12 Magnetotactic Bacteria

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Abstract

Prokaryotes that exhibit magnetotaxis, collectively known as the magnetotactic bacteria, are those whose direction of motility is influenced by the Earth's geomagnetic and externally applied magnetic fields. These ubiquitous, aquatic microorganisms represent a morphologically, phylogenetically, and physiologically diverse group that biomineralize unique organelles called magnetosomes that are responsible for the cells' magnetotactic behavior. Magnetosomes consist of magnetic mineral crystals, either magnetite (Fe_3O_4) or greigite (Fe_3S_4), each enveloped by a phospholipid bilayer membrane that contains proteins not present in other membranes. While there are several different magnetite and greigite crystal morphologies, mature crystals of both minerals are always in the single magnetic domain size range, about 35–120 nm, thus having the highest possible magnetic moment per unit volume. In most magnetotactic bacteria, magnetosomes are arranged as a chain within the cell thereby maximizing the magnetic dipole moment of the cell causing the cell to passively align along magnetic field lines as it swims. Magnetotaxis is thought to function in conjunction with chemotaxis in aiding magnetotactic bacteria in locating and maintaining an optimal position in vertical chemical concentration gradients common in stationary aquatic habitats, by reducing a three-dimensional search problem to one of a single dimension.

Although the detection of magnetotactic bacteria in samples collected from natural environments is relatively easy, the magnetotactic bacteria are a fastidious group of prokaryotes and special culture conditions are necessary for their isolation and cultivation. Phylogenetically, most known cultured and uncultured magnetotactic bacteria are associated with the *Alpha-*, *Gamma-*, and *Deltaproteobacteria* classes of the *Proteobacteria* phylum and the *Nitrospirae* phylum. All cultured species are either microaerophiles or anaerobes or both. Most cultured species of the *Alpha-* and *Gammaproteobacteria* classes are microaerophiles that grow chemolithoautotrophically using reduced sulfur compounds as electron sources and the

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Calvin-Benson-Bassham cycle or the reverse tricarboxylic acid cycle for autotrophy and chemoorganoheterotrophically using organic acids as electron and carbon sources. Those in the *Deltaproteobacteria* are sulfate-reducing anaerobes that only grow chemoorganoheterotrophically. Almost all cultured species exhibit nitrogenase activity and thus fix atmospheric nitrogen and many denitrify. Magnetotactic bacteria thus show a great potential for iron, nitrogen, sulfur, and carbon cycling in natural environments.

Genetic determinants for magnetosome synthesis, the *mam* and *mms* genes, are organized as clusters in the genomes of all magnetotactic bacteria examined. These clusters are in close proximity to each other within the genomes and are surrounded by genomic features that suggest that magnetosome genes are organized as a magnetosome gene island that might be transmitted to many different bacteria through horizontal gene transfer. Through the development of genetic systems in some magnetotactic bacteria, the functions of several magnetosome membrane proteins in the biomineralization of the magnetite magnetosome chain have been demonstrated although the roles of most remain unknown.

Bacterial magnetosomes have novel physical and magnetic properties and also geological significance and have been used in a large number of commercial and medical applications.

Introduction

Magnetotactic bacteria are gram-negative, motile prokaryotes that synthesize intracellular, membrane-bounded crystals of magnetic iron oxide or iron sulfide minerals. The mineral crystals together with their associated membrane are called magnetosomes (Balkwill et al. 1980) and cause the bacteria to orient and swim along external magnetic and geomagnetic field lines. These microorganisms were first described by Salvatore Bellini in 1963 in a publication of the Instituto di Microbiologia of the University of Pavia, Italy (Bellini 1963, 2009a, b). He observed large numbers of bacteria that swam in a consistent, single, northward direction and referred to them as "batteri magnetosensibili" (magnetosensitive bacteria) and speculated that the magnetic behavior of the cells was due to an internal "magnetic compass." This internal "magnetic compass" was later confirmed by Richard P. Blakemore who independently rediscovered magnetotactic bacteria in 1974 and was the first to observe magnetosomes within cells of these microorganisms (Blakemore 1975).

Magnetotactic bacteria are indigenous in sediments or chemically stratified water columns where they occur predominantly at the oxic-anoxic interface/transition zone (OAI or OATZ) and the anoxic regions of the habitat or both. They represent a diverse group of microorganisms with respect to morphology, phylogeny, and physiology. Despite the efforts of a number of different research groups, few representatives of this group of bacteria have been isolated in axenic culture since their discovery, and even fewer have been adequately described in the literature. Therefore, little is known about their metabolic plasticity, whereas their diverse morphology and phylogeny has been analyzed to some extent by culture-independent methods. To date, the only validly described species of magnetotactic bacteria are members of the genus *Magnetospirillum*. Representatives of this genus have been isolated reproducibly from various aquatic environments, can now be grown relatively easily in mass culture, and are genetically tractable. Thus, much of the knowledge regarding the metabolism, genetics, and biochemistry of magnetotactic bacteria is derived from studies involving strains of this genus.

Ecology

Magnetotactic bacteria are cosmopolitan in distribution and ubiquitous in sediments of freshwater, brackish, marine, and hypersaline habitats as well as in chemically stratified water columns of these environments (Bazylinski and Frankel 2004). They have also been found in some wet soils (Fassbinder et al. 1990) although it is not known whether their presence is common in them. The occurrence of magnetotactic bacteria appears to be dependent on the presence of an oxic-anoxic interface (OAI) that represents opposing gradients of oxygen from the surface and reduced compounds (usually reduced sulfur species) in sediments or water columns. Generally, the highest numbers of magnetotactic bacteria are observed at the OAI of sediments or chemically stratified water columns (Moskowitz et al. 2008). Moreover, within the OAI, different species of magnetotactic bacteria occupy different positions that are also probably dependent on specific chemical conditions. Magnetotactic bacteria are known to biomineralize two magnetic minerals: the iron oxide magnetite (Fe_3O_4) or the iron sulfide greigite (Fe_3S_4). Most magnetotactic bacteria produce only one mineral although there is a group that synthesizes both. Typically, the magnetite-producers are found at the OAI proper while the greigite-producers are found below the OAI when the anoxic zone is sulfidic (Moskowitz et al. 2008). Magnetotactic bacteria can thus be considered as typical examples of gradient organisms.

For many years, magnetotactic bacteria were thought to be restricted to habitats with pH values near neutral and at ambient temperature. Very recently, however, Lefèvre et al. described an uncultured, moderately thermophilic magnetotactic bacterium in hot springs in northern Nevada (Lefèvre et al. 2010b) with a probable upper growth limit of about 63 °C. In addition, this same group isolated several strains of obligately alkaliphilic magnetotactic bacteria from different aquatic habitats in California including the hypersaline, extremely alkaline Mono Lake (Lefèvre et al. 2011b). These strains have an optimal growth pH ~ 9.0. None yet have been found in habitats that are strongly acidic (e.g., acid mine drainage).

Detection and Collection of Magnetotactic Bacteria from Natural Environments

The detection of magnetotactic bacteria in environmental water and sediment samples is relatively easy due to their magnetic behavior in turn due to their permanent magnetic dipole

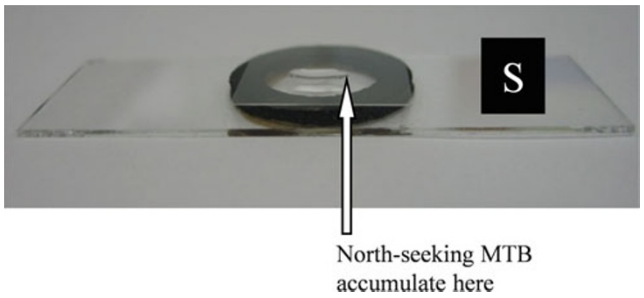


Fig. 12.1
Image of the "hanging drop" setup used for the detection of magnetotactic bacteria in water and sediment samples (Schüler 2002). A drop of water/sediment is placed on a cover slip and inverted and then set on a small rubber o-ring on a microscope slide. The south pole (S) of a bar magnet is placed next to one side of the drop, here the *right side*. North-seeking magnetotactic bacteria will swim to the right edge of the drop (shown at *arrow*) and accumulate making them easy to detect and observe microscopically. Using this technique, perturbation of the drop by air currents and evaporation of the drop is reduced. In addition, sediment in the drop settle to the drop's lowest point, leaving the edge of the drop clear to view the bacteria. The hanging drop was used in the video Figs. 12.5, 12.12, 12.13, and 12.15

Fig. 12.2
Magnetic enrichment of magnetotactic bacteria from a water and sediment sample in a jar by applying the south pole of a magnet (*M*) outside the jar several centimeters above the water-sediment interface for about 30 min. Magnetotactic bacteria that accumulate at the magnet are shown as a dark spot at the arrow. Interestingly, although one would expect only north-seeking magnetotactic bacteria to accumulate near the magnet, those of both polarities collect near the magnet

moment. A simple method is to put a drop of water or sediment on a microscope slide which is then set on the microscope stage. A bar magnet is now placed on the microscope stage near the drop so the axis of the magnet is parallel to the plane of the slide and passes through the center of the drop. The magnetic field at the drop should be at least a few gauss and the bar magnet should be oriented so that the "south" magnetic pole (the pole that attracts the north end of a magnetic compass needle) is on the microscope stage in such a way that all the magnetotactic bacteria are guided in one direction until they reach and accumulate at the edge of the drop of water and/or sediment where they can be observed. If the magnet is rotated 180°, the bacteria will also rotate and swim away from the edge of the drop. The use of phase contrast or differential interference contrast microscopy provides much better contrast than bright field microscopy making cells much easier to observe.

A commonly used modification of the procedure described above is the so-called hanging drop technique in which a drop of water/sediment is placed on a cover slip and inverted and then set on a small rubber o-ring on the microscope slide (Fig. 12.1; Schüler 2002). This technique eliminates perturbation of the drop by air currents and reduces evaporation of the drop. It also allows sediment in the drop to settle to the drop's lowest point, leaving the edge of the drop clear to view the bacteria.

Both procedures work well if there are good concentrations of magnetotactic bacteria in the samples. To ensure visualization of cells if concentrations are low, one can magnetically enrich for higher numbers of cells by placing the south pole (in the Northern Hemisphere; the north pole of the magnet is used in the Southern Hemisphere) of a bar magnet adjacent to the outer wall of a jar filled with sediment and water. If magnetotactic bacteria are abundant in the sample, a brownish or grayish to white spot consisting mainly of magnetotactic bacteria will form next to the inside of the glass wall closest to the south pole of the bar magnet (Fig. 12.2). Cells can be easily removed from the jar with a Pasteur pipette and examined as described above.

An extension and scale-up of the magnetic collection method was recently described (Jogler et al. 2009b). By using larger "magnetic traps" holding up to several liters of sediment slurry, large numbers (about 10^8 cells per liter of sediment) of diverse uncultivated magnetic cells can be selectively harvested from large volumes of sediment samples. In this method, bacteria are magnetically directed toward the tips of collection tubes, from which they can be conveniently collected.

Magnetotactic bacteria commonly enrich (increase in numbers) in sediment samples in jars or aquaria stored in dim light at room temperature for several weeks to months. In several studies, successions of different magnetotactic bacterial morphotypes have been observed during the enrichment process. Surprisingly, magnetotactic bacteria sometimes remain active for several years in aquaria without addition of nutrients. Characterization of the large ovoid *Nitrospirae*, *Candidatus* Magnetoovum mohavensis, was only possible due to its enrichment in samples incubated for several months after collection (Lefèvre et al. 2011a).

It is important to note that all methods commonly used for the detection and collection of uncultivated magnetotactic bacteria are inherently selective for cells which are highly motile, abundant, and at least temporarily tolerate exposure to atmospheric concentrations of oxygen. Thus, modifications to these techniques to detect, collect, and cultivate environmental magnetotactic bacteria that are at very low concentrations in the sample, that swim very slowly, or that are poisoned quickly by oxygen potentially may reveal an even greater diversity.

Diversity and Physiology of the Magnetotactic Bacteria

Even before the advent of molecular phylogenetics, the great diversity of magnetotactic bacteria was obvious to most investigators that study them because of the large number of different, sometimes unique, morphotypes observed in environmental samples of water and sediment. Commonly observed morphotypes include coccoid to ovoid cells, rods, vibrios, and spirilla of various dimensions. Two of the more unique morphotypes include a group of apparently multicellular bacteria referred to as MMPs and a very large rod provisionally named *Candidatus* Magnetobacterium bavaricum.

Regardless of their morphology, all magnetotactic bacteria studied thus far are motile by means of flagella and have a cell wall structure characteristic of typical gram-negative bacteria with one exception: uncultured, freshwater magnetotactic bacteria belonging to the *Nitrospirae* phylum appear to have a more complex cell wall structure (Jogler et al. 2011; Lefèvre et al. 2011a). The arrangement of flagella differs and can be either polar, bipolar, or in tufts. Another trait which shows considerable diversity is the arrangement of magnetosomes within the cell. In the majority of magnetotactic bacteria, the magnetosomes are aligned in chains of various lengths and numbers along the long axis of the cell, which is magnetically the most efficient orientation. However, dispersed aggregates or clusters of magnetosomes occur in some magnetotactic bacteria usually at one side of the cell, which often corresponds to the site of flagellar insertion (Moench and Konetzka 1978; Moench 1988; Cox et al. 2002). Besides magnetosomes, large inclusion bodies containing elemental sulfur, polyphosphate, or poly-β-hydroxybutyrate (PHB) are common in magnetotactic bacteria collected from natural environments and in pure culture (Bazylinski et al. 2004; Schultheiss et al. 2005). In cultivated magnetospirilla, PHB granules were found to be associated with phasin-like proteins as in other PHB-producing bacteria (Schultheiss et al. 2005).

The most commonly observed type of magnetotactic bacteria present in environmental samples are coccoid to ovoid cells, the so-called magnetococci, possessing two flagellar bundles on one somewhat flattened side. This bilophotrichous type of flagellation resulted in the provisional name "*Bilophococcus*" for the genus of these bacteria (Moench 1988). Interestingly, marine magnetococci possess a sheath surrounding their flagellar bundles (Lefèvre et al. 2010c). Two representative strains of this morphotype are now in axenic culture (Frankel et al. 1997; Lefèvre et al. 2009); one now named *Magnetococcus marinus* (Schübbe et al. 2009; Bazylinski et al. 2012a).

The phylogenetic diversity of magnetotactic bacteria, including both those in axenic culture and those collected from natural environments, is also considerable and based on the sequence of their 16S rRNA genes. To date, representatives of the magnetotactic prokaryotes are phylogenetically associated with four major lineages within the domain bacteria and three within the *Proteobacteria*. No magnetotactic bacterium phylogenetically associated with the Archaea has yet been discovered. Although most known cultured and uncultured magnetotactic bacteria belong to the *Alpha-*, *Gamma-*, and *Deltaproteobacteria* classes of the *Proteobacteria* phylum, several uncultured species are affiliated with the *Nitrospirae* phylum and one, assigned strain SKK-01, to the candidate division OP3, part of the Planctomycetes-Verrucomicrobia-Chlamydiae (PVC) bacterial superphylum (Kolinko et al. 2012) (*Fig. 12.3*).

The physiology of known magnetotactic bacteria, including that determined experimentally with cultured strains and that inferred from uncultured types, is also quite diverse. In general, however, the physiology of magnetotactic bacteria in almost all cases suggests that they are important in the cycling of key elements including iron, sulfur, nitrogen, and carbon in natural habitats.

Alphaproteobacteria Class

Magnetotactic bacteria in the *Alphaproteobacteria* are only known to biomineralize magnetite and include: all cultured species of the freshwater genus *Magnetospirillum* (Burgess et al. 1993; Schüler et al. 1999); all of the bilophotrichous magnetotactic cocci including the cultured *Magnetococcus marinus* strain MC-1 (DeLong et al. 1993; Schübbe et al. 2009; Bazylinski et al. 2012a) and strain MO-1 (Lefèvre et al. 2009) and numerous uncultured types (Spring et al. 1994, 1998; Lin and Pan 2009), the marine vibrios *Magnetovibrio blakemorei* strains MV-1 and MV-2 (DeLong et al. 1993; Bazylinski et al. 2012b), and the marine spirilla *Magnetospira thiophila* strain MMS-1 and strain QH-2 (Williams et al. 2012; Zhu et al. 2010). Using in situ hybridization with fluorescently labeled oligonucleotide probes, it was demonstrated that members of the *Alphaproteobacteria* class represent the dominant proportion of uncultured magnetotactic bacteria in many freshwater and marine environments (Spring et al. 1992, 1994, 1998). Because many uncultured magnetotactic *Alphaproteobacteria* contain intracellular sulfur globules (Moench 1988; Cox et al. 2002), autotrophy and/or mixotrophy based on the oxidation of reduced sulfur compounds is thought to be a common feature of these organisms. The ability to fix atmospheric nitrogen was found in all those tested (Bazylinski and Williams 2007).

All cultured magnetotactic *Alphaproteobacteria* are obligate microaerophiles or anaerobes or both (Bazylinski and Frankel 2004; Bazylinski and Williams 2007). Those that tolerate relatively high concentrations of oxygen do not synthesize magnetite under these conditions. They are mesophilic with regard to growth temperature, and none grow much higher than 30 °C.

Magnetospirillum species have a respiratory form of metabolism and are chemoorganoheterotrophic using organic acids as a source of carbon and electrons (Schleifer et al. 1991). *M. gryphiswaldense* is also capable of autotrophic and mixotrophic growth using reduced sulfur compounds as a source of electrons (Geelhoed et al. 2010). Although the pathway of autotrophy was not determined, it seems likely that carbon dioxide fixation occurs through the

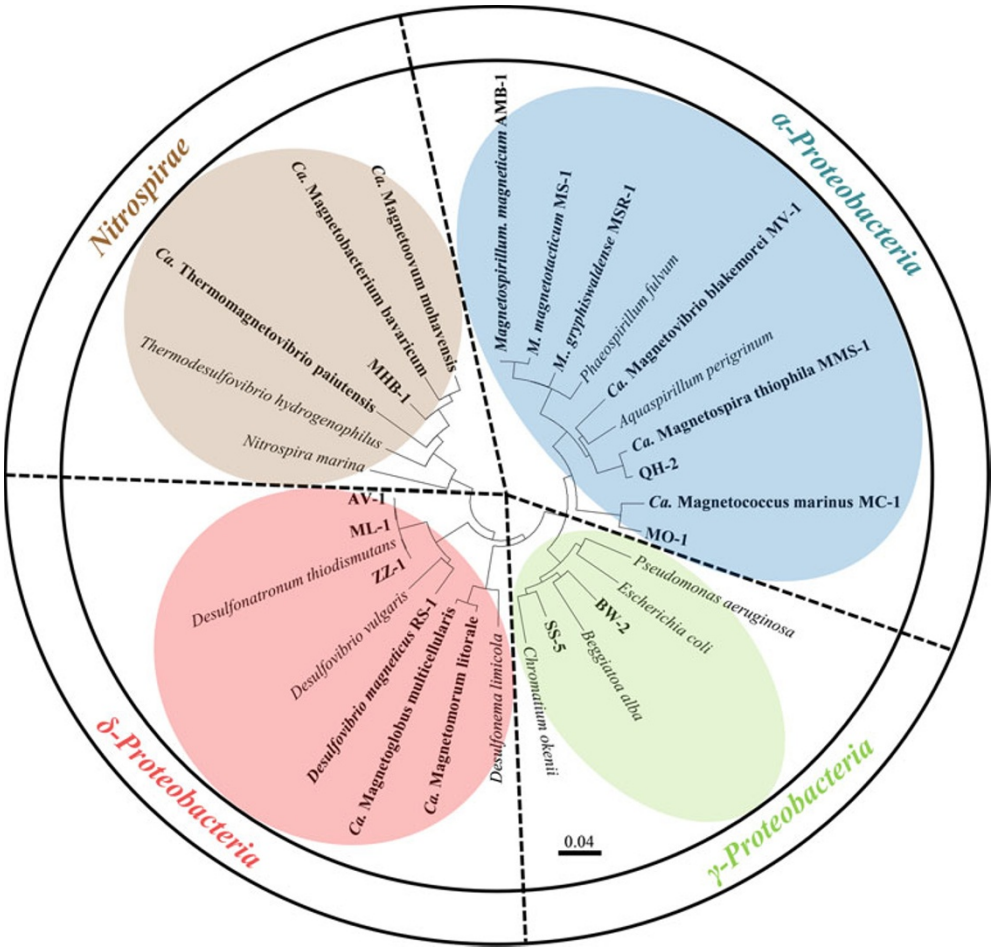


Fig. 12.3

Phylogenetic distribution of cultured and some uncultured magnetotactic bacteria in the *Alpha-*, *Gamma-*, and *Deltaproteobacteria* classes of the *Proteobacteria* phylum, the *Nitrospirae* phylum and the candidate division OP3. Magnetotactic bacteria are in *bold*. The uncultured organisms include: *Candidatus* Magnetoglobus multicellularis and *Ca.* Magnetomorum litorale, both forms of the MMP, of the *Deltaproteobacteria*; all strains in the *Nitrospirae*; and SKK-01 of the candidate division OP3. All others are cultured

Calvin-Benson-Bassham cycle since a form II ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) gene was found in the genome of *M. magnetotacticum* (Bazylinski et al. 2004). While most species are facultative anaerobes that utilize nitrate as an alternate terminal electron acceptor to oxygen, *M. magnetotacticum* appears to be an obligate microaerophile that requires oxygen even when growing with nitrate (Bazylinski and Blakemore 1983a; Blakemore et al. 1985). In *Magnetospirillum* species, magnetite synthesis only occurs at very low levels of oxygen or under anaerobic conditions when nitrate is the alternate terminal electron acceptor to oxygen (Bazylinski and Blakemore 1983a; Blakemore et al. 1985; Schüler and Baeuerlein 1998; Heyen and Schüler 2003). All three described species of *Magnetospirillum* show dinitrogen-dependent growth and show nitrogenase activity (the reduction of acetylene to ethylene in nitrogen-free medium) demonstrating that they are capable of nitrogen fixation (Bazylinski and Blakemore 1983b; Bazylinski et al. 2000). In further support of this, a full series of *nif* genes is present in the genomes of *M. magnetotacticum* and *M. magneticum*.

The marine vibrio, *Magnetovibrio blakemorei* strain MV-1, also has a respiratory metabolism using oxygen, nitrate and nitrous oxide as terminal electron acceptors (Bazylinski et al. 1988, 2012b). It grows chemoorganoheterotrophically with organic and some amino acids as carbon and electron sources (Bazylinski et al. 1988, 2012b; Bazylinski and Williams 2007) and also chemolithoautotrophically using reduced sulfur compounds as an electron source (Bazylinski et al. 2004, 2012b). This strain utilizes the Calvin-Benson-Bassham cycle for autotrophy: cell-free extracts display RubisCO activity and the strain possesses a form II 326 RubisCO gene (Bazylinski et al. 2004). MV-1 also grows chemoorganoautotrophically with formate as the electron donor (Bazylinski et al. 2004, 2012b). This strain shows nitrogenase activity under both heterotrophic and autotrophic conditions (Bazylinski and Williams 2007; Bazylinski et al. 2012b).

