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CHAPTER 1.26

Magnetotactic Bacteria

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Introduction

Magnetotactic bacteria are Gram-negative, motile prokaryotes that synthesize intracellular crystals of magnetic iron oxide or iron sulfide minerals. These apparently membrane-bounded crystals are called magnetosomes (Balkwill et al., 1980) and cause the bacteria to orient and migrate along geomagnetic field lines. Magnetotactic bacteria are indigenous in sediments or stratified water columns where they occur predominantly at the oxic-anoxic transition zone (OATZ) and the anoxic regions of the habitat or both. They represent a diverse group of microorganisms with respect to morphology, physiology and phylogeny. Despite the efforts of a number of different research groups, only a few representatives of this group of bacteria have been isolated in axenic culture since their discovery by (Richard P. Blakemore, 1975), 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 and can be grown relatively easily in mass culture. Therefore, most of the knowledge about the metabolism and biochemistry of magnetotactic bacteria relies on results obtained with strains of this genus.

Ecology

Magnetotactic bacteria are ubiquitous and common in sediments of freshwater or marine habitats, but also in stratified water columns (Bazylinski et al., 1995) and wet soils (Fassbinder et al., 1990). The occurrence of magnetotactic bacteria appears to be dependent on the presence of opposing gradients of reduced and oxidized compounds, usually represented by reduced sulfur species and oxygen, in the sediments or water columns. It has been shown in some cases that the distribution and abundance of these bacteria in the environment might also be dependent on the availability of soluble iron (Stolz et al., 1986). The highest numbers of magnetotactic bacteria are observed at the OATZ of sediments or stratified water columns. Magnetotactic bacteria can therefore be considered as typical examples of gradient organisms. In one study (Fig. 1), the number of a morphologically-distinct magnetotactic bacterium, "*Magnetobacterium bavaricum*," at the OATZ in a freshwater sediment was determined to be up to 7×10^5 live cells per cm^3 (Spring et al., 1993).

"*Magnetobacterium bavaricum*" is very large (average volume ca. 25.8 ± 4.1 μm^3) and could account for approximately 30% of the microbial biovolume in this layer of the sediment. In some environments, magnetotactic bacteria, particularly those that produce iron sulfide minerals, can also be detected in the anoxic region of the habitat, but are only rarely found at sites or regions of water columns or sediments exposed to high levels of oxygen.

The detection of magnetotactic bacteria in environmental samples is relatively easy due to their permanent magnetic dipole moment. One simple method is to put a drop of water or sediment on a microscope slide and place a bar magnet 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 a drop of water and/or sediment where they can be visualized. Alternatively, it is possible to 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 sometimes grayish to white (if the cells contain elemental sulfur globules) 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. This material can easily be removed from the jar with a Pasteur pipette and

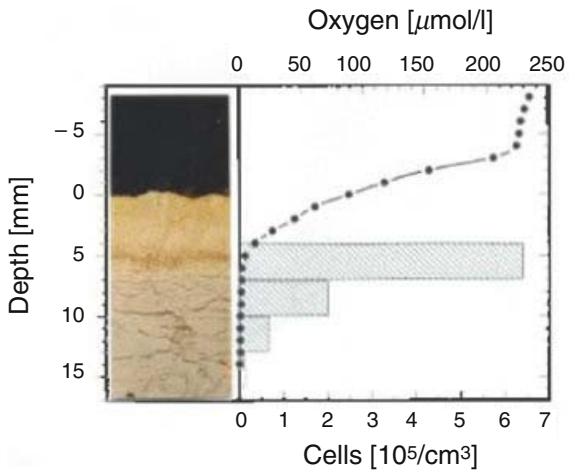

Fig. 1. Vertical distribution of "*Magnetobacterium bavaricum*" in the stratified sediment of a freshwater lake in Bavaria, Germany (Lake Chiemsee). The analyzed sediment sample was stored in an aquarium for several weeks before the measurements. The crosshatched bars indicate counts for successive depth fractions (3 mm intervals). Oxygen was measured every 1 mm (solid circles). A color photograph of the corresponding section of the aquarium is shown on the left. The upper brownish gray layer followed by a thin reddish brown and then a gray layer is characteristic of Lake Chiemsee sediments. The water-sediment interface corresponds to 0 mm.

