Guaymas-like spatial distribution of orange mats with white peripheries (Wirsen et al. 1992; Sassen et al. 1994; Larkin and Henk 1996;

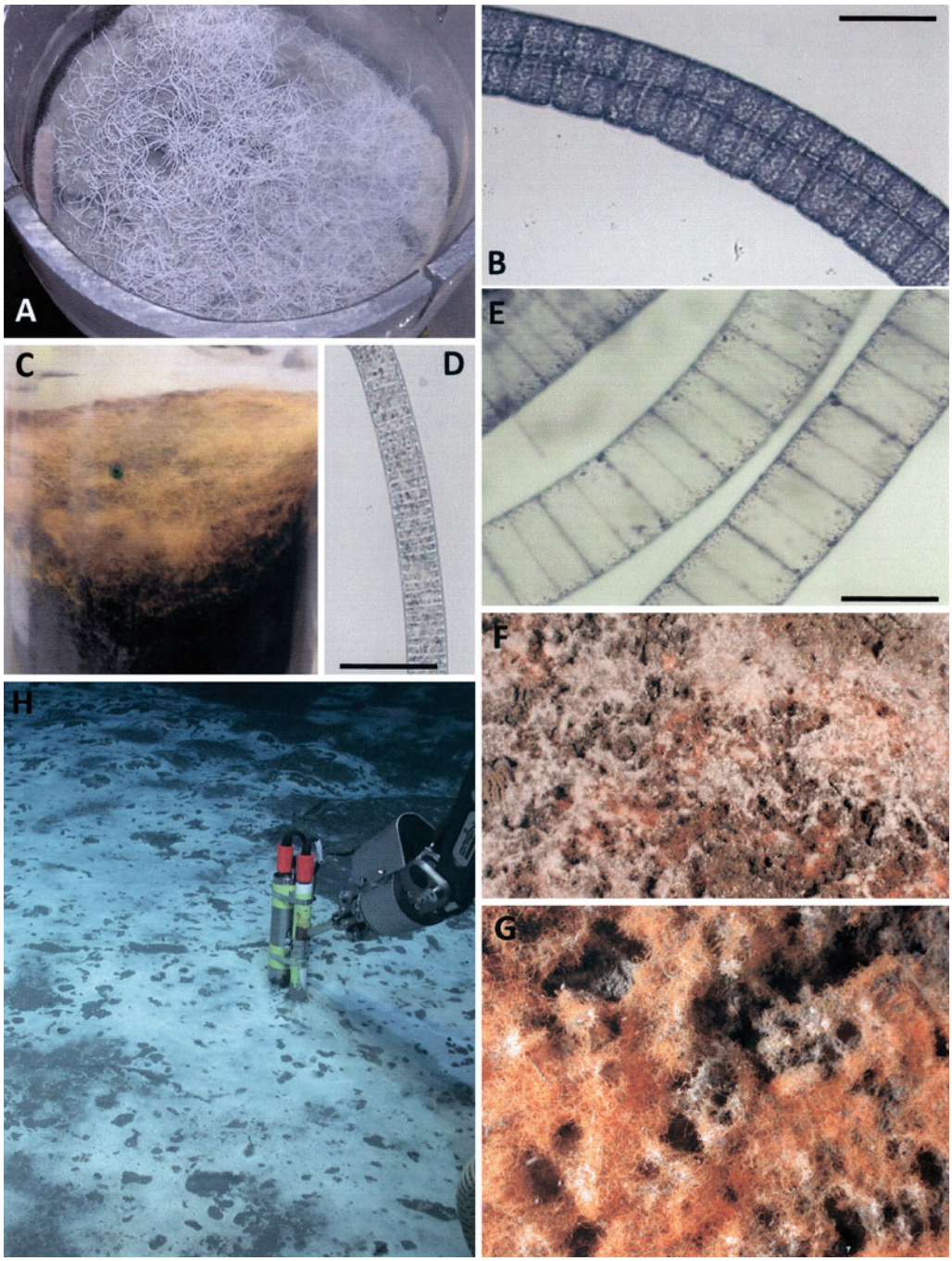

Fig. 6.10

***Cold seep Beggiatoaceae*.** (**a**) Sediment core, 6 cm diameter, with mat of white filaments collected on Alvin dive 4645 near Garden Banks 425 mud volcano in the Gulf of Mexico (N 27°33.140, W 92°32.437; 597 m depth). (**b**) Phase contrast microphotograph of single white filament from this mat; filament diameters range from 78 to 94 μm. Note central ''pipeline''-like structure running through the filament. Scale bar 100 μm. (**c**) Orange mat in sediment core, 6 cm diameter, collected on Alvin dive 4653 near Green Canyon 233 brine lake (N 27°43.429′, W. 91°16.777, 633 m depth). Scale bar 100 μm. (**d**) Transmission microphotograph of individual filament from this mat, average diameter 38 μm. Scale bar 100 μm. (**e**) Transmission microphotograph of large, vacuolated, white filaments (collected on Alvin dive 4652 in Green Canyon 426 Mud volcano area (N 27°42.098, W 90°38.887; 837 m depth), average diameter 119 μm. Scale bar 100 μm. (**f**, **g**) In situ close-up photograph of predominantly orange filamentous mats at Green Canyon 185, a hydrate-rich seep site in the Gulf of Mexico (N 27°44.930, W 91°30.450; 540 m depth). Note the complex mat architecture containing spherical sulfur bacteria (most likely *Thiomargarita* spp.) and white and orange filaments growing in tufts. (**h**) ROV sampling of white filamentous mats (''*Candidatus* Halobeggiatoa'') at Håkon Mosby mud volcano, Barents Sea (Niemann et al. 2006; Grünke et al. 2012) (Photos (**a**–**e**) Andreas Teske, University of North Carolina; (**f**, **g**) Ian McDonald, Florida State University; (**h**) MARUM, Bremen University)

Nikolaus et al. 2003). Hydrocarbon analysis of sediments under *Beggiatoaceae* mats revealed that, in comparison to white filaments, adjacent orange filaments reside over sediments with elevated concentrations of unresolved petroleum hydrocarbons, and 1–3 orders of magnitude higher methane and ethane concentrations (Sassen et al. 1994). A recent survey in the Gulf of Mexico identified colorless, vacuolated, large filaments with diameters near 90–120 µm and smaller, vacuolated, orange-colored filaments near 35 µm (Fig. 6.10a–e); these coloration and size classes resembled those of the Guaymas Basin *Beggiatoaceae* (Teske, unpublished results). Rates of hydrocarbon seepage could control the composition of Gulf of Mexico mats similarly as hydrothermal seepage in Guaymas Basin.