The cultured marine spirilla, *Magnetospira thiophila* strain MMS-1 and strain QH-2, both appear to be obligate microaerophiles that grow with organic acids as carbon and electron sources (Williams et al. 2012; Zhu et al. 2010). Chemolithoautotrophic growth is also supported in *M. thiophila* by thiosulfate but not sulfide (Bazylinski and Williams 2007). This species also displays nitrogenase activity under heterotrophic and autotrophic conditions (Bazylinski and Williams 2007).

The known cultured and uncultured magnetotactic cocci are not closely related to other *Alphaproteobacteria* and form their own clade within the *Alphaproteobacteria* that is basal to the rest of the group (◗ *Fig. 12.3*). Many uncultured magnetotactic cocci contain sulfur globules even when sulfide is not apparent or measureable in the sample from which they were collected (Moench 1988; Cox et al. 2002) suggesting an autotrophic or mixotrophic metabolism based on the oxidation of reduced sulfur compounds. The two cultured magnetococci, *Candidatus* Magnetococcus marinus and strain MO-1, are obligately microaerophilic and grow autotrophically on sulfide and thiosulfate (Williams et al. 2006; Lefèvre et al. 2009). *Ca.* Magnetococcus marinus utilizes the reverse (reductive) tricarboxylic acid cycle for carbon dioxide fixation and autotrophy (Williams et al. 2006). It also grows chemoorganoheterotrophically with acetate as the carbon and electron source and is capable of nitrogen fixation based on the strain exhibiting nitrogenase activity and the presence of a full suite of *nif* genes in its genome (Bazylinski and Williams 2007; Schübbe et al. 2009).

Gammaproteobacteria Class

Simmons et al. (2004) provided some evidence for a putative greigite-producing rod belonging to the *Gammaproteobacteria*. However, the mineral present in its magnetosomes was never identified and a thorough examination of the phylogenetic relationship of this organism raised doubts to its affiliation with this group (Amann et al. 2007). Only recently have magnetotactic bacteria, specifically two strains designated BW-2 and SS-5, been reported to unequivocally belong to the *Gammaproteobacteria* (Lefèvre et al. 2012), and thus, there is little information regarding the extent of the diversity of magnetotactic bacteria in this class. Both organisms are mesophilic, microaerophilic rods and biomineralize magnetite. BW-2 and SS-5 are not closely related: BW-2 belongs to the Thiotrichales order whereas SS-5 to the Chromatiales. Very recently, a large group of uncultured and one cultured greigite-producing rods were found to be phylogenetically affiliated with the *Deltaproteobacteria* (Lefèvre et al. 2011d).

Strain BW-2 was isolated from sediment and water collected from a brackish, sulfidic spring at Badwater Basin at Death Valley, California in which the dominant magnetotactic bacteria were greigite-producing rods (Lefèvre et al. 2012). Cells are motile by a single polar, unsheathed bundle of seven flagella. This strain is only known to grow chemolithoautotrophically using sulfide and thiosulfate as electron donors. Cells produce intracellular sulfur globules, and thiosulfate is oxidized completely to sulfate. Cells show nitrogenase activity.

Strain SS-5 was isolated from sediment and water collected from the southeastern shore of the hypersaline Salton Sea, California (Lefèvre et al. 2012). Cells possess a single polar flagellum. Like those of BW-2, cells grow chemolithoautotrophically with sulfide and thiosulfate (oxidized completely to sulfate) but also show potential for heterotrophic growth on succinate. Although they do not produce intracellular sulfur globules, they synthesize large deposits of phosphate-rich inclusions. Unlike all magnetotactic bacteria tested, SS-5 did not show nitrogenase activity.

Deltaproteobacteria Class

The *Deltaproteobacteria* contain both magnetite- and greigite-producing magnetotactic bacteria and include: the various forms of the uncultured MMP which biomineralize either or both minerals (DeLong et al. 1993; Keim et al. 2003; Abreu et al. 2007; Simmons and Edwards 2007); a group of uncultured and two cultured (strains BW-1 and SS-2), large, rod-shaped bacteria that biomineralize either or both minerals (Lefèvre et al. 2011d); the magnetite-producing, rod-shaped, sulfate-reducer *Desulfovibrio magneticus* strain RS-1 isolated from a freshwater river in Japan (Sakaguchi et al. 1993, 2002); and several similar strains of obligately alkaliphilic, sulfate-reducing, magnetite-producing rods isolated from extremely alkaliphilic habitats in California, USA, that, based on 16S rRNA gene sequence identity, likely represent new strains of *Desulfonatronum thiodismutans*, a known non-magnetotactic Deltaproteobacterium (Lefèvre et al. 2011b). All magnetotactic *Deltaproteobacteria* are mesophilic based on their growth temperature or the temperature of their habitats.

The MMP. One of the most interesting and unusual examples of prokaryotic morphology is that of the organisms known as magnetotactic multicellular prokaryotes (MMPs; also known as the magnetotactic multicellular aggregate (MMA) (Farina et al. 1983; Lins and Farina 1999), the magnetotactic multicellular organism (MMO) (Keim et al. 2004a), and magnetotactic multicellular bacteria (Shapiro et al. 2011). The acronym MMP originally represented many-celled magnetotactic prokaryote (Rodgers et al. 1990a, b) because it was difficult to prove that the organism was truly multicellular. Because of a number of recent findings suggesting that individual cells interact and/or communicate with each other, many researchers now use MMP for multicellular magnetotactic prokaryote (e.g., Wenter et al. 2009). Three MMPs have been tentatively named: *Candidatus* Magnetoglobus multicellularis (Abreu et al. 2007), *Ca.* Magnetomorum litorale (Wenter et al. 2009), and *Ca.* Magnetananas tsingtaoensis (Zhou et al. 2012). Interestingly, despite their unique morphology, if not for its magnetotactic behavior, it is unlikely that the MMP would have been discovered.

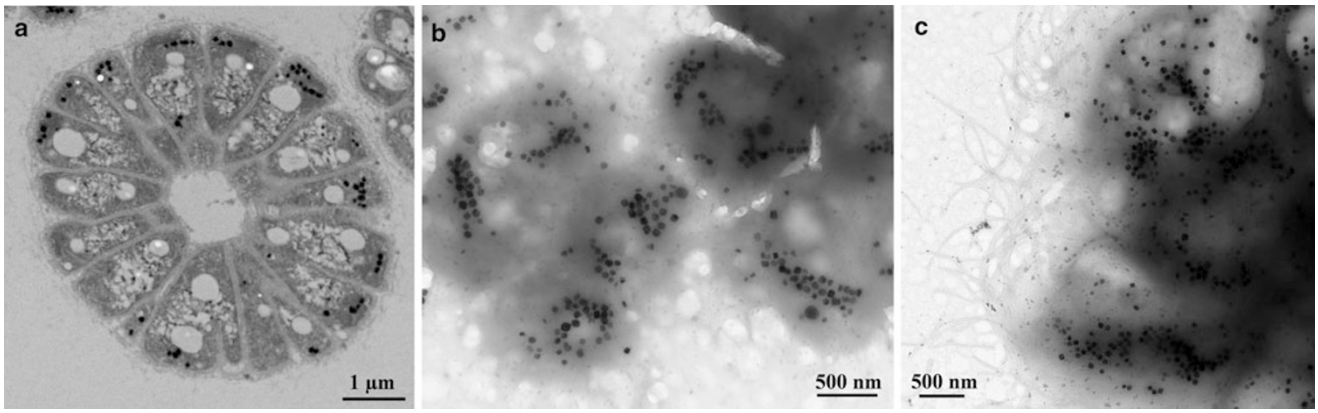


Fig. 12.4

Brightfield transmission electron microscopy (TEM) images of the multicellular magnetotactic prokaryote (MMP). (a) Thin-section of an MMP showing its many-celled nature and the acellular compartment in the center of the rosette of cells. Electron-lucent vacuoles may represent poly-β-hydroxybutyrate (PHB) granules (Micrograph courtesy of F. Abreu) (b) Unstained cells of an MMP revealing the numerous greigite-containing magnetosomes within the organism mostly arranged in short chains. (c) Outer surface of an unstained MMP. Flagella are distributed asymmetrically on each cell and cover the cell on one side

MMPs are relatively large for prokaryotic microorganisms and range from about 3- to 12-µm in diameter (Bazylinski et al. 1990; Rodgers et al. 1990a, b) (*Fig. 12.4*). It is best described as an aggregation of about 10–60 g-negative, genetically similar cells that swim only as an intact unit and not as individual cells (Bazylinski et al. 1990; Rodgers et al. 1990a, b; Simmons and Edwards 2007). Cells that become separated from the intact unit die quickly (Abreu et al. 2006). Cells are asymmetrically flagellated, the surface of the cell exposed to the surrounding environment covered with numerous flagella (Rodgers et al. 1990a, b; Silva et al. 2007). Most described MMPs are spherical (Bazylinski et al. 1990; Rodgers et al. 1990a, b; Keim et al. 2004a, 2007; Abreu et al. 2007; Wenter et al. 2009), although some are ovoid or pineapple-shaped in morphology (Lefèvre et al. 2007; Zhou et al. 2012), and all appear to possess a central, acellular compartment (Keim et al. 2004a, 2007). The MMP divides as aggregates without an individual cell stage (Keim et al. 2004b, 2007; Zhou et al. 2012).

MMPs are cosmopolitan in distribution in numerous saline aquatic environments, ranging from brackish to hypersaline (Keim et al. 2004a, b; Abreu et al. 2007; Martins et al. 2009). In all cases, the salinity is due to the input of seawater, and many have considered these organisms indigenous only to marine environments (Simmons and Edwards 2007). Recently, non-magnetotactic forms of MMP (referred to as nMMPs) were found in springs and lakes with relatively low salinities (~5–11 ppt) and no marine input (Lefèvre et al. 2010a). The nMMPs have typical MMP morphology but contain up to 60 cells per aggregate. They are phylogenetically closely related to MMPs (Lefèvre et al. 2010a). Little is known regarding the physiology but it seems very likely that the MMPs are sulfate-reducing bacteria based on the fact that their closest phylogenetic relatives are sulfate-reducers (DeLong et al. 1993; Simmons and Edwards 2007) and that the genes for dissimilatory sulfite reductase (*dsrAB*) and dissimilatory adenosine-5′-phosphate reductase (*aprA*) were detected in purified MMP samples (Wenter et al. 2009).

The magnetic mineral greigite in magnetotactic bacteria was first discovered in MMPs (Farina et al. 1990; Mann et al. 1990b). Since then, they have also been found to contain nonmagnetic precursors to greigite (Pósfai et al. 1998a, b), magnetite (Zhou et al. 2011, 2012), or both magnetite and greigite magnetosomes (Keim et al. 2003; Lins et al. 2007). The greigite crystals in magnetosomes of MMPs are generally pleomorphic although cuboctahedral, elongated-prismatic, and bullet-shaped particles have been observed (Mann et al. 1990b; Pósfai et al. 1998a, b; Wenter et al. 2009) (see later section on "Magnetosomes"). Only bullet-shaped magnetite crystals have yet been found in magnetosomes of MMPs (Keim et al. 2003; Lins et al. 2007; Zhou et al. 2011, 2012). Magnetosomes are usually loosely arranged in short chains or clusters in individual cells (Mann et al. 1990b; Pósfai et al. 1998a, b; Lins et al. 2007; Wenter et al. 2009) although there is a general enough consensus in magnetosome arrangement that there is a magnetic dipole to the entire unit (Bazylinski and Frankel 2000; Wenter et al. 2009). It has been also shown that magnetosome chain polarities of individual cells contribute coherently to the total magnetic moment of the MMP in a highly coordinate fashion, suggesting a remarkable degree of magnetic optimization, which is likely inherited by individual cells by a sophisticated reproduction mechanism (Winklhofer et al. 2007).

The total magnetic moments of MMPs from different collecting sites ranged from 5×10^{-16} to 1×10^{-15} A m^2 for one group (Rodgers et al. 1990a, b) and 9–20 $\times 10^{-15}$ A m^2 for *Candidatus* Magnetoglobus multicellularis (Perantoni et al. 2009), which are sufficient for an effective magnetotactic response. However, magnetic measurements of greigite-containing MMPs showed that hysteresis loops of these

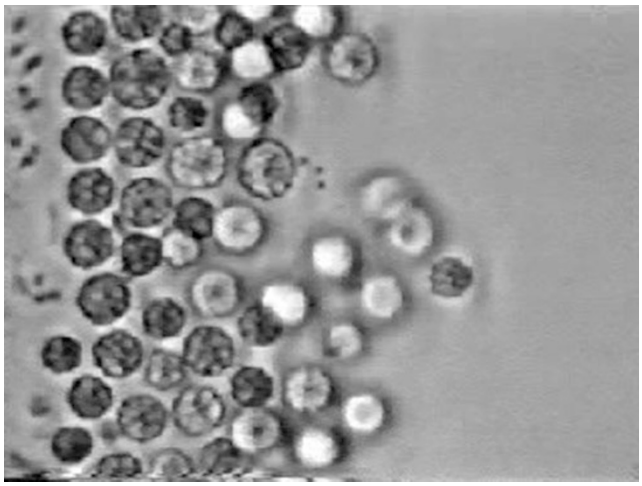


Fig. 12.5
Sequence showing the typical "ping-pong" motility of the MMP. For the video, see the online version of *The Prokaryotes*

organisms are not square indicating that MMPs, unlike magnetotactic bacteria that contain a single chain of magnetite magnetosomes, can be demagnetized (Penninga et al. 1995).

The type of magnetotaxis displayed by the MMP appears to be polar (see "Magnetotaxis, Chemotaxis, Aerotaxis, and Phototaxis" section below), although they have been observed to reverse direction in a magnetic field (it is unknown whether they physically turn around). Under oxic conditions in a uniform magnetic field, the swimming speed of the MMP in the preferred direction averages about 105 μm/s. After reaching the edge of a water drop, they sometimes spontaneously swim in the opposite direction and show short excursions of 100–500 mm at twice the speed of the forward motion in the opposite direction of their polarity after which they return to the same edge of the drop at a slower speed (Rodgers et al. 1990a, b) as shown in *Fig. 12.5*. MMPs exhibit this so-called "ping-pong" motility (Rodgers et al. 1990a, b) in magnetic fields at least several times stronger than the Earth's magnetic field (~0.5 G) (Greenberg et al. 2005). A detailed study of this behavior in hanging drops (Greenberg et al. 2005) revealed that the outward and return excursions show a uniform deceleration and acceleration, respectively. In addition, the probability per unit time of an MMP undergoing a ping-pong increases monotonically with an increase in the strength of the magnetic field. Outward excursions show an unusual minimum distance distribution, also dependent on the magnetic field strength, in which the higher the field strength, the lower the minimum excursion distance.

Desulfovibrio magneticus strain RS-1 is an obligate anaerobe that grows and respires with sulfate and fumarate as electron donors (Sakaguchi et al. 1993, 2002). Like all *Desulfovibrio* species, cells are curved rods (vibrios) that possess a single polar flagellum and show no potential for autotrophic growth. Small organic molecules and some organic acids support heterotrophic growth in this organism. It is the only known cultured magnetotactic bacterium to be capable of fermentation: pyruvate is fermented to acetate and hydrogen (Sakaguchi et al. 2002).

While magnetotactic bacterium have never been associated with extremophilic conditions, recently, three strains of obligately alkaliphilic, anaerobic, sulfate-reducing, magnetotactic bacteria belonging to the *Deltaproteobacteria* with optimal growth pH's of 9.0–9.5 were isolated and grown in axenic culture (Lefèvre et al. 2011b). All strains biomineralize bullet-shaped crystals of magnetite, are closely related to each other, and appear to be strains of *Desulfonatronum thiodismutans*, a known alkaliphilic sulfate-reducing bacterium that does not biomineralize magnetosomes (Pikuta et al. 2003) based on their very high sequence identities of their 16S rRNA genes (Lefèvre et al. 2011b). Like *D. thiodismutans*, cells are vibrioid to helicoid in morphology and possess a single polar flagellum. All strains grow autotrophically and possibly mixotrophically with hydrogen as electron donor. Formate is also utilized as electron donor.

Strain BW-1, recently isolated from a saline spring at Badwater Basin, Death Valley National Park (California), and strain SS-2 isolated from the Salton Sea (California) are two members of a group of large, rod-shaped bacteria that biomineralize greigite and/or magnetite (*Fig. 12.6*). BW-1 grows chemoheterotrophically using sulfate as a terminal electron acceptor and produces both minerals, the dominant mineral present being dependent upon culture conditions (e.g., sulfide concentration). The greigite crystals appear to be pleomorphic, while the magnetite crystals are bullet-shaped like those of all other deltaproteobacterial magnetotactic bacteria. Both organisms belong to a previous unrecognized group of sulfate-reducing bacteria in the family *Desulfobacteraceae* (Lefèvre et al. 2011d).

Nitrospirae Phylum

Thus far, no magnetotactic *Nitrospirae* have been isolated in axenic culture. However, four different uncultured magnetotactic bacteria phylogenetically associated with this phylum have been described in some detail. The large rod, *Candidatus* Magnetobacterium bavaricum, is the most studied and was first discovered in sediment samples from Lake Chiemsee and Lake Ammersee in southern Germany (Vali et al. 1987; Petersen et al. 1989). Another magnetotactic *Nitrospirae*, a small rod-shaped bacterium collected from sediment of the Waller See, Germany, was described by Flies et al. (2005b) and designated strain MHB-1. Recently, Lefèvre et al. (2010b, 2011a) described two new *Nitrospirae*: a moderately thermophilic species tentatively named *Candidatus* Thermomagnetovibrio paiutensis strain HSMV-1 found in brackish hot springs within the Great Boiling Springs geothermal field in Gerlach, Nevada, USA, and a large ovoid-shaped organism tentatively named *Candidatus* Magnetoovum mohavensis strain LO-1 from freshwater sediments of Lake Mead, Nevada. An organism closely related to strain LO-1, designated MWB-1, isolated from Lake Beihai in Beijing, China, was recently described (Lin et al. 2012). All known

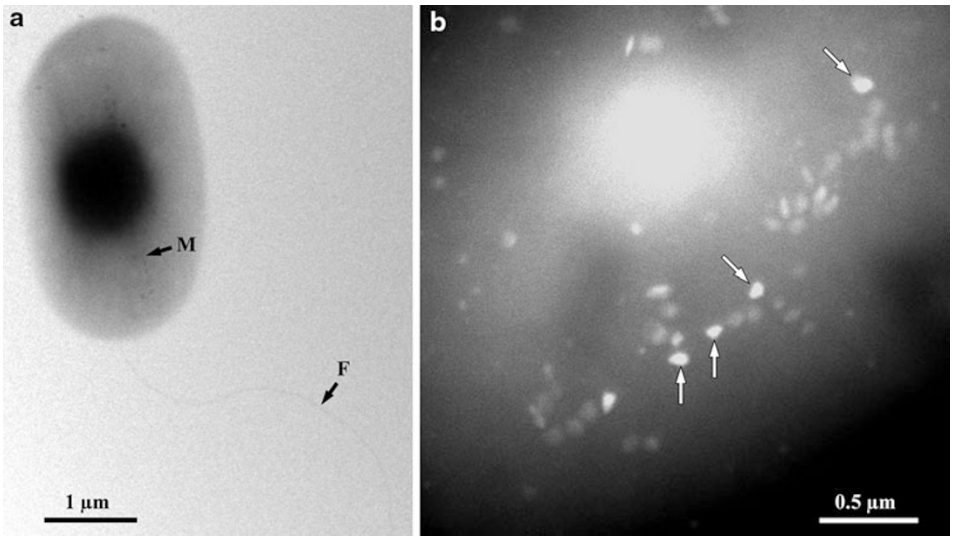


Fig. 12.6

TEM images of the cultured strain BW-1, a deltaproteobacterial magnetotactic bacterium that biomineralizes greigite and magnetite. (a) Brightfield TEM image of a cell of strain BW-1 showing chain of magnetosomes (*M*) and single polar flagellum (*F*). (b) Dark field TEM image of magnetosomes in a cell of BW-1. Crystals at *arrows* are magnetite, the others are greigite

magnetotactic *Nitrospirae* biomineralize bullet-shaped crystals of magnetite.

Candidatus Magnetobacterium bavaricum. This cell morphotype was first observed in samples of littoral sediments collected from Lake Chiemsee and Lake Amersee in southern Germany (Vali et al. 1987; Petersen et al. 1989). *Ca.* M. bavaricum-like cells have also been found in Brazil (Lins et al. 2000), France (Isambert et al. 2007), and China (Lin et al. 2009; Lin and Pan 2009; Li et al. 2010). Because of its large size, volume (average volume ca. 25.8 ± 4.1 μm^3), and relative abundance, *Ca.* M. bavaricum may account for approximately 30 % of the microbial biovolume in the microaerobic zone of sediments and may, therefore, be a dominant fraction of the microbial community in this zone of Lake Chiemsee (Spring et al. 1993). In addition, 16S rRNA sequences very similar to that of *Ca.* M. bavaricum (>99 % identity) have been retrieved from a number of freshwater and marine habitats and biological reactor columns (Jogler et al. 2010).

Cells of *Candidatus* Magnetobacterium bavaricum are large rods having dimensions of 1–1.5 × 6–9 µm and are motile by a single polar tuft of flagella (*Fig. 12.7*). Cells contain between 600 and 1,000 magnetosomes that contain bullet-shaped crystals of magnetite that range from 110 to 150 nm in length and are arranged as several braid-like bundles (usually 3–5 per cell) of multiple chains (Hanzlik et al. 1996, 2002; Jogler et al. 2010; Li et al. 2010). Many of the crystals display a kink or hook-like feature. The average total magnetic moment per cell was experimentally determined to be approximately 3×10^{-14} A m^2, which is about an order of magnitude higher than that of most other magnetotactic bacteria. Large amounts of bullet-shaped magnetite crystals have been found in some sediments where *Ca.* M. bavaricum is present suggesting to some that magnetite from this organism accounts for a large proportion (up to 10 %) of the total magnetization in these sediments (Petersen et al. 1989; Pan et al. 2005).

Candidatus Magnetobacterium bavaricum displays polar magnetotaxis, and in a uniform magnetic field, cells swim forward with an average speed of about 40 µm/s with the flagella wound around the rotating cell. Gradients of some chemical substances lead to a reversal of the sense of flagellar rotation resulting in a swimming in the opposite direction for a short time (Spring et al. 1993).

Because *Candidatus* Magnetobacterium bavaricum is mainly found in the microaerobic zone (OAI) of sediments and contains sulfur-rich globules, it is thought to be a microaerophilic, sulfide-oxidizing bacterium (Spring et al. 1993; Jogler et al. 2010). In addition, a putative large type IV ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) subunit gene was found in a 34 kb genomic region of *Ca.* M. bavaricum, and although these RubisCO-like proteins do not exhibit RubisCO enzymatic activity (Hanson and Tabita 2001), it may be linked to sulfur metabolism in this organism (Jogler et al. 2010).