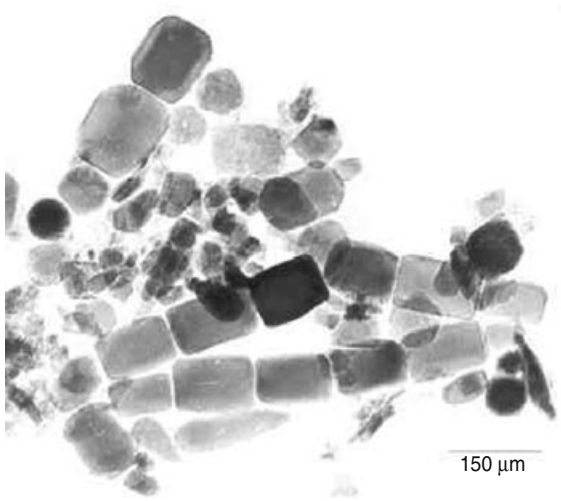

Fig. 2. Brightfield TEM image of a magnetic separate from surface sediments collected from the Irish Sea. Note the presence of parallelepipedal, cubo-octahedral, and tooth-shaped crystals of magnetite, presumed "magnetofossils" left from magnetotactic bacteria. Courtesy of Z. Gibbs.

examined as described above. Magnetotactic bacteria commonly enrich (i.e., increase in numbers) in sediment samples in jars or aquaria stored in dim light at room temperature. The process may take several weeks to months, however. In several cases, successions of different magnetotactic bacterial morphotypes have been observed during the enrichment process. Astonishingly, magnetotactic bacteria sometimes remain active for several years in the aquaria without addition of any nutrients.

When magnetotactic bacteria die and lyse, magnetosome crystals sometimes remain stable in sediments at sites where these bacteria were abundant, resulting in a change of the remanent magnetization of those sediments. Fossil magnetosome crystals consisting of magnetite ("magnetofossils") have been retrieved from many sites including deep sea sediments up to 50 million years old (Petersen et al., 1986) and ancient consolidated sediments up to 2 billion years old (Chang and Kirschvink, 1989a; Chang et al., 1989b). The bacterial origin of particles like those shown in Fig. 2 is assumed based on the unique morphology and size distribution of these magnetite crystals (see Magnetite-type Magnetosomes in this Chapter). Magnetite particles of similar sizes and shapes to those of magnetotactic bacteria have also been discovered within the Martian meteorite ALH84001 where they are thought to be the result of biological processes and therefore have been used as evidence for the presence of life on ancient Mars (McKay et al., 1996).

Nanometer-sized magnetite particles have also been found in soil samples. There the occurrence of magnetite seems to be strongly dependent on environmental conditions favoring the growth of magnetotactic bacteria, a fact which is also of some archaeological interest. It has been speculated that changes in the magnetic susceptibility of top soils, which frequently indicate locations of buried remains of archaeological objects, are caused by magnetotactic bacteria (Fassbinder and Stanjek, 1993). It is thought that the decay of wooden posts or palisades in wet soil leads to localized sites rich in organic material where the growth of magnetotactic bacteria is stimulated, thereby leading to the localized production and accumulation of magnetite. However, it is also possible that the magnetite is produced by iron-reducing bacteria through a biologically-induced mineralization process. In this case, Fe^{2+}, resulting from the bacterial enzymatic reduction of Fe^{3+}, reacts chemically with ferrihydrite to form extracellular magnetite.