The physiological capacity of hydrocarbon seep-associated *Beggiatoaceae* is an open research field. White filaments from Gulf of Mexico seeps assimilate CO_2 autotrophically, whereas orange filaments show strongly reduced capacity for CO_2 uptake and appear to be heterotrophs (Wirsen et al. 1992; Nikolaus et al. 2003). When methane-derived DIC or low molecular weight organic compounds reach the sediment surface, *Beggiatoaceae* mats can take up ^{13}C-depleted CO_2 derived from methane oxidation and acquire the isotopically light signature ($\delta^{13}C$ in the range of −50 to −60‰) of partially methane-derived biomass (Paull et al. 1992; Orphan et al. 2002). In most cases, mixed *Beggiatoaceae* mat samples collected at cold seeps in the Gulf of Mexico have $\delta^{13}C$ isotopic signatures near −26 to −29‰, indicating that carbon sources of planktonic, photosynthetic origin are assimilated (Zhang et al. 2005).

In contrast to the commonly studied filamentous mats at hydrocarbon seeps, *Beggiatoaceae* occurring at a methane seep off Costa Rica have an attached habitus (Fig. 6.4f). Nonfilamentous sulfur bacteria (certain *Thiomargarita* sp. and *Thiopilula* sp.) are attached to solid surfaces like rocks, shells, or the byssus of mussels, where they are exposed to turbulent mixing of alternating sulfidic and oxygenated water (Bailey et al. 2011).

Mud Volcanoes

When fluidized mud flows from the subsurface reach the seafloor, they form extensive mud volcanoes characterized by high gas flow and an unstable sediment–water interface. At the center of mud volcanoes, the highly dynamic sediment–water interface does not allow the formation of sulfide-oxidizing microbial mats; more quiescent regions towards the periphery of the mud volcano provide the stable sediment–water interface that is required for growth of filamentous sulfide-oxidizing bacteria (Niemann et al. 2006; de Beer et al. 2006; Grünke et al. 2011; Girnth et al. 2011) (Fig. 6.10h).