Another magnetotactic *Nitrospirae*, a small rod-shaped bacterium collected from sediment of the Waller See, Germany, was described by Flies et al. (2005b) and designated strain MHB-1. This organism is a slow moving, rod-shaped bacterium that contains a single bundle of multiple chains of magnetite magnetosomes whose crystals are also bullet-shaped.

The uncultured *Candidatus* Thermomagnetovibrio paiutensis strain HSMV-1 was found in a series of brackish hot springs with temperatures between 32 °C and 63 °C within the Great Boiling Springs geothermal field in Gerlach, Nevada USA (Lefèvre et al. 2010b). Cells are small vibrios with a single polar flagellum. The upper limit of growth of this bacterium is probably around 63 °C as it was not present in those springs with higher temperatures.

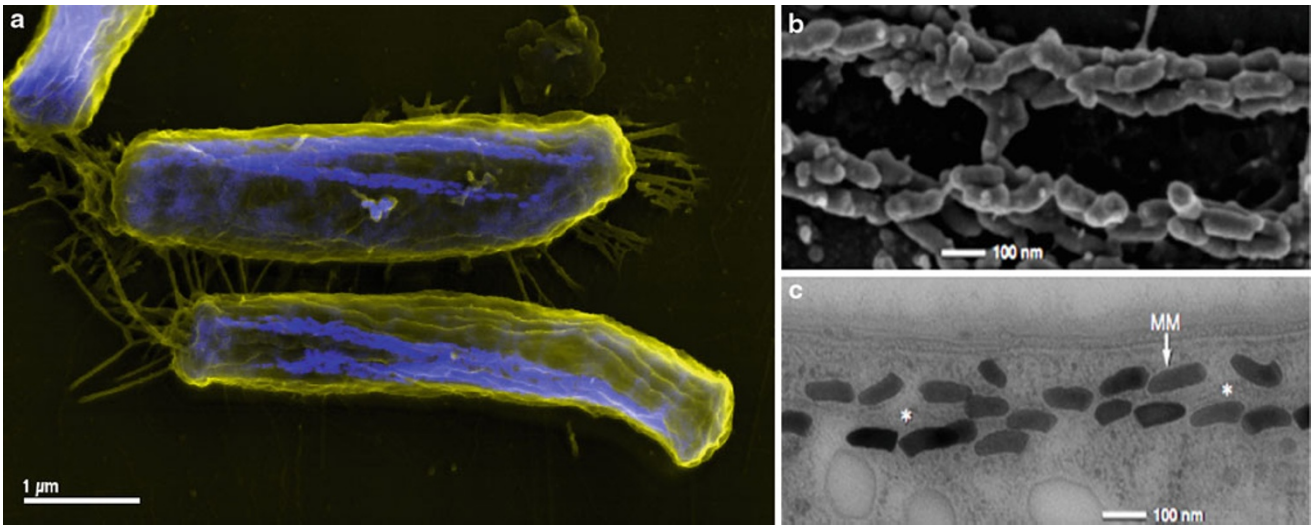


Fig. 12.7
Scanning electron microscope (SEM) and TEM images of cells of *Candidatus* Magnetobacterium bavaricum. (a) SEM image obtained by simultaneous detection of secondary (*yellow*) and backscattered electrons (*blue*). Multiple chains of magnetite crystals are visible (*blue*). (b) SEM image of a cryofractured cell showing two bundles of magnetosome strands. (c) TEM image of an ultrathin section of high-pressure frozen and freeze-substituted cells showing strands of magnetosomes aligned parallel to a tubular filamentous structure (at *asterisks*). *MM* magnetosome membrane) (Micrographs courtesy of Gerhard Wanner, LMU München [with kind permission])

Candidatus Magnetoovum mohavensis strain LO-1 was discovered in freshwater sediments of Lake Mead, Nevada USA (Lefèvre et al. 2011a). This bacterium is relatively large, ovoid in morphology, has a single polar bundle of sheathed flagella, and biomineralizes braid-like bundles (usually three) of multiple chains of bullet-shaped magnetosomes. Although the organism is likely gram-negative, it appears to have an unusual three-layered cell wall. This organisms may be widely distributed as similar organisms have been observed and collected from freshwater and estuarine environments including the Exeter River, New Hampshire USA (Mann et al. 1987a, b); the Pettaquamscutt Estuary, Rhode Island USA (Bazylinski and Frankel 2003); several sites in Germany (Flies et al. 2005a; Amann et al. 2007); and freshwater lagoons (Jacarepiá Lagoon, Saquarema, Brazil) and brackish waters (Lagoa de Cima, Rio de Janeiro) in southeastern Brazil (Lins et al. 2000). Like those of *Candidatus* Magnetobacterium bavaricum, cells of *Ca.* Magnetoovum mohavensis contain sulfur-rich inclusions suggesting a metabolism based on the oxidation of reduced sulfur compounds. The distribution of cells in a natural microcosm was also similar to that found for *Ca.* Magnetobacterium bavaricum (Jogler et al. 2010) in that the majority of cells were found at the OAI and the upper layer of the anaerobic zone. In semi-solid oxygen-gradient medium, however, cells immediately migrated to the anoxic zone and remained viable for several days. These results indicate that LO-1 is likely an anaerobe that tolerates low concentrations of oxygen. These studies also suggest that this organism is mesophilic. A magnetotactic bacterium morphologically similar to *Ca.* Magnetoovum mohavensis, strain MWB-1, was isolated from Lake Beihai in Beijing, China, and shares 95% 16S rRNA gene sequence identity with *Ca.* Magnetoovum mohavensis (Lin et al. 2012). The watermelon-shaped MWB-1 appears to account for more than 10 % of the natural remanent magnetization of the surface sediment of Lake Beihai (Lin et al. 2012).

Other Groups

Recently, by using single cell-based techniques, a magnetotactic bacterium of low abundance was found to belong to the candidate OP3 division of bacteria which so far lacks any cultured representatives (Hugenholtz et al. 1998), based on 16S and 23S rRNA gene sequences (Kolinko et al. 2012). This might indicate that the diversity and phylogenetic distribution of magnetotactic bacteria is underestimated and may extend toward other unrecognized groups.

Evolution of Magnetotaxis and the First Magnetosomes

The early initial discovery that greigite- and magnetite-producing magnetotactic bacteria were affiliated with two evolutionary distinct lines of descent, the *Deltaproteobacteria* and the *Alphaproteobacteria*, respectively, led DeLong et al. (1993) to suggest that the trait of magnetotaxis based on iron sulfide and iron oxide had multiple evolutionary origins. At the present, however, considering the now considerable genomic and new phylogenetic information, it seems more likely that the magnetotactic trait is monophyletic originating from a common ancestor regardless of magnetosome mineral composition (Abreu et al. 2011; Jogler et al. 2011) and that it has been passed to many

diverse prokaryotes through horizontal gene transfer (discussed later in the chapter). Strong evidence for this hypothesis was recently found by the discovery of putative magnetosome (*mam*) genes in uncultivated MMP from the *Deltaproteobacteria* (Abreu et al. 2011) and in *Candidatus* Magnetobacterium bavaricum belonging to the deeply branching *Nitrospirae* phylum (Jogler et al. 2011), which are homologous to those previously found in the remotely related magnetotactic *Alphaproteobacteria.*

Interestingly, there is a strong correlation with the phylogeny of magnetotactic bacteria and the composition and morphology of the magnetosome mineral crystal they produce (Abreu et al. 2011; Lefèvre et al. 2011c, 2012). Magnetotactic *Alphaproteobacteria* only biomineralize morphologically consistent, well-defined crystals of magnetite that include cuboctahedra and elongated prisms (appear as rectangular in projection in electron micrographs). The only two known magnetotactic *Gammaproteobacteria* also synthesize these types of magnetite crystals. While magnetotactic *Deltaproteobacteria* biomineralize magnetite or greigite or both, the magnetite crystals are always bullet- or tooth-shaped and show much more morphological variation and defects (e.g., kinks) than those produced by the *Alphaproteobacteria.* The magnetotactic *Nitrospirae* are only known to biomineralize magnetite crystals whose morphologies are very similar, if not identical, to those found in the *Deltaproteobacteria.* The great variation in the magnetite crystals of these latter two groups suggests that there is less control over the biomineralization process in these organisms which may be due to the fact that species in these groups appear to possess less magnetosome genes than those in the *Alphaproteobacteria* (Lefèvre et al. 2012). Because of this and the fact that the *Nitrospirae* and the *Deltaproteobacteria* are the more deeply branching phylogenetic groups, it has been suggested that bullet-shaped magnetite crystals might represent the first magnetosome mineral phase (Abreu et al. 2011).

Cultivation and Isolation

Magnetotactic bacteria have generally proven to be fastidious with respect to growth, and the inability to isolate new strains of magnetotactic bacteria and the lack of specific enrichment and isolation media for them have frustrated potential and current researchers in this area for many years. This is in part because these organisms are ubiquitous in freshwater and marine habitats and because many different cell morphotypes can be present in relatively high numbers in collected samples that can be easily visualized. Moreover, many morphotypes actually enrich in samples of mud and water collected in jars or in microcosms that are simply exposed to dim light and left at room temperature without special treatments such as the addition of nutrients (Blakemore 1982; Flies et al. 2005a). Lastly, based on their ecology and those species already in culture, magnetotactic bacteria are clearly gradient-loving or gradient-requiring organisms (e.g., Frankel et al. 1997). Phylogeny of specific morphotypes of magnetotactic bacteria might provide clues as to their physiology which might be helpful in their isolation and axenic culture. For example, the phylogeny of the MMPs strongly suggests that these organisms are anaerobic sulfate-reducing bacteria (DeLong et al. 1993; Abreu et al. 2007; Simmons and Edwards 2007; Wenter et al. 2009). It is risky, however, to infer physiological capabilities solely on the basis of phylogenetic affiliation.

A major problem in the isolation of magnetotactic bacteria is the lack of an effective enrichment medium. Several media have been constructed for the isolation of magnetotactic bacteria, and most that have proven successful are based on the formulation of Blakemore et al. (1979) for freshwater magnetotactic spirilla. While cells of most magnetospirilla grow well in this medium, the medium does not enrich for them when water and/or mud samples containing magnetotactic bacteria are used as an inoculum as non-magnetotactic organisms rapidly outcompete them. However, magnetotactic bacteria can be quite efficiently and selectively separated from sediment particles and contaminating microorganisms by making use of their active magnetically directed motility. Schüler et al. (1999) developed an improved technique for the enrichment and isolation of magnetotactic spirilla by exploiting their magnetotactic behavior, the idea using magnetotactic bacterial cells as inocula that were rendered free of non-magnetotactic contaminants by magnetically separating them using the magnetic capillary "racetrack" of Wolfe et al. (1987). In this technique (modified slightly from the original), a Pasteur pipette is sealed at its thin end in a flame and a cotton plug set at where the wide-mouthed end of the pipette tapers to the thin portion (*Fig. 12.8*). The pipette is sterilized, after which the sealed end is filled with filter-sterilized (0.2 μm) water from the original sample until the cotton plug is wetted. Sediment and/or water containing magnetotactic bacteria are placed on top of the sterile, wetted cotton plug in the wide-mouthed end of the pipette. The south pole of a bar magnet is placed near the sealed tip of the capillary furthest from the reservoir, and the north pole of an additional bar magnet set near the entrance of the wide-mouthed end of the pipette. Migration to and accumulation of magnetotactic cells at the end of the capillary can be observed by using a dissecting microscope with the lighting set up for dark field. Generally, most fast-swimming cells of magnetotactic bacteria (cocci) will reach the sealed tip in about 20–30 min. When enough cells have accumulated for a reasonable inoculum, the tip of the pipette is broken off and the cells are removed aseptically using a thin syringe needle. The cells are then subsequently transferred to appropriate enrichment media. Although the magnetic capillary racetrack method is quite useful for the separation of larger, faster swimming magnetotactic bacteria such as some large spirilla and the ubiquitous magnetotactic cocci, it can take much longer periods of time for smaller, slower swimming organisms (e.g., cells *Magnetovibrio blakemorei* strain MV-1) to reach the sealed end of the pipette. Moreover, it is difficult to determine whether these small cells are present at the end of the capillary using a dissecting microscope. After about 30 min, it is not uncommon for motile non-magnetotactic contaminants to reach the end of the sealed capillary. Although this technique has

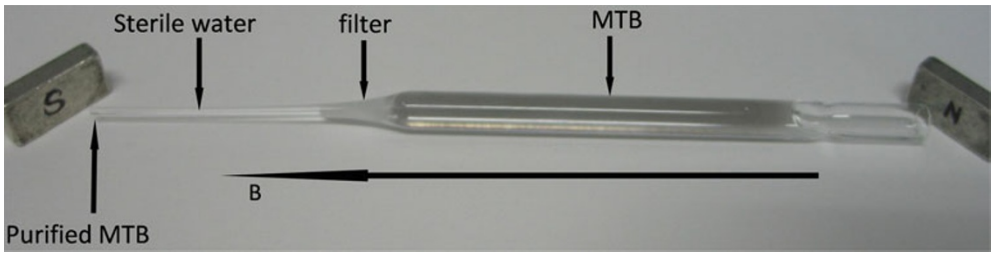


Fig. 12.8

Image of the magnetic "racetrack" described by Wolfe et al. (1987). In this technique (modified slightly from the original), a Pasteur pipette is sealed at its thin end in a flame and a cotton plug placed where the wide-mouthed end of the pipette tapers to the thin portion. The pipette is sterilized (by autoclaving) after which the sealed end is filled with filter-sterilized (0.2 μm) water from the original sample until the cotton plug is wetted. Sediment and/or water containing magnetotactic bacteria are placed on top of the sterile, wetted cotton plug in the wide-mouthed end of the pipette. North-seeking cells are purified by placing the south pole of a bar magnet near the sealed tip of the capillary furthest from the reservoir and the north pole of an additional bar magnet set near the entrance of the wide-mouthed end of the pipette thereby directing north-seeking cells to the sealed end of the capillary containing sterile water. Accumulation of magnetotactic cells at the end of the capillary can be observed by using a dissecting microscope with the lighting set up for dark field. Once enough cells have accumulated at the tip, the sealed pipette tip is broken off and cells are removed aseptically using a thin syringe needle

proven effective in a number of studies, it does not guarantee a homogenous population unless only one type of magnetotactic bacterium is present in the original sample. There is a report questioning whether cells purified by this technique reflect the diversity of magnetotactic bacteria in the original environmental samples (Lin et al. 2008). This should be assumed considering the very diverse swimming speeds of different magnetotactic bacteria. This limitation of magnetic collection can be circumvented by the application of single cell techniques, such as microscopically controlled micromanipulation and cell sorting, by which any conspicuous morphotype can be targeted and separated from mixed environmental communities of magnetotactic bacteria (Kolinko et al. 2012).

All known magnetite-producing magnetotactic bacteria are microaerophiles (atmospheric oxygen concentrations are inhibitory to growth), anaerobes, or facultatively anaerobic microaerophiles. Most media used for the growth of these organisms are semi-solid oxygen concentration gradients or liquid anaerobic media. In general, relatively low concentrations of nutrients appear more favorable for the isolation of magnetotactic bacteria compared to richer media containing higher concentrations of carbon and nitrogen sources. Although some species, including *Desulfovibrio magneticus* and some greigite-producing species (e.g., strain BW-1), are obligate anaerobes, most magnetotactic bacteria tolerate short exposures to oxygen during magnetic purification and inoculation, making the strict exclusion of oxygen during cell manipulations unnecessary. However, it is not clear if this is true for all other uncultivated species, and the strict exclusion of atmospheric oxygen from all sampling, enrichment, and cultivation steps wherever possible might increase the success of isolation.

Magnetite-producing magnetotactic bacteria are not only sensitive to high concentrations of oxygen but are also redox-sensitive, that is, they do not grow from small inocula in growth medium without the addition of a reducing agent. Thus, formation of the oxygen/redox gradient in the growth medium is crucial for the growth of magnetite-producing magnetotactic bacteria. Redox buffering by the addition of reducing agents such as sodium thioglycolate, sodium sulfide, ascorbic acid, or cysteine at concentrations of 0.1–0.4 g L^{-1} or dithiothreitol at 1 mM to the medium is required for growth of these microaerophilic or anaerobic species (Bazylinski et al. 1988; Schüler et al. 1999). The inclusion of resazurin, a redox indicator that is colorless when fully reduced, is very helpful in the determination of whether a liquid growth medium is totally reduced or whether an oxygen concentration-redox gradient has formed in semi-solid medium. In the latter case, the surface of the medium should be oxidized and pink and the anoxic zone at the lower part of the tube should be reduced and colorless. The formation of semi-solid media containing inverse oxygen and sulfide concentration double gradients has been used for the successful enrichment of freshwater and marine magnetotactic bacteria (Bazylinski and Williams 2007; Schüler et al. 1999). The formulation for this gradient medium, a modification of the medium originally developed by Nelson and Jannasch (1983) for the enrichment and isolation of the microaerophilic, filamentous, sulfide-oxidizer *Beggiatoa*, is described in detail in Schüler et al. (1999). In this medium, the sulfide gradient is the result of the diffusion from sulfide from a solid sulfide agar plug at the bottom of the tube. Growth of magnetite-producing species in all oxygen concentration growth medium initially occurs as a well-defined microaerophilic band of cells at the OAI (the pink: colorless interface) (*Fig. 12.9*). As the band thickens and number of cells in the band increases, cells deplete oxygen at the OAI and the band of motile cells moves toward the surface.

Many magnetite-producing magnetotactic bacteria are heterotrophic but facultatively chemolithoautotrophic (Bazylinski et al. 2004; Williams et al. 2006; Geelhoed et al. 2010) or are obligately chemolithoautotrophic (Lefèvre et al. 2012). Oxygen concentration gradient medium can be used for both

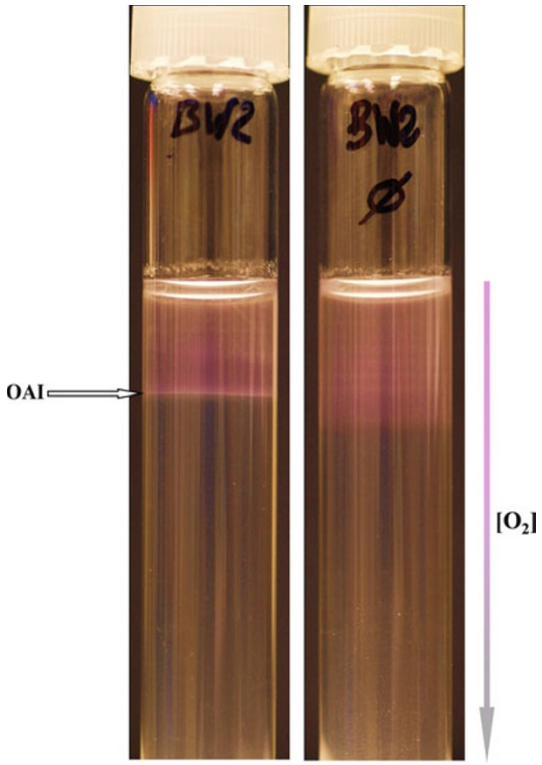


Fig. 12.9
Growth of the magnetite-producing microaerophilic magnetotactic bacterium strain BW-2 in semi-solid oxygen concentration gradient medium. Cells initially grow as a microaerophilic band of cells at the oxic-anoxic transition zone (OATZ; also known as the oxic-anoxic interface (*OAI*)) which here is shown as the *pink*:colorless interface (tube on the *left*). As the band thickens and number of cells in the band increases, cells deplete oxygen at the OAI and the band of motile cells moves toward the surface. The tube on the right is uninoculated and the OATZ will gradually move downward in the tube as the medium oxidizes

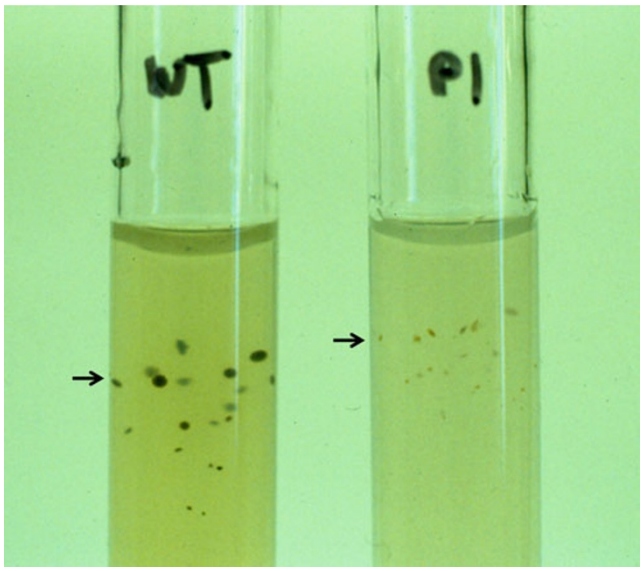
chemolithoautotrophic and chemoorganoheterotrophic growth. For the former, bicarbonate must be included in the medium and all organic compounds should not be present with the possible exception of the reducing agent (e.g., cysteine) and vitamins if required. The best known electron donors for this medium are sulfide and thiosulfate. Sulfide can be supplied as a solid agar plug as described earlier or directly as a sterile solution after autoclaving (Lefèvre et al. 2012). A problem here is that bands of elemental sulfur often form in this medium as oxygen chemically oxidizes the sulfide over time. In growth medium used to confirm chemolithoautotrophic growth, highly purified agar should be used such as Agar Noble (Difco) or even high-quality agarose because many typical agars contain impurities that might be inhibitory to autotrophic growth. For chemoorganoheterotrophic growth, a carbon source is required and the best choices are organic acids (e.g., succinate, malate) and some amino acids as no magnetotactic bacterium has been shown to utilize any other type of organic compound as a carbon source. The concentration of the carbon source should initially be kept low ($\leq$2 mM) to allow magnetotactic bacteria to compete with possible contaminants (Schüler et al. 1999).

Only recently has a greigite-producing magnetotactic bacterium been grown in axenic culture. Strain BW-1 was isolated from a saline spring at Badwater Basin in Death Valley National Park (California) (Lefèvre et al. 2011d). Cells of this organism were magnetically separated using the magnetic racetrack as described earlier and inoculated into different types of growth medium. BW-1 appears to be an obligate, sulfate-reducing, chemoorganoheterotrophic anaerobe. Interestingly, BW-1 biomineralizes both magnetite and greigite and the proportion of the minerals within magnetosomes appears to be dependent on chemical conditions in the growth medium, for example, the concentration of sulfide (Lefèvre et al. 2011d).

