20 The Family *Leucotrichaceae*

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Abstract
The family *Leucotrichaecae* contains filamentous, rosette-forming, aerobic or microaerophilic, neutrophilic, sulfide-oxidizing or heterotrophic bacteria that span the physiological range from obligate sulfur-based chemolithoautotrophy to obligately heterotrophic growth without any supplemental role for sulfur oxidation. In contrast to their cousins of the family *Beggiatoaceae*, the *Leucotrichaceae* filaments are non-motile and feature a very interesting dimorphic life cycle that involves differentiation into small motile cells, called gonidia, which attach to each other and to surfaces, creating a rosette-like cluster of elongating filaments. Their systematic position was debated for a long time; as some members of the *Beggiatoaceae*, the *Leucotrichaceae* were regarded as nonphotosynthetic versions to filamentous cyanobacteria. The *Leucotrichaecae* are now placed as a distinct lineage among the *Gammaproteobacteria* based on 5S, 16S, and 23S rRNA phylogenies. Currently, the *Leucotrichaceae* contain the two genera *Leucothrix* and *Thiothrix*, which differ mostly by the extent of their sulfur metabolism. While reduced sulfur sources provide auxiliary electron donors for the essentially heterotrophic genus *Leucothrix*, they sustain mixotrophic or lithotrophic growth of *Thiothrix*.

Taxonomy, Historical and Current

Early systematic considerations of non-motile, attached, rosette-forming filaments did not always attribute these bacteria in the same group of organisms. Characteristics shared with filamentous bacteria of other taxa placed the genus *Leucothrix* in close relationship with the *Oscillatoriaceae* (Oersted 1844), and the genus *Thiothrix* with the *Beggiatoaceae* (Rabenhorst 1865; Buchanan and Gibbons 1974). The establishment of the family *Leucotrichaceae* (Buchanan 1957) eventually provided a taxonomic home for the genera *Thiothrix* and *Leucothrix*, consolidating previously recognized similarities in morphology and physiology (Harold and Stanier 1955).

The genus *Thiothrix* was among the first to be studied by ribosomal RNA sequence analyses. Initially, 5S rRNA-based phylogeny studies placed *Thiothrix* into the *Gammaproto-bacteria*, separately from the filamentous sulfur oxidizer *Beggiatoa* (Stahl et al. 1987). *Thiothrix* was also placed into the *Gammaproteobacteria* in the first 16S rRNA sequencing survey of sulfur-oxidizing bacteria (Lane et al. 1992). The first 16S rRNA gene phylogeny that included the filamentous sulfur-oxidizing bacteria *Thiothrix nivea*, *Beggiatoa alba* and *Thiopoca ingrica* showed that *Thiothrix* on the one hand, and *Beggiatoa* and *Thioploca* on the other, form two separate lineages within the *Gammaproteobacteria* (Teske et al. 1995). Subsequently, a comprehensive 16S rRNA gene sequencing study showed that the genera *Thiothrix* and *Leucothrix* are sister taxa, and form a monophyletic lineage within the *Gammaproteobacteria*; the *Thiothrix*/*Leucothrix* lineage separates from the gammaproteobacterial lineage represented by *Beggiatoa* and *Thioploca* (Howarth et al. 1999). All later additions to the genera *Leucothrix* and *Thiothrix* fall consistently into the *Leucothrix* and *Thiothrix* branches of this lineage (*Fig. 20.1*). This phylogenetic framework is remarkably consistent with the validly published family *Leucotrichaceae* (Buchanan 1957; Brock 1974) that contains the genera *Leucothrix* and *Thiothrix*, and it therefore forms the basis for the family *Leucotrichaceae* as covered in this chapter. The *Leucotrichaceae* provide a clearly defined, monophyletic framework that can accommodate novel genera and species in the future.

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, the *Thiotrichaceae* comprise physiologically and phylogenetically divergent bacteria, including the type genera (*Beggiatoa*, *Leucothrix*, and *Achromatium*) of the validly published families *Beggiatoaceae* (Migula 1894; Leadbetter 1974; Strohl 1989; Salman et al. 2011), *Leucotrichaceae* (Buchanan 1957; Brock 1974) and *Achromatiaceae* (Van Niel 1948) that are based on distinct cell morphology and physiological characteristics and hold up well in the light of modern molecular taxonomy. For these reasons, the polyphyletic family designation *Thiotrichaceae* should no longer be used.

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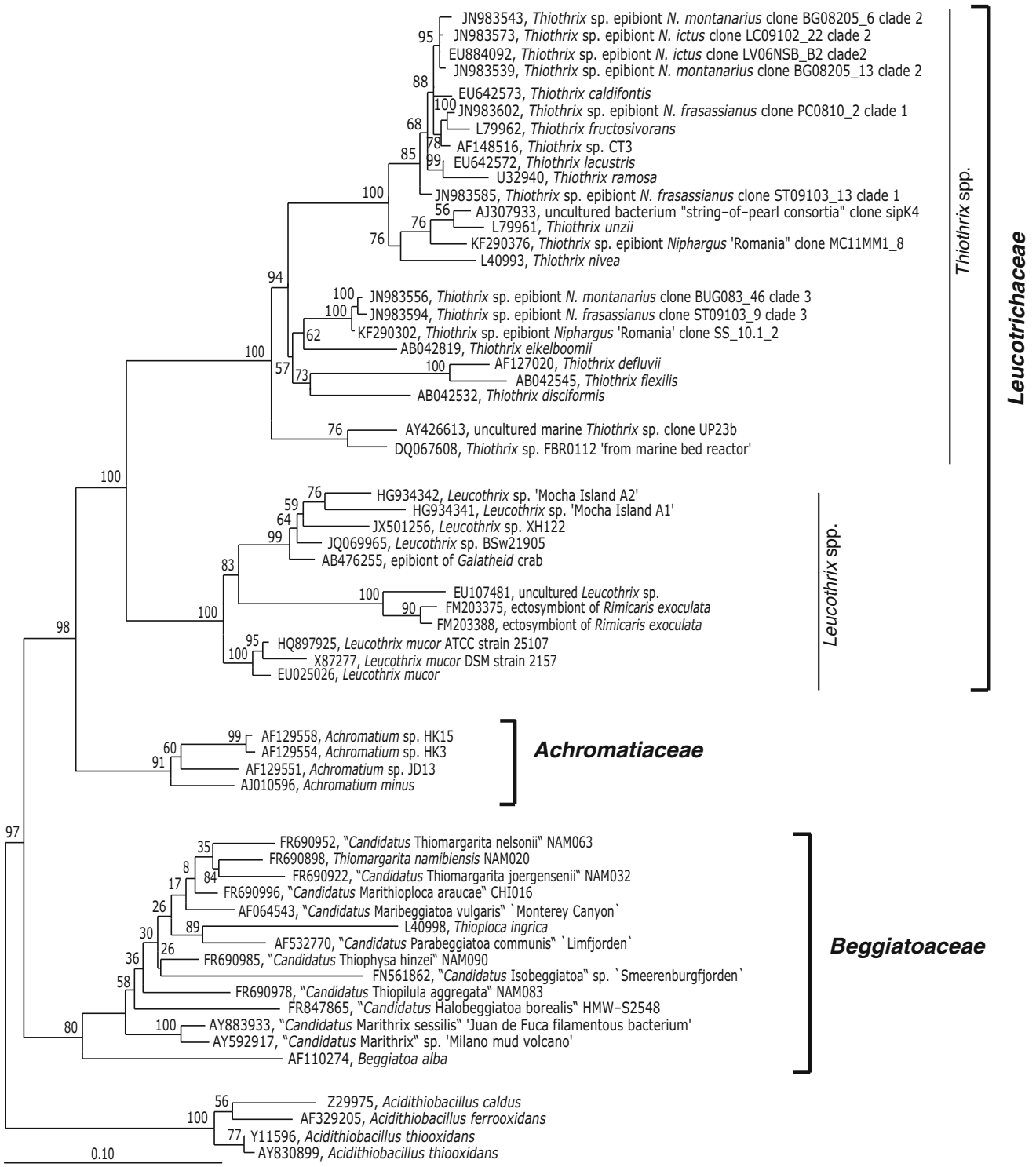

Fig. 20.1

Phylogeny of the family *Leucotrichaeceae*. The phylogenetic tree is based on near-complete 16S rRNA genes sequences, and shows the two sister genera *Leucothrix* and *Thiothrix* that constitute the *Leucotrichaceae* in relation to large sulfur bacteria of the sister families *Beggiatoaceae* and *Achromatiaceae*

This chapter provides an overview of the family *Leucotrichaecae* that synthesizes published taxonomic descriptions, physiology based on pure cultures and field samples, cell and filament morphology, and 16S rRNA gene sequences obtained from pure cultures and single cells.

Leucotrichaceae Buchanan 1957

Leu.co.trich.ac'e.ae. M.L. fem.n. *Leucothrix* type genus of the family; *-aceae* ending to denote a family; M.L. fem.pl.n. *Leucotrichaceae* the *Leucothrix* family.

Type genus: *Leucothrix* Oersted 1844.

Leu'co.thrix. Gr. adj. *leucus* clear, light; Gr. n. *thrix*, *trichis* hair; M.L. fem. n. *Leucothrix* colorless hair.

Molecular Analyses

Family *Leucotrichaceae*. The family *Leucotrichaceae* contains two genera of rosette-forming filamentous bacteria, the marine heterotroph *Leucothrix* that does not accumulate sulfur globules intracellularly, and the sulfur oxidizer *Thiothrix* that includes diverse autotrophic and heterotrophic representatives, forms intracellular sulfur globules and usually occurs in freshwater habitats. Based on 16S rRNA sequence analysis, both genera form a monophyletic lineage within the *Gammaproteobacteria* that is independent of the *Beggiatoaceae* (Howarth et al. 1999; *Fig. 20.1*). The *Leucotrichaceae* forms two major branches, one consisting of *Leucothrix mucor* and diverse *Leucothrix* phylotypes and environmental enrichments, the other including diverse *Thiothrix* species and strains that differentiate themselves into increasingly distal branches (*Fig. 20.1*). Due to historical precedent, *Leucothrix mucor* remains the type species and genus of the family (Brock 1974).

***Genus Leucothrix*.** The only described species and type species in the genus *Leucothrix*, *L. mucor* DSM 2157, has been placed at the basis of the *Leucothrix*/*Thiothrix* clade by 16S rRNA analysis (Ludwig et al. 1995; Howarth et al. 1999). Previously, *Leucothrix mucor* had been included in early 16S rRNA oligonucleotide surveys, and shown to be a member of the *Gammaproteobacteria* (Woese et al. 1985), but unrelated to filamentous *Cyanobacteria*, *Chloroflexi* (*Herpetosiphon*) and *Betaproteobacteria* (*Vitreoscilla*) (Reichenbach et al. 1986). In addition to the 16S and 23S rRNA genes of *L. mucor* str. DSM 2157 (X87277 [identical to NR_044870], X87285), the gyrB genes for gyrase subunit B (HQ897924), the soxB genes for sulfate thioesterase/sulfate thiohydrolase (EF618586), as well as an assembled genome (GB Projects PRJNA218889 and PRJNA81139) are available from Genbank. Functional gene data also exist for *Leucothrix mucor* strain DSM 621 (soxB gene, EF618580).

Although only the type species, *L. mucor*, is currently recognized, the genus *Leucothrix* does not necessarily lack diversity. Several strains of *L. mucor* isolated from different Atlantic and Pacific coastal locations had similar G+C% values (with a single exception, 48.0–49.5 %), indicating that the physiological requirements and the morphology of the organism are sufficiently specific to yield closely related strains of the same species as long as a consistent isolation procedure is maintained (Brock and Mandel 1966). However, it is very likely that diverse *Leucothrix*-like bacteria exist in nature, as shown by preliminary studies of isolated strains (Williams and Unz 1985, 1989), and by the G+C range of 46–51 mol% found among more than 30 different strains (Kelly and Brock 1969b). New 16S rRNA data from environmental studies, including epibionts of marine invertebrates, demonstrate expanding phylogenetic diversity within the genus (*Fig. 20.1*). The metadata of published database entries of *Leucothrix* environmental 16S rRNA gene clones show a preference of this bacterium for organic-rich marine habitats; examples include marine *Leucothrix* clones from a coastal algal bloom (AF195464; Kelly and Chistoserdov 2001), an arctic fjord (JQ069965), mangrove rhizosphere (JQ965724), ectobionts of cold seep and hydrothermal invertebrates, such as *Shinkaia crosnieri* and *Rimicaris exoculata* (EU107481, FM203375, FM203388, AB476255; Watsuji et al. 2010), and from larvae of the Chinese mitten crab, *Eriocheir sinensis* (EU025026). A fresh examination of *Leucothrix* laboratory strains and new isolates with modern physiological and molecular methods is overdue.