Magnetotaxis, Chemotaxis and Aerotaxis

Soon after the discovery of magnetotactic bacteria, a model was proposed to explain the function of the bacterial magnetosome and the biological advantage of magnetotaxis. The original theory,

proposed by Blakemore (1975), was based on the assumption that all magnetotactic bacteria are microaerophilic and indigenous in sediments. Richard B. Frankel and co-workers clearly showed that these bacteria passively align and actively swim along the inclined geomagnetic field lines as a result of their magnetic dipole moment. Blakemore called this behavior magnetotaxis and proposed that magnetotaxis helps to guide the cells down to less oxygenated regions of aquatic habitats at the surface of sediments. Once cells have 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. By this mechanism, a conventional aerotactic response which is a three dimensional search problem could be reduced to an one dimensional search problem in which cells only swim downward, thereby increasing the efficiency of the organism in finding an optimal oxygen concentration in the sediments. This theory is supported by the predominant occurrence of magnetotactic bacteria that are North-seeking (i.e., swim in the direction indicated by the North-seeking pole of a magnetic compass needle) under oxic conditions in the Northern hemisphere whereas bacteria are predominately South-seeking in the Southern hemisphere. Due to the negative and positive sign of the geomagnetic field inclination in the Northern and Southern hemispheres, respectively, magnetotactic bacteria in both hemispheres therefore swim downward toward the sediments (Blakemore, 1982).

Recent findings, including the discovery of large populations of magnetotactic bacteria at the OATZ of chemically stratified aquatic habitats and the isolation of obligately microaerophilic, coccoid magnetotactic bacterial strains, make it necessary to rethink this view of magnetotaxis. The traditional model does not completely explain how bacteria in the anoxic zone of a water column benefit from magnetotaxis, nor does it explain how the 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 (with pure cultures of magnetite-producing magnetotactic bacteria) that magnetotaxis and aerotaxis work together in these bacteria (Frankel et al., 1997). The behavior observed in these strains has been referred to as "magneto-aerotaxis," which is a more accurate description than magnetotaxis because these cells do not try to reach a distinct magnetic pole or field as the term magnetotaxis implies. Thus, the term magnetotaxis is a misnomer.

The traditional model also fails to explain the various types of magnetotactic behavior which had been observed by several authors 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 using thin, flattened capillaries (Frankel et al., 1997), it became clear that two types of mechanisms have been observed, which apparently occur in different bacteria, termed polar and axial.

The distinction can be seen by examination of cells in wet mounts under oxic conditions using a microscope and a magnet of a few gauss parallel to the plane of the slide (Fig. 3). Polar magnetotactic bacteria, particularly the magnetotactic cocci, swim persistently along the 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. Bacteria from Northern hemisphere habitats 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 discovery of magnetotactic bacteria by Blakemore (1975). On the other hand, axial magnetotactic bacteria, especially the freshwater spirilla, orient and swim in both directions along the magnetic field lines with frequent reversals of swimming direction and some accumulating in approximately equal numbers on both sides of the water drop (Fig. 3a).

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 in Fig. 3b) and the other sealed, a single oxygen 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 *M. magnetotacticum* also rotate 180° in these capillaries but the band of cells does not separate and remains intact (Fig. 3). In the second situation (not shown), where both ends of the capillary

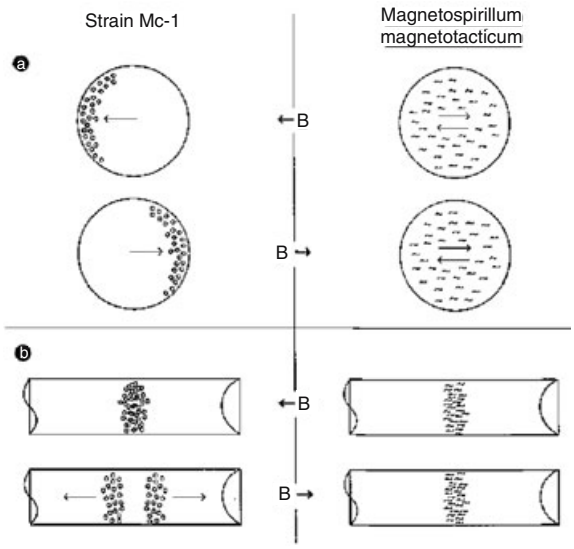

Fig. 3. Two types of magnetotaxis. (a) Depictions of the polar magnetotactic behavior of strain MC-1 and axial magnetotactic behavior of *Magnetospirillum magnetotacticum* in water drops under oxic conditions on a microscope slide (B, magnetic field; arrow points northward). Cells of strain MC-1 swim 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 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).

tubes are open, diffusion of oxygen into the ends of the tubes creates an oxygen 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 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.