Iron is required for magnetosome synthesis and, therefore, it must be present in the growth medium. The type of iron source is not critical, however, as long as it is kept soluble at neutral pH either by the presence of chelating agents (particularly if the iron is supplied as Fe(III)) or reducing agents which reduce Fe(III) to the much more soluble Fe(II). Ferrous or ferric salts at concentrations between 20 and 50 μM are generally sufficient to allow for both growth and magnetosome formation (Schüler and Baeuerlein 1996, 1998)), which match the concentration range of free soluble iron found in environmental sediment horizons where magnetic bacteria are most abundant (Flies et al. 2005a). Remarkably, the growth of cultivated *Magnetospirillum* species is inhibited at iron concentrations of >200 μM (Schüler and Baeuerlein 1996), indicating that intracellular magnetite biomineralization is not an adaptation specific to iron-rich environments. Ferric citrate and ferric quinate are the most often used iron source for growth and magnetite biomineralization, as they can be easily prepared and autoclaved together with other medium components usually without problems with precipitation (Blakemore et al. 1979; Schüler et al. 1999). It is important to understand that Fe(II) and Fe(III) inverse concentration gradients form in the oxygen concentration gradient medium described in the previous paragraph due to the presence of the chemical reducing agents. The formation of sulfide in anaerobic cultures of sulfate-reducing bacteria appears to pose a problem regarding iron availability to cells for magnetosome formation (Lefèvre et al. 2011b). Several strains of obligately alkaliphilic, sulfate-reducing magnetotactic bacteria were isolated but displayed a weak or no magnetotactic response apparently due to scavenging of iron by sulfide produced during sulfate reduction resulting in the precipitation of black iron sulfides. To obtain a stronger magnetotactic response, the iron concentration was increased from 20 to 200 μM and the headspace of the cultures purged every other day with oxygen-free argon gas to decrease the concentration of hydrogen sulfide in the cultures (Lefèvre et al. 2011b). This iron availability issue may be true for other non-alkaliphilic, sulfate-reducing magnetotactic bacteria such as *Desulfovibrio magneticus* since this organism produces very few magnetosomes when grown anaerobically with sulfate compared to fumarate (Pósfai et al. 2006).

For marine strains and those from other saline habitats, the concentration of salts in the growth medium is important. An artificial seawater (ASW) formula that has been used successfully for the isolation of various different morphotypes of marine magnetotactic bacteria can be found in Bazylinski et al. (1994). For magnetotactic bacteria from brackish environments, the salinity can be determined with a hand-held refractometer and the seawater diluted with distilled water to the appropriate salinity. For those from nonmarine saline environments, the same artificial seawater is effective. In the case of salinities higher than that of seawater, using a salt mixture with approximately the same ratio of the salts in the ASW only at higher concentrations seems to work well. Because of the high amounts of magnesium and calcium in these media, the phosphate concentration should be kept low ($\leq$1 mM) and should be added from a sterile stock solution to the medium after autoclaving to prevent it from precipitating.

The media as described above are effective for the growth of magnetotactic bacteria but are not useful for their isolation. Two general methods have been used to isolate magnetotactic bacteria in pure culture. The first involves the formation of individual colonies. This has been achieved using agar plates of appropriate media such as activated charcoal agar (ACA) (Schultheiss and Schüler 2003). This technique has proven effective for the growth of *Magnetospirillum* species on solid medium. Activated charcoal scavenges and decomposes toxic-free oxygen radicals and peroxides thought to inhibit the growth of many microaerophiles (Hoffman et al. 1983; Krieg and Hoffman 1986). Once inoculated, ACA plates are incubated under microaerobic or anaerobic conditions under special gas mixtures (e.g., 1 % oxygen in nitrogen) or oxygen-free gases depending upon the organism (Schultheiss and Schüler 2003; Dubbels et al. 2004). A second method for obtaining individual colonies is through the use of solid medium in shake tubes (Bazylinski et al. 1988). This is useful for those organisms that will not form colonies on plates. Both oxygen concentration gradient and anaerobic shake tubes (◘ *Fig. 12.10*) can be made using air or oxygen-free gas in the headspace, respectively. Using either agar plates or shake tubes, colonies of magnetotactic bacteria are usually brown or black in color due to the formation of magnetite (◘ *Fig. 12.10*; Schultheiss and Schüler 2003; Dubbels et al. 2004). For those organisms that do not form colonies on either plates or in shake tubes, pure cultures can be obtained by a repeated series of dilution to extinction in many of the media described here as long as the dominant bacterium present in the original culture is the one targeted for isolation.



◘ **Fig. 12.10**
Oxygen concentration gradient shake tubes of wild-type (tube labeled *WT*) and a non-magnetotactic mutant (tube labeled *P1*) of *Magnetovibrio blakemorei*. The headspace of the tubes is air. Note that colonies form in a band about a centimeter below the meniscus at the oxic-anoxic transition zone (OATZ). Colonies of the wild-type are *black* due the production of magnetite and mutants that do not biomineralize magnetite (e.g., *P1*) form *cream-to-pink colored* colonies

Magnetotaxis, Chemotaxis, Aerotaxis, and Phototaxis

After Blakemore's rediscovery of magnetotactic bacteria, he proposed a model to explain the function of the bacterial magnetosome (Blakemore 1975). The model was based on the assumption that all magnetotactic bacteria are microaerophilic and indigenous in sediments. Frankel and Blakemore (1980) showed that these bacteria passively align and actively swim along the inclined geomagnetic field lines as a result of their magnetic dipole moment. Blakemore referred to this behavior as magnetotaxis and proposed that magnetotaxis helps to guide cells to swim downward to less oxygenated regions of aquatic habitats presumably to the surface of or within sediments. Once cells reached their preferred microhabitat, they would presumably stop swimming and adhere to sediment particles until conditions changed, as for example, when additional oxygen was introduced. This notion was supported by an observed predominant occurrence of north-seeking magnetotactic bacteria (i.e., swim in the direction indicated by the North-seeking pole of a magnetic compass needle) under oxic conditions in the Northern.

Hemisphere while in the Southern Hemisphere, south-seeking bacteria appear to predominate (Blakemore et al. 1980; Blakemore 1982). Due to the inclination of geomagnetic field lines in both the Northern and Southern Hemispheres and the direction of downward being reversed, magnetotactic bacteria in both hemispheres therefore swim downward toward sediments (Blakemore 1982).

Later findings, including the discovery of large populations of magnetotactic bacteria in the water columns of chemically stratified aquatic habitats and the isolation of obligately microaerophilic, coccoid magnetotactic bacterial strains, made it necessary to modify this view of magnetotaxis. The traditional model did not completely explain how magnetotactic bacteria in

the anoxic zone of a water column benefit from magnetotaxis, nor did it explain how magnetotactic cocci form microaerophilic bands of cells in semi-solid oxygen-gradient medium. Spormann and Wolfe (1984) showed earlier that magnetotaxis is somehow controlled by aerotaxis in some magnetotactic bacteria, but this alone does not help to explain all observed effects of magnetotaxis. More recently, it was demonstrated (using pure cultures of different types of magnetotactic bacteria) that magnetotaxis and aerotaxis work in conjunction in these bacteria (Frankel et al. 1997). The behavior observed in these strains is now referred to as "magneto-aerotaxis," which appears to be a more accurate description than magnetotaxis. Moreover, "magnetotaxis" is a misleading term (a misnomer) in that cells do not swim toward or away from a magnetic field as the term implies.

The traditional model also failed to explain various types of apparently unusual magnetotactic behavior observed by a number of investigators but without recognizing the fundamental differences between these behaviors (Moench and Konetzka 1978; Blakemore et al. 1980; Spormann and Wolfe 1984). Only when distinct morphotypes of magnetotactic bacteria were isolated and grown in pure culture for detailed studies in using thin, flattened capillaries (Frankel et al. 1997), it became clear that two general types of mechanisms were observed, apparently occurring in different bacteria, termed polar and axial.

The distinction between polar and axial behavior can be seen by observing cells in wet mounts under oxic conditions using a microscope and a bar magnet of a few gauss parallel to the plane of the slide (*Fig. 12.11*). Polar magnetotactic bacteria, particularly the magnetotactic cocci, swim persistently along magnetic field lines without reversing their direction or turning. If the magnetic field is reversed, the bacteria reverse their swimming direction and continue swimming persistently in the same direction relative to the magnetic field. Polar magnetotactic bacteria from Northern Hemisphere habitats appear to predominately swim parallel to the magnetic field, corresponding to northward migration in the geomagnetic field. Bacteria from the Southern hemisphere swim antiparallel to the magnetic field. It was this consistent swimming behavior that led to the discoveries of magnetotactic bacteria by both Bellini and Blakemore (1975). In contrast, axial magnetotactic bacteria, especially the freshwater spirilla grown in liquid culture, orient along magnetic field lines and swim in both directions displaying frequent reversals of swimming direction with some cells accumulating or getting stuck in approximately equal numbers on both the north and south edges of the water drop (*Fig. 12.11a*).

The distinction between polar and axial magneto-aerotaxis can also be seen in flattened capillary tubes containing suspensions of cells in reduced medium with one or both ends of the capillary tube open. In the first situation, where one end of the capillary is open (the right end of the capillaries *Fig. 12.11b*) and the other sealed, a single oxygen concentration gradient forms beginning at the open end of the capillary. Cells of strain MC-1 in these capillaries rotate 180° after a reversal of B, the magnetic field, and the band separates into groups of cells swimming in opposite directions along B, away from the position of the band before the reversal. A second reversal results in the reformation of a single band. Cells of *Magnetospirillum magnetotacticum* also rotate 180° in these capillaries, but the band of cells does not separate and remains intact (*Fig. 12.11*). In the second situation (not shown), where both ends of the capillary tubes are open, diffusion of oxygen into the ends of the tubes creates an oxygen concentration gradient at each end of the tube, oriented in opposite directions. Polar magnetotactic bacteria incubated in a magnetic field oriented along the long axis of the tube form an aerotactic band at only one end of the tube, whereas axial magnetotactic bacteria form bands at both ends of the tube. Thus, for polar magnetotactic bacteria, the magnetic field provides an axis and

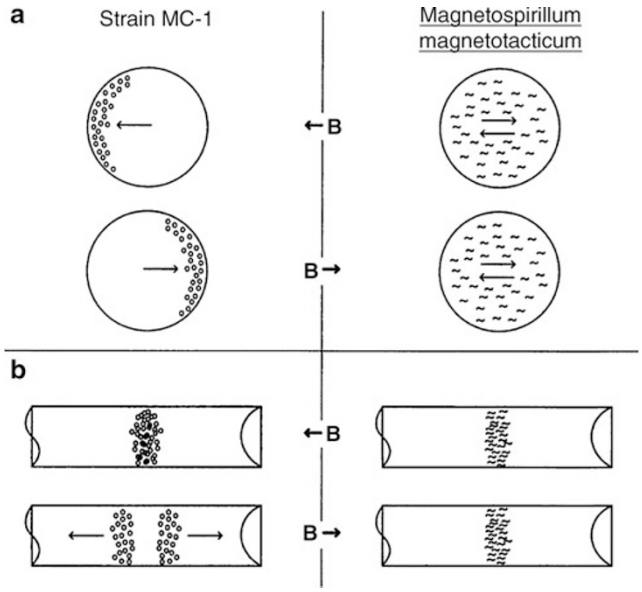


Fig. 12.11
Two types of magnetotaxis. (a) Depictions of the polar magnetotactic behavior of strain MC-1 and axial magnetotactic behavior of *Magnetospirillum magnetotacticum* in water drops under oxic conditions on a microscope slide (*B*, magnetic field; *arrow points northward*). Cells of strain MC-1 swim persistently parallel to *B* (north-seeking motility) and accumulate at the edge of the drop. When *B* is reversed, cells continue to swim parallel to *B* (north-seeking motility) and accumulate at the other side of the drop. Cells of *M. magnetotacticum* swim in either direction relative to *B* and continue to do so when the field is reversed. (b) Illustrations of aerotactic bands of strain MC-1 and *M. magnetotacticum* in flat glass capillaries. The right ends of the capillaries are open to air and the left ends are sealed. After reversal of *B*, cells of strain MC-1 rotate 180° and the band separates into groups of cells swimming in opposite directions along *B*, away from the position of the band before the reversal. A second reversal results in the reformation of a single band. Cells of *M. magnetotacticum* also rotate 180° but the band of cells remains intact (Figure adapted from Frankel et al. (1997))

direction for motility, whereas for axial magnetotactic bacteria, the magnetic field only provides an axis of motility, pointing to different magneto-aerotactic mechanisms occurring in two types of bacteria. Nonetheless, regardless of the form of magneto-aerotaxis, it appears to function in magnetotactic bacteria by aiding them to more efficiently locate and maintain position in an optimal position in chemical concentration (e.g., oxygen) gradients by reducing a three-dimensional search problem to that of a single dimension, that is, once cells are aligned along inclined geomagnetic field lines, they only have to swim up or down (Frankel et al. 1997).

Axial Magneto-aerotaxis

Almost all cultured magnetotactic spirilla exhibit axial magneto-aerotaxis (◉ *Figs. 12.11* and ◉ *12.12*) after repeatedly grown in liquid media. In most environmental samples, however, magnetospirilla appear to display polar magneto-aerotaxis (see next section), and there is only a single report of magnetospirilla exhibiting axial magneto-aerotaxis in an environmental sample (Spormann and Wolfe 1984). Thus, axial magnetotactic bacteria may represent only a very small fraction of the total count of magnetotactic bacteria in natural samples, although these organisms are harder to detect in wet mounts or hanging drops using a microscope. Cells representing this type of magnetotaxis were referred to as two-way swimmers, because in a homogeneous medium, they swim in either direction along the magnetic field, B (◉ *Fig. 12.12*). In the presence of an oxygen concentration gradient, cells swim parallel or antiparallel to B with aerotaxis determining the direction of migration. Therefore, an aerotactic band of cells forms at both ends of the tube in capillaries where both ends are open, whereas cells displaying a polar magnetotaxis form only one band at the end of the tube corresponding to their magnetic polarity. The aerotactic, axial magnetotactic spirilla appear to use a temporal sensory mechanism for oxygen detection as do most microaerophilic bacteria studied so far (Frankel et al. 1998). Changes in oxygen concentration measured during swimming determine the sense of flagellar rotation. Cells moving away from the optimal oxygen concentration consequently reverse their swimming direction. In this model, changes in oxygen concentration are measured within short intervals implying that these bacteria must be actively motile in order to quickly measure and respond to concentration gradients in their habitat. The combination of a passive alignment along geomagnetic field lines with an active, temporal, aerotactic response provides the organism with an efficient mechanism to locate the microoxic or suboxic zone in its habitat. Therefore, the term magneto-aerotaxis is also an appropriate descriptive term for this tactic behavior.

Polar Magneto-aerotaxis

The large majority of uncultured, naturally occurring magnetotactic bacteria display polar magnetotaxis most notably

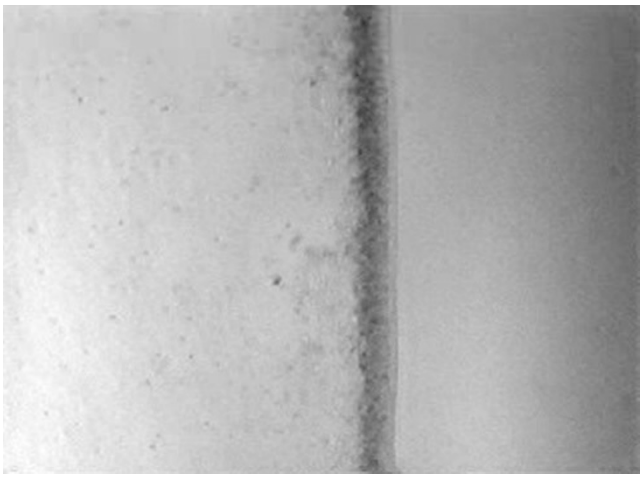


Fig. 12.12
Sequence showing magnetotactic spirilla displaying axial magnetotaxis. For the video, see the online version of *The Prokaryotes*

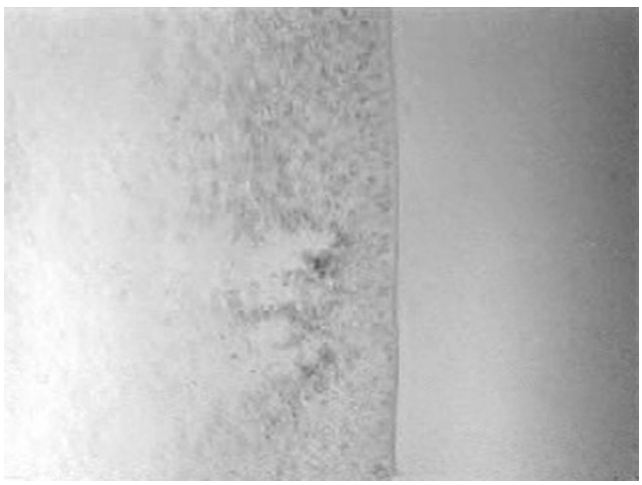


Fig. 12.13
Sequence showing magnetotactic cocci displaying polar magnetotaxis. For the video, see the online version of *The Prokaryotes*

the magnetococci (◉ *Figs. 12.11* and ◉ *12.13*). However, as indicated in the previous section, polar magnetotaxis has also been observed in several freshly isolated strains of *Magnetospirillum* from environmental samples (Schüler et al. 1999). Although individual cells swam back and forth, they had a preference for one direction over the other, and the entire population migrated with a predominantly N-seeking polarity and accumulated at one edge of a hanging drop in magnetic fields. Magnetic polarity, however, was lost upon repeated subcultivation of the new isolate under laboratory conditions, presumably due to the absence of a selective pressure for polarity.

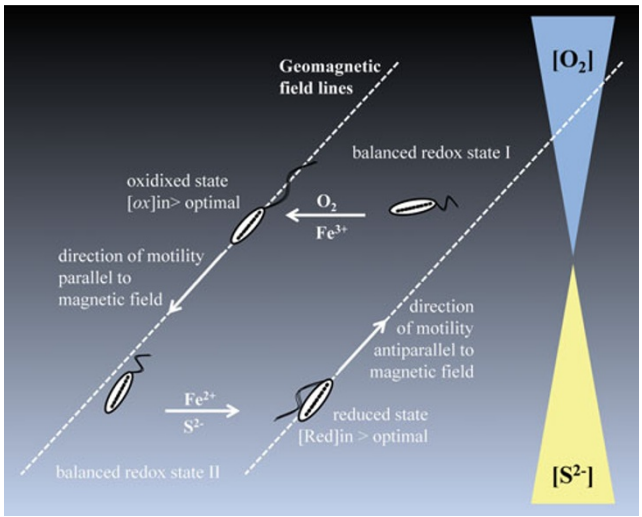
The following mechanism for polar magnetotaxis was proposed based on experimental data obtained with an axenic

culture of the marine magnetotactic coccus *Candidatus* Magnetococcus marinus strain MC-1 (Frankel et al. 1997). These cocci were shown to swim in both directions along a static magnetic field, B, apparently without the need of physically turning around, by reversing the sense of flagellar rotation. It seems that a two-state sensory mechanism determines the sense of flagella rotation leading to parallel or antiparallel swimming along the geomagnetic field lines.

Under higher than optimal oxygen tensions, the cell is presumably in an "oxidized state" and swims persistently parallel to B (◉ *Fig. 12.13*), that is, downward in the Northern Hemisphere. Under reducing conditions or suboptimal oxygen concentrations, the cell switches to a second state, the "reduced state," which leads to a reversal of the flagellar rotation and to a swimming antiparallel to B (upward in the Northern Hemisphere). This two-state sensing mechanism results in an efficient aerotactic response, provided that the oxygen-gradient is oriented correctly relative to B, so that the cell is guided in the right direction to find either reducing or oxidizing conditions. This is especially important because adaptation, which would lead to a spontaneous reversal of the swimming direction, was never observed in controlled experiments with the cocci. The redox sensor, which controls this two-state response, might be similar to the FNR (fumarate and nitrate reduction) transcription factor found in *Escherichia coli* and other bacteria. The FNR factor is sensitive to oxygen and activates gene expression in the reduced state thereby promoting the switch between aerobiosis and anaerobiosis in *E. coli* (de Graef et al. 1999). The sensory mechanism in the examined magnetotactic cocci is not only affected by oxygen. Cells exposed to light of short wavelengths (≤500 nm) also showed a response similar to a switch to the "oxidized state" (Frankel et al. 1997).

Revised Model of Magnetotaxis: Redoxtaxis

In this section, we extend the current model of magneto-aerotaxis to a more complex redoxtaxis model. In this case, the unidirectional movement of magnetotactic bacteria in a drop of water would be only one aspect of a sophisticated redox-controlled response. Many magnetotactic bacteria are now known to be chemolithoautotrophic using reduced sulfur compounds as a source of electrons (Bazylinski et al. 2004; Bazylinski and Williams 2007; Williams et al. 2006; Lefèvre et al. 2012). Oxygen is the terminal electron acceptor, but it should be stressed that atmospheric levels of oxygen are toxic to these obligate microaerophiles (Bazylinski and Frankel 2004). Thus, in natural environments, energy conservation in these organisms is strongly dependent on the uptake of reduced sulfur compounds which, in many habitats, are present only in deeper regions at or below the OAI due to the rapid chemical oxidation of these reduced chemical species by oxygen or other oxidants in the upper layers. To overcome the problem of separated pools of electron donor and acceptor, several strategies have been developed by sulfide-oxidizing bacteria. *Thioploca* spp., for example, use nitrate as a terminal electron acceptor, which is stored



◘ Fig. 12.14
Hypothetical model of the function of polar magnetotaxis in bacteria (in the Northern hemisphere). Cells are guided along the geomagnetic field lines depending on their "redox state" either downward to the sulfide-rich zone or upward to the microoxic zone, thereby enabling a shuttling between different redox layers

intracellularly (most of the internal space of the cell is vacuolar) to oxidize sulfide and have developed vertical sheaths in which bundles of motile filaments are located. *Thioploca* is thought to use these sheaths to efficiently move in a vertical direction in the sediment, thereby accumulating sulfide in deeper layers and nitrate in upper layers (Huettel et al. 1996). For some magnetotactic bacteria, it might also be necessary for them to perform excursions to anoxic zones of their habitat in order to accumulate reduced sulfur compounds. The model shown in ◉ *Fig. 12.14* illustrates how polar magnetotaxis might help to guide bacteria, depending on their internal redox state, either downward to accumulate reduced sulfur species or upward to oxidize stored sulfur with oxygen. Thus, we hypothesize that magnetotactic bacteria displaying polar magnetotaxis alternate between two internal redox states. The "oxidized state" would result from the almost complete consumption of stored sulfur, the electron donor. In this state, cells seek deeper anoxic layers where they could replenish the depleted stock of electron donor using nitrate or other compounds as alternative electron acceptor. Eventually, they would reach a "reduced state" in which they would have accumulated a large amount of sulfur which cannot be efficiently oxidized under anaerobic conditions leading to a surplus of reduction equivalents. Cells must therefore return to the microoxic zone where oxygen is available to them as an electron acceptor. In addition, low concentrations of oxygen have been shown to be necessary for the synthesis of magnetosomes in some magnetotactic bacteria (e.g., Blakemore et al. 1985). The advantage of polar magnetotaxis is that an oxygen gradient is not necessary for efficient orientation in the anoxic zone, thereby enabling a rapid return of the cell along large distances to the preferred microoxic conditions. A further benefit would be that cells avoid wasting energy by constantly

moving along gradients but instead can attach to particles in preferred microniches until they reach an unfavorable internal redox state that triggers a magnetotactic response either parallel or antiparallel to the geomagnetic field lines. In any case, greater than optimal concentrations of oxygen would switch cells immediately to an "oxidized state" provoking the typical down-seeking response of magnetotactic bacteria observed in oxic water drops under the microscope. This model is summarized in ◉ *Fig. 12.14*).