Genus *Thiothrix*. Numerous isolations, physiological studies, 16S rRNA sequencing surveys and species descriptions have greatly expanded the known species diversity within the genus *Thiothrix*. Currently, nine species are described, with the type species *Thiothrix nivea* (Larkin and Shinabarger 1983). The first comprehensive phylogeny of the genus *Thiothrix* (Howarth et al. 1999) included the type species *T. nivea* (L40993; Teske et al. 1995), the well-documented but not yet validated strain '*T. ramosa*' (U32940; Polz et al. 1996), the species *T. eikelboomii* str. AP3^T (L79965, also AB042819 and NR_024758), *T. unzii* str. A1^T (L79961), *T. fructosivorans* strains I and Q (L79962 [type strain Q] and L79963), and *T. defluvii* (AF217020; synonymous with *Thiothrix* I str. Ben57^T). The genus was subsequently enlarged with the species *T. disciformis* (AB042532 [type strain B3-1] to AB042538) and *T. flexilis* (AB042543 [type strain EJ2M-B] to AB042545), both isolated from wastewater treatment plants and capable of growth without reduced sulfur (Aruga et al. 2002), and with the two hot spring species *T. caldifontis* (EU642573, type strain G1) and *T. lacustris* (EU642572, type strain BL) which can grow as facultative lithoautotrophs with reduced sulfur sources (Chernousova et al. 2009).

Increasingly, the genus *Thiothrix* is being analyzed with functional protein-coding genes, including the gyrB gene for DNA gyrase subunit B (Chernousova et al. 2009), the cpn60 gene for the 60 kDa heat shock chaperonin (Dumonceaux et al. 2006), and the narG gene for dissimilatory nitrate reductase (Trubitsyn et al. 2013). Phylogenetic analysis based on deduced

amino acid sequences of the gyrase B subunit showed a monophyletic genus *Thiothrix*, a result that is consistent with 16S rRNA analysis (Chernousova et al. 2012). The species *T. lacustris* strains AS and BL^T, *T. caldifontis* G1^T, *T. unzii* A1^T, and *T. eikelboomii* AR3^T reduce nitrate to nitrite with thiosulfate as electron donor, and harbor the narG gene, which encodes the alpha subunit of respiratory nitrate reductase NarGHI; gene expression was demonstrated for *T. lacustris* (Trubitsyn et al. 2013). *Thiothrix nivea* (strain DSM 5205^T) and *Thiothrix* sp. strain CTD (DSMZ 12750) possess the genes (aprBA) encoding for dissimilatory adenosine-5′-phosphosulfate reductase; this key enzyme of the dissimilatory sulfate-reducing pathway is postulated to operate in the reverse direction in sulfur-oxidizing prokaryotes, oxidizing sulfite to adenosine-5′-phosphosulfate (Meyer and Kuever 2007; Meyer et al. 2007). The genome of *Thiothrix nivea* DSM 5205 has been fully sequenced at JGI (JGI Project ID 4086502; http://www.jgi.doe.gov) and is available on Genbank (Lapidus et al. 2011).

Species of the Genus *Leucothrix*

Leucothrix mucor. Oerstedt 1844. Leu'co.thrix. Gr. *leucus* clear, light; Gr. N. *thrix*, *trichis* hair; M.L. femn. *Leucothrix* colorless hair. mu'cor. L. n. *mucor* mold; M.L. n. *mucor* a genus of molds.

The type species of the genus *Leucothrix*, *Leucothrix mucor*, is the only recognized species of the genus. *L. mucor* grows in filaments of variable length, often longer than 100 µm, with a diameter of 2–3 µm. Sulfur granules are not formed. Filaments are colorless, unbranched, nonmotile, and are lacking a sheath. Filaments often grow intertwined or in dense tangles, and are attached to solid substrates by means of an inconspicuous holdfast. Individual cells within filaments round up and form ovoid to spherical gonidia, which when released acquire a jerking gliding motility. Gonidia frequently aggregate in cultures, probably chemotactically, to form rosettes. *L. mucor* is obligately aerobic and heterotrophic, does not require growth factors, grows on glutamate as sole source of carbon, nitrogen and energy, uses sugars, organic acids, and other amino acids as carbon and energy sources, and NH_4^+ as nitrogen source. Thiosulfate is used as auxiliary electron donor for lithoheterotrohic growth (Grabovich et al. 1999). Growth requires Na^+: optimal 1.5 % NaCl, minimum 0.3 %, maximal 7 %. The growth temperature optimum is 25–28 °C, the maximum is 32–35 °C; *L. mucor* also grows at 0 °C to form visible colonies within 1–2 weeks. After its first successful isolation by Harold and Stanier (1955), *L. mucor* has been reisolated by other investigators; currently nine pure culture strains are available at the American Type Culture Collection, and two strains at the German Type Culture Collection. The neotype strain is ATCC 25107/DSM 2157, isolated as an epiphyte from seaweed (*Monostroma*) in Friday Harbor, Washington (Brock 1969) and represented by 16S and 23S rRNA gene sequencing (Ludwig et al. 1995).

The mol% G+C of the type strain was estimated as 49 by buoyant density (Brock and Mandel 1966), and was determined as 47.8 by genome sequencing. The genome of *L. mucor* 2157 constitutes 5.19 Mb.

Type strain: ATCC 25107, DSM 2157.

Genbank accession numbers of 16S rRNA gene sequence: X87277.

Other taxa. The species *L. cohaerens* was isolated by Pringsheim (1957) but has been lost soon after and remains unvalidated. The more recently proposed but also unvalidated species *L. thiophila* remains to be placed by 16S rRNA gene sequencing (Dul'tseva et al. 1996).

Species of the Genus *Thiothrix*

Thiothrix nivea. (Rabenhorst 1865) Winogradsky 1888.

Thi'o.thrix Gr. neut. n. *theion* (Latin transliteration *thium*), sulfur; Gr. n. *thrix* hair; M.L. fem. n. *Thiothrix* sulfur hair; ni've. a. L. adj. *nivea*, snow-white.

The type species of the genus *Thiothrix* is mixotrophic, requires carbon substrates (acetate, pyruvate, malate, oxalacetate) for growth, and sulfide or thiosulfate as reduced sulfur source for energy supply. *T. nivea* is aerobic, but prefers reduced oxygen concentrations (ca. 10 % saturation), and can also reduce nitrate to nitrite. Rod shaped cells, about 1.0–1.5 µm in diameter, seriate in multicellular filaments (trichomes) of uniform diameter. Filaments are ensheathed and non-motile. Attachment with holdfast structure, form gonidia successively at filament end. Rosettes are formed by gonidia when in high density. Gonidia are motile by gliding, about 1–2 µm/min. Stores sulfur in the periplasm and PHA in the cytoplasm. Produces cytochrome oxidase but no catalase. Gram-negative. Optimum temperature for growth is 25–30 °C, maximum about 32–34 °C, minimum about 6–8 °C. The type strain was isolated from sulfide-containing well water in John Pennekamp State Park in Key Largo, Florida (Larkin and Shinabarger 1983).

The mol% G+C of the DNA is: 52 (T_m).

Type strain: JP2, DSM 5202.

Genbank accession numbers of 16S rRNA gene sequence: L40093.

Thiothrix caldifontis. Chernousova, Cridneva, Grabovich, Dubinina, Akimov, Rosetti and Kuever 2009.

cal.di.fon'tis. L. adj. *caldus* hot; L. n. fons, *fontis* a spring; N.L. gen. n. *caldifontis* from a hot spring, pertaining to the source of isolation of the first strains.

Mixotrophic, aerobic; chemoheterotrophic growth with a variety of organic acids, e.g. lactate, and amino acids used as carbon and energy sources; lithotrophic growth in the presence of reduced sulfur compounds, such as sulfide and thiosulfate. Rod-shaped cells with rounded ends, seriate in multicellular filaments (trichomes) with polysaccharide sheaths. Gram-negative. Cells are 0.9–2.2 µm in diameter and 3.2–6.5 µm long. Filaments are non-motile. Gliding gonidia are produced from the apical ends of the filaments. Gonidia can form rosettes. At early stages of exponential growth, a spiral form of filaments is often observed. The top cells of short filaments sometimes form pin-like bulges during the stationary growth phase. The temperature range for growth is

7–37 °C, with optimum growth at 25 °C. The pH range for growth is 7.0–8.6, with optimum growth at pH 8.0. The type strain was isolated from the sulfide spring Petushok at 33–40 °C in the Northern Caucasus region, Russia.

The mol% G+C of the DNA is 52 (T_m).

Type strain: GI, DSM21228, VKM B-2520.

Genbank accession numbers of 16S rRNA gene sequence: EU642573.

Thiothrix defluvii. Howarth, Unz, Seviour, Blackall, Pickup, Jones, Yaguchi and Head 1999.

de.flu'vi.i. L. neut. n. *defluvium* sewage; L. gen.n. *defluvii* of sewage.

Mixotrophic, aerobic; isolates are extremely slow growing and biochemical properties of this organism have not been determined. Rods, variable in shape (cylindrical, barrel-shaped, frequently elongate and swollen) in multicellular filaments (trichomes) with base to tip differentiation, apical cells are 1.0–2.0 μm in diameter and 5.0–10.0 μm in length, whereas cells at the base of trichomes are 2.0–4.0 μm in diameter and 0.5–10 μm in length. Trichomes are unsheathed but may form knots; rosettes, holdfasts, and gliding gonidia are present in some strains. Gram negative or gram-variable. Growth occurs in the temperature range of 10–30 °C but not at 4 °C or 37 °C. Deposits intracellular sulfur but no PHA. Isolated from an activated sludge treatment plant in Australia.

The mol% G+C of the DNA is: unknown.

Type strain: Ben57.

Genbank accession numbers of 16S rRNA gene sequence: AF127020.

Thiothrix disciformis. Aruga, Kamagata, Kohno, Hanada, Nakamura and Kanagawa 2002.

dis.ci.for'mis. L. masc. n. *discus* a disc; L.n. *forma* shape; N.L. adj. *disciformis* disc-shaped, the main cell form.

Mixotrophic, aerobic; reduced inorganic sulfur is not required for growth, but internal storage of sulfur and production of sulfuric acid are observed when present. Glucose, fructose, mannose, sucrose, maltose, trehalose, mannitol, succinate, pyruvate, acetate, malate, butyrate, hydroxy-butyrate, glutamate, glycerol and aspartate are utilized as sole carbon sources. Almost all strains utilize citrate and alanine. Growth is inhibited by 0.5 % (w/v) NaCl. Cells are rods and form slightly bent multicellular filaments. Filaments are more than 0.5 mm long and can reach several millimeters. Colonies are fingerprint-like. Cell morphology in filaments is variable, particularly for length, and discoid to ovoid shaped cells are often observed. Cells of the major form are 1.2–3.0 μm in diameter and 0.5–3.0 μm in length. Elongate and swollen cells are sometimes present in filaments. Gram-negative. A sheath is absent. Sudanophilic granules are observed. Growth occurs in the temperature range 14–32 °C and pH range 7.0–7.9, but there is no growth at 4 or 37 °C. Optimum temperature 25–30 °C. Oxidase-positive and catalase-positive (violent bubble generation). Nitrate is not reduced. The G+C content of the DNA is 44–45 mol% (HPLC). Isolated from activated sludge suffering from bulking.

The mol% G+C of the DNA is 43.9–44.7.

Type strain is B3-1^T = DSM 14473^T = JCM 11364^T.