Mud volcanoes with periodic flows of subsurface-derived brines (for example, the Amon mud volcano on the Nile Deep Sea fan in the Eastern Mediterranean; Girnth et al. 2011) present a special habitat. The brine flows cover the sediment surface at irregular intervals with dense, highly sulfidic brine; under these conditions, stationary *Thiomargarita* spp. have an advantage against "Maribeggiatoa" and "Marithioploca." The latter two would spend energy by chemotactic responses to the shallow brine flow, whereas the stationary *Thiomargarita* cells avoid this energy expenditure and await the end of the sulfidic brine flow episode (Girnth et al. 2011). This strategy resembles the stationary survival mode of *Thiomargarita namibiensis*, which relies on environmental perturbations for sulfide and nitrate exposure and uptake (Schulz 2006). Attached *Beggiatoaceae* ("Marithrix" spp.) have a similar ecological advantage under the highly fluctuating regimes of oxidized and reduced bottom waters and passing brine flows (Heijs et al. 2005; Grünke et al. 2011, 2012).

Nearshore Upwelling Areas: The Chilean Continental Shelf

Nearshore upwelling areas are characterized by oxygen-depleted or anoxic bottom water overlying organic-rich sediments; sulfate reduction in the sediments produces sulfide that is oxidized by microaerophilic and nitrate-reducing mats of large filamentous sulfide oxidizers (Fig. 6.3a–c). By geographical extent, these mats are probably the most widespread sulfide-oxidizing mat ecosystem on earth. Currently, the ecologically and oceanographically best documented mat systems are the complex sulfide-oxidizing bacterial mats on the Pacific continental shelf of Chile and Peru dominated by the large nitrate-accumulating filaments of "*Candidatus* Marithioploca" (Gallardo 1963, 1977a, b; Fossing et al. 1995; Gallardo and Espinoza 2007) (Fig. 6.3a–f).

The predominantly vertically oriented "Marithioploca" filaments can bridge and exploit the vertically separated pools of sediment sulfide and seawater nitrate (Hüttel et al. 1996; Schulz et al. 1996) and thus improve on the "holding your breath" strategy of nitrate accumulation and respiration of large, marine "Maribeggiatoa" spp. Large "Marithioploca" spp. from the Chilean continental shelf have turnover times of 8–10 days for their intracellular nitrate and sulfur reserves (Otte et al. 1999). For long-term survival, "Marithioploca" filaments require just the right balance of nitrate availability in oxygen-depleted bottom water and sulfide availability in the sediment. The annual fluctuations in mat abundance during a seasonal upwelling cycle (Schulz et al. 2000) can be exacerbated by prolonged summer anoxia, when the mats cannot cope with increased sulfate reduction and sulfide inundation; the result is mat die-off (Gallardo 1992) and high porewater sulfide concentrations in previously sulfide-free surficial sediments (Holmkvist et al. 2010). On the other hand, increased oxygen exposure and decreased water column productivity and organic matter input during El Niño years adversely affect the "Marithioploca" mats. During such events, bottom water oxygen concentrations increase from near detection limit (<2 µM) to ca. 20–40 µm; at the same time, "Marithioploca" biomass decreases considerably, from up to 160 g m^{-2} (Schulz et al. 2000) to <1 to 5 g m^{-2} (Schubert et al. 2000; Neira et al. 2001).

The spatial structure of the Chilean "Marithioploca" mats is conducive to the microbial lifestyle of bridging sulfide and nitrate pools. The densest mat matrix of randomly oriented filaments and bundles is found in the uppermost centimeter layer, while predominantly vertically oriented, less densely packed "Marithioploca" bundles reach down to a depth of generally 4–8 cm; they peter out at approx. 10–15 cm (Schulz et al. 1996) and are only rarely found in deeper sediment layers. The surface layer of the mat is generally well supplied with nitrate; it can penetrate several centimeters into the hydraulically conductive, porous, and soft "Marithioploca" mat sediments (Hüttel et al. 1996). The upper 1–5 cm of the sediment also shows the highest sulfate reduction rates, up to 1,500 nmol cm^{-3} d^{-1}, which are extremely high rates for marine sediments. Nevertheless, efficient in situ reoxidation of sulfide keeps the sulfide concentrations in the "Marithioploca" mat sediments low, mostly in the range of 5–50 µm, while sulfate concentrations were never depleted below bottom water concentrations (Ferdelman et al. 1997; Thamdrup and Canfield 1996). "Marithioploca" mats contribute significantly to in situ anaerobic oxidation of sulfide produced by sulfate reduction; their share can range from 6 to 91 %, but most measurements indicate a contribution between 20 % and 30 % (Fossing et al. 1995; Thamdrup and Canfield 1996; Ferdelman et al. 1997; Otte et al. 1999). The sulfide-oxidizing activity of "Marithioploca" is most significant in the upper 4 or 5 cm of sediment and quickly declines towards deeper sediment layers (Zopfi et al. 2008).