Although the model of magneto-aerotaxis for some magnetotactic bacteria appears to make sense, there are still many unanswered questions regarding why bacteria are magnetotactic and biomineralize magnetosomes (Frankel and Bazylinski 2004). For example, Simmons et al. (2006) discovered a population of a new magnetotactic bacterial morphotype in the water column of chemically stratified Salt Pond (Woods Hole, Massachusetts USA) whose cells were greater than 90% south-seeking at specific depths. In addition, even the majority of MMPs were south-seeking at certain depths at Salt Pond. Shapiro et al. (2011) also found a majority of south-seeking MMPs in sites at the Little Sippewissett salt marsh (Woods Hole, Massachusetts, USA). Based on the current model of polar magnetotaxis, these organisms would swim southward/upward toward surface waters containing toxic levels of oxygen and would presumably die. In this way, north-seeking bacteria would be selected for and those of the opposite polarity would be selected against. Other significant questions involve the ability of non-magnetotactic mutants of cultured species to form microaerophilic bands of cells in oxygen-gradient medium similar to the wild type and why some cultured species biomineralize far more magnetite under anaerobic conditions when no gradient is present in the medium (e.g., *Magnetovibrio blakemorei*). It is important to understand that the magnetotaxis model presented above does not preclude other reasons for the organisms' ability to biomineralize magnetite and/or greigite. While it seems logical that there is a physiological explanation (e.g., magnetite is known to decompose oxygen radicals such as hydrogen peroxide produced during oxygen respiration (Blakemore 1982)), few hypotheses have been put forward and none have been generally accepted.

Phototaxis

Some MMPs and nMMPs show a strong negative phototactic response to white light and wavelengths of light ≤480 nm (Lefèvre et al. 2010a; Shapiro et al. 2011) (◉ *Fig. 12.15*). Because shorter wavelengths of light, ≤480 nm (blue to violet), are those that generally penetrate the water column the deepest (Braatsch et al. 2004), this negative phototactic response might function similarly to magnetotaxis in that, if light causes MMPs and nMMPs in nature to swim more or less vertically, then, like magnetotaxis (Frankel et al. 1997), it would at least partially reduce a three-dimensional search problem to a one-dimensional search problem for an organism that must locate and maintain an optimal position in vertical chemical and redox gradients common in aquatic habitats. Negative phototaxis in this case might increase the efficiency of chemotaxis as does magnetotaxis (Frankel et al. 1997). Alternatively, light might simply drive MMPs and nMMPs downward toward anoxic conditions which are likely favorable to them as it has been inferred from phylogenetic data that they are likely sulfate-reducing bacteria (DeLong et al. 1993; Simmons and Edwards 2007; Wenter et al. 2009).

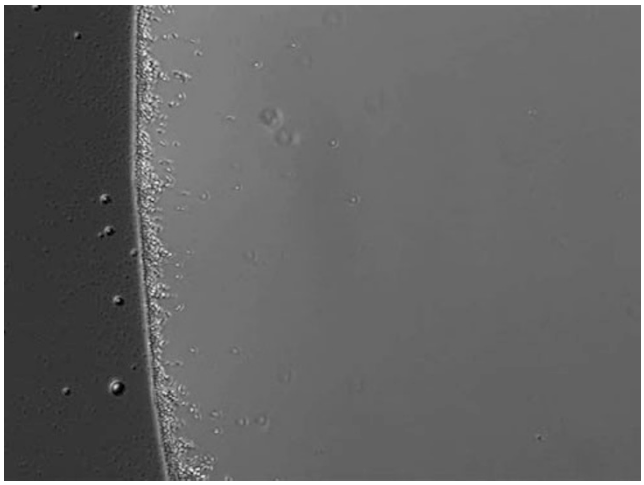


Fig. 12.15
Sequence demonstrating the negative phototactic response of the nMMPs. Differential interference microscopy of a hanging drop containing nMMPs and magnetotactic rod-shaped bacteria collected from a pool at ambient temperature at the Great Boiling Springs geothermal field in Gerlach, Nevada, demonstrating the negative phototactic response of the nMMPs. The drop is exposed to a magnetic field and initially the microscope is focused at the edge of the north side of the drop. Note the presence of the magnetotactic rod-shaped bacteria that migrated and accumulated at the edge of the drop. After 2 s, the magnetic field is reversed and the magnetotactic bacteria reverse their swimming direction. At 5 s, the field is reversed again. At about 7 s, the point of focus is moved to the opposite, dark side (the *south side*), far edge of the drop where the nMMPs have accumulated. Note that they move toward the north side of the drop (toward the *left*) when exposed to the light. The magnetic field direction is consistent throughout this part of the video. nMMPs continue to move away from the light when followed by the light source of the microscope. For the video, see the online version of *The Prokaryotes*

Magnetosomes

Magnetosomes have an organic membrane and an inorganic mineral phase. The magnetosome mineral phase consists of tens-of-nanometer-sized crystals of an iron oxide and/or an iron sulfide. The mineral composition of the magnetosome in some magnetotactic bacteria is specific enough for it to be likely under genetic control, in that cells of several cultured

magnetite-producing species still synthesize an iron oxide and not an iron sulfide, even when hydrogen sulfide is present in the growth medium (Meldrum et al. 1993a, b). However, there are some magnetotactic bacteria (in addition to the MMP), specifically a group of large, slow-swimming, rod-shaped types phylogenetically affiliated with the *Deltaproteobacteria* (Lefèvre et al. 2011d), that produce both magnetite and greigite magnetosome crystals aligned within the same chain or chains in the cell (Bazylinski et al. 1993b, 1995). There is some evidence to suggest that environmental conditions, that is, whether the cells are under oxic or anoxic conditions, affect what and how much of each mineral is biomineralized in these organisms (Bazylinski et al. 1995; Lefèvre et al. 2011d).

Magnetic and Mineral Properties of Magnetosomes

The size of the magnetosome mineral crystals also appears to be under control of the organism because almost all magnetotactic bacteria contain crystals that display only a very narrow size range, from about 35 to 120 nm (Frankel et al. 1998). Magnetite and greigite particles in this range are stable single magnetic domains (Butler and Banerjee 1975; Diaz-Ricci and Kirschvink 1992; Frankel and Moskowitz 2003). Smaller particles are superparamagnetic at ambient temperature and do not have stable, remanent magnetization. Larger particles tend to form multiple domains, reducing the remanent magnetization. However, exceptionally large magnetite magnetosomes have been observed in some uncultured cocci from the Southern Hemisphere, having dimensions well above the theoretically determined size limits of single domain magnetite (Farina et al. 1994; Spring et al. 1998; McCartney et al. 2001; Lins et al. 2005). Nonetheless, as evidenced by magnetic holography in the transmission electron microscope (TEM), even these large crystals behave as single magnetic domains when they are present in the cell in a chain configuration where they are magnetized by neighboring crystals (McCartney et al. 2001).

In contrast to chemically synthesized magnetite and greigite crystals, biologically produced magnetosome mineral particles display a range of well-defined morphologies which can be classified as distinct idealized types (*Fig. 12.16*). These morphologies and the consistent narrow size range (Devouard et al. 1998) of intracellular magnetosome particles represent typical characteristics of a biologically controlled mineralization and are clear indications that the magnetotactic bacteria exert a high degree of control over the biomineralization processes involved in magnetosome synthesis (Bazylinski and Frankel 2003).

Iron Oxide-Type Magnetosomes. The iron oxide-type magnetosomes consist solely of magnetite, Fe_3O_4. The particle morphology of the magnetite crystals in magnetotactic bacteria varies but is generally extraordinarily consistent within cells of a single bacterial species or strain (Bazylinski et al. 1994). Three general morphologies of magnetite particles have been observed in magnetotactic bacteria using transmission electron microscopy (*Fig. 12.16*) (Mann et al. 1990a; Bazylinski et al. 1994). They include: (1) roughly cuboidal (cuboctahedral; Balkwill et al. 1980; Mann et al. 1984a, b), (2) parallelepipedal or elongated-prismatic (rectangular in the horizontal plane of projection; Moench and Konetzka 1978; Towe and Moench 1981; Moench 1988; Bazylinski et al. 1988), and (3) bullet- or tooth-shaped (also described as anisotropic meaning these crystals lack a center of inversion symmetry; Mann et al. 1987a, b; Thornhill et al. 1994; Lefèvre et al. 2011c).

High resolution TEM and selected area electron diffraction studies have revealed that the magnetite particles within magnetotactic bacteria are of relatively high structural perfection and have been used to determine their idealized crystal morphologies (Matsuda et al. 1983; Mann et al. 1984a, b, 1987a, b; Meldrum et al. 1993a, b). In crystallographic notation, numbers in square brackets (e.g., [100]) denote a direction vector. Coordinates in angle brackets (also referred to as chevrons), such as <111>, denote a family of directions related by the symmetry of the crystal structure. The family of directions is called directions of a form. For cubic crystal structures, <111> comprises eight directions (all the possible combinations of 1 and -1 taken three at a time). Numbers in parentheses such as (111) denote a particular plane of the crystal structure; the numbers are referred to as the Miller indices. Indices in curly brackets, such as {100}, represent a family of symmetry-related planes similar to the way angle brackets denote a family of directions (Borchardt-Ott 2011). Magnetotactic bacterial morphologies are derived from combinations of {111}, {110}, and {100} forms with suitable distortions (Devouard et al. 1998). The roughly cuboidal crystals are cuboctahedra ({100} + {111}), and the elongated, parallelepipedal crystals are either pseudo-hexahedral or pseudo-octahedral prisms derived from {100} + {110} + {111}.

Examples are shown in *Fig. 12.17a–d*. The cuboctahedral crystal morphology preserves the symmetry of the face-centered cubic spinel structure in which all equivalent crystal faces develop equally. The pseudo-hexahedral and pseudo-octahedral prismatic particles represent anisotropic growth in which equivalent faces develop unequally (Mann and Frankel 1989; Devouard et al. 1998).

Synthesis of the bullet- and tooth-shaped magnetite particles (*Fig. 12.17e–h*), the most anisotropic of the magnetotactic bacterial magnetite crystals, appears to be more complex than that of the other types. These crystals can be further subdivided into those with one pointed end and one flat end (flat-top shape; fts) and those with two pointed ends (double-triangular shape; dts) which appear as two isosceles triangles sharing a common base (*Fig. 12.18*) (Lefèvre et al. 2011c). In the dts crystals, both projected triangles appear to have the same width, although one triangle is longer than the other in mature crystals.

The first of the anisotropic magnetosome crystals to be examined were fts type from an uncultured magnetotactic bacterium collected from the Exeter River in New Hampshire, USA. It was proposed that growth of these crystals occurred in two stages and that they have an idealized, six-sided prismatic, magnetosome habit comprising four {111} and two {100} faces capped by two faces of {111} with associated {111} and {100} corner faces. Crystal growth of a nascent cuboctahedron

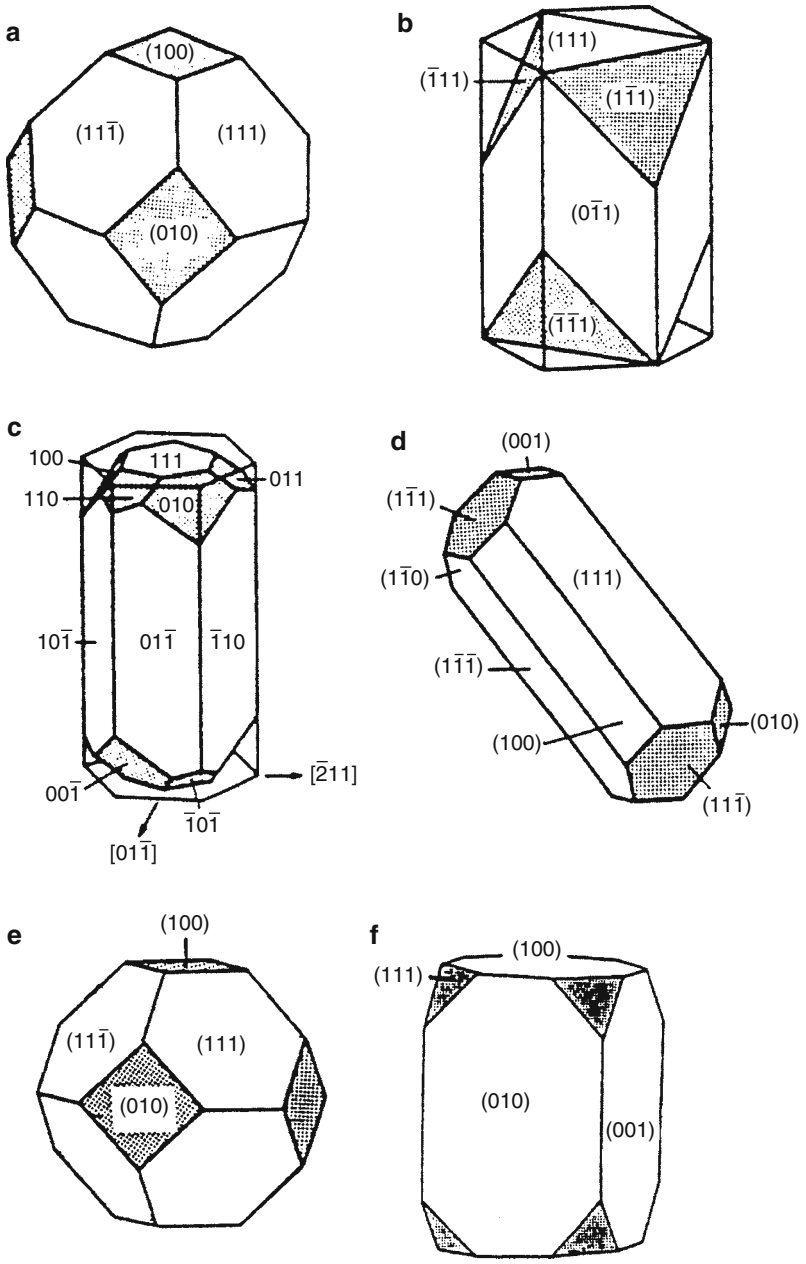


Fig. 12.16

Idealized magnetite (a–d) and greigite (e–f) crystal morphologies derived from high resolution TEM studies of magnetosome crystals from magnetotactic bacteria: (a) and (e) cuboctahedrons; (b), (c), and (f) variations of elongated pseudohexagonal prisms; (d) elongated cuboctahedron. *Numbers within parentheses* refer to the faces of the crystal lattice planes on the surface of the crystal (Figure adapted from Heywood et al. (1991) and Mann and Frankel (1989))

presumably commences via nucleation on the magnetosome membrane and continues until the width of the crystal is about 40 nm. In this first stage, there is proportional growth between width and length of the crystal. In the second stage, anisotropic growth commences with subsequent elongation parallel to <112> while the crystal width remains relatively constant (Mann et al. 1987a, b).

In magnetite magnetosome crystals with elongated-prismatic habits, the axis of elongation is the <111> axis of orientation which is considered the "easy" (lowest energy) direction of magnetization in single magnetite crystals (Frankel et al. 2007). When these particles are in a chain within a magnetotactic bacterial cell, they are oriented with the <111> long crystal axis parallel to the chain axis. While elongated-anisotropic magnetosomes are also usually oriented with their long axes parallel to the chain axis, the axis of elongation varies among the <100>, <110>, <111>, and <112> axes (Lefèvre et al. 2011c).

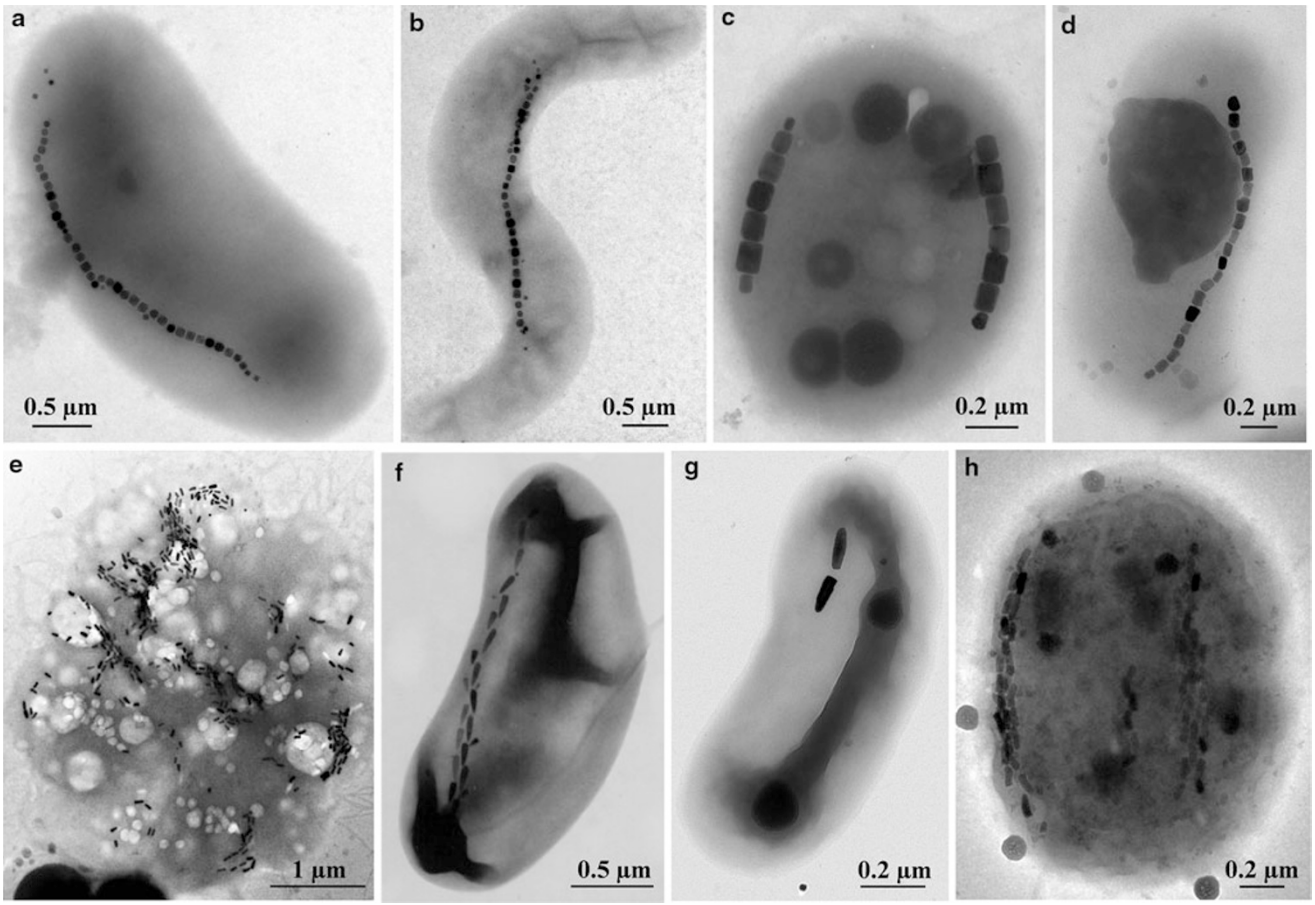


Fig. 12.17

Brightfield TEM images of magnetite (Fe_3O_4) crystals in magnetosomes of different magnetotactic bacteria. (a) and (b), cuboctahedral crystals in the cultured gammaproteobacterium strain BW-2 isolated from Badwater Basin, Death Valley National Park, California (Lefèvre et al. 2012); and from a spirillum, strain CB-1, isolated from Lake Mead, Nevada, respectively. (c) and (d), elongated-prismatic crystals from an uncultured magnetotactic bacterium found in sediment of the Mediterranean Sea collected at Marseille, France; and from the cultured, gammaproteobacterium strain SS-5 isolated from the Salton Sea, California (Lefèvre et al. 2012), respectively. (e) through (h), elongated-anisotropic (*bullet-shaped*) crystals from a variety of different magnetotactic bacteria. (e) and (f), an uncultured, multicellular magnetotactic prokaryote (MMP) and an uncultured rod-shaped magnetotactic bacterium both found in sediment of the Mediterranean Sea collected at Marseille, France (Lefèvre et al. 2007), respectively. (g), the uncultured, moderately thermophilic vibrio, *Candidatus* Thermomagnetovibrio paiutensis strain HSMV-1 (Lefèvre et al. 2010b). (h), the uncultured ovoid magnetotactic bacterium, *Ca.* Magnetoovum mohavensis strain LO-1 found in sediment of Lake Mead, Nevada (Lefèvre et al. 2011a)

There is evidence that in some cultured alkaliphilic magnetotactic bacteria, individual anisotropic crystals may partially result from aggregation of multiple magnetite crystals perhaps arising from multiple nucleation events in the magnetosome membrane vesicle (Lefèvre et al. 2011c).

Whereas the cuboctahedral form of magnetite can occur in inorganically formed magnetites (Palache et al. 1944), the prevalence of elongated-prismatic and elongated-anisotropic habits in magnetosome crystals imply anisotropic growth conditions, for example, a temperature gradient, a chemical concentration gradient, or an anisotropic ion flux (Mann and Frankel 1989). This aspect of magnetosome particle morphology has been used to distinguish magnetosome magnetite from detrital or magnetite produced by biologically induced mineralization (by the anaerobic iron-reducing bacteria), using electron microscopy of magnetic extracts from sediments (e.g., Petersen et al. 1986; Chang and Kirschvink 1989; Chang et al. 1989; Stolz et al. 1986, 1990; Stolz 1993).