Axial Magneto-Aerotaxis

Almost all magnetotactic spirilla available in axenic culture and grown in liquid media, exhibit axial magneto-aerotaxis (Figs. 3 and 4). Other bacteria that show axial magnetotaxis are microaerophilic or anaerobic chemoheterotrophs, or facultative chemolithoautotrophic bacteria that are either monopolarly or bipolarly flagellated. In most habitats, axial magnetotactic bacteria appear to represent only a very small fraction of the total count of magnetotactic bacteria, although these organisms are harder to detect in wet mounts 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. 4). In the presence of an oxygen 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 find the microoxic or suboxic zone in its habitat. Therefore the term magneto-aerotaxis is also an appropriate descriptive term for this tactic behavior.

Fig. 4. Sequence showing magnetotactic spirilla displaying axial magnetotaxis. For the video, see the online version of *The Prokaryotes*.

Polar Magneto-Aerotaxis

The large majority of naturally-occurring magnetotactic bacteria display polar magnetotaxis (Figs. 3 and 5). The following mechanism for polar magnetotaxis was proposed based on experimental data obtained with an axenic culture of a marine magnetotactic coccus. It was demonstrated that these cocci can swim in both directions along a static magnetic field, B, without the need of 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. 5), i.e., 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).

Fig. 5. Sequence showing magnetotactic cocci displaying polar magnetotaxis. For the video, see the online version of *The Prokaryotes*.

Revised Model of Magnetotaxis

Based on these observations, we would like to extend the current model of a magnetically-guided aerotaxis (magneto-aerotaxis) to a more complex redoxtaxis. 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. One hint for the possible function of polar magnetotaxis could be that most of the representative microorganisms are characterized by possessing either large sulfur inclusions or magnetosomes consisting of iron-sulfides. Therefore, it may be speculated that the metabolism of these bacteria, being either chemolithoautotrophic or mixotrophic, is strongly dependent on the uptake of reduced sulfur compounds which occurs in many habitats only in deeper regions at or below the OATZ 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. Microorganisms belonging to the genus *Thioploca*, for example, use nitrate, which is stored 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. It is assumed that *Thioploca* uses 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 to perform excursions to anoxic zones of their habitat in order to accumulate reduced sulfur compounds. In our model, shown in Fig. 6, we propose that polar magnetotaxis helps 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 assumed 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. Finally, they would reach a "reduced state." According to our model, cells in this redox state would have accumulated a large amount of sulfur which cannot be efficiently oxidized under anaerobic conditions leading to a surplus of reduction equivalents. Therefore, cells must return to the microoxic zone where oxygen is available to them as an electron acceptor. In addition, oxygen may be necessary for the synthesis of magnetosomes in some bacteria (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 the waste of energy by constant movement 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 visible under the microscope. The observation of significant numbers of microaerophilic, magnetotactic bacteria in the anoxic zone of some sediments and the attachment of "*Magnetobacterium bavaricum*" to sediment particles in

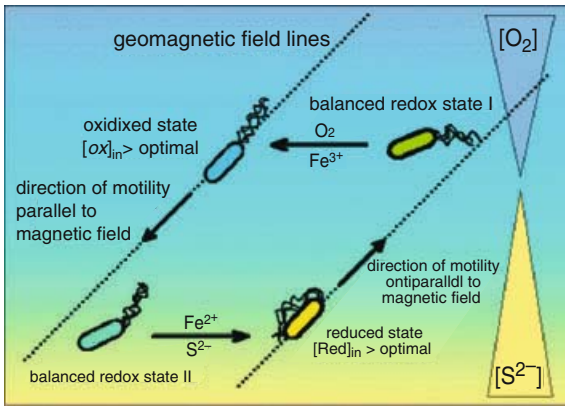

Fig. 6. Hypothetical model of the function of polar magnetotaxis in bacteria (Northern hemisphere). Cells are guided along the geomagnetic field lines depending on their "redox-state" either downward to the sulfide-rich zone or upward to the microoxic zone, thereby enabling a shuttling between different redox layers.

microcolony-like aggregates, fits well with this model, which is summarized in the following Fig. 6.