Sulfur recycling within the "Marithioploca" mats most likely benefits from the close spatial association between sulfate-reducing and sulfide-oxidizing bacteria in "Marithioploca" mats. Mat biomass and cultivable MPN numbers of sulfate-reducing bacteria both peak in the surface layer of the mat (Teske et al. 2009). Filamentous sulfate reducers of the genus *Desulfonema* grow on and within the "Marithioploca" sheaths and thus contribute to a cycle of sulfate reduction and reoxidation within a single "Marithioploca" bundle (Fukui et al. 1999; Teske et al. 2009).

Nearshore Upwelling Areas: The Benguela Upwelling System

The survival strategy of "Marithioploca" spp. contrasts with the ecophysiology of its relative *Thiomargarita namibiensis*. This immobile, giant sulfide oxidizer, the largest known prokaryote by volume, relies on its enormous storage capacity for sulfur and nitrate, to carry it through irregular natural fluctuations of sulfide and nitrate availability in its sedimentary habitat in the Benguela upwelling region offshore Namibia (Schulz et al. 1999). The high input of diatom-dominated phytoplankton debris fuels extremely high sulfate reduction rates that deplete sulfate within a few centimeters of the sediment surface and generate extremely sulfidic conditions (up to 20 mM) in the extremely soft diatomaceous ooze bottom sediments (*Fig. 6.4a*) (Brüchert et al. 2003). In this habitat, *Thiomargarita* cells reach a biomass density of up to 170 g m^{-2} sediment, similar to the "Marithioploca" mats offshore Chile (Brüchert et al. 2003). Filamentous sulfur bacteria, free-living or bundled, are scarce in the sulfidic Namibian liquid sediments; although previous reports suggest the possibility, only a few specimens were found in recent surveys (Gallardo et al. 1998; Salman et al. 2013). In contrast to the oxygen-sensitive Chilean "Marithioploca" spp., *Thiomargarita namibiensis* tolerates prolonged oxygen exposure and, in addition to nitrate, appears to be able to use oxygen for sulfide oxidation if acetate is provided (Schulz and de Beer 2002). This respiratory flexibility in combination with its large intracellular storage capacity helps *Thiomargarita namibiensis* to tolerate fluctuations of sulfide, nitrate, and oxygen during irregular resuspension episodes due to massive sulfide and methane outgassing events in its natural habitat (Emeis et al. 2004; Weeks et al. 2002, 2004).

Ecosystem Roles of *Beggiatoaceae*

The *Beggiatoaceae* serve as indicator organisms of beginning or advanced oxygen depletion and sulfidic bottom conditions in aquatic habitats; these conditions go generally together with increased oxygen demand due to seasonal biomass degradation (Bernard and Fenchel 1995). Point sources of anthropogenic pollution (Elliott et al. 2006) and fish farm eutrophication (Bissett et al. 2007; Gallardo and Espinoza 2007; Aranda et al. 2010) favor the development of *Beggiatoaceae* mats; sea grass beds (*Zostera marina*) are replaced by sulfidic bottom mud with bacterial mats (Elliott et al. 2006).

As a result of their growth pattern at the sediment–water interface, *Beggiatoaceae* mats play a significant role for the benthic–pelagic exchange of sulfur in the marine environment. They act as a sulfide trap that prevents sulfide from entering the water column; during this process they enrich the sulfur content of surface sediments and allow resuspension and recycling of partially oxidized sulfur species in the water column (Grant and Bathmann 1987).

A significant ecosystem service of *Beggiatoaceae* mats, catalyzing phosphorous retention in benthic sediments, was recently proposed in a geochemical modelling study (Yekta and Rahm 2011). Sulfide oxidation by *Beggiatoaceae* contributes to shifting the redox balance of iron in surficial marine sediments from Fe-II to Fe-III and changes the balance of the resulting iron solid phases in surficial sediments from iron sulfide and pyrite to ferric oxyhydroxides; the latter absorb and immobilize phosphate in surficial sediments. This mechanism could provide a strategy for phosphorus retention in hypoxic marine sediments affected by eutrophication, a widespread problem in the Baltic Sea (Yekta and Rahm 2011). It remains a matter of debate whether the sulfur-oxidizing activities and biomass of *Beggiatoaceae* in situ are sufficient to turn around the redox state of the sedimentary iron pool (Preisler et al. 2007).