Iron Sulfide-Type Magnetosomes. Almost all known freshwater magnetotactic bacteria biomineralize magnetite as the mineral phase of their magnetosomes. In contrast, others, particularly many marine, estuarine, and salt marsh species, produce iron sulfide-type magnetosomes consisting primarily of the magnetic iron sulfide, greigite, and Fe_3S_4 (Heywood et al. 1990, 1991; Mann et al. 1990b; Pósfai et al. 1998a, b) although these organisms have recently been found in nonmarine environments (Lefèvre et al. 2011d). Early reports of nonmagnetic iron pyrite (FeS_2; Mann et al. 1990b) and magnetic pyrrhotite (Fe_7S_8; Farina et al. 1990) have never been confirmed and likely represent misidentifications of additional iron sulfide species

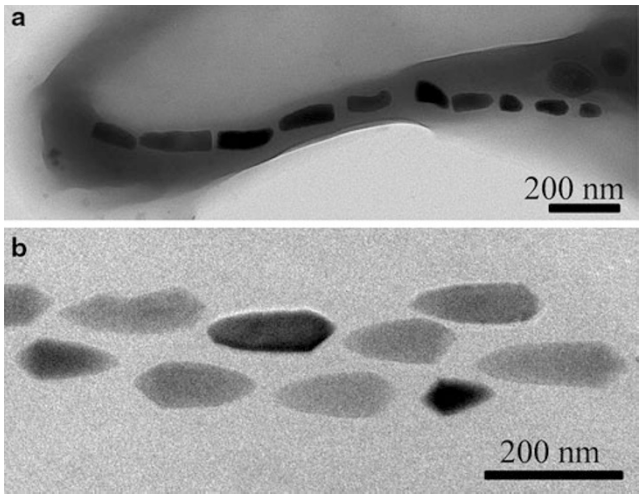


Fig. 12.18
High magnification brightfield TEM images of elongated-anisotropic magnetite crystals in magnetotactic bacteria. These type of crystals can divided into those with one pointed end and one flat end (flat-top shape; fts) (a) and those with two pointed ends (*double-triangular shape*; dts) which appear as two isosceles triangles sharing a common base (b) (Lefèvre et al. 2011c). In the dts crystals, both projected triangles appear to have the same width, although *one triangle* is longer than the other in mature crystals. The crystals in (a) are from the uncultured, moderate thermophilic magnetotactic bacterium, *Candidatus* Thermomagnetovibrio paiutensis strain HSMV-1 (Lefèvre et al. 2010b) and those in (b) from a cell collected from sediment of an alkaline spring in California, of the now cultured obligately alkaliphilic magnetotactic bacterium strain AV-1 (Lefèvre et al. 2011b)

occasionally observed with greigite in cells (Pósfai et al. 1998a, b). Currently recognized greigite-producing magnetotactic bacteria includes the MMP (Farina et al. 1983; Rodgers et al. 1990a, b; DeLong et al. 1993) and a variety of relatively large, rod-shaped bacteria (Bazylinski et al. 1990, 1993a, b; Heywood et al. 1990, 1991; Bazylinski and Frankel 1992; Lefèvre et al. 2011d).

The iron sulfide-type magnetosomes contain either particles of greigite (Heywood et al. 1990, 1991) or a mixture of greigite and transient nonmagnetic iron sulfide phases that appear to represent mineral precursors to greigite (Pósfai et al. 1998a, b). These phases include mackinawite (tetragonal FeS) and a possible sphalerite-type cubic FeS (Pósfai et al. 1998a, b). Based on TEM observations, electron diffraction, and known iron sulfide chemistry (Berner 1967, 1970, 1974), the reaction scheme for greigite formation in the magnetotactic bacteria appears to be: cubic FeS → mackinawite (tetragonal FeS) → greigite (Fe_3S_4) (Pósfai et al. 1998a, b).

The de novo synthesis of nonmagnetic crystalline iron sulfide precursors to greigite aligned along the magnetosome chain indicates that chain formation within the cell does not involve magnetic interactions. Interestingly, under the strongly reducing, sulfidic conditions at neutral pH in which the greigite-producing magnetotactic bacteria are found (Bazylinski et al. 1990; Bazylinski and Frankel 1992), greigite particles might be expected to transform into pyrite (Berner 1967, 1970). However, pyrite has never been unequivocally identified in any magnetotactic bacterium.

The same general morphologies of magnetite crystals in magnetotactic bacteria are also those observed for greigite (◉ *Fig. 12.19*): (1) cuboctahedral (the equilibrium form of face-centered cubic greigite) (Heywood et al. 1990, 1991), (2) elongated-prismatic (◉ *Fig. 12.16f*) (Heywood et al. 1990, 1991), and (3) bullet- and tooth-shaped (Pósfai et al. 1998a, b). Like that of their magnetite counterparts, morphology of the greigite particles appears to be species- and/or strain-specific, although confirmation of this observation requires controlled studies of pure cultures of greigite-producing magnetotactic bacteria. One clear exception to this rule is the MMP (Farina et al. 1983; Bazylinski et al. 1990, 1993a; Mann et al. 1990b; Rodgers et al. 1990a, b; Bazylinski and Frankel 1992). This unusual microorganism contains pleomorphic, elongated-prismatic, bullet-shaped, and cuboctahedral greigite particles. Some of these particle morphologies are shown in ◉ *Figs. 12.4* and ◉ *12.19c*. Therefore, the biomineralization process appears to be more complicated in this organism than in the rods with greigite-containing magnetosomes or in magnetite-producing, magnetotactic bacteria.

Arrangement of Magnetosomes Within Cells of Magnetotactic Bacteria

In cells of most magnetotactic bacteria, the magnetosomes are usually positioned as one or more chains that traverse the long axis of the cell (Bazylinski 1995; Bazylinski and Moskowitz 1997; Frankel and Moskowitz 2003) (◉ *Figs. 12.17* and ◉ *12.18*). In the chain arrangement of the single magnetic domain crystal magnetosomes, the magnetic dipole moment of the cell is maximized because magnetic interactions between the magnetosomes cause each magnetosome moment to spontaneously orient parallel to the others along the chain axis by minimizing the magnetostatic energy (Frankel 1984; Frankel and Moskowitz 2003). Therefore, the total magnetic dipole moment of the chain and the cell is the algebraic sum of the moments of the individual crystals in the chain. This has been confirmed repeatedly using a number of techniques including direct magnetic measurements (Penninga et al. 1995), magnetic force microscopy (Proksch et al. 1995; Suzuki et al. 1998), and electron holography (Dunin-Borkowski et al. 1998, 2001). The significance of this is that the chain of magnetosomes in a magnetotactic bacterium functions like a single magnetic dipole rather than a collection of individual dipoles and causes the cell to behave similarly. Magnetotaxis results from this magnetic dipole imparted by the chain of magnetosomes which cause the cell to passively align along geomagnetic field lines while it swims (Frankel 1984; Frankel and Moskowitz 2003). Living cells are neither attracted nor pulled toward either geomagnetic pole, and dead cells, like living cells, also align along

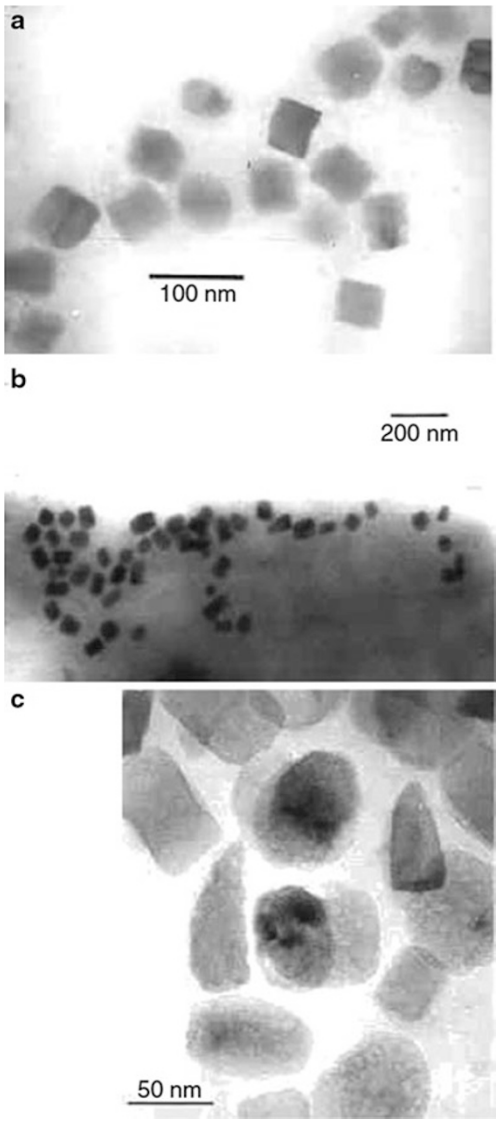


Fig. 12.19
Morphologies of intracellular greigite (Fe_3S_4) particles produced by magnetotactic bacteria. (a) Brightfield scanning transmission electron microscope (STEM) image of cuboctahedra in an unidentified rod-shaped bacterium collected from the Neponset River estuary, Massachusetts, USA. (b) Brightfield STEM image of rectangular prismatic particles in an unidentified rod-shaped bacterium collected from the Neponset River estuary, Massachusetts, USA. (c) Brightfield TEM image of tooth-shaped and rectangular prismatic particles from the multicellular magnetotactic prokaryote (MMP) (Courtesy of M. Pósfai and P. R. Buseck)

geomagnetic field lines but do not swim. Magnetosomes must be anchored in place within the cell to function as described as if they were free-floating in the cell, they would likely clump, causing a significant reduction in the cellular dipole moment. This is accomplished by dedicated cytoskeletal structures and close attachment to the inner cell membrane (see later section on "The Magnetosome Membrane"). In addition to biological control, assembly of magnetosome chains involves magnetostatic interaction, and magnetic "docking" to stable magnetic single domain particles is a key mechanism for building the functional cellular magnetic dipole (Faivre et al. 2010).

Biomineralization of Magnetosomes

Because little is known regarding the biomineralization of greigite magnetosomes at the molecular level except that there is evidence that similar genes and proteins are involved (Abreu et al. 2011; Lefèvre et al. 2011d), this section is focused on magnetite magnetosome synthesis. Biomineralization of the bacterial magnetosome appears to be a complex process that involves several steps that temporally overlap during the lifetime of the cell (*Fig. 12.20*).

The first step is invagination of the cell membrane and the possible formation of a bona fide, pinched off magnetosome membrane vesicle, an important question that remains unresolved. Using electron cryotomography, the magnetosome membrane in *Magnetospirillum* species has clearly been shown to originate as an invagination of the cytoplasmic membrane (CM) and that magnetite precipitation occurs after the invagination is formed (Komeili et al. 2006; Katzmann et al. 2010). Presumably, there is some sorting of magnetosome membrane proteins during the invagination and/or membrane vesicle formation process (Murat et al. 2010) as it is clear that magnetosome membranes contain proteins that are not present in the CM. Different stages of magnetite precipitation were observed within magnetosome membrane invaginations/vesicles. Cells grown under iron limitation contained empty magnetosome invaginations/vesicles arranged in a chain engaged to the CM (Komeili et al. 2006). Only 35% of the magnetosomes examined showed the magnetosome membrane to be an invagination of the CM suggesting that the invaginations pinch off and become true vesicles. Alternatively, this may be a result of a technical problem involving the technique (Komeili 2007a, b). It is also not known if this is a common characteristic of magnetite magnetosomes in all magnetotactic bacteria. In parallel experiments with *M. gryphiswaldense*, Scheffel et al. (2006) found empty magnetosome membrane vesicles in cells grown under iron limitation and also that magnetic cells contain, in addition to magnetite filled magnetosome vesicles, many empty vesicles inside the cell. As in *M. magneticum*, vesicles in *M. gryphiswaldense* were shown by cryo-electron microscopy to invaginate from the CM (Katzmann et al. 2010). However, most mature vesicles appeared to be no longer connected to the CM, and it was therefore hypothesized that nascent magnetosome particles become detached during maturation of magnetite crystals in this organism (Faivre et al. 2007). The mature magnetosome membrane invaginations/vesicles probably become aligned in the chain motif during their formation.

Iron uptake by the cell is certainly required for magnetosome synthesis and is likely occurring continually as long as it is available. Cells of cultured magnetotactic bacteria are extremely proficient at iron uptake as they have been shown to consist of

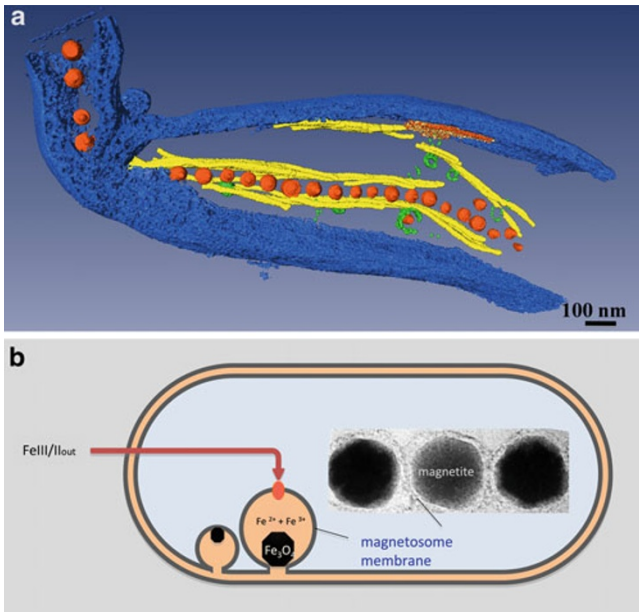


Fig. 12.20
(a) Cryo-electron tomogram of a section of cell of *Magnetospirillum gryphiswaldense* showing its intracellular organization. Magnetite crystals (*orange*) are closely adjacent to and aligned along bundles of the cytoskeletal magnetosome filament (*yellow*) formed by the actin-like MamK protein. Several vesicles of the magnetosome membrane (*green*) are visible. *Blue* represents outer and inner membranes. Figure modified from Katzmann et al. (2010). (b) Schematic representation of intracellular magnetosome formation in a magnetotactic bacterial cell. Extracellular iron (as ferrous or ferric ions) is taken up and transported into the cell. Biomineralization of magnetite crystals then occurs in specific compartments provided by the magnetosome membrane, in which the physico-chemical conditions required for magnetite crystallization are controlled. Magnetosome membrane vesicles originate by invagination from the inner membrane prior to magnetite synthesis. Mature magnetosome crystals are then assembled and concatenated into linear chains by the action of the magnetosome filaments, facilitated by magnetostatic interactions between particles

greater than 3 % iron on a dry weight basis, a value several orders of magnitude over non-magnetotactic bacterial species (Blakemore 1982; Heyen and Schüler 2003). In addition, iron uptake for magnetite synthesis appears to occur relatively quickly (Schüler and Baeuerlein 1998; Heyen and Schüler 2003). It appears that both Fe(II) and Fe(III) can be taken up by cells of magnetotactic bacteria for magnetite synthesis although not necessarily simultaneously (Matsunaga and Arakaki 2007; Schüler and Baeuerlein 1996; Suzuki et al. 2006). How iron is taken up by magnetotactic bacteria is unknown but it would seem that there would be multiple mechanisms for this in a single bacterium as has been found in other non-magnetotactic bacteria. Thus far, siderophores, low molecular weight ligands produced by the cell that chelate and solubilize Fe(III) (Neilands 1984, 1995), have been implicated in iron uptake by magnetotactic bacteria (Paoletti and Blakemore 1986; Calugay et al. 2003; Dubbels et al. 2004) as well as a putative copper-dependent iron uptake system similar to that found in the yeast *Saccharomyces cerevisae* (Dubbels et al. 2004).

By correlation of iron uptake rates with results from TEM analysis, it has been demonstrated that the morphology of magnetite crystals is not only determined by biological control through biological regulation at the magnetosome compartments but to some degree also by the rates of iron uptake by magnetotactic bacteria (Faivre et al. 2008). These observations imply that the biological control over magnetite biomineralization can be disturbed by environmental parameters.

Iron then would have to enter the magnetosome invagination/vesicle. If the magnetite crystals are truly formed in permanent invaginations of the CM, then iron would only have to be transported through the outer membrane (OM) and enter the periplasm since any invagination of the CM would be open to the periplasm. This situation might only be temporary, however, if true independent vesicles are formed. In this case, iron may have to be transported across the CM and then through the magnetosome membrane to enter the vesicle. Several magnetosome membrane proteins have been implicated in this process (discussed in a later section). Based on Mößbauer spectroscopic analysis of *Magnetospirillum gryphiswaldense*, a mechanism was proposed by which iron required for magnetite biomineralization is processed throughout the CM directly to the magnetosome membrane without iron transport through the cytoplasm, suggesting that pathways for magnetite formation and biochemical iron uptake are distinct (Faivre et al. 2007). Magnetite formation occurs via membrane-associated crystallites, whereas the final step of magnetite crystal growth is possibly spatially separated from the CM. This work also suggests that cellular iron pools required for biochemical synthesis and magnetite biomineralization are distinct. This latter suggestion has been further substantiated by the analysis of a *M. gryphiswaldense* strain, in which the gene for a Fur-like iron uptake regulator was deleted (Uebe et al. 2010). This revealed that Fur is involved in global iron homeostasis, probably by balancing the competing demands for biochemical iron supply and magnetite biomineralization. In a very similar study, Qi et al. (2012) confirmed that Fur in *M. gryphiswaldense* directly regulates genes involved in iron and oxygen metabolism thereby influencing magnetosome biomineralization.

Finally, there is nucleation and controlled maturation of the magnetite crystal within the magnetosome invagination/vesicle. Magnetite precipitation might occur through the reduction of hydrated ferric oxide(s) (Frankel et al. 1979, 1983; Schüler and Baeuerlein 1998). However, when cells of *Magnetospirillum gryphiswaldense* were shifted from iron-limited to iron-sufficient conditions, they showed no delay in magnetite production (Heyen and Schüler 2003) suggesting that there are no mineral precursors to magnetite during biomineralization or that they are unstable and convert to magnetite extremely quickly.

The specificity for iron into the magnetosome mineral crystal appears to be very high. However, there are a number of

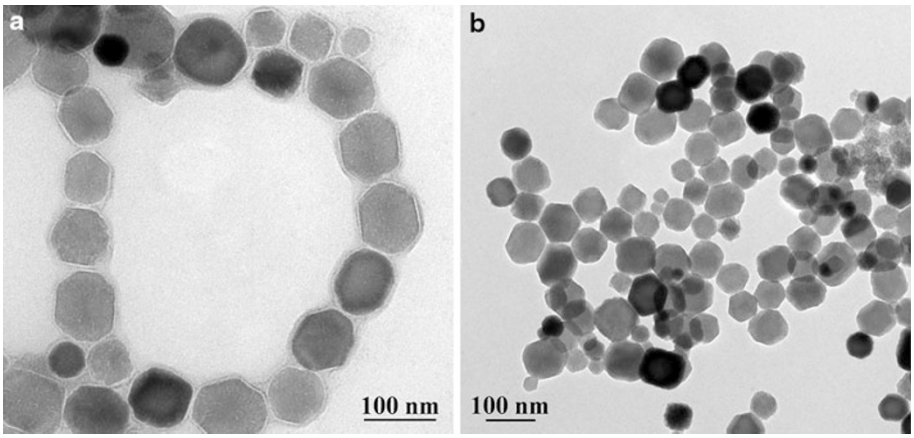


Fig. 12.21
(a) Purified magnetosomes of *Candidatus* Magnetococcus marinus negatively stained with uranyl acetate. Magnetosome membranes are represented by the electron-lucent layer surrounding each crystal. Note presence of chains. (b) After treatment with 1 % sodium dodecyl sulfate (SDS). Note electron-lucent layer on crystals is no longer present and the absence of chains

reports of the presence of other transition metals ions in magnetite and greigite magnetosome crystals in both cultured and uncultured magnetotactic bacteria. Trace amounts of titanium were found in magnetite particles of an uncultured freshwater magnetotactic coccus collected from a wastewater treatment pond (Towe and Moench 1981). The incorporation of small amounts of cobalt in surface layers of magnetosome magnetite crystals was demonstrated in three *Magnetospirillum* species (Staniland et al. 2008). Cells grown in cobalt-containing media showed very small changes in their magnetic properties, including the Verwey transition compared to a control culture. These results indicate that cobalt was not incorporated in the lattice structure of the magnetite crystals (Staniland et al. 2008). Uncultured magnetotactic bacteria in microcosms were exposed to $MnCl_2$, and up to 2.8 % atomic manganese in ultrathin sectioned cells and magnetosomes was detected via localized energy dispersive X-ray analysis (Keim et al. 2009). Magnetic properties of these cells and their magnetosomes were not examined. Elemental maps of thin sections of magnetite magnetosomes showed a higher concentration of manganese at the edges of the crystals suggesting that, like cobalt in the previous study, manganese incorporation was limited to the surface of the crystals. Significant amounts of copper were found in greigite magnetosome crystals of some uncultured MMPs collected from a salt marsh in California (Bazylinski et al. 1993a). The concentration of copper was extremely variable and ranged from about 0.1 at.% to 10 at.% relative to iron. Again, copper appeared to be mostly concentrated on the surface of the crystals.

The Magnetosome Membrane

The magnetosome membrane which encloses magnetite crystals (Gorby et al. 1988; Schüler and Baeuerlein 1997) in magnetosomes appears to be the locus of control and regulation of magnetite biomineralization in magnetotactic bacteria (Schüler 2008) (◉ *Fig. 12.21*). It appears to be a universal feature of magnetotactic bacteria, although it has been speculated that at least one species, *Desulfovibrio magneticus*, may not possess magnetosome membranes around their magnetite crystals because of the inability to visualize them by several electron microscopic techniques (Byrne et al. 2010). Interestingly, despite this speculation, there has been a proteomic study of magnetosomes of *D. magneticus* to determine magnetosome membrane-associated proteins (Matsunaga et al. 2009). The magnetosome membranes of *Magnetospirillum magnetotacticum* and *M. gryphiswaldense* are lipid bilayers consisting of components typical of this type of membrane including proteins, fatty acids, glycolipids, sulfolipids, and phospholipids (Gorby et al. 1988; Grünberg et al. 2004). This is in contrast to other intracellular inclusions in prokaryotes which are generally surrounded by a single layer of protein. Phospholipids make up 58–65 % of the total lipids of the magnetosome membrane of *M. magneticum* (Nakamura and Matsunaga 1993), and the predominant phospholipids in all *Magnetospirillum* species are phosphatidylserine, phosphatidylglycerol, and phosphatidylethanolamine (Gorby et al. 1988; Nakamura and Matsunaga 1993; Grünberg et al. 2004). A comparison of the fatty acids of the magnetosome membrane, the CM and the outer membrane (OM) showed that the composition of the magnetosome membrane is similar to the CM but distinct from the OM (Tanaka et al. 2006) suggesting that the magnetosome membrane is derived from the CM. In addition, magnetite magnetosomes are almost always located adjacent to the CM in *Magnetospirillum* species (Bazylinski and Schübbe 2007; Katzmann et al. 2010).

Magnetosome Membrane Proteins

Magnetosome membranes can be easily removed from magnetosomes using detergents such as sodium deodecyl sulfate

(SDS) for protein analysis (Fig. 12.21). Protein profiles of the magnetosome membrane are distinct from other cell fractions (the soluble periplasmic and cytoplasmic fractions, and the cell and outer membrane fractions) in currently recognized *Magnetospirillum* species, in *Desulfovibrio magneticus*, and in *Magnetovibrio blakemorei* (Gorby et al. 1988; Okamura et al. 2000; Grünberg et al. 2001; Dubbels et al. 2004; Tanaka et al. 2006; Matsunaga et al. 2009). In addition, there are also differences in the magnetosome membrane protein profiles between these organisms and even between the species of *Magnetospirillum* (Grünberg et al. 2004). Because the magnetosome membrane contains proteins that are unique to it, it seems very probable that these proteins play the key roles in magnetite biomineralization in magnetosomes. Most of the focus of investigators in magnetite biomineralization by magnetotactic bacteria is on these proteins and the genes that encode for them. These proteins and genes are generally referred to as the Mam (magnetosome membrane) or Mms (magnetic particle membrane specific) proteins and the *mam* or *mms* genes, respectively, although a gene called *magA* has been described as coding for a magnetosome membrane protein (Matsunaga et al. 1992; Nakamura et al. 1995b). All *mam* and *mms* genes have been found to be clustered within several operons of the conserved genomic magnetosome island (see below). Identifying the function of the magnetosome membrane proteins appears to be key to understanding magnetosome biomineralization. Putative functions of these proteins, based on comparisons of similar proteins through blast searches and through mutagenesis experiments, include iron uptake into the cell and/or the magnetosome vesicles, crystal nucleation and biomineralization of magnetite, and the arrangement of the magnetosomes in the chain motif. The putative roles of a number of magnetosome membrane proteins follow, although this list is not complete. It should also be kept in mind that each magnetotactic bacterium appears to have genes within their magnetosome gene islands that are specific to them that might encode for proteins involved in biomineralization that have little or no homology with any known proteins.