Genbank accession numbers of 16S rRNA gene sequence: AB042532.

Taxonomic comment: Additional strains of *T. disciformis* are B4-1 (=JCM 11365), B2-7 (=JCM 11362), SCM-A (=JCM 11132), B5-1 (=JCM 11366), B2-8 (=JCM 11363) and OS-F (=JCM 11131); their 16S rRNA gene sequences are available from Genbank under accession numbers AB042532 to AB042538 (Aruga et al. 2002).

Thiothrix eikelboomii. Howarth, Unz, Seviour, Blackall, Pickup, Jones, Yaguchi and Head 1999.

ei.kel.boom'i.i. M.L. gen. n. *eikelboomii* of Eikelboom, named for D.H. Eikelboom, who pioneered morphological identification of filamentous bacteria in wastewater.

Mixotrophic, aerobic; uses the widest variety of organic compounds among *Thiothrix*. Forms internal sulfur deposits but does not require reduced sulfur for growth. Rods may vary in shape (cuboidal, barrel-shaped, cylindrical, discoid, bead-like) depending on location in filaments; apical cells are 0.6–0.8 × 1.0–1.5 μm and frequently bead-like, whereas cells at the base of filaments are discoid, with 1.0–3.0 μm in diameter and 0.4–0.7 μm in length. Filaments have no sheath but may form knots, rosettes and a holdfast. The type strain does not form rosettes. Gliding gonidia occur only in rosette-forming strains and have a tuft of monopolar fimbriae but lack flagella. Gram negative or gram variable. Growth occurs in the temperature range of 10–33 °C but not at 37 °C. Growth pH range is 6.5–8.5. Isolated from activated sludge treating domestic wastewater (Williams and Unz 1985).

The mol% G+C of the DNA is 44.1–46.1.

Type strain: AP3, ATCC 49788.

Genbank accession numbers of 16S rRNA gene sequence: AB042819.

Taxonomic comment: Numerous strains of *T. eikelboomii* have been isolated mostly from wastewater treatment plants, which resulted in a correspondingly large number of 16S rRNA sequences deposited in Genbank. Initially, *T. eikelboomii* consisted of a cluster of several mutually closely related strains (Genbank entries L79965, AF126148, AF126150, AF126151, AF126153, AF126154, AF126155) from the USA, Australia and Japan (Howarth et al. 1999). Additional isolates (strain Tl-4 [=JCM 11127], Genbank entry AB042540; strain Tl-2 [=JCM11128], Genbank entry AB042541; strain COM-A [=JCM11133], Genbank entry AB042542; and strain KR-A [=JCM11129], Genbank entry AB042539) have amended and enlarged the species to its current definition (Kanagawa et al. 2000; Aruga et al. 2002).

Thiothrix flexilis. Aruga, Kamagata, Kohno, Hanada, Nakamura and Kanagawa 2002.

Fle.xi'lis. L. adj. *flexilis* pliable.

Mixotrophic, aerobic; reduced inorganic sulfur is not required for growth. When reduced inorganic sulfur compounds are present, only few sulfur granules are deposited, and frequently absent in situ. No or only slight production of sulfuric acid. Glucose, fructose, mannose, sucrose, maltose, trehalose, mannitol, lactate, propionate, succinate, pyruvate, acetate, malate, hydroxybutyrate, glutamate and aspartate are utilized

as sole carbon sources. Almost all strains utilize citrate and alanine. Cells are rod-shaped and form slightly bent multicellular filaments. Filaments are more than 0.5 mm long and can reach several millimetres. Colonies are fingerprint-like. Cell morphology in filaments is variable, particularly for length, discoid to ovoid-shaped cells are often observed. Cells are 1.0–4.0 μm in diameter and 0.5–5.5 μm in length. Elongate and swollen cells are sometimes present in filaments. Gram-negative. A sheath is absent. Rosettes and holdfasts are observed in some but not all strains. Gliding gonidia are produced from the end of the filaments only in rosette-forming strains. Sudanophilic granules are observed. Growth occurs in the temperature range 14–37 °C and pH range 7.0–7.9, but there is no growth at 4 °C or 42 °C. Optimum temperature 20–30 °C. Oxidase and catalase-positive. Good growth occurs in 0.1 % NaCl and growth is inhibited slightly in 2 % (w/v) NaCl. Nitrate is reduced to nitrite. Isolated from activated sludge suffering from bulking.

The G+C content of the DNA is 44 mol%.

Type strain: EJ2M-B^{T} = DSM 14609^{T} = JCM11135^{T}.

Genbank accession numbers of 16S rRNA gene sequence: AB042545.

Taxonomic comment: Additional strains of *T. flexilis* are EJ1M-B (=JCM 11134; Genbank entry AB042544) and SNR-3 (=JCM 11130, Genbank entry AB042543) (Aruga et al. 2002).

Thiothrix fructosivorans. Howarth, Unz, Seviour, Blackall, Pickup, Jones, Yaguchi and Head 1999.

fruc.to.si.vor'ans. M.L. neut. n. *fructosum* fructose; L. part. pres. *vorans*, eating; M.L. adj. *fructosivorans* fructose-eating.

Mixotrophic, aerobic; no requirement for reduced sulfur compounds for growth, but deposits internal sulfur when present. Grows on fructose, sucrose, melezitose, pyruvate, succinate, malate, acetate, lactate, and propionate as carbon and energy source, and hydrolyses gelatin. Stores volutin inclusions, sudanophilic granules and PHA. Sole nitrogen sources are ammonia, nitrate, proline, and cys-glucosamine. Rod-shaped cells are 1.2–2.5 × 2.7–4.5 μm, in multicellular filaments. Gram-negative, ensheathed, forming rosettes and holdfast, gliding gonidia with monopolar fimbriae but no flagella. Oxidase positive and weakly catalase positive. Growth occurs in the temperature range of 4–28 °C but not at 33 °C. Growth at pH range 6.5–8.5. Isolated from activated sludge treating domestic wastewater (Williams and Unz 1985).

The mol% G+C of the DNA is 51.5 (Chernousova et al. 2009).

Type strain: Q, ATCC 49748.

Genbank accession numbers of 16S rRNA gene sequence: L79962.

Taxonomic comment: a second strain of *T. fructosivorans*, strain I (ATCC 49749; Genbank accession No. L79963) is closely related to the type strain and was isolated from the same wastewater treatment plant (Williams and Unz 1985).

Thiothrix lacustris. Chernousova, Cridneva, Grabovich, Dubinina, Akimov, Rosetti and Kuever 2009.

la.cus'tris. N.L. fem. adj. *lacustris* belonging to a lake, referring to the site from where the type strain was isolated.

Mixotrophic, aerobic; chemoheterotrophic growth with a variety of organic acids and amino acids used as carbon and energy sources; lithotrophic growth in the presence of reduced sulfur compounds produces sulfuric acid. Maximum cell counts obtained during mixotrophic growth in the presence of thiosulfate, lactate and other organic substrates. Oxidase positive, catalase negative. Nitrogen sources are peptone, yeast extract ammonium, nitrate and nitrite. Rod-like cells with rounded ends, 0.9–2.3 μm in diameter and 4.4–6.3 μm long, occur in sheathed, non-motile filaments. Gram-negative. Gliding gonidia are produced from the apical ends of the filaments. Gonidia can produce rosettes. At early stages of exponential growth, a spiral form of filaments is often observed. During the stationary growth phase, pin-like bulges can appear on the ends of short filaments. The temperature range for growth is 5–32 °C, with optimum growth at 24 °C. The pH range for growth is 6.2–8.2, with optimum growth at pH 7.0. The type strain was isolated from a low-temperature lake of the Blue Lake system (Kabardino-Balkaria).

The mol% G+C of the DNA is 51.4.

Type strain: BL, DSM 21227, VKM B-2521.

Genbank accession numbers of 16S rRNA gene sequence: EU642572.

Thiothrix unzii. Howarth, Unz, Seviour, Blackall, Pickup, Jones, Yaguchi and Head 1999.

Un'zi.i. M.L. gen.n. unzii of Unz, named for R.F. Unz.

Mixotrophic, aerobic; requires reduced inorganic sulfur for growth. Gelatin and casein are hydrolyzed. Carbon sources are pyruvate, succinate, acetate, lactate and propionate. Weak growth with malate. Stores sulfur, volutin, lipids and PHA. Oxidase positive, catalase negative. Nitrogen sources are ammonia, nitrate, asparagine, glutamine, aspartate, glutamate, and glucosamine. Nitrate is reduced to nitrite. Rods 0.7–1.5 × 1.5–3.0 μm, in multicellular filaments. Gram-negative; filaments without sheaths, rosettes formed, holdfast present, gliding gonidia with monopolar fimbriae but no flagella. Growth occurs in the temperature range of 4–33 °C but not at 37 °C. Growth at pH range 6.5–8.5. Isolated from activated sludge treating domestic wastewater (Williams and Unz 1985).

The mol% G+C of the DNA is 49.3 (Chernousova et al. 2012).

Type strain: A1, ATCC 49747.

Genbank accession numbers of 16S rRNA gene sequence: L79961.

Other taxa: a second strain of *T. unzii*, strain TN (not deposited in ATCC; Genbank accession No. KF720709) is closely related to the type strain and was isolated from a sulfide spring in the North Caucasus (Belouscova, Dubinina and Grabovich, unpublished).

"***Thiothrix ramosa***". Odintsova and Dubinina 1990a.

Ra.mo'sa. L. fem. adj. ramosa, full of boughs, having many branches, branching, branchy.

This isolate is physiologically and molecularly well-studied (Odintsova and Dubinina 1990a, b, 1993; Odintsova et al. 1993; Polz et al. 1996), but has not yet been included in the list of prokaryotic names with standing in nomenclature (Parte 2014).

"*Thiothrix ramosa*" is capable of aerobic, mixotrophic and autotrophic growth, and can grow in purely lithoautotrophic medium. Oxidizes sulfide, thiosulfate, tetrathionate and carbon disulfide, when lactate is present as carbon source, it also grows mixotrophically on the sulfur sources methanethiol and diethyl sulfide. Substituted thiotherpenes can also be used as sole substrates. Isolated from a spring in Latvia.

The mol% G+C of the DNA is 51–52 mol%.

Type strain: A1, ATCC 49747.

Genbank accession numbers of 16S rRNA gene sequence: U32940.

Additonal *Thiothrix* isolates have not yet been validated as new species: The facultatively autotrophic *Thiothrix* strain CT3, from activated sludge, is a close relative of *T. fructosivorans* (AF148516) (Rosetti et al. 2003). The isolate "*Thiothrix arctophila*" remains to be placed by 16S rRNA sequencing (Dul'tseva and Dubinina 1994).

Phenotypic Analyses

General characteristics of the genus *Leucothrix*. This short characterization of the genus *Leucothrix* follows the succinct chapter on *Leucothrix* by Brock (2006). *Leucothrix* is identified on the basis of morphological examination with the light microscope. The filaments are of large diameter (greater than 2 µm; average, 2–3 µm), and each filament is composed of short cylindrical or ovoid cells, with cross-walls clearly visible (*Fig. 20.2c*). Cell division is not restricted to either end but occurs throughout the length of the filament, as shown by autoradiography with tritiated thymidine (Brock 1967). The filaments are colorless, unbranched, and of variable length; they may reach lengths of 0.1–0.5 cm. The filaments do not taper, but there may be variation in diameter along the length of the filaments. The free filaments never glide (thus distinguishing them from many other filamentous gliding bacteria, such as *Beggiatoa* and *Vitreoscilla*), although they occasionally wave back and forth in a jerky fashion. Rosette formation is a key diagnostic characteristic; it is not possible to easily identify an isolate as *Leucothrix* without observation of the presence of rosettes. *Leucothrix* is found widely in the littoral zone in marine environments worldwide, growing primarily as an epiphyte of micro- and macroscopic algae or animals (*Fig. 20.2a, b*). Although all *Leucothrix* strains that have been isolated so far are marine and require NaCl for growth, the possible existence of freshwater strains should not be ruled out.