Morphologic and Phylogenetic Diversity

Morphotypes

The diversity of magnetotactic bacteria is reflected by the high number of different morphotypes found in environmental samples of water or sediment. Commonly observed morphotypes include coccoid to ovoid cells, rods, vibrios and spirilla of various dimensions. One of the more unique morphotypes is an apparently multicellular bacterium referred to as the MMP many-celled magnetotactic prokaryote.

Regardless of their morphology, all magnetotactic bacteria studied so far are motile by means of flagella and have a cell wall structure characteristic of Gram-negative bacteria. 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 inside the bacterial cell. In the majority of magnetotactic bacteria, the magnetosomes are aligned in chains of various lengths and numbers along the cell's 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. Besides magnetosomes, large inclusion bodies containing elemental sulfur, polyphosphate, or poly-β-hydroxybutyrate are common in magnetotactic bacteria collected from the natural environment and in pure culture.

The most abundant type of magnetotactic bacteria occurring in environmental samples, especially sediments, are coccoid cells possessing two flagellar bundles on one somewhat flattened side. This bilophotrichous type of flagellation gave rise to the tentative genus "*Bilophococcus*" for these bacteria (Moench, 1988). One representative strain of this morphotype is in axenic culture (see Other Magnetotactic Strains in Pure Culture in this Chapter). In contrast, two of the morphologically more conspicuous magnetotactic bacteria, regularly observed in natural samples but never isolated in pure culture, are the MMP and a large rod containing large numbers of hook-shaped magnetosomes ("*Candidatus Magnetobacterium bavaricum*").

The MMP, a Many-Celled Magnetotactic Prokaryote A magnetotactic aggregation of cells that swims as an entire unit and not as separate cells was first reported and described by Farina et al. (1983). Similar morphotypes were later found also in sulfide-rich marine and brackish waters and in sediments along the coasts of North America and Europe (Mann et al., 1990a). The MMP (for many-celled magnetotactic prokaryote) consists of about 10 to 30 coccoid to ovoid Gram-negative cells, roughly arranged in a sphere with a diameter ranging from approximately 3 to 12 μm (Fig. 7).

Cells are asymmetrically multiflagellated on their outer surfaces exposed to the external surroundings. Magnetosomes consist of the magnetic iron-sulfide greigite, Fe_3S_4, and several nonmagnetic precursors to greigite (see "Iron-Sulfide Type Magnetosomes"). The magnetosome crystals are generally pleomorphic although cubo-octahedral, rectangular prismatic, and tooth-shaped particles have also been observed in cells. They are usually loosely arranged in short chains or clusters in individual cells. The total magnetic moment of the MMP was determined and ranges from 5×10^{-16} to 1×10^{-15} Am^2, which is sufficient for an effective magnetotactic response. The type of magnetotaxis displayed by the MMP appears to be polar, but aggregates have been observed to reverse direction. Under oxic conditions in a uniform magnetic field, the swimming speed in the preferred direction averages 105 μm/s. After reaching the edge of a water drop, aggregates sometimes spontaneously reverse their swimming direction and show short excursions of 100 to 500 μm with twice the speed of the forward motion in the opposite direction of their polarity (Rodgers et al., 1990) as shown in Fig. 8. This so