The MagA protein of *Magnetospirillum magneticum* has low similarity to the *Escherichia coli* potassium efflux protein KefC and its transcription reported to be upregulated by low iron concentrations in the growth medium (Nakamura et al. 1995b). Based on the putative phenotype of a non-complemented transposon mutant, a potential function is as a magnetosome-directed ferrous iron transporter having an essential role in magnetosome formation in *M. magneticum* (Nakamura et al. 1995a). However, a recent study showed that targeted deletion *magA* mutants of *M. magneticum* and *M. gryphiswaldense* still biomineralize wild type-like magnetosomes and have no obvious growth defects, thus unambiguously showing that *magA* is not involved in magnetosome formation in magnetotactic bacteria (Uebe et al. 2012).

Based on genetic studies in *Magnetospirillum magneticum*, four genes (*mamI, mamL, mamQ, and mamB*) seem to be absolutely essential for the formation of magnetosomes (Murat et al. 2010) but were not sufficient to support magnetosome formation in the absence of other magnetosome genes. MamI and MamL are unique to magnetotactic bacteria and were implicated in the invagination of the magnetosome membrane from the CM, since in Δ*mamI* and Δ*mamL* mutants, no structures resembling empty magnetosome compartments could be detected. However, the mechanism by which this is mediated is unclear, and MamI and MamL lack any significant homology to eukaryotic proteins known to be involved in deformation of cellular membranes.

The *mamA* (corresponds to *mam22* and *mms24* in different magnetotactic bacteria) gene is present in the genomes of all magnetotactic bacteria examined (Okuda et al. 1996; Grünberg et al. 2001; Komeili et al. 2004; Matsunaga et al. 2005; Schübbe et al. 2009; Nakazawa et al. 2009; Abreu et al. 2011). The amino acid sequences of the MamA proteins show high similarity to proteins of the tetratricopeptide repeat (TPR) protein family (Okuda et al. 1996). MamA is thought to be important in protein-protein interactions that might occur in the synthesis of magnetosomes and the magnetosome chain (Okuda et al. 1996; Okuda and Fukumori 2001) since multiple copies of TPRs are known to form scaffolds within proteins to mediate protein-protein interactions and to coordinate the assembly of proteins into multisubunit complexes (Ponting and Phillips 1996). A deletion of *mamA* in *Magnetospirillum magneticum* resulted in shorter magnetosome chains, this leading to the suggestion that MamA activates magnetosome vesicles and is involved in magnetite crystal maturation (Komeili et al. 2004; Murat et al. 2010).

Genes for the proteins MamB and MamM are also present in the genomes of all magnetotactic bacteria examined (Grünberg et al. 2001; Matsunaga et al. 2005; Schübbe et al. 2009; Nakazawa et al. 2009; Abreu et al. 2011) and show strong similarity to heavy metal transporting proteins of the cation diffusion facilitator family. An additional magnetosome membrane protein, MamV, also appears to be in this family, but its gene is only present in *M. magnetotacticum* and *M. magneticum* and not in other magnetotactic bacteria. Proteins in this family display an unusual degree of size variation, sequence divergence, and polarity, can catalyze the influx or efflux of metal ions, and include a ferrous iron transport system (Paulsen et al. 1997; Grass et al. 2005; Haney et al. 2005). For this reason, these Mam proteins might be involved in the transportation of the iron into the magnetosome vesicle (Grünberg et al. 2001). As demonstrated by a recent study in *M. gryphiswaldense*, MamB and MamM form heterodimers and also interact with other magnetosome proteins, indicating that the functions of these two proteins are complex and involved in the control of different key steps of magnetosome formation (Uebe et al. 2012).

Genes for the MamE, MamO, and MamP proteins are present in all magnetotactic bacteria investigated to date. MamE is required for magnetite biomineralization in *Magnetospirillum magneticum* (Murat et al. 2010). These proteins show sequence similarity to HtrA-like serine proteases but little similarity to each other. HtrA (also known as DegP) is a heat-shock-induced, envelope-associated serine protease first discovered in *Escherichia coli* (Lipinska et al. 1989). The main role of HtrA

seems to be in the degradation of misfolded proteins in the periplasm (Pallen and Wren 1997). These proteases are also known to be involved in nondestructive protein processing and modulation of signaling pathways by degrading important regulatory proteins and are characterized by the inclusion of one or two PDZ-domains (Fanning and Anderson 1996) and a trypsin-like protease domain. These proteins could function as chaperones in magnetosome formation (Grünberg et al. 2001). In *M. magneticum*, MamE may be important in arranging proteins in the magnetosome membrane while MamO may be involved in iron uptake and magnetosome magnetite crystal initiation in the magnetosome invagination (Murat et al. 2010). Both MamE and MamO have been shown to be essential for magnetite biomineralization in *M. gryphiswaldense* based on results from gene deletion experiments (Yang et al. 2010).

The magnetosome membrane proteins MamC (Mms13, Mam12 (Arakaki et al. 2003; Taoka et al. 2006)), MamD (Mms7; Fukuda et al. 2006), MamF, MamG, MamQ, MamR, and MamS are unique to some magnetotactic bacteria, and homologues of these proteins have not been found in non-magnetotactic bacteria (Grünberg et al. 2004). All recognized *Magnetospirillum* species contain the genes for these proteins. *Candidatus* Magnetococcus marinus contains all but *mamG* and *mamR* (Schübbe et al. 2009). *Magnetovibrio blakemorei* contains all but *mamG* in its magnetosome gene island, but the presence of the other genes in the genome cannot be excluded at this time (genome of this organism is not complete; Jogler et al. 2009a). *Desulfovibrio magneticus* possesses only *mamQ* (Nakazawa et al. 2009). *Ca.* Magnetoglobus multicellularis and *Ca.* Magnetobacterium bavaricum contain only *mamQ*, the latter species has two copies, but the presence of the other genes cannot be excluded as the genomes are not complete Abreu et al. 2011; Jogler et al. 2011). MamC is an abundant protein in the magnetosome membranes of *M. magnetotacticum* (Taoka et al. 2006), *M. gryphiswaldense* (Grünberg et al. 2001), and *Magnetovibrio blakemorei* (Dubbels et al. 2004). The hydrophobic proteins, MamC and MamF, contain predicted transmembrane helices. MamD and MamG are partially identical and are similar to another magnetosome membrane protein, Mms6 of *M. magneticum*. All three proteins contain large repeating leucine-glycine (L-G) motifs present in other proteins known to be involved in biomineralization. Mms6, an amphiphilic protein consisting of an N-terminal LG-rich hydrophobic region and a C-terminal hydrophilic region containing repeats of acidic amino acids, has been shown to affect the crystal morphology of crystals when present during the chemical precipitation of magnetite (Arakaki et al. 2003; Prozorov et al. 2007). The proteins MamGFDC, in concert, comprise about 35% of the protein associated with the magnetosome membrane and, although not essential for magnetite biomineralization, have been shown to regulate the size of magnetosome magnetite crystals in *M. gryphiswaldense* (Scheffel et al. 2008). In *M. magneticum*, MamR and MamS appear to be involved in magnetite crystal maturation while MamQ in the invagination of the cytoplasmic membrane to form the magnetosome vesicle (Murat et al. 2010; Quinlan et al. 2011).

MamN, the gene of which is not present in the genomes of *Candidatus* Magnetococcus marinus and *Desulfovibrio magneticus* (Schübbe et al. 2009; Nakazawa et al. 2009) and also not in the putative magnetosome gene islands of *Ca.* Magnetoglobus multicellularis and *Ca.* Magnetobacterium bavaricum (Abreu et al. 2011; Jogler et al. 2011), shows some similarity to certain transport proteins, some of which transport protons leading to an idea that this protein might function as a proton pump transporting protons accumulating during magnetite precipitation (Jogler and Schüler 2007). In *Magnetospirillum magneticum*, MamN, like MamM, is thought to be involved in iron uptake and initiation of magnetite crystal formation (Murat et al. 2010).

The gene for MamT is present in all magnetotactic bacteria studied (Grünberg et al. 2001; Matsunaga et al. 2005; Schübbe et al. 2009; Nakazawa et al. 2009; Abreu et al. 2011) except in the putative magnetosome gene island of *Candidatus* Magnetobacterium bavaricum (Jogler et al. 2011). MamT contains two possible binding sites for the heme group present in cytochrome c and, therefore, might be involved in redox reactions within the magnetosome vesicle (Grünberg et al. 2004) that might be important in magnetite crystal maturation (Murat et al. 2010).

The genes *mamJ* and *mamK* are located within the *mamAB* gene cluster in *Magnetospirillum* species and are cotranscribed (Schübbe et al. 2006). The *mamK* gene has been found in all magnetotactic bacteria studied except in the putative magnetosome gene island of *Candidatus* Magnetobacterium bavaricum (Jogler et al. 2011), while *mamJ* is present only in recognized *Magnetospirillum* species (Nakazawa et al. 2009; Schübbe et al. 2009; Jogler et al. 2009a, 2011; Abreu et al. 2011). MamJ is a strongly acidic protein with a repeating glutamate-rich section in its central domain (Scheffel et al. 2006) that is reminiscent to certain other acidic proteins (Grünberg et al. 2004) involved in biomineralization processes such as calcium carbonate biomineralization in shells (Baeuerlein 2003). Carboxy groups of the acidic amino acids are recognized to have a high affinity for metal ions, and because of this, magnetosome proteins with these characteristics have been thought to be involved in the initiation of magnetite crystal nucleation (Arakaki et al. 2003). However, deletion of *mamJ* in *M. gryphiswaldense* had no effect on the biomineralization of magnetite but resulted in cells in which magnetosome crystals were organized in agglomerated clusters instead of regular chains (Scheffel et al. 2006), whereas in *M. magneticum*, the phenotype of a co-deletion of *mamJ* along with the paralogous *limJ* gene was less severe and resulted in interrupted magnetosome chains (Draper et al. 2011). MamK shows some homology to actin-like proteins including MreB (Schübbe et al. 2003), which have important functions in the control of cell morphology and elongation, peptidoglycan synthesis, and portioning of plasmids in many bacteria (Jones et al. 2001; Figge et al. 2004; Carballido-Lopez 2006; Cabeen and Jacobs-Wagner 2010; Garner et al. 2011; Dominguez-Escobar et al. 2011). MamK proteins in magnetotactic bacteria are more similar to each other than they are to MreB homologues (Komeili et al. 2006; Derman et al. 2009). In addition, assembly of

MamK filaments appears to be independent of MreB (Pradel et al. 2006). Experiments involving gene knockout mutants of *mamJ* in *M. gryphiswaldense* and *mamK* in *M. magneticum* showed that the products of these genes are responsible for magnetosome chain formation but did not inhibit magnetosome formation in these organisms (Komeili et al. 2006; Scheffel et al. 2006). MamJ is thought to function by anchoring magnetosomes to MamK filaments in *Magnetospirillum* species (Komeili et al. 2006; Scheffel et al. 2006; Scheffel and Schüler 2007), whereas MamK is involved in dynamic assembly, positioning and segregation of the magnetosome chain during cell cycle rather than merely providing a rigid mechanical scaffold (Katzmann et al. 2010, 2011). However, the observed differences between mutant phenotypes in different magnetospirilla suggest that the functions of *mamK* and *mamJ* may be somewhat distinct in different species depending on their genetic context.

Genomics and Genetics of Magnetotactic Bacteria

The genome sequences of several magnetotactic bacteria are now complete or nearly so and are available for examination. Analysis of these genome sequences has provided valuable insight into how magnetosome genes are organized in different magnetotactic bacteria. The genome of *Magnetospirillum magnetotacticum* strain MS-1 consists of a single, circular chromosome about 4.3 Mb in size with a possible extrachromosomal element as determined by pulsed-field gel electrophoresis (Bertani et al. 2001). The genome of this bacterium is partially sequenced and annotated and is available at the Joint Genome Institute's website (http://genome.jgi-psf.org/draft_microbes/magma/magma.home.html). *M. magneticum* strain AMB-1 contains a circular chromosome slightly larger than that of *M. magnetotacticum* at 4.97 Mb (Matsunaga et al. 2005) and likely a cryptic plasmid (Okamura et al. 2003). The genome sequence of this species is available at the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp) under accession number AP007255. The genome of *M. gryphiswaldense* strain MSR-1 is comprised of a circular chromosome about 4.3 Mb in size and also contains a native plasmid (Jogler and Schüler 2007; Richter et al. 2007). The genome of the marine coccus *Candidatus* Magnetococcus marinus strain MC-1 consists of a singular, circular chromosome about 4.5 Mb in size and there is no evidence for the presence of plasmids. The genome sequence of this species is complete (Schübbe et al. 2009) and can be viewed at http://genome.jgi-nsf.org/draft_microbes/magm1/magm1.home.html. The genomes of the marine vibrios *Magnetovibrio blakemorei* strains MV-1 and MV-2 are approximately 3.7 and 3.6 Mb in size, respectively, based on pulsed-field gel electrophoresis. Data suggest genomes of both strains consist of a single, circular chromosome with no evidence of plasmids (Dean and Bazylinski 1999). The genome of MV-1 is almost complete. The genome sequence for *Desulfovibrio magneticus* strain RS-1 is complete (Nakazawa et al. 2009) and is available at the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp) under accession numbers AP010904 to AP010906.

In an attempt to identify magnetotaxis-specific genes by bioinformatic analysis, cross-comparisons of the complete or nearly complete genomes of the magnetotactic *Alphaproteobacteria* including *Magnetospirillum magneticum*, *M. magnetotacticum*, *M. gryphiswaldense*, and *Candidatus* Magnetococcus marinus revealed a core genome of about 890 genes which are shared by all four strains. In addition to a set of approximately 152 genus-specific genes shared by the three *Magnetospirillum* strains, 28 genes were identified as group specific, that is, which occur in all four analyzed magnetotactic *Alphaproteobacteria* but exhibit no (magnetotactic bacterial-specific genes) or only weak (magnetotactic bacterial-related genes) similarity to any genes from non-magnetotactic organisms and which, besides various novel genes, included nearly all *mam* and *mms* genes that were previously shown to control magnetosome formation. If the genome sequence of the sulfate-reducing, deltaproteobacterium *Desulfovibrio magneticus* was available at the time for inclusion in this comparison, the number of signature genes conserved in all five magnetotactic *Proteobacteria* decreased to only nine.

Molecular Organization of Magnetosome Genes

In all magnetotactic bacteria examined, the *mam* and *mms* genes are present as clusters that are in relatively close proximity to one another within the genome and are organized as a genomic "magnetosome island (MAI)" (see below). The *mamA* and *mamB* genes in *Magnetospirillum gryphiswaldense* are present in a segment of DNA about 16.4 kb in length in collinear order with 15 other genes that comprise the *mamAB* cluster (Grünberg et al. 2001). Recent deletion studies in *M. magneticum* and *M. gryphiswaldense* demonstrated that the *mamAB* cluster is the only operon-containing genes that are absolutely essential for magnetosome formation and magnetite biomineralization, whereas the other operons have important accessory functions in controlling the synthesis of regularly shaped and sized crystals that are functional for magnetic orientation (Murat et al. 2010; Ullrich and Schüler 2010; Lohße et al. 2011). One of such accessory operons, the *mamGFDC* cluster is about 2.1 kb in length located about 15-kb upstream of the *mamAB* operon and is composed of four genes which encode a group of abundant magnetosome membrane proteins involved in size control of magnetite crystals (Scheffel et al. 2008). The 3.6 kb *mms6* cluster is located 368 bp upstream of the *mamGFDC* operon and contains five genes (Schübbe et al. 2003). Another gene encoding for a magnetosome membrane protein, *mamW*, is not present in these three operons but is located about 10-kb upstream of the *mms6* operon (Ullrich et al. 2005). All *mam* and *mms* genes are located on a segment of DNA about 45 kb in length in *M. gryphiswaldense*.

The operon-like, collinear organization of the *mamAB*, *mamGFDC*, and *mms6* clusters suggests that they are transcribed as single long mRNAs, and experimental evidence provides support for this polycistronic transcriptional unit. The

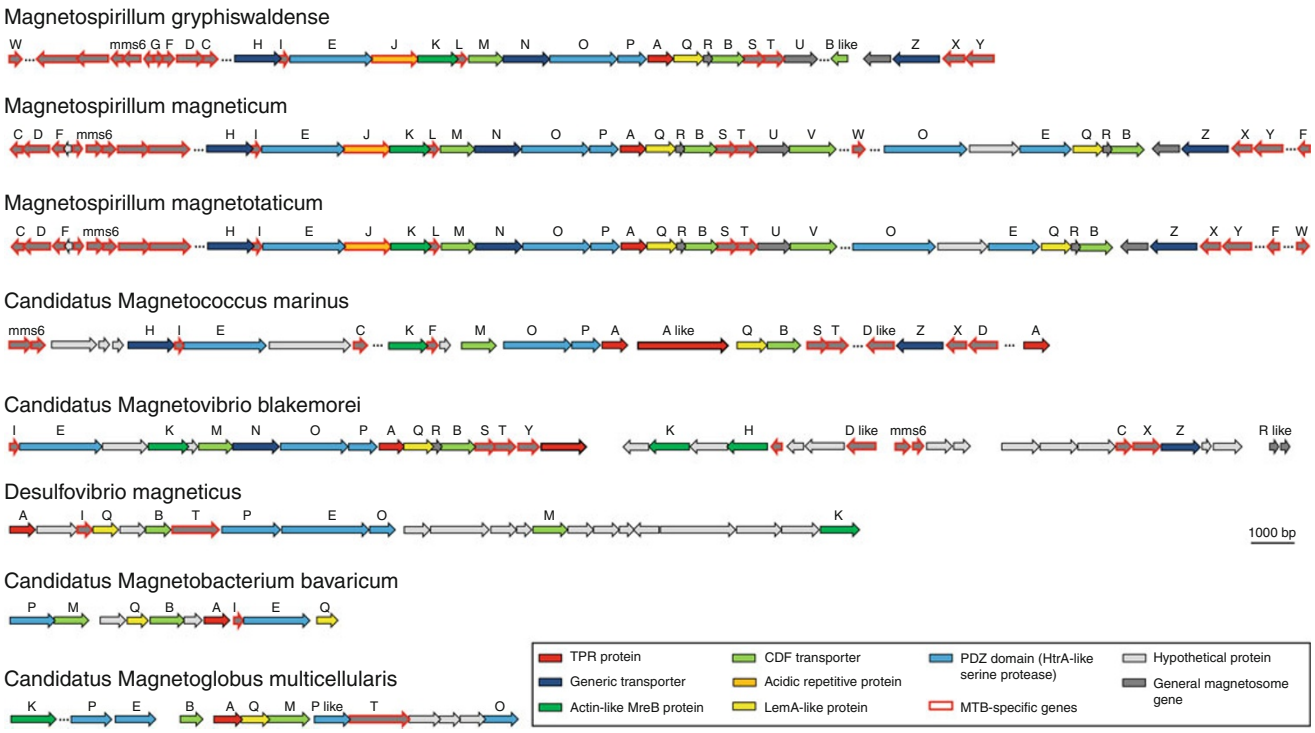


Fig. 12.22

Organization of magnetosome genes in the putative magnetosome gene islands (MAIs) of different magnetotactic bacteria. All organisms are cultured with the exception of *Candidatus* Magnetobacterium bavaricum and *Ca.* Magnetoglobus multicellularis. Letters above genes indicate *mam* genes (e.g., *A mamA*)

transcription starting points of the *mamAB*, *mamGFDC*, and *mms6* operons were mapped closely upstream of the first genes in the operons, respectively (Schübbe et al. 2006).

The organization of the *mam* and *mms* genes is relatively well conserved in *Magnetospirillum* strains. In addition, there are high similarities for specific Mam and Mms proteins and their encoding genes, respectively, in recognized *Magnetospirillum* species. The organization and sequence of the magnetosome genes is less conserved in other unrelated magnetotactic strains (Schübbe et al. 2003, 2009; Ullrich et al. 2005; Jogler et al. 2011).

The genomic region that contains the magnetosome genes in *Magnetospirillum gryphiswaldense* also contains 42 mobile elements as transposases of the insertion sequence type and integrases (Ullrich et al. 2005). These mobile elements are common, important features in genomic islands (Mahillon and Chandler 1998; Mahillon et al. 1999). Other characteristics of gene islands include the presence of tRNA genes that can act as insertion sites for integrases (Blum et al. 1994; Reiter and Palm 1990) and a different guanine + cytosine (G + C) content compared to the rest of the genome (Dobrindt et al. 2004). In *M. gryphiswaldense*, the magnetosome gene region is about 130 kb in size, contains three tRNA genes upstream of the *mms* operon, has a slightly different G + C content versus the rest of the genome, and contains many hypothetical genes and pseudogenes (Schübbe et al. 2003; Ullrich et al. 2005) which apparently have no function as their deletions had no obvious effect on either growth or magnetosome formation (Lohße et al. 2011). Therefore, it seems very likely that this genomic region represents a large magnetosome gene island (MAI) which appears to be present with variations in other cultured and uncultured magnetotactic bacteria (Fukuda et al. 2006; Richter et al. 2007; Nakazawa et al. 2009; Schübbe et al. 2009; Abreu et al. 2011; Jogler et al. 2011). A comparison of the putative MAIs of different cultured and uncultured magnetotactic bacteria is shown in ❯ *Fig. 12.22*. ❯ *Table 12.1* lists all the magnetosome genes present in the putative MAIs of all magnetotactic bacteria in which one has been identified.

Gene or genomic islands are reported to be distributed to different bacteria through horizontal gene transfer and thus may be a major pathway for the evolution of bacterial genomes (Juhas et al. 2009). In addition, genomic islands are thought to undergo frequent gene rearrangements (Juhas et al. 2009). Gene rearrangements, gene deletions, and duplications may be the reason for the frequent development of spontaneous nonmagnetic mutants of various strains. Spontaneous deletions that lead to a loss of the magnetic phenotype with a frequency of 10^{-2} were observed under starvation conditions in late stationary phase cultures of *Magnetospirillum gryphiswaldense* and most likely caused by RecA-dependent homologous recombination between numerous repeats present in the MAI (Ullrich et al. 2005; Kolinko et al. 2011). One of these mutants, designated *M. gryphiswaldense* strain MSR-1B, showed poorer growth in the presence of increased iron concentration and lower iron uptake compared to the wild-type strain (Schübbe et al. 2003). Frequent

nonmagnetic mutants that do not synthesize magnetosomes were also observed in *Magnetovibrio blakemorei* (Dubbels et al. 2004) and *M. magneticum* (Fukuda et al. 2006; Komeili et al. 2006).