Life cycle of *Thiothrix* and *Leucothrix*. *Leucothrix*, as well as *Thiothrix*, are characterized by a dimorphic lifecyle that includes the transition from a static multicellular filamentous form to a motile unicellular form (*Fig. 20.3*). Under environmental conditions unfavorable to rapid growth, such as low temperature or low nutrient concentration, individual cells of the filaments become round and form ovoid structures called "gonidia," which are either released individually, often from the tips of the filaments, or, as more typically, the entire filaments disintegrates (*Fig. 20.3*). The gonidia are able to glide in a jerky manner,

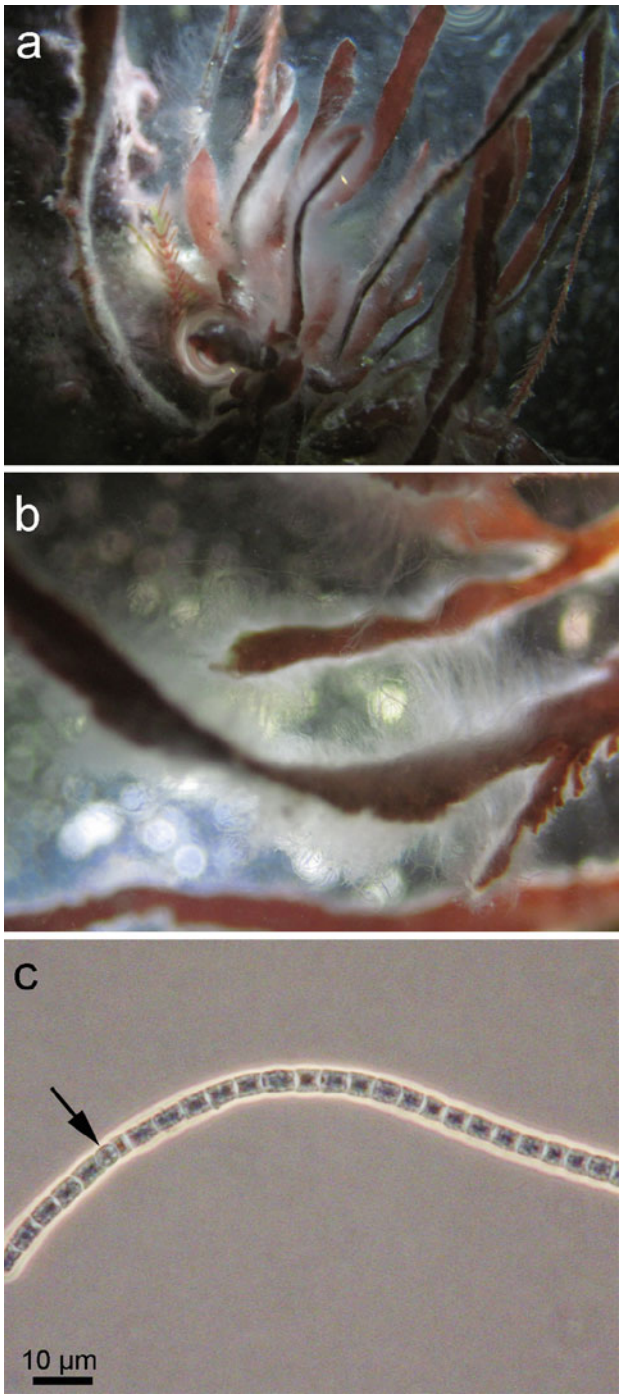

Fig. 20.2
Natural enrichment of *Leucothrix* spp. These *Leucothrix* enrichments are growing on red algae at shallow-water methane seeps of Mocha Island, Chile (Jessen et al. 2011; Sellanes et al. 2011). (**a**) *Leucothrix* filaments cover the algal fronds (*Gelidium lingulatum*) in a thick epiphyte layer. (**b**) Close-up view of the colorless *Leucothrix* filaments; the algal fronds are ca. 3–5 mm wide. (**c**). Individual *Leucothrix* filament; note the absence of sulfur globules, and the round shape of some cells (*arrow*) that most likely represent the initial stage of gonidia formation (Photos courtesy of Eduardo Tejos, Universidad de Concepcion, Chile (**a**, **c**), and adapted from Sellanes et al. 2011 (**b**))

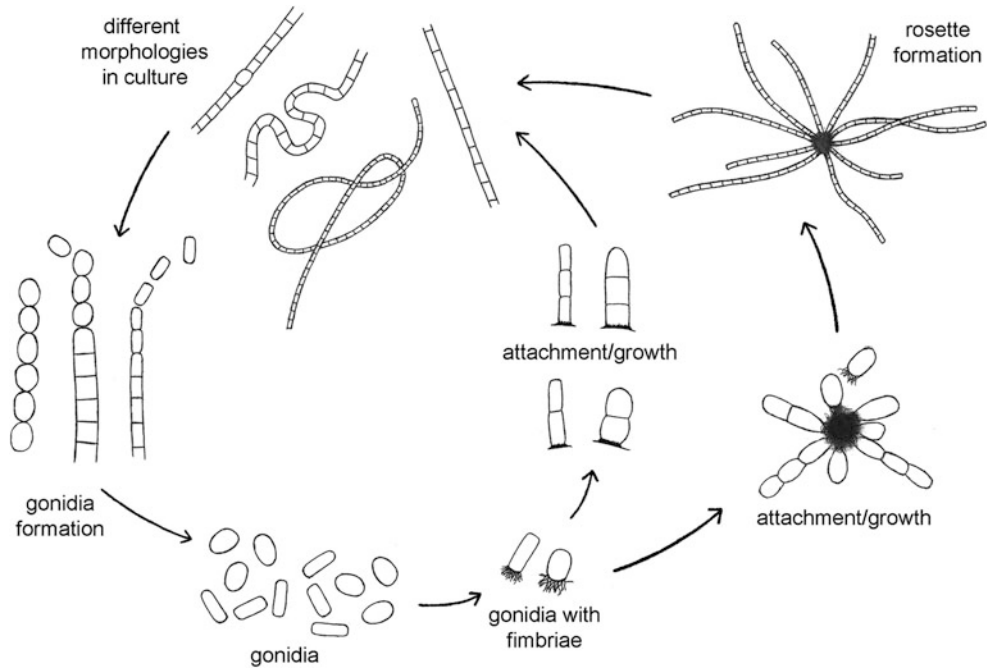

Fig. 20.3

Life cycle drawing of *Thiothrix* and *Leucothrix* as observed in culture studies. Attached filaments form apical gonidia that are released into the medium and are typically motile; they form fimbriae on one pole and attach to surfaces where they grow out into new filaments. Rosettes are formed when gonidia attach to each other when a high density of gonidia is present (Adapted from Brock 2006)

form fimbriae on one pole, and attach when they come into contact with a solid surface. They settle down on biotic or abiotic surfaces, synthesize a holdfast matrix, and form new filaments through growth and successive cell divisions. Attachment to other gonidia causes rosette formation (*Fig. 20.3*). The holdfast structure is inconspicuous, but fluoresces red when stained with primulin and viewed under blue light. In nature, the gonidia are presumably elements of dispersal and enable the organism to spread to other areas. Brock (2006) emphasizes that the term "gonidia," first used by Winogradsky (1888) for *Thiothrix*, should not be considered as the designation of some sort of unique structure; the gonidium of *Leucothrix* and *Thiothrix* corresponds functionally and structurally to the motile dispersal structure or hormogonium of the cyanobacteria, which is formed by the rounding up of a vegetative cell or group of cells in a filament.

Gonidia may form by terminal differentiation of a filament tip as in *Thiothrix*, or by division and dissociation of a fully formed filament, as in *Leucothrix*. Gonidia do not have a holdfast when first formed, but make it only in response to the proper environmental conditions. Individual gonidia may aggregate if they occur in sufficiently high concentrations. A new filament grows out from each gonidium, eventually resulting in a large and striking structure, a rosette (*Figs. 20.3* and *20.4a*). Rosette formation is found in both *Leucothrix* and *Thiothrix* and is an important means of distinguishing these organisms from many other filamentous bacteria.

Another interesting characteristic of *Leucothrix* is the ability of filaments to grow in such a way that knots are formed (*Figs. 20.3* and *20.4b*; Brock 1964). Knots occur mainly when the organism is growing in rich liquid culture media, where filamentous growth is rapid. Knot formation is frequent enough in *Leucothrix* cultures to be used as a taxonomic characteristic. Filaments in culture often form true knots, and the presence of knots in a culture may be considered indicative of *Leucothrix* even without the formation of rosettes. However, the density of knots is never high, and a number of microscopic fields must be searched to ascertain if knots are present. Knot formation is most frequent when growth occurs to a high cell density in a relatively rich culture medium. Larger structures, bulbs, may form in knotty cultures, probably as result of fusion of cells in the region of the knots (Brock 1964; Pringsheim 1957; Snellen and Raj 1970).

Physiology of *Leucothrix*. *Leucothrix* is chemoorganotrophic, strictly aerobic, and never deposits sulfur. It can use a wide variety of sugars and other organic compounds, particularly glutamate, mannose, and peptone, but not xylose as sources of nitrogen and/or carbon and energy (Bland and Brock 1973; Harold and Stanier 1955; Raj 1967). Most *L. mucor* strains require vitamin B12 for growth (Kelly and Brock 1969a, b). The deletion of calcium from the growth medium prevents rosette formation, but filaments tend to bundle (Snellen and Raj 1970). Lewin (1959) found that both Ca^{++} and K^{+} are required for growth. Ammonium can be used as nitrogen source. Na^{+} is required for growth, optimum is 1.5 % NaCl, minimum 0.3 %, maximum 7 %. Optimum temperature is 25–28 °C, maximum 32–35 °C, and it grows at 0 °C to form visible colonies within 1–2 weeks.