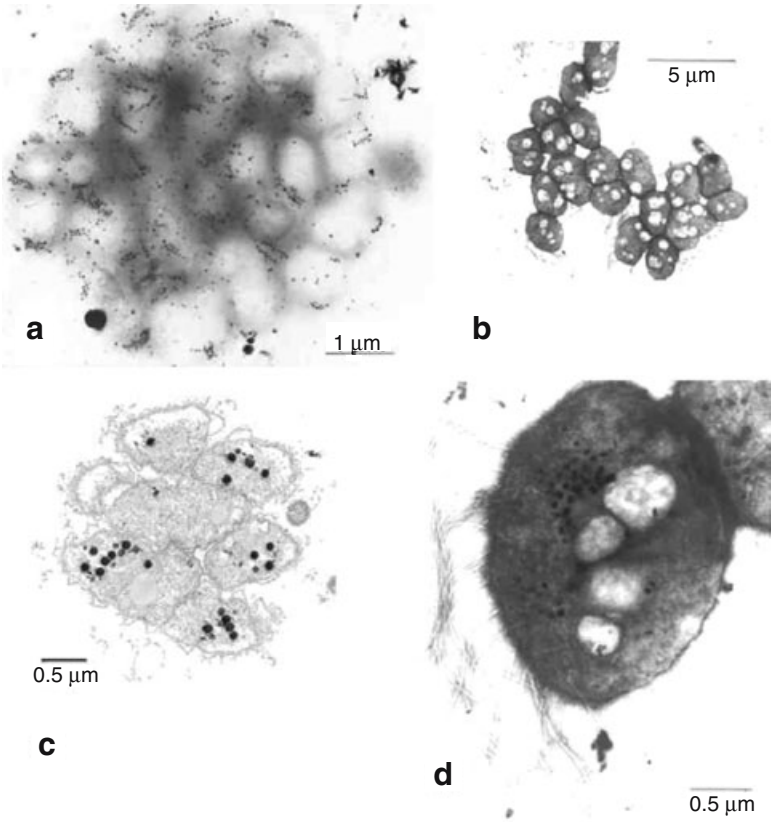

Fig. 7. Brightfield TEM micrographs of the many-celled magnetotactic prokaryote (MMP). (a) An unstained, single MMP revealing the numerous greigite-containing magnetosomes within the organism mostly arranged in short chains. (b) Negatively-stained preparation (2.5% ammonium molybdate, pH 7.0) of a single MMP that is disrupted to reveal separated individual cells. (c) Thin-section of an MMP again showing its many-celled nature. (d) Negatively-stained individual cell of the MMP. Note the asymmetric distribution of flagella which cover the cell on one side, the pleomorphism of the greigite-containing magnetosomes, and the electron-lucent vacuoles resembling poly-β-hydroxybutyrate (PHB) granules.

called "ping pong" motion seems to be a peculiarity of this organism.

Fig. 8. Sequence showing the typical "ping-pong" motility of the MMP. For the video, see the online version of *The Prokaryotes*.

In one study, it was reported that individual cells within the aggregate are connected by intercellular membrane junctions (Rodgers et al., 1990a; 1990b). However, the cohesive force among individual cells seems to be relatively weak because a lowering of the osmotic pressure leads to an immediate disruption of the aggregate into single nonmotile cells.

"*Candidatus Magnetobacterium bavaricum*" The first phenotypic description of this morphotype by Vali et al. (1987) was based on cells collected from material retrieved from the littoral sediments of a large freshwater lake in Southern Germany (Lake Chiemsee). Later, similar bacteria were also found in sediments of other freshwater habitats in Germany and Brazil. After the determination of its phylogenetic relationship (Spring et al., 1993), this organism was given the candidatus status due to its unusual phenotypic traits which distinguish it from all other magnetotactic bacteria. "*Magnetobacterium bavaricum*" displays polar magnetotaxis and is preferentially found in the microoxic zone of sediments, although significant numbers are also found in anoxic regions of their habitat (Fig. 1). In situ hybridizations using a specific fluorescently-labeled oligonucleotide probe targeting the 16S rRNA of this organism enabled the detection of microcolonies of this bacterium on microscope slides immersed into sediment for several weeks (Fig. 9). Thus, there appears to be a tendency for "*Magnetobacterium bavaricum*" to adhere to particles located in microsites with preferred environmental conditions.

Cells of "*M. bavaricum*" are large rods having dimensions of 1–1.5 x 6–9 µm and are motile by a polar tuft of flagella. The most impressive trait of this bacterium is the extremely high number of magnetosomes per cell. A single cell may contain up to a thousand hook-shaped magnetosomes usually arranged in 3–5 rope-shaped bundles oriented parallel to the long axis of the cell (Fig. 10).