Rioux et al. (2010) identified a separate group of *mam*-like genes in the genome of *Magentospirillum magneticum* strain AMB-1. These genes, including *mamKDLJEFQ*-like genes, are clustered in a genomic islet distinct and distant from the known magnetosome genomic island. In this study, they demonstrate that the *mamK*-like gene is transcribed and that the gene product is protein filaments as is MamK. Thus far, this is the only report of a functional *mam* gene located outside of the magnetosome genomic island. There is also some evidence for the presence of magnetosome membrane protein genes on a plasmid rather than a genome in magnetotactic bacterium (Matsunaga et al. 2009). Genes encoding for two homologous

Table 12.1

Magnetosome genes present in the putative magnetosome gene islands of different cultured and uncultured magnetotactic bacteria

Gene	MSR-1[a]	MS-1	AMB-1	MC-1	MV-1	RS-1	*Ca.* M. bav.	*Ca.* M. mult.
mamA	+[b]	+[c]	+[d]	+	+	+	+	+
mamB	+	+	+	+	+	+	+	+
mamC	+	+[e]	+[d]	+	+	–	/	/
mamD	+	+	+[d]	++	+	–	/	/
mamE	+	+	+	+	+	+	+	+
mamF	+	+	+	+	+	–	/	/
mamG	+	+	+	–	/	–	/	/
mamH	+	+	+	+	+	–	/	/
mamI	+	+	+	+	+	+	+	/
mamJ	+	+	+	–	/	–	/	/
mamK	+	+	+	+	++	+	/	+
mamL	+	+	+	–	+	–	/	/
mamM	+	+	+	+	+	+	+	+
mamN	+	+	+	–	+	–	/	/
mamO	+	+	+	+	+	+	/	+
mamP	+	+	+	+	+	+	+	+
mamQ	+	+	+	+	+	+	++	+
mamR	+	+	+	–	+	–	/	/
mamS	+	+	+	+	+	–	/	/
mamT	+	+	+	+	+	+	/	+
mamU	+	+	+	–	/	–	/	/
mamV	–	+	+	–	/	–	/	/
mamW	+	–	+	–	/	–	/	/
mamX	+	+	+	+	+	–	/	/
mamY	+	+	+	–	+	–	/	/
mamZ	++	++	++	+	+	–	/	/
mgl462	+	+	+	–	/	–	/	/
mms6	+	+	+	+	+	–	/	/
mgl459	+	+	+	+	+	–	/	/
mgl458	+	+	+	–	/	–	/	/
mgl457	+	+	+	–	/	–	/	/
mamE/S-like	+	+	+	+	/	–	/	/
mamF-like	+	+	+	+	/	–	/	/
mamH-like	+	+	+	+	/	–	/	/
mamA-like	–	–	–	++	/	–	/	/
mamP-like	–	–	–	–	–	–	–	+
mgr4150	+	+	+	–	/	–	/	/

Table 12.1 (continued)

Gene	MSR-1[a]	MS-1	AMB-1	MC-1	MV-1	RS-1	*Ca.* M. bav.	*Ca.* M. mult.
mgr0208	+	+	+	+	/	–	/	/
mgr0207	+	+	+	+	/	–	/	/
mgr0206	+	+	+	+	/	–	/	/
mgr3500	+	+	+	+	/	–	/	/
mgr3499	+	+	+	–	/	–	/	/
mgr3497	+	+	+	+	/	–	/	/
mgr3495	+	–	+	–	/	–	/	/

[a]Organisms: MSR-1, *Magnetospirillum gryphiswaldense* (Ullrich et al. 2005); MS-1, *M. magnetotacticum* (Bertani et al. 2001); AMB-1, *M. magneticum* (Matsunaga et al. 2005); MC-1, *Candidatus* Magnetococcus marinus (Schübbe et al. 2009); MV-1, *Magnetovibrio blakemorei* (Jogler et al. 2009a); RS-1, *Desulfovibrio magneticus* (Nakazawa et al. 2009); *Ca. M. bav*. *Ca.* Magnetobacterium bavaricum (Jogler et al. 2011); *Ca. M. mult*. *Ca.* Magnetoglobus multicellularis (Abreu et al. 2011)
[b]Symbols: +, homologue present in genome; ++, two paralogues in genome; -, homologue absent from genome; /, homologue absent from putative magnetosome island but genome sequence has not been completed
[c]In *M. magnetotacticum* strain MS-1, *mamA* = *mam22* (Okuda et al. 1996)
[d]In *M. magneticum* strain AMB-1, *mamA* = *mms24*, *mamC* = *mms13* and *mamD* = *mms7* (Fukuda et al. 2006)
[e]In *M. magnetotacticum* strain MS-1, *mamC* = *mam12* (Taoka et al. 2006)

magnetosome proteins of *Candidatus* Magnetococcus marinus were found on a cryptic plasmid (pDMC1) of *Desulfovibrio magneticus* (Matsunaga et al. 2009).

Strain BW-1, the only greigite-producing magnetotactic bacterium known to be in pure culture, biomineralizes both greigite and magnetite and contains two sets of magnetosome genes although it is not known whether they are on separate gene islands (Lefèvre et al. 2011d). Because one set of genes is more similar to the magnetite-producing *Desulfovibrio magneticus* and *Candidatus* Magnetococcus marinus and the other to the greigite-producing *Candidatus* Magnetoglobus multicellularis, it was suggested that the first set is responsible for greigite biomineralization and the second for magnetite production (Lefèvre et al. 2011d). Because the proportion of the different minerals produced is affected by external conditions in the growth medium, perhaps the two sets of genes are regulated separately.

Distribution of the MAI through horizontal gene transfer would explain the phylogenetic diversity of the magnetotactic bacteria, while variations of the MAI in different magnetotactic bacteria may be the result of rearrangements within the MAI occurring over time.

Genetic Manipulation of Magnetotactic Bacteria

Because of the difficulty in growing magnetotactic bacteria on agar plates as individual colonies, it took many years to develop tractable genetic systems in these organisms. In general, they do not form colonies unless the agar plates contain compounds to scavenge toxic radicals (e.g., activated charcoal) or they are incubated under low concentrations of oxygen. The most common way of assigning definitive functions to specific genes in prokaryotes is through single gene knockouts with subsequent analysis of the mutant phenotype, and this approach has proved very useful in the determination of the functions of a number of magnetosome genes. Tractable genetic systems have now been developed for *Magnetospirillum gryphiswaldense* strain MSR-1 (Schultheiss and Schüler 2003) and *M. magneticum* strain AMB-1 (Matsunaga et al. 1992). It is relatively easy to detect non-magnetotactic mutants of magnetotactic bacteria unable to synthesize magnetosomes since magnetite-forming colonies are generally dark brown or black versus those of nonmagnetic mutants which are white or cream colored (Dubbels et al. 2004; Schultheiss and Schüler 2003). In addition, the degree of a magnetic response or its absence related to the number of magnetosomes per cell can be easily tested by light-scattering measurements of cell suspensions in variable magnetic fields by "C_{mag}" values (Schüler et al. 1995; Zhao et al. 2007).

Conjugational transfers of replicative plasmids were accomplished with frequencies of 1 and $3-4.5 \times 10^{-3}$ for *Magnetospirillum gryphiswaldense* and *M. magneticum*, respectively (Matsunaga et al. 1992; Schultheiss and Schüler 2003). Mutants of both strains were generated using Tn5 transposon mutagenesis as well as broad host range replication (pBBRMCS, IncQ) and suicide vectors (pK19mobsacB, pMB1) (Komeili et al. 2004; Matsunaga et al. 1992; Schultheiss and Schüler 2003; Schultheiss et al. 2004). The development of genetic systems for these strains has allowed for the extrachromosomal expression of genes and the integration of reporter genes like luciferase or green fluorescent protein genes (*gfp*) and their derivatives. In turn, these techniques have allowed for studies involving the subcellular localization of proteins putatively involved in magnetite magnetosome biomineralization (Komeili et al. 2004; Matsunaga et al. 2000a, b; Nakamura et al. 1995b; Schultheiss et al. 2004). General transposon mutagenesis is random but can generate nonmagnetic mutants that make it possible to identify genes in the genome involved in magnetite biomineralization. Suicide vectors together with genomic data now allow for the integration of these vectors at specific sites on the genome to generate site-directed gene knockouts for the definitive

determination of precise roles of specific genes in magnetite magnetosome biomineralization (Komeili et al. 2004, 2006; Pradel et al. 2006; Scheffel et al. 2006; Murat et al. 2010). Related techniques based on the Cre-*lox* system have also allowed the targeted deletion of large (up to 60 kb and more) regions from the genome of *M. gryphiswaldense*, which facilitates functional analysis and genomic engineering (Ullrich and Schüler 2010; Lohße et al. 2011).

Applications of Magnetotactic Bacteria, Magnetosomes, and Magnetosome Crystals

Cells of magnetite-producing magnetotactic bacteria and their magnetic inclusions have been shown to have novel magnetic, physical, and optical properties that can and have been exploited in a variety of scientific, commercial, and other applications (reviewed in Lang and Schüler 2006; Lang et al. 2007; Matsunaga and Arakaki 2007; Arakaki et al. 2008; Xie et al. 2009). While the number of applications and patents involving magnetotactic bacteria appears to be ever increasing, a major problem is the mass culture of these organisms and the subsequent efficient harvesting of magnetosomes. However, there has been significant progress in this area in the last decade.

Mass Culture of Magnetotactic Bacteria

Considering that the amount of magnetic materials from magnetotactic bacteria required for most applications is relatively high, obtaining higher yields of magnetotactic bacterial cells and magnetosomes from cultures poses a significant obstacle. In order to produce enough cells, magnetosomes, and magnetite crystals for these applications, cells therefore must be grown in mass culture where conditions for growth and magnetite synthesis must be optimized. In almost all cases, the focus of these studies involved modification of growth media and the conditions under which cultures are incubated. *Magnetospirillum* species are the only magnetotactic bacteria used in these studies. In some cases, it is difficult to compare yields directly as some studies focus on magnetosomes and it is unclear whether magnetosome membranes are included in the yield values.

In an early study of this type, Matsunaga et al. (1990) grew *Magnetospirillum magneticum* in a 1,000-l fermenter and recovered 2.6 mg of magnetosomes per liter of culture. Culture optimization experiments were later conducted in fed-batch cultures of the same organism but did not result in a higher yield of cells or magnetosomes (Matsunaga et al. 1996, 2000a). A recombinant *M. magneticum* strain harboring the plasmid pEML was grown in a pH-regulated fed-batch culture system where the addition of fresh nutrients was feedback-controlled as a function of the pH of the culture (Yang et al. 2001a). Here, the magnetosome yield was maximized by adjusting the rate of addition of the major iron source. Providing ferric quinate at 15.4 mg/min resulted in a magnetosome yield of 7.5 mg/l. Different iron sources and the addition of various nutrients and chemical reducing agents (e.g., L-cysteine, yeast extract, polypeptone) were also later shown to have significant effects on magnetosome yield by *M. magneticum* grown in fed-batch culture (Yang et al. 2001b).

More precise control over the growth of *Magnetospirillum* species was achieved using an oxygen-controlled fermenter (Heyen and Schüler 2003; Lang and Schüler 2006). Three species were grown using this method, *M. gryphiswaldense*, *M. magnetotacticum*, and *M. magneticum*, and 6.3, 3.3, and 2.0 mg magnetite per liter per day were obtained from each species, respectively (Heyen and Schüler 2003). Using a similar type of fermenter, except that dissolved oxygen was controlled to an optimal level using the change of cell growth rate rather than from a direct measurement from the sensitive oxygen electrode, Sun et al. (2008) obtained a cell density of OD_{565} of 7.24 for *M. gryphiswaldense* after 60 h of culture. The cell yield (dry weight) was 2.17 g/l, and the yield of magnetosomes (dry weight) was 41.7 mg/l and 16.7 mg/l/day. By decreasing the amount of carbon and electron source (lactate) in the same fermenter, Liu et al. (2010) reported later growth and magnetosome yields of OD_{565nm} of 12 and 55.49 mg/l/day, respectively, after 36 h of culture again using *M. gryphiswaldense*.

Applications of Cells of Magnetotactic Bacteria

Both live and dead magnetotactic bacterial cells have proven useful in medical, magnetic, and environmental applications. They have been used to magnetically separate granulocytes and monocytes after having been phagocytized by them (Matsunaga et al. 1989). Because of the relatively easy separation of magnetic cells, the use of magnetotactic bacteria in the uptake and remediation of heavy metals and radionucleotides from wastewater has been discussed and investigated (Bahaj et al. 1993, 1998a, b, c; Arakaki et al. 2002). Cells of polar magnetotactic bacteria have been used to determine south magnetic poles in meteorites and rocks containing fine-grained (<1 mm) magnetic minerals (Funaki et al. 1989, 1992) and for nondestructive magnetic domain analysis on soft magnetic materials (Harasko et al. 1993, 1995).

Applications of Magnetosomes

Magnetosomes contain single magnetic domain crystals that have useful magnetic and physical properties. Like magnetotactic bacterial cells, magnetosomes can be used for cell separation (Kuhara et al. 2004). Importantly, the organic, phospholipid magnetosome membrane that surrounds the crystals allows for the attachment of biological molecules including proteins and nucleic acids on their surfaces. Magnetite magnetosomes have been used in the immobilization of enzymes (Matsunaga and Kamiya 1987) and in the formation of magnetic antibodies useful in various fluoroimmunoassays (Matsunaga et al. 1990) involving the detection of allergens

(Nakamura and Matsunaga 1993) and squamous cell carcinoma cells (Matsunaga 1991) and the quantification of immunoglobulin G (Nakamura et al. 1991). Bacterial magnetite crystals have been used in the detection and removal of cells of *Escherichia coli* with a fluorescein isothiocyanate-conjugated monoclonal antibody immobilized on magnetosomes (Nakamura et al. 1993). Magnetite magnetosomes have been used to detect single nucleotide polymorphism based on a fluorescence resonance energy transfer (FRET) technique in which double-stranded labeled DNA synthesized by PCR and immobilized to the magnetosomes hybridizes to target DNA and a fluorescence signal is detected (Maruyama et al. 2004; Nakayama et al. 2003; Ota et al. 2003; Tanaka et al. 2003; Yoshino et al. 2003).

Magnetosomes can be used to detect biomolecular interactions in medical and diagnostic analyses. Biotin and other molecules attached to a monolayer-modified substrate can be detected by streptavidin immobilized to magnetosomes using magnetic force microscopy (Arakaki et al. 2004). For example, streptavidin-modified magnetosomes have been used for the immobilization of biotin-modified antibodies (Amemiya et al. 2005). Other biomedical applications include the use of magnetosomes in drug delivery after attachment of the drug in question (Matsunaga et al. 1997).

Protein displays have been designed using specific magnetosome membrane proteins as anchor molecules for the assembly of foreign proteins on the surface of magnetite magnetosomes. These proteins included MagA, MpsA, Mms16, and Mms13 (same as MamC and Mam12) (Arakaki et al. 2003; Matsunaga and Takeyama 1998; Matsunaga et al. 1999, 2000b, 2002; Nakamura et al. 1995a, b; Okamura et al. 2001; Yoshino and Matsunaga 2005, 2006) that were fused to the chemiluminescent protein luciferase (Matsunaga et al. 2000a, 2002; Yoshino and Matsunaga 2006) to determine the stability of the anchor proteins. The most stable anchor protein among those tested was Mms13 (MamC, Mam12), based on the fact that this fusion resulted in 400–1,000 times the luminescence activity observed for Mms16 or MagA fusions (Yoshino and Matsunaga 2006). The MamC protein has been used as an anchor for a paraoxonase to the surface of magnetosomes causing them to have phosphohydrolase activity effective in the degradation of ethyl-paraoxon, an organophosphate pesticide (Ginet et al. 2011). The result was the production of functionalized magnetic nanoparticles efficient as a reusable nanobiocatalyst for pesticide bioremediation in contaminated effluents (Ginet et al. 2011). Oligomeric proteins have also been expressed by genetic fusion to the MamC protein in *Magnetospirillum gryphiswaldense* (Ohuchi and Schüler 2009).

Magnetosomes have been shown to be useful in the isolation of nucleic acids. Magnetosomes have been modified using compounds such as hyperbranched polyamidoamine dendrimers or amino silanes for the extraction of DNA (Yoza et al. 2002, 2003a, b). An efficient means of isolating mRNA using oligo(dT)-modified magnetosomes has also been described (Sode et al. 1993).

Chemically produced magnetic nanocrystals have been shown to be useful in a number of medical applications. The specificity, affinity, and binding capacity of magnetic nanoparticles depend on their size, form, dispersion, and surface chemistry. When conjugated to antibodies, they have been shown to enhance magnetic resonance imaging (MRI) sensitivity for detection of cancer markers compared with other types of probes currently available (Lee et al. 2007) and of acute brain inflammation in multiple sclerosis (McAteer et al. 2007). Functional antibody fragments ("nanobodies") have also been coupled in vivo by genetic fusion to magnetosomes (Pollithy et al. 2011). These types of conjugated magnetic nanoparticles can also be linked to genes or drugs and could be used as carriers of these molecules for targeted therapy of tumors (Chertok et al. 2007; Zhao et al. 2010). Magnetosomes may prove to be useful and perhaps superior to chemically produced magnetite nanoparticles in the same types of applications. Initial studies suggest that purified, sterilized magnetosomes from magnetotactic bacteria are nontoxic for mouse fibroblasts in vitro (Xiang et al. 2007) and may be useful as carriers of genes or drugs for cancer therapy or other diseases (Sun et al. 2007).

Another interesting characteristic of magnetic nanoparticles is that heat is produced when they are in the presence of an oscillating (alternating) magnetic field (Hergt et al. 2002; Duguet et al. 2006; Dutz et al. 2005, 2007; Glöckl et al. 2006) presumably as a result of hysteresis losses (Hergt et al. 1998). This feature has led to the idea that magnetic nanoparticles might be helpful in the destruction or elimination of tumors through hyperthermia or thermoablation (Hilger et al. 2001, 2005; Ito et al. 2006; Ciofani et al. 2009). Bacterial magnetite magnetosomes have also been shown to possess this characteristic (Hergt et al. 2005, 2006) even when the magnetite has oxidized to maghemite (Alphandéry et al. 2011, b).

Geological Significance of Magnetotactic Bacteria and Magnetosomes

The discovery of magnetotactic bacteria has had great impacts on geology and paleomagnetism (Bazylinski and Moskowitz 1997). When magnetite-producing magnetotactic bacterial die and lyse, their magnetosome crystals are released into the surrounding environment where they can persist or undergo dissolution (e.g., through iron(III) reduction) and/or transformation into other minerals (e.g., iron sulfides) (Vali and Kirschvink 1989; Bazylinski and Moskowitz 1997). The small size of magnetosome magnetite crystals makes them particularly susceptible to relatively rapid dissolution when exposed to reducing environments because of their large surface to volume ratio (Abrajevitch and Kodama 2011). In a system where sediment accumulation and reductive diagenesis are coupled, magnetosome magnetite crystals are not likely to be preserved (Vali and Kirschvink 1989; Snowball 1994; Abrajevitch and Kodama 2011). However, in habitats where the crystals persist for some time, magnetotactic bacterial magnetite has been shown to be an important, sometimes the primary, carrier of magnetic remanence in some oceanic and lake sediments (Snowball 1994; Oldfield and Wu 2000; Snowball et al. 2002; Kim et al. 2005). This fine-grained magnetic material records the

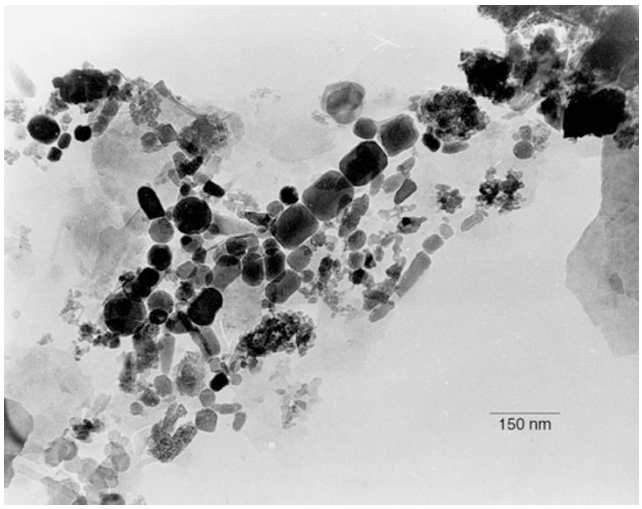


Fig. 12.23
Brightfield TEM image of a magnetic separate from surface sediments collected from the Irish Sea. Note the presence of elongated-prismatic, cuboctahedral, and elongated-anisotropic (*tooth-shaped*) crystals of magnetite, presumed "magnetofossils" *left* from magnetotactic bacteria (Figure courtesy of Z. Gibbs)

Earth's magnetic field at the time it was deposited. Using isotopic dating and other technologies, investigators can determine approximately when sediments were deposited and track changes in the magnetic field, which in turn provides information about the origin of the geomagnetic field and properties of the deep Earth, history of plate motions and magnetic reversals, and even magnetic proxy records of paleoenvironments and paleoclimate (Verosub and Roberts 1995; Evans and Heller 2003). Very little is known regarding the deposition and significance of greigite in sediments although magnetotactic bacterial greigite appears to have been recovered from such habitats (Demitrack 1985; Snowball and Thompson 1988; Snowball 1991).

Magnetite magnetosome crystals have also had a major impact on astrobiology. Magnetite crystals morphologically similar to those present in some magnetosomes, specifically the elongated-prismatic and elongated-anisotropic forms, of magnetotactic bacteria living in the present have been found in ancient and modern sediments (*Fig. 12.23*) (Chang and Kirschvink 1989; Chang et al. 1989) and in the martian meteorite ALH84001 (Thomas-Keprta et al. 2000, 2001, 2002; Clemett et al. 2002). These crystals, referred to as "magnetofossils," have been used as evidence for the past presence of magnetotactic bacteria in sediments and in meteorite ALH84001. The presence and interpretation of these crystals in martian meteorite ALH84001, in particular, have instigated great controversy and debate. If the magnetite crystals were indeed biogenic, the implication was that bacterial life had existed on ancient Mars (McKay et al. 1996; Thomas-Keprta et al. 2000, 2001, 2002; Buseck et al. 2001; Clemett et al. 2002; Weiss et al. 2004). In turn, this debate has led to a number of criteria to be used to distinguish biogenic magnetite from inorganically produced magnetite (Thomas-Keprta et al. 2000; Arató et al. 2005; Kopp and Kirschvink 2008; Jimenez-Lopez et al. 2010; Gehring et al. 2011; Kind et al. 2011). In addition, nanosized greigite crystals may be used as a biomarker but biogenically produced greigite must also be distinguishable from that produced inorganically (Pósfai et al. 2001). The debate over these and other putative microbial fossils illustrates the need for and the ability to recognize and reliably distinguish abiotic formations versus authentic prokaryotic fossils (Bazylinski and Frankel 2003; Jimenez-Lopez et al. 2010).

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