Local "hot spots" of decaying biomass that sustains mat-forming *Beggiatoaceae* can range from whale carcasses (Smith et al. 1989; Deming et al. 1997) to coral heads, where

environmental stressors can induce the formation of mucus, which is then colonized by sulfate reducers and filamentous sulfur bacteria (Mitchell and Chet 1975). In black band coral disease, cyanobacterial mats and diverse heterotrophic bacteria are overgrowing and degrading coral tissue; colorless sulfur bacteria are a significant component of these mats growing on necrotic coral tissue (Carlton and Richardson 1995). Functional gene studies using the widely distributed sulfur oxidation key gene soxB show that uncultured *Alphaproteobacteria* are the dominant component of these mats; the *Beggiatoaceae* are morphologically conspicuous but appear as a minority in functional gene surveys (Bourne et al. 2013) or remain undetected in standard 16S clone library surveys (Sekar et al. 2006).

Many associations of *Beggiatoaceae* with other organisms benefit the partner organisms. The addition of cultured *Beggiatoa* filaments and tufts to different soils with rice plant seedlings reduced hydrogen sulfide levels in the flooded soil and increased oxygen production by the rice seedlings (Pitts et al. 1972; Joshi and Hollis 1977). Sulfide removal and detoxification has also been invoked to explain the conspicuous association of filamentous marine *Beggiatoaceae* with protists and nematodes in highly reducing marine sediments (Bernhard et al. 2003). Specific nematodes also inhabit *Beggiatoaceae* mats and the underlying sediment at the Håkon Mosby mud volcano and shape a meiofaunal community that is taxonomically distinct from and less diverse than its counterpart outside of the mat area (Van Gaever et al. 2010). On the Pacific continental shelf offshore Chile, benthic invertebrates (polychaetes, crustaceans, mollusks, anthozoa) occurred in greater abundance and diversity at sampling locations with well-developed "Marithioploca" mats than at sites where mats were sparse or absent (Carrasco et al. 1999). The Chilean "Marithioploca" mats also provide nursery habitat for marine invertebrates, such as squat lobster larvae (Roa et al. 1995; Gallardo et al. 1994). These effects are not only attributable to food source availability but also to sulfide sequestration.

Due to nitrate reduction and ammonification by "*Candidatus* Marithioploca," ammonia accumulates at high rates in "Marithioploca"-harboring sediment surface layers (Thamdrup and Canfield 1996). The "Marithioploca" mats turn the sediments from a denitrifying nitrogen sink into an ammonia-producing nitrogen source; ammonia would constitute a readily utilized and recycled nitrogen source for the water column (Farias et al. 1996; Farias 1998). Similar findings have been reported for mats of large, vacuolated *Beggiatoa*-like filaments in Tokyo Bay (Sayama 2001) and in Aarhus Bay marine sediments (Sayama et al. 2005). Here, *Beggiatoaceae* separate the sulfide and nitrate porewater pools by pushing the nitrate-reducing sulfide oxidation horizon down into the sediment; in parallel, the predominant nitrate and nitrite reduction pathway in the *Beggiatoa*-inoculated sediment shifts from denitrification to N_2 towards dissimilatory nitrate reduction to ammonia (Sayama et al. 2005). The product of anaerobic sulfide oxidation, sulfur, is then transported to the sediment surface and oxidized aerobically to sulfate. This spatial separation of anaerobic and aerobic sulfur oxidation pathways, and parsimonious use of nitrate only for the initial oxidation step (of sulfide to sulfur), counteracts nitrate depletion during complete oxidation of sulfide to sulfate.

In a fascinating twist, microbial associations of "Marithioploca" filaments and sheath-associated anaerobic ammonia-oxidizing bacteria ("*Candidatus* Scalindua") can recycle ammonia by anaerobic oxidation to dinitrogen via conproportionation with nitrite (Prokopenko et al. 2006, 2013). Freshly generated bioavailable reduced nitrogen in the sediments would be lost in a coupled nitrate ammonification/anammox process that, from the outside, looks like straightforward denitrification. In a detailed case study of the Soledad basin offshore Baja California, the "Marithioploca"-catalyzed nitrogen loss from the sediment was very similar to the measured efflux of ammonia; anammox rate measurements and geochemical modelling showed that the Marithioploca/Scalindua consortium contributed ca. 20–57 % of the total N_2 production. In this way, "Marithioploca" catalyzes nitrogen loss in organic-rich, highly reducing sediments where otherwise limited diffusion of the oxidants nitrate and nitrate into the sediment limits denitrification (Prokopenko et al. 2011, 2013). Thus, benthic mats of nitrate-accumulating, sulfur-oxidizing *Beggiatoaceae* represent simultaneously a source and a sink of bioavailable nitrogen, and the relative contributions of these processes will depend to a large extent on the redox state and organic carbon load of benthic sediments.

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