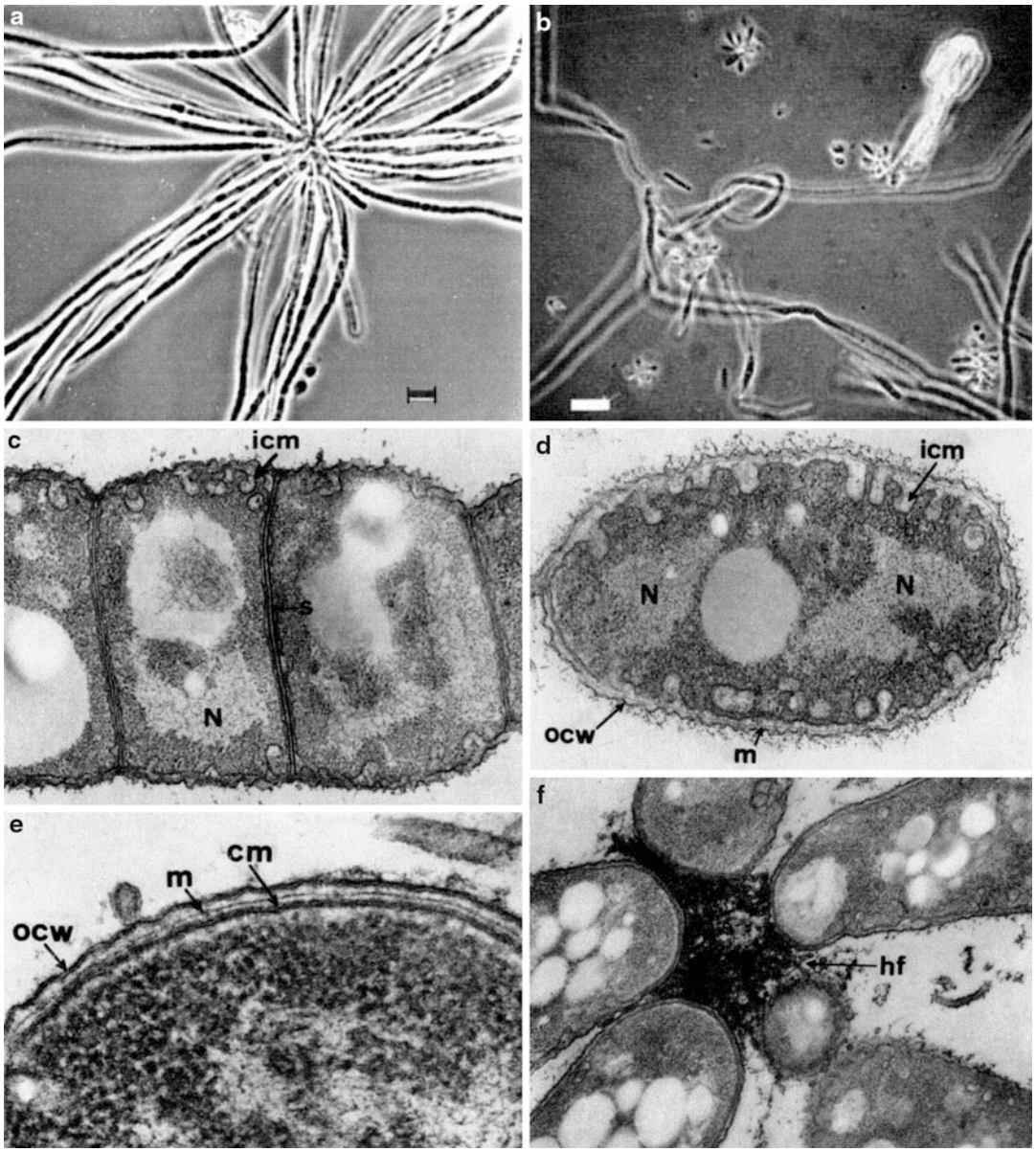

Fig. 20.4

Microscopic images of *Leucothrix spp*. (a) Rosette composed of several *Leucothrix* filaments. Phase-contrast photomicrograph. Bar = 10 μm. (b) Knots formed by filaments of *Leucothrix mucor*, and gonidia aggregates as rosettes. Phase-contrast photomicrograph. Bar = 5 μm. (c) Filament of *Leucothrix mucor* with prominent invagination of the cytoplasmatic membrane (icm) that occur on the periphery of the cells but can also be found along the cross walls that separate cells. *N* nuclear body, *s* cross wall septum, magnification ×55,000. (d) Cross sections of gonidial cell with numerous invaginations of the cytoplasmic membrane. The gonidia retain the cell envelope structure of the filaments and the fine structure of the cytoplasm. *ocw* outer cell wall, *m* middle layer of cell wall, most likely peptidoglycan, magnification ×84,000. (e) Electron micrograph of the cell envelope region illustrating that the middle layer (*m*) is single, whereas the outer cell wall layer (*ocw*) and cytoplasmic membrane (*cm*) are double unit membranes. Magnification ×155,500. (f) Cross sections through rosette of *Leucothrix mucor* filaments; the holdfast (*hf*) appears as the electron-dense material between the basal cells of the rosette. Magnification ×40,000. Photomicrographs (a) and (b), Brock 2006. Electron micrographs c–f, Brock and Conti 1969

While *Leucothrix* is characterized by a heterotrophic, aerobic metabolism, its growth is also stimulated by the presence of reduced sulfur sources that serve as auxiliary electron donors, as shown for L. *mucor* (Grabovich et al. 1999). In lithoheterotrophic growth, metabolic energy could be obtained by both substrate-linked phosporylation via adenosin-5′-phosphosulfate reductase (operating in oxidative direction from sulfite to sulfate), as well as through oxidative phosphorylation involving membrane-bound cytochromes that oxidize thiosulfate and sulfite (Grabovich et al. 1999). Yet, thiosulfate oxidation is not accompanied by the formation of intracellular sulfur globules (Grabovich et al. 1999). Informal reports of sulfur-accumulating

Leucothrix strains (as included in Brock 2006, and Bland and Brock 2005) have so far remained unconfirmed by published physiological studies or molecular analyses. Therefore, periplasmic sulfur inclusions remain a characteristic of the genus *Thiothrix*, but not *Leucothrix*.

Cell structure of *Leucothrix*. The ultrastructure of *Leucothrix* is typical prokaryotic (Brock and Conti 1969; Snellen and Raj 1970; Webster et al. 1968). The cell wall is typical gram-negative (*Fig. 20.4e*); it is multilayered, in contrast to gram-positive cell walls, running unparallel to its more nearly planar cytoplasmic membrane and the outer wall layer. The peptidoglycane layer is single and ca. 2 nm wide. The convoluted outer wall layer is double and ca. 9 nm wide, and consists of lipid, polysaccharide, and protein with the hydrophilic portion made of sugars and the hydrophobic portion of a unique lipid material (Brock 1974; Brock and Conti 1969). During the life cycle of the organism, little ultrastructural differentiation can be observed. There are no significant changes in cell wall structure during cell division, or when gonidia or rosettes are formed (*Fig. 20.4c, d*). However, the filaments in the process of knot formation show contorted cell walls and marked deformation of the cell wall septa (Brock and Conti 1969). Cell division is initiated by transverse septation, typically perpendicular to the long axis of the dividing cell. Every cell in the filament can initiate division independently. The formation and biological function of bulbs that are occasionally found along the filaments of *Leucothrix* are unknown until today. In rare cases "bulb-tubes" have been observed, describing a thin projection from one side of the bulb having a continuous connection of cell wall, cytoplasmic membrane and cytoplasm with the main body of the bulb (Snellen and Raj 1970).

All *Leucothrix* filaments show fimbrillar nuclear material, ribosomes, and some storage granules in the cytoplasmic matrix (*Fig. 20.4c–f*). Invaginations occur most frequently at the periphery of the cell, not at the septa. In electron micrographs from thin sections, the cytoplasm includes invaginations, cavities and vacuolar bodies (*Fig. 20.4c, d, f*), which are suggested to contain carbon storage compounds such as poly-ß-hydroxyalcanoates and lipids (Brock and Conti 1969). Thin sections through rosettes show the typical holdfast structure at the bottom of each attached filament as electron dense material (*Fig. 20.4f*), possibly consisting of polysaccharides that are peripheral to the outer cell wall layer (Brock 1966; Brock and Conti 1969).

General characteristics of the *genus Thiothrix*. The genus characterization by Unz and Head (2005) is amended here in the light of new species descriptions (Aruga et al. 2002; Chernousova et al. 2009) and physiological studies (Trubitcyn et al. 2013). *Thiothrix* typically occur in sewage water or attached as saprophytes to macroalgae, stones or other solid substrates in fast-flowing sulfidic streams or springs (*Fig. 20.5a*). All known species are mesophiles and neutrophiles. Depending on the species, *Thiothrix* cells are rod-shaped ranging from 0.7–4.0 µm width and 1.2–6.3 µm length, and seriate in rigid, unbranched, multicellular filaments of uniform or slightly tapering diameter. The filaments can reach lengths of over

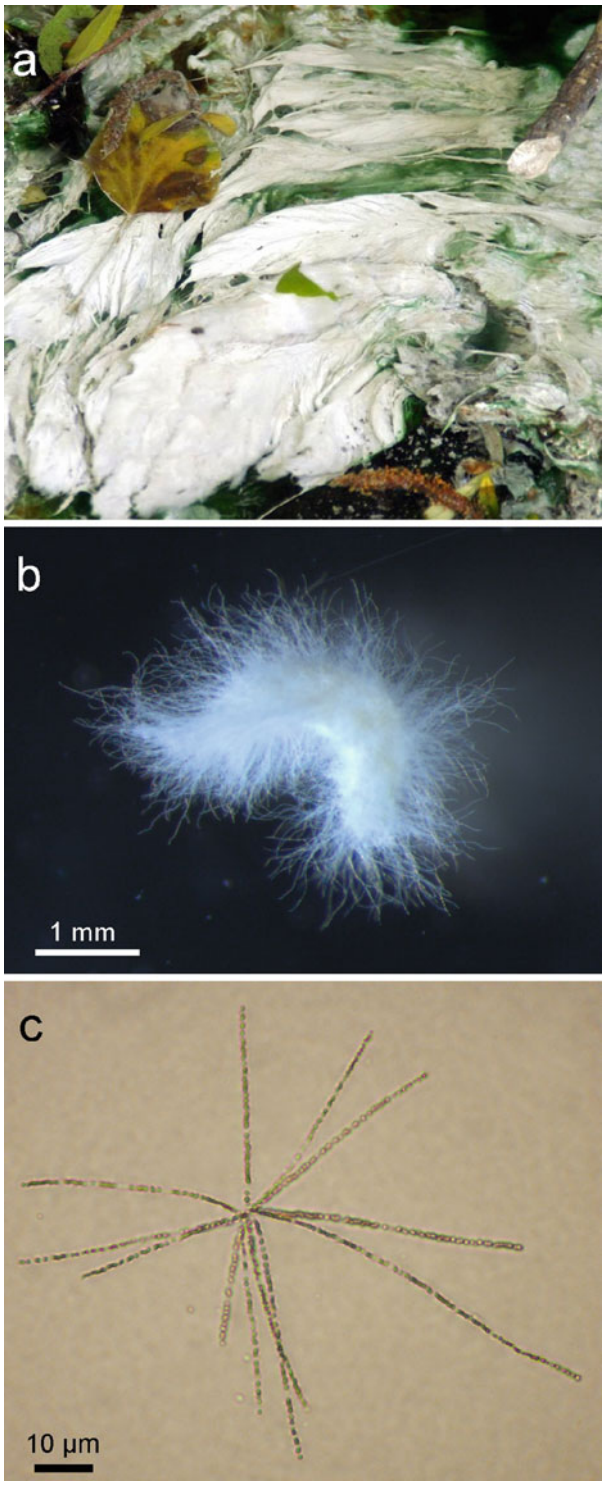

Fig. 20.5
Natural enrichment of *Thiothrix* spp. (a) White streamers of *Thiothrix* overgrowing leaf litter in the sulfidic stream near the entrance to Frasassi cave, Italy. (b) Floc of white *Thiothrix* filaments attached to small plant particle from this location. (c) Light microphotograph of rosette-forming *Thiothrix* filaments from the Frasassi location, with numerous sulfur inclusions (Photos courtesy of Lubos Polerecky, University of Utrecht, The Netherlands (a), and Verena Salman, UNC Chapel Hill (b, c))

500 µm (uniform) or 100–200 µm (tapered filaments), and can be ensheathed in some species. Rod-shaped gonidia with gliding motility are formed at the apical ends of filaments. Filaments often grow as rosettes and are anchored to a common holdfast (Fig. 20.5b, c). Only rosette-forming filaments produce gonidia. Resting stages are not known. Flagella are absent from the filaments, but the gonidia are motile due to a tuft of monopolar fimbriae.

All species grow as aerobes or microaerophiles, sometimes also by nitrate reduction to nitrite, and show facultatively autotrophic, chemoorganotrophic and mixotrophic metabolisms. The mixotrophic species require any of several small organic compounds, such as acetate, lactate, propionate, pyruvate, succinate, fumarate and oxalacetate, as well as reduced sulfur sources. Cells grown in the presence of an inorganic reduced sulfur source have intracellular sulfur globules. The sulfur globules appear to be internal by light microscopy, and electron microscopy indicates intracellular deposition within invaginations of the cytoplasmic membrane.

Physiology of *Thiothrix*. The genus *Thiothrix* includes filamentous, rosette-forming sulfur oxidizing bacteria that range in their physiology from predominantly heterotrophic species and strains (*T. eikelboomii*, *T. flexilis*, *T. disciformis*; Aruga et al. 2002) that use sulfide only as an auxiliary electron donor (Williams and Unz 1985, 1989), to species that are capable of sulfur-oxidizing autotrophic growth; the latter include '*T. ramosa*', (Odintsova et al. 1993) *Thiothrix* CT3 (Tandoi et al. 1994), *T. caldifontis* and *T. lacustris* (Chernousova et al. 2009), and a strain that was identified phenotypically as *T. nivea* (McGlannan and Makemson 1990). The neotype strain *Thiothrix nivea* has previously been described as an obligate mixotroph since pure cultures required both a reduced sulfur compound and an organic substrate for growth (Larkin and Shinabarger 1983; Strohl and Schmidt 1984; Nelson 1989); the results by McGlannan and Makemson (1990) suggest that autotrophic strains of *T. nivea* exist in nature. High rates of sulfide oxidation to sulfate (Strohl and Schmidt 1984) and oxidation of sulfur globules and sulfide to sulfate have also been reported for *T. nivea* (Schmidt et al. 1987).