The magnetosomes consist of magnetite (Fe_3O_4) and have a length of 110–150 nm. The average total magnetic moment per cell was experimentally determined to be approximately 3×10^{-14} Am^2, which is about an order of magnitude higher than that of most other magnetotactic bacteria. The presence of large sulfur inclusions is typical for this bacterium and seems

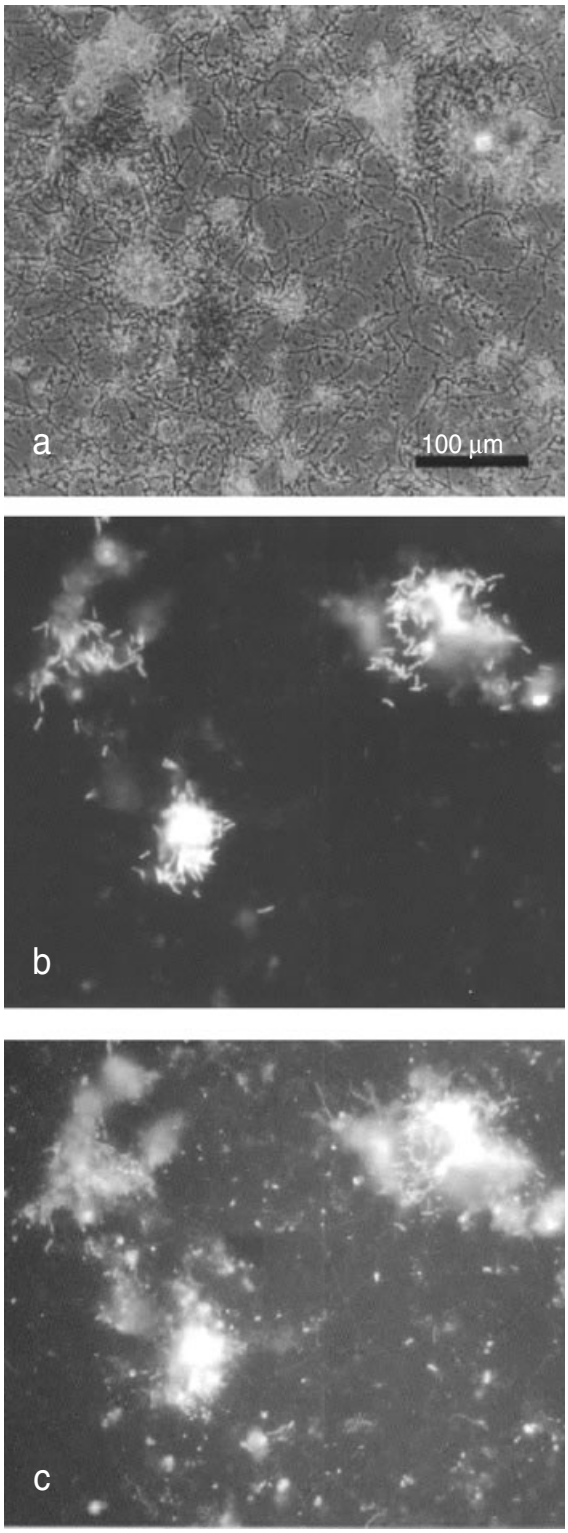

Fig. 9. In situ hybridization of a microscope slide grown over with sediment bacteria using fluorescently labeled oligonucleotide probes. (a) Phase contrast micrograph. (b+c) Same field viewed with epifluorescence microscopy enabling the detection of a specific probe binding to a signature region of the 16S rRNA of "*M. bavaricum*" (b), and of a probe with broad specifity hybridizing with the 16S rRNA of most known bacteria (c).