The species of the genus *Thiothrix* can be divided into phylogenetically and physiologically defined clusters (Fig. 20.1) that show contrasting sulfur and carbon utilization patterns. The first is the "*T. nivea*" group, a monophyletic branch containing the species *T. nivea*, *T. unzii*, "*T. ramosa*", *T. fructosivorans*, *T. caldifontis*, and *T. lacustris*, where the first three require sulfur as electron donor for growth and the others prefer reduced sulfur compounds for mixotrophic growth.

The second major group is the "Eikelboom type 021N" filaments (Eikelboom 1975, 1977) represented by the species *T. eikelboomii*, *T. disciformis*, *T. defluvii*, and *T. flexilis*. The Eikelboom type 021N strains grow heterotrophically on a wide range of carbohydrates without requiring reduced sulfur (Aruga et al. 2002), while *T. nivea* JP2^T and *T. unzii* A1^T require reduced sulfur for growth (Larkin and Shinabarger 1983; Williams and Unz 1985). The filaments of Eikelboom type 021N bacteria are very long and reach several millimeters in length, while those of members of the *T. nivea* group are less than 1 mm long (Eikelboom 1975; Kanagawa et al. 2000; Williams and Unz 1985). The Eikelboom type 021N strains form fingerprint-like colonies, a characteristic different from members of the *T. nivea* group (Williams and Unz 1985; Kanagawa et al. 2000). In contrast to the Eikelboom type 021N strains, *T. nivea* JP2^T and *T. unzii* A1^T have no catalase activity (Larkin and Shinabarger 1983; Williams and Unz 1985). *T. nivea*, '*T. ramosa*' and *T. fructosivorans* form sheaths (Larkin and Shinabarger 1983; Odintsova and Dubinina 1990b; Williams et al. 1987), while the Eikelboom type 021N strains do not.

Cell structure of *Thiothrix*. The following summary of *Thiothrix* cell structure is based on the microscopic and ultrastructural studies of Bland and Staley (1978), and Williams et al. (1987) that should be consulted for further information. Multiple filaments, each consisting of several hundred seriate cells, are growing as a rosette emerging from a central branching point (Figs. 20.5c and 20.6e). Multicellular *Thiothrix* filaments consist of generally cylindrical cells; barrel-shaped, cuboidal, bead-like or discoid cell morphologies can also be found (Fig. 20.6a–d). The filaments are attached to each other and/or a solid surface (Fig. 20.5b) by an extracellular holdfast matrix that appears to be secreted by the basal cells of the rosette-forming filaments (Fig. 20.6e, f). The tips of individual filaments may divide into gonidial cells that, upon release, disperse, divide and then form the nucleus of new filaments, or aggregate into small rosettes of gonidia that grow into new rosettes of full-length filaments (Fig. 20.3). The gonidia have tufts of fimbriae that are located predominantly around one pole of the cell; these fimbriae attach to each other and establish intracellular contact (Larkin and Nelson 1987). The filaments of some *Thiothrix* species are surrounded by sheaths (Fig. 20.6b, g, h); in contrast to other sheathed sulfur-oxidizing bacteria, for example the genus *Thioploca*, each sheath contains only a single filament in *Thiothrix*. An unusual structural feature of *Thiothrix* filaments that requires additional documentation and follow-up study are extensive filamentous appendages that appear to grow on the fully developed filaments (sampled from a natural sulfur spring) like a coat of bristles (Morita and Burton 1965; Larkin 1980).

Thiothrix cells contain sulfur globules that are surrounded by two membranes; the vacuolar membrane immediately adjacent to the sulfur granule is surrounded by a distinct cytoplasmatic membrane that is sometimes connected to the cytoplasma membrane by invaginations (Fig. 20.6i). The sulfur globules are therefore topologically located in the periplasmic space. As in the family *Beggiatoceae*, sulfur globules in *Thiothrix* function as an electron donor reservoir; they are formed and consumed depending on the availability of reduced sulfur sources in the environment. Polyhydroxyalkanoates (Fig. 20.6a, d) and polyphosphates (Fig. 20.6f) form additional cytoplasmatic cell inclusions.

Each cell of *Thiothrix* is surrounded by a cytoplasmic membrane, followed by a peptidoglycan layer located within the periplasmic space, and an outer membrane of variable thickness due to additional membrane layers that appear in some species (Fig. 20.6j–m); in some species the sheath completes the cell envelope (Fig. 20.6m). Detailed chemical studies have

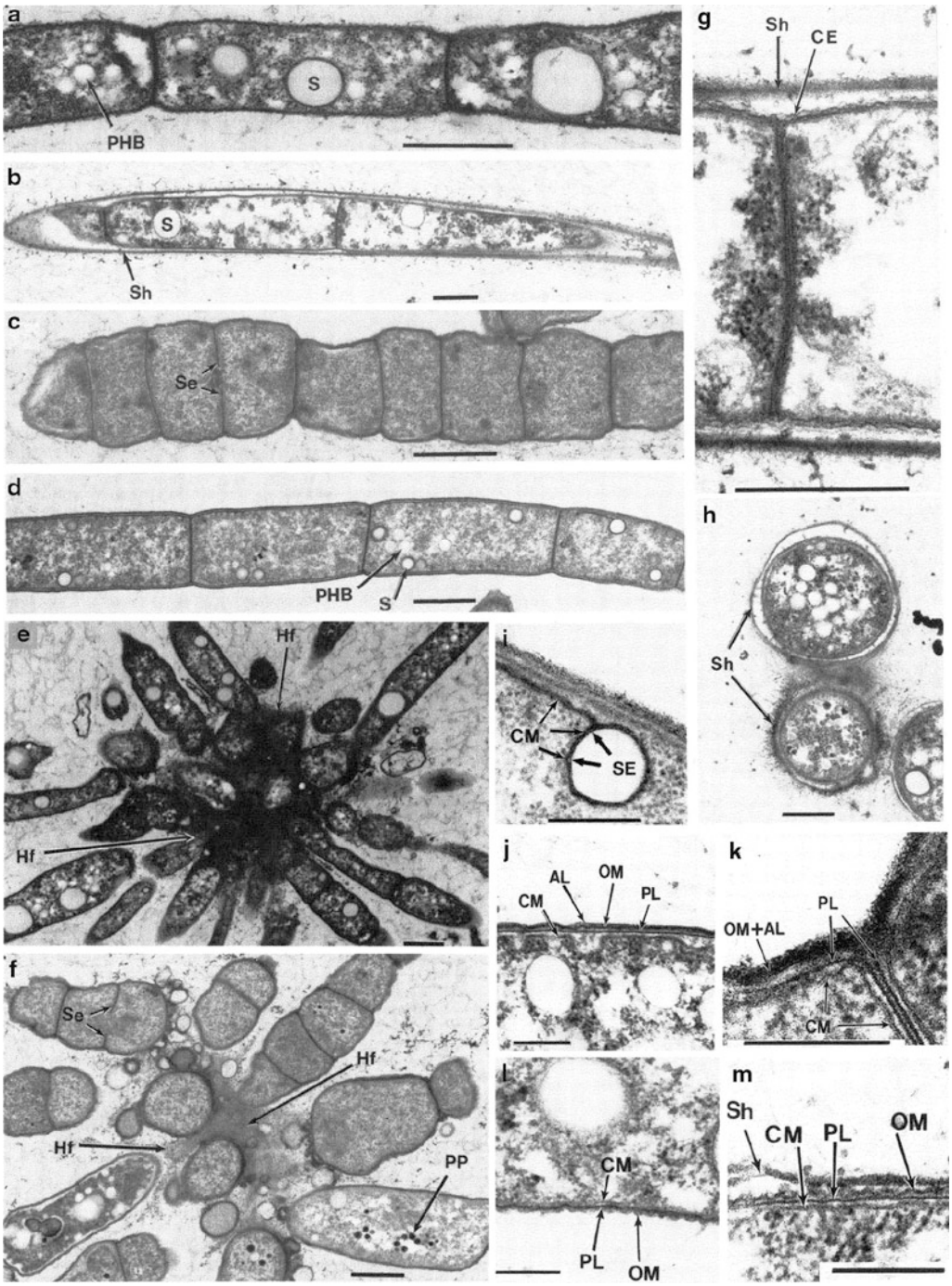

Fig. 20.6

Microscopic images of *Thiothrix*. General ultrastructural characteristics of filaments of *Thiothrix* and ''type 021N'' bacteria. (**a**) *Thiothrix unzii* strain A1. (**b**) *Thiothrix fructosivorans* strain Q. (**c**) *Thiothrix* ''type 021N'' strain N7. (**d**) *Thiothrix* ''type 021N'' strain N2. (**e**) Thin section through rosette in *Thiothrix unzii* strain A1. (**f**) Thin section through rosette in *Thiothrix* ''type 021N'' strain N7. (**g**) Sheath characteristics in *Thiothrix fructosivorans* strains Q; the sheath is visible along filament with septum separating two cells. (**h**) The sheath is shown surrounding a filament in cross section. (**i**) sulfur inclusions in *Thiothrix* ''type 021N'' strain N2, with two membrane layers consisting of sulfur inclusion membrane (*inside*) and cytoplasmic membrane (*outside*), the latter form extensions into the cytoplasm and are likely to continue towards the outer cytoplasmic membrane. Panels **j**–**m**: Cell membrane and envelope structure in *Thiothrix* ''Type 021N'' strain N7 (**j**) and strain N2 (**k**), *Thiothrix unzii* strain A1 (**l**) and *Thiothrix fructosivorans* strain Q (**m**). Abbreviations: *AL* additional outer layers, *CE* cell envelope, *CM* cytoplasmic membrane, *Hf* holdfast material, *PP* polyphosphate, *Se* cell septum, *PHB* polyhydroxybutyrate, *PL* peptidoglycan layer of cell wall, *PP* polyphosphate, *OM* outer membrane, *S* sulfur inclusion, *Se* septum, *SE* sulfur inclusion envelope, *Sh* sheath. Scale bars **a**–**f**, 1.0 μm. Scale bars (**g**, **h**), 0.5 μm. Scale bars (**l**–**m**), 250 nm (Electron micrographs modified from Williams et al. 1987)

investigated the complex polysaccharide structure of these sheaths in *T. nivea* (Takeda et al. 2012) and *T. fructosivorans* (Kondo et al. 2013). Cell septa consist of four distinct layers, the cytoplasmic membrane and the periplasmic layer on each side (Fig. 20.6k). During cell division within a filament, cell septa grow from opposite sides of the peripheral cell membrane into the cytoplasm until they meet in the center of the dividing cell and separate it into two daughter cells; as the new cell septum tightens and closes, electron-dense material that contrasts visually with the cytoplasm (potentially genomic DNA) congregates near the closing hole at the septa and appears to be redistributed between the separating daughter cells (Fig. 20.6c, f).

Structural similarities and differences among *Leucothrix and Thiothrix*. Most observations on *Thiothrix* morphology and cell structure also apply to *Leucothrix*. The gonidia-forming filaments and rosettes of *Leucothrix* (Fig. 20.4a, b) resemble those of *Thiothrix* (Fig. 20.5c). Individual filaments of *Leucothrix* often form knots, which is not observed in *Thiothrix*; this presumably results from asymmetrical growth on different sides of an individual filament that causes the filament tip to curve during filament elongation until genuine knots are completed (Figs. 20.3 and 20.4b). Also, in contrast to *Thiothrix*, sulfur globules are absent in cells of the genus *Leucothrix*; however, their filament cells and gonidial cells show extensive cytoplasmic invaginations (Fig. 20.4c, d). The multilayered cell envelope of cytoplasmic membrane, periplasmatic membrane (most likely a peptidoglycan layer) and outer cell wall resembles that of *Thiothrix* (Fig. 20.4e). The electron-dense holdfast structure surrounding the basal cells of rosette-forming *Leucothrix* filaments (Fig. 20.4f) is also closely reminiscent of its counterpart in *Thiothrix* (Fig. 20.6e).

Isolation, Enrichment and Maintenance Procedures

Enrichment and isolation of *Leucothrix*. Isolates of *Leucothrix* can be obtained using a basal salt medium (Brock 2006) with a low phosphate concentration, as this compound was reported to inhibit the growth of *Leucothrix*. The basal salt medium contains (per liter of deionized water) 11.75 g NaCl, 5.35 g $MgCl_2$ * 6 H_2O, 2.0 g Na_2SO_4, 0.75 g $CaCl_2$ * 2 H_2O, 0.35 g KCl, 0.5 g Tris(hydroxymethyl)aminomethane, 0.05 g $NaHPO_4$; the pH is adjusted to 7.6. Glutamate suffices as the sole source of carbon, nitrogen and energy for most strains, and—by adding 0.1 % monosodium glutamate (MSG) to basal salts and 2 % agar—allows the isolation of strains from environmental materials. Other useful supplements include 0.1 % MSG plus a vitamin mixture, 0.1 % MSG plus 0.01 % yeast extract, and 0.1 % tryptone plus 0.1 % yeast extract. The concentration of organic compounds should be kept low to avoid overgrowth by non-filamentous bacteria.

For isolation, single algal filaments are streaked directly (or after washing in sterile salts) onto agar plates, which are incubated at 20–25 °C overnight. Within 12–18 h after streaking, the plates are examined under 125× magnification for the characteristic coiled rope or thumbprint morphology of *L. mucor* colonies. Brock (2006) suggests a combination of a 12.5× eyepiece and 10× phase-contrast microscope objective for early identification of *Leucothrix* filaments, before nascent colonies are overgrown by bacterial epibionts. These colonies are picked by touching them with a sterile insect pin and transferring them to fresh agar plates of the same composition. This enrichment and microscopic monitoring strategy allows to isolate *Leucothrix* colonies directly from filaments still attached to seaweed fronds, and to identify the precise source habitat of an isolate; such information is of considerable value in studies on the molecular evolution of *Leucothrix* (Kelly and Brock 1969b).

During transfer of colonies to liquid culture, the inoculum often grows best (overnight) when placed in a small (1–2 ml) volume of medium. These pre-cultures can serve as the inoculum for the buildup of large-volume cultures in large flasks. In liquid medium, growth is best when the flasks are shaken gently, such as on a wrist-action shaker or slowly on a rotary shaker. With the latter kind of shaker, growth rate is increased if the flasks contain small internal baffles, made by pushing in the sides of the flasks during heating with an oxygen flame. For growing high-density cultures, a medium containing 1 % MSG, 0.2 % sodium lactate, and 0.01 % yeast extract has proved suitable; the yeast extract provides growth factors needed by some strains, and the sodium lactate substantially increases the yield of most cultures.

Enrichment and Isolation of *Thiothrix*. Multiple protocols have been developed for the isolation of *Thiothrix* spp.: Enrichment in slide culture (Bland and Staley 1978), physical separation and plate streaking of *Thiothrix* tufts (two different procedures based on Strohl and Larkin 1978 or Williams and Unz 1985), and pre-enrichment in agar tubes (Williams and Unz 1985). The media formulations described here favor strains with heterotrophic capabilities; the enrichment and isolation of a broader spectrum of facultatively autotrophic, sulfur-oxidizing strains (Chernousova et al. 2009) may require different media (Armbruster 1969).

1. *Slide culture.* Slide culture has been used to enrich *Thiothrix* from natural sulfur spring habitats; *Thiothrix* rosettes and filaments growing on the glass slides provide suitable material for microscopy (Bland and Staley 1978). Slides are coated with the medium of Morita and Burton (1965) containing per liter of natural sulfur spring water: 2 g yeast extract, 0.1 g $CaCl_2$, 0.5 g sodium acetate, and 15 g agar. Slides inoculated with *Thiothrix* material were covered with a thin, gas-permeable Teflon membrane, placed in a desiccator containing water and 5 g of sodium sulfide, and incubated at 14 °C. The slides are periodically checked by phase contrast microscopy for filaments and rosettes that are developing attached to the agar-coated slide (Bland and Staley 1978).
2. *Physical separation and plate streaking I.* Fine-tipped forceps select for tufts of suspected *Thiothrix* filaments from *Thiothrix*-containing material contained in a Petri dish while under observation with a dissecting microscope and transmitted light (Larkin 1989). Bundles of filaments are

transferred to a second Petri dish containing about 5–10 ml of a salt solution SS-1 (Strohl and Larkin 1978), agitated by forceps, and then transferred to another petri dish containing salt solution SS-1. This salt solution contains (per liter) 200 mg NH_4Cl, 10 mg $MgSO_4$, 10 mg $CaSO_4$, and 5 ml trace element solution (per liter: 10 mg $ZnSO_4 * 7H_2O$, 20 mg $MnSO_4 * 4\ H_2O$, 0.5 µg $CuSO_4 * 5\ H_2O$, 10 mg H_3BO_3, 1 mg $Co(NO_3)_2$, 1 mg Na-Molybdate, 200 mg EDTA, 700 mg $FeSO_4 * 7\ H_2O$). The procedure is repeated through four or five transfers. A few drops from each dilution are transferred with a Pasteur pipette to either MP agar (SS-1 plus 0.01 % each of sodium acetate nutrient broth powder, and yeast extract, plus 0.03 % Na_2S, and 1.5 % agar; Strohl and Larkin 1978) or MY agar (SS-1 plus 0.01 % each of sodium acetate, nutrient broth agar, and yeast extract, plus 0.03 % Na_2S, and 1.5 % agar; Larkin 1980) in separate Petri dishes. Each dish is held at an angle so that the drops will flow across the agar surface. The excess is then withdrawn from the other side with the pipette. The dishes are incubated at about 20–30 ºC and are examined daily under transmitted light with the aid of a dissecting microscope. Colonies with a hairy or filamentous edge are transferred with sterile toothpicks to fresh media and are restreaked until pure.

3. *Physical separation and plate streaking II.* A washing-sonication pretreatment step helps to increase the density of filamentous bacteria in the inoculum and to reduce the content of adventitious microorganisms. Several loopfuls of enrichment culture surface film or activated sludge (10^{-1} dilution) samples are transferred to small glass Petri dishes containing 7 ml of sterile Mineral salts vitamin mix (MSV; see paragraph below). With the aid of a stereomicroscope at 15–45-fold magnification, approximately 40–50 single filaments or rosettes are individually transferred with sterile glass micropipettes through a series of six to seven washings in fresh MSV. The inoculum of washed filaments is transferred to 3 ml of MSV and both spread and streak-plated on glucose-sulfide or LT medium. Glucose-sulfide medium contains (per liter): 0.15 g glucose, 0.5 g $(NH_4)_2SO_4$, 0.01 g $Ca(NO_3)_2$, 0.05 g $K_2HPO_4 * 7\ H_2O$, 0.05 g KCl, 0.1 g $CaCO_3$, 0.187 g $Na_2S * 9\ H_2O$, 1.0 ml vitamin mix, 15 g agar; final pH is 7.5. LT medium contains per liter of MSV: 0.5 g sodium lactate, 0.5 g $Na_2S_2O_3 * 5\ H_2O$, and 12 g agar. All media are adjusted with NaOH to pH 7.2–7.5. Plates are incubated at 20–22 °C and examined periodically (15–45-fold magnification) for evidence of filamentous colonies. Suspect colonies are transferred three times on primary isolation media and once on SCY and CGY media (see Williams and Unz 1985) to ascertain purity.
4. *Enrichment in agar tubes.* This approach has been used for the isolation of *Thiothrix*, *Leucothrix*, and *Beggiatoa* spp. (Williams and Unz 1985). Enrichment cultures can provide inocula for pure culture isolation if *Thiothrix* strains cannot be isolated by direct plating of mixed liquid samples. 1 ml of activated sludge fluids are transferred to test tubes containing 5 ml of solid media (per liter: 1.5 g glucose and/or 0.6 g $Na_2S * 9\ H_2O$ plus 2 % Bacto-Agar) and an overlay of 15 ml of mineral salts-vitamin mix (MSV) containing the following ingredients per liter: 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 * 7\ H_2O$, 0.05 g $CaCl_2 * 2\ H_2O$, 0.11 g K_2HPO_4, 0.085 g KH_2PO_4, 0.002 g $FeCl_3 * 6\ H_2O$, 0.003 g EDTA, and 1 ml vitamin solution (Eikelboom 1975). Cultures are incubated at final pH of 7.2–7.5 and at a temperature of 22–25 °C, and examined microscopically for evidence of *Thiothrix* trichomes in the surface biomass.
5. *Strain maintenance.* The type species can be maintained in semisolid (0.15 % agar) deeps of either MP or MY medium, with transfer intervals of ca. 3–4 weeks. Axenic cultures of other *Thiothrix* spp. can be maintained by cryopreservation at −83 °C to −90 °C, or at 10 °C by monthly transfers on LTH medium, which contains the following per liter of MSV: 1.0 g sodium lactate, 0.5 g $Na_2S_2O_3 * 5\ H_2O$, and 0.01 M HEPES buffer (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid). If required, the pH is adjusted to about 7.5 with NaOH (Unz and Head 2005).
6. *Testing for special characteristics.* MP broth or appropriate modifications are used to test sole carbon (0.05 %), nitrogen (0.02 %) and sulfur (0.03 %) sources in support of sustained growth of *T. nivea* strains through three successive transfers (Larkin 1989). Other *Thiothrix* species were nutritionally evaluated in MSV; optical density equal to more than four-fold greater than the controls counted as positive result (Williams and Unz 1989).

Ecology

Host associations of *Leucothrix*. In its marine habitat, *Leucothrix* filaments grow typically as epiphytes on marine macrophytes, and in association with marine invertebrates that offer suitable colonization surfaces for bacterial epibionts (reviewed in Raj and Ordal 1977). *Leucothrix* colonizes marine algae, arthropods, or rocky shores aerated by wave action (Brock 1966; Harold and Stanier 1955; Kelly and Brock 1969a; Pringsheim 1957). Due to its aerobic nature, *Leucothrix* is never found is slow-moving or stagnant waters, and the attachment to motile animals or flexible algal filaments constitutes an ecological advantage for the non-motile *Leucothrix*, interplaying winds, waves, currents and nutrients (Raj and Ordal 1977). *Leucothrix* overgrowth can cause an extensive infestation of benthic crustacea and fish eggs (Johnson et al. 1971), and has become a problem in the field of aquaculture, especially in the cultivation of lobsters.

Especially in temperate waters, *Leucothrix* growth density on seaweeds can be substantial (Kelly and Brock 1969a). *Leucothrix* occurs on red, green, and brown algae, and prefers intertidal rather than subtidal location (Bland and Brock 1973). In special cases host specificity can be observed; under laboratory conditions a preference of *L. mucor* for the red algae *Antithamnion sarniense* and *Bangia fuscopurpurea*, and the brown alga *Sphacelaria* sp., but not the red alga *Rhodochorton* sp., was observed (Bland and Brock 1973; Brock 1966). Several marine algae are known to produce significant amounts of fixed carbons

and other compounds, supporting growth of a number of different more or less associated heterotrophic bacteria, but some algal species also produce antimicrobial agents such as sulfuric acid or acrylic acid. Their cuticle or mucilaginous covering typically consists of mannans and mannose, with a wall composed of xylan. *L. mucor* cannot metabolize xylan but mannose, which is why it attaches only to the cuticle but does not penetrate to the wall. At night, when the alga stops the synthesis of its cuticle material, large numbers of *L. mucor* gonidia are released (Bland and Brock 1973).

Unusually dense growth on the filamentous red alga *Bangia fuscopurpurea* that is exposed to air for extended periods of time during low tide highlights the preference of *Leucothrix* for ample aeration, and the recruitment of larger structures as medium to reach full-air regions of the intertidal zone. At low tide, *Leucothrix* is found at the underside of the dry algae mats, maintaining moist conditions. Furthermore, there seems to be a correlation of *Leucothrix* density and algal age, indicating true growth on the alga and not incidental attachment. Brock (1966) showed that *Leucothrix* can grow on substances liberated by the alga, and a few years later Bland and Brock (1973), after conducting a variety of ecological studies, found that *Leucothrix* receives most of its nutrients from the algae and not from seawater. The attachment to its algal host is thus virtually obligate for survival, and it is the gonidium that initiates the association (Bland and Brock 1973; Harold and Stanier 1955).