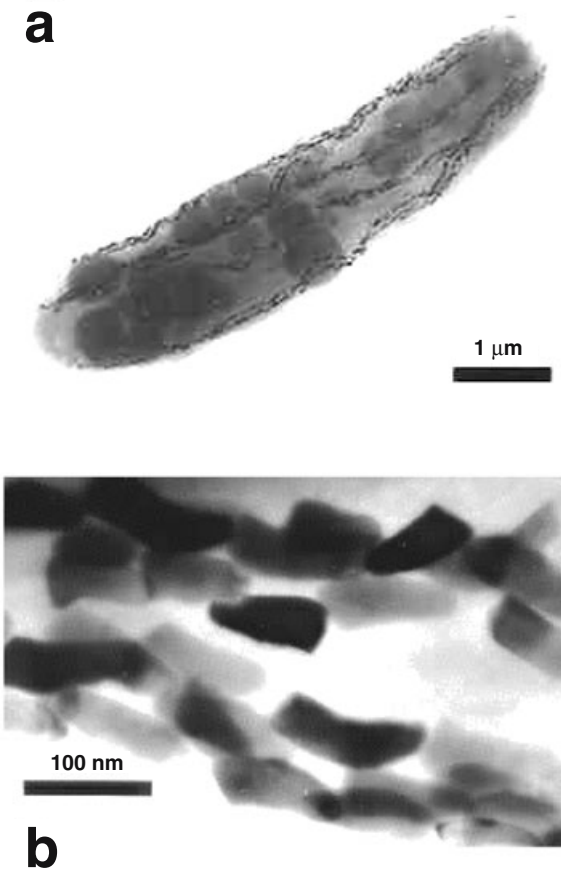

Fig. 10. Brightfield transmission electron microscope (TEM) micrographs of "*M. bavaricum*." (a) Whole cell displaying bundles of magnetosome chains and sulfur globules. (b) Hook-shaped magnetite-type magnetosomes. Courtesy of M. Hanzlik.

to be dependent on environmental conditions. In an unidirectional magnetic field, cells swim forward (i.e., northward in the Northern Hemisphere) with an average speed of 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.

Composition and Structure of Magnetosome Crystals

The magnetosome mineral phase in magnetotactic bacteria are tens-of-nanometer-sized crystals of an iron oxide and/or an iron sulfide. The mineral composition of the magnetosome is specific enough for it to be likely under genetic control, in that cells of several cultured magnetite-producing magnetotactic bacteria still synthesize an iron oxide and not an iron sulfide, even when hydrogen sulfide is present in the growth medium

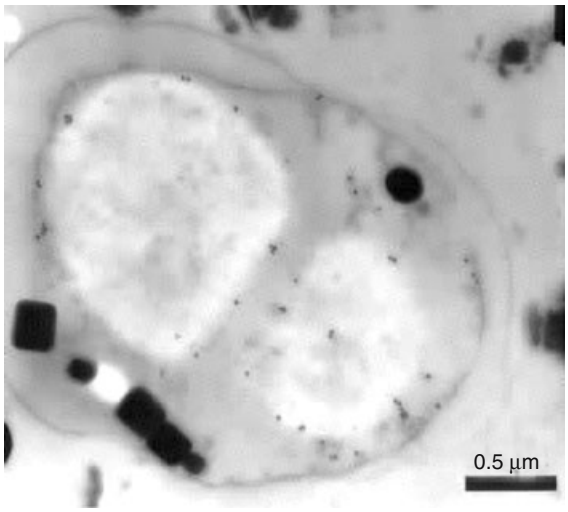

Fig. 11. Unusually large magnetite crystals identified in coccoid magnetotactic bacteria retrieved from a lagoon near Rio de Janeiro, Brazil. Small black dots represent gold-labeled antibodies detecting a specifically bound polynucleotide probe complementary to a highly variable region of the 16S rRNA of these cells.

(Meldrum et al., 1993a; Meldrum et al., 1993b). The size of the magnetosome mineral crystals also appears to be under control of the organism because the large majority of magnetotactic bacteria contain crystals displaying 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-Rizzi and Kirschvink, 1992). Smaller particles would be superparamagnetic at ambient temperature and would not have stable, remanent magnetization. Larger particles would tend to form multiple domains, reducing the remanent magnetization. However, in some uncultured bacteria from the Southern Hemisphere exceptionally large magnetite-magnetosomes have been observed in some uncultured bacteria from the Southern hemisphere (Fig. 11), having dimensions well above the theoretically determined size limits of single domain magnetite (Spring et al