Studies of the association of *Leucothrix* filaments with the red alga *Gelidium linguatum* at the methane seep area west of Mocha Island, off central Chile (*Fig. 20.2*), demonstrate that the direct exposure to methane seepage can result in assimilation of carbon derived from methane into *Leucothrix* (Jessen et al. 2011; Sellanes et al. 2011). The *Leucothrix* filaments show an unusually light carbon isotopic signature ($\delta^{13}C$ value of -39.2 ± 2.5 ‰) indicating that assimilated carbon was derived from local methane seepage. The filaments in turn provide an important source of methane-derived carbon for grazing invertebrates (Sellanes et al. 2011); the filaments are grazed by the tanaids *Nototanais dimorphus* and *Zeuxo marmoratus*, and by other amphipods (Sellanes et al. 2011, Sellanes personal observation). The filaments were identified by 16S rRNA sequencing and affiliate with other epiphytic filamentous *Leucothrix* spp. (*Fig. 20.1*).

Leucothrix does not only form associations with algae but also colonizes the eggs of commercially important fish (e.g. from cod fish and winter flounder), the larvae and carapaces of benthic marine crustacea; it overgrows the antenna of hermit crabs and the gills of horseshoe crabs, and forms heavy infestations on zooplankton, gravid rock crabs, small unidentified prawns and other benthic copepod and decapod crustaceans (Raj and Ordal 1977). Although *L. mucor* is not a pathogen in the sense that it affects human physiology, it can cause environmental damage; for example it triggers high crustacean mortalities by causing eggs to sink below the surface and by interfering with the filter apparatus of larvae. Details about the associations of *Leucothrix* and crustaceans remain to be studied, however, the frequent observation of feeding of their bacterial films by the host suggests a contribution of the epiphyte to the hosts daily nutrition (Johnson et al. 1971; Raj and Ordal 1977). Studies of the association of different kinds of bacteria, including *Leucothrix* filaments, with the septae of the Galatheid crab *Shinkaia crosnieri* showed that labeled bicarbonate was assimilated into the epibiotic microbial communities on the specialized tissues of the deepsea invertebrate. Interestingly, the incorporation of $^{13}HCO3^-$ into the epibiotic microbial communities was stimulated by the addition of sulfide and thiosulfate, but not by molecular hydrogen, strongly suggesting primary production by facultative thiotrophic (sulfur-oxidizing) energy metabolism (Watsuji et al. 2010), but whether this also includes the *Leucothrix* filaments needs to be verified.

Although *Leucothrix* attaches to a wide variety of substrates in nature, any association with abiotic surfaces such as rocks seems to be short-lived, and the filaments prefer a living host for permanent colonization. This observation is congruent with the requirement of most *Leucothrix mucor* isolates for vitamins (Kelly and Brock 1969a), which they might receive from the host or from other associated microorganisms.

The surface-attached lifestyle of *Leucothrix* suggests that this bacterium competes effectively with other epibionts, and might be a source as well as a recipient of signaling compounds and growth inhibitors. *Leucothrix* is sensitive to antibiotics and its growth can be inhibited by penicillin (0.1 mg/l), streptomycin (5.0 mg/l), or chloromycetin (0.7–0.9 mg/l) (Raj and Ordal 1977). *Leucothrix* strain N11, isolated from industrial chemical wastewater (Williams and Unz 1985), did not grow under exposure to >0.125 mg/l of streptomycin, gentamicin, tetracycline, ampicillin, and penicillin G. The same strain is resistant to sulfanilamide and lincymycin at >0.64 mg/l, and is sensitive to chloramphenicol (0.5 mg/l) and bacitracin (4.0 mg/l).

Host associations of Thiothrix. Its attached growth and filamentous morphology allows *Thiothrix* to form opportunistic or symbiotic associations with other microorganisms in aquatic habitats. An intriguing example are the "String-of-pearl" colonies that consist of *Thiothrix* filaments associated with uncultured archaea that grow attached to plant material in cold sulfidic freshwater springs (Rudolph et al. 2001; Moissl et al. 2002, 2003). The *Thiothrix* symbiont (Genbank accession number of 16S rRNA sequence, AJ307933; related to *T. unzii*) can be replaced by other, presumably sulfur-oxidizing filamentous bacteria, for example uncultured epsilonproteobacteria (Rudolph et al. 2004). The archaeal community is also variable and includes specific members of the euryarchaeota (the SM1 lineage) as well as diverse crenarchaeota (MG-1 and other lineages) (Koch et al. 2006). As a working hypothesis, the "String-of-pearl" colonies appear to remain viable in different taxonomic composition as long as biogeochemical functionality is maintained; however, the biochemical basis of this association and its possible symbiotic nature are not yet understood.

Members of the genus *Thiothrix* form associations with invertebrates that are host- and strain-specific, as shown by the best-studied example, the epibiotic association of *Thiothrix* with

the freshwater amphipod *Niphargus*. *Thiothrix* filaments growing attached to the exoskeleton of the amphipod *Niphargus* are alternately exposed to sulfide and oxygen and can assimilate CO_2, as the host amphipod spends most of its time in oxygenated waters but also dives to the sulfide/oxygen interface (Dattagupta et al. 2009). The amphipod host could also benefit from its chemosynthetic epibiont as a possible food source, but this symbiotic linkage remains to be proven. Molecular studies have shown that two different species of the amphipod *Niphargus* occur associated with three distinct populations of *Thiothrix* ectosymbionts in the sulfidic waters of the Frasassi cave system in Italy (Bauermeister et al. 2012); the ectobiont phylotypes are distinct from free-living *Thiothrix* sp. in the cave waters, and indicate intra- or interspecific inoculation among *Niphargus*. Similar results were found for *Thiothrix*/*Niphargus* associatons in sulfidic aquifers and the sulfidic Movile cave ecosystem in Romania; the *Niphargus*-specific *Thiothrix* ectobiont clusters did not overlap with free-living *Thiothrix* populations from Movile cave (Flot et al. 2013).

These *Thiothrix*/host associations may be to a large extent opportunistic. A *Thiothrix* population colonizes the outer surface of a motile aquatic host organism as long as this carrier shuttles between sulfidic and oxygenated waters and provides suitable redox conditions for *Thiothrix*. Without sulfide exposure, the host may not harbor any *Thiothrix* epibionts. For example, *Thiothrix* filaments cover the larvae of the mayfly (*Drunella grandis*) in a specific location where a sulfidic spring enters the freshwater creek habitat of the mayfly larvae. The *Thiothrix* filaments grow as epiphytes on the larvae, and appear to host intracellular bacterial parasites that remain so far unidentified (Larkin et al. 1990).

Under certain conditions, marine habitats can also harbor *Thiothrix*; their 16S rRNA sequences branch off at the base of the *Thiotrix* cluster (*Fig. 20.1*). For example, seasonal benthic sulfur-oxidizing mats on decaying plant material in a brackish coastal lagoon in Greenland contained abundant *Thiothrix* filaments; possibly, meltwater input in the arctic summer freshens the water in the lagoon sufficiently to allow the growth of *Thiothrix* mats (Glud et al. 2004). A marine amphipod crustacean harbored an epibiotic *Thiothrix* population that, by 16S rRNA gene sequence, constitutes a sister lineage to *T. eikelboomii* and *T. disciformis* (AY426613, Gillan and Dubilier 2004). A related *Thiothrix* population was forming flocs in the wastewater treatment system of a mariculture facility supplied with artificial seawater of 20 ppt salinity (DQ067608, Cytryn et al. 2006). Another marine occurrence could be the population of *Thiothrix*-like filamentous bacteria found as ectobionts on the cecum nodule of a deposit-feeding echnoid, *Echinocardium cordatum*, identified by filamentous morphology and by immunostaining with a *Thiothrix*-targeted antibody. Untypically, sulfur globules were not reported, and 16S rRNA sequencing would be required to substantiate the genus identification (Brigmon and de Ridder 1998). Similarly to *Leucothrix*, these marine *Thiothrix* populations could have relevance as a link in the food web that recycle secondary chemosynthetic production into higher trophic levels.

The oxidized niche of *Thiothrix*. Freshwater habitats characterized by mixed sulfidic and oxic fluids, including sulfur springs, vents, and irrigation ditches, provide suitable habitats for a variety of sulfur bacteria, but they constitute the primary habitat for *Thiothrix* (Bland and Staley 1978; Larkin and Strohl 1983; Strohl and Schmidt 1984; Macalady et al. 2008; Konkol et al. 2010). *Thiothrix*-dominated sulfur-oxidizing mats occur frequently in sulfidic waters of limestone caves (karst caves), where they oxidize reduced sulfur species to sulfate, and generate acidity that contributes to limestone dissolution (Brigmon et al. 1994; Engel et al. 2010; Steinhauer et al. 2010). A systematic comparison of the geochemical niches and growth forms of *Beggiatoa* and *Thiothrix* in a sulfidic karst cave stream (Frasassi cave complex, Italy) showed that *Thiothrix* filaments grow predominantly as streamers exposed to flowing water, and prefer lower sulfide (100–200 μM) and higher oxygen concentrations (up to 10–15 μM); in contrast, *Beggiatoa* filaments grow as stable biofilms under higher sulfide concentrations (mostly 150–500 μM) and lower oxygen concentrations (max. 5 μM; Macalady et al. 2008). Relatively oxidizing conditions and convective mixing of sulfidic and oxygenated waters select for the surface-attached rosette-forming growth of *Thiothrix* filaments, similar to the attached, rosette-forming sulfur oxidizer "*Candidatus* Marithrix" that forms a distinct phylogenetic lineage within the *Beggiatoaceae* (Teske and Salman 2014). Attached, rosette-forming, filamentous sulfur-oxidizing bacteria have therefore evolved twice—*Thiothrix* and *Leucothrix* in the *Leucotrichaecae*, and "*Candidatus* Marithrix" in the *Beggiatoaceae*—and have adapted to the same ecological niche of surface-attached growth sustained by well-mixed aerobic and sulfidic waters.

Applications

Role of Thiothrix in wastewater treatment. *Thiothrix* are conspicuous microbial community members in activated sludge in wastewater treatment plants; this man-made dynamic habitat is characterized by strong fluctuations in carbon substrates, sulfur sources, and oxygen and nitrate availability. Large numbers of *Thiothrix* filaments and flocs in the sludge interfere with sludge settling and lead to filamentous sludge bulking. *Thiothrix* filaments are well suited to the wastewater plant environment due to their versatile carbon assimilation patterns that can be examined using microautoradiography (Andreasen and Nielsen 1997) and FISH hybridization (Wagner et al. 1994; Nielsen et al. 1998, 1999). Several physiological findings stand out (Nielsen et al. 2000): (1) *Thiothrix* in activated sludge can grow heterotrophically as well as autotrophically and assimilate DIC as well as acetate; (2) DIC incorporation is strongly stimulated by acetate addition, indicating that acetate serves not only as a carbon source, but also as an energy source that facilitates autotrophic carbon fixation; (3) both uptake rates are increased further in the presence of the electron donor thiosulfate; (4) after thiosulfate addition and oxidation, sulfur accumulation in the cytoplasm proceeds not only aerobically,

but also with nitrate as electron acceptor; (5) under anaerobic conditions in the absence of nitrate, *Thiothrix* appears to use its intracellular sulfur globules as an electron acceptor reserve, similar to freshwater *Beggiatoa* spp. (Nelson and Castenholz 1981). Similarly, *Thiothrix*-dominated flocs accumulate transiently during sulfidic episodes within the wastewater treatment steps of a marine fish culture system and show high rates of sulfide oxidation under oxic as well as anoxic conditions in the presence of nitrate (Cytryn et al. 2006). These results from wastewater treatment facilities in different locations (Denmark and Israel) suggest that *Thiothrix* populations can persist as nitrate-reducing or sulfur-reducing facultative anaerobes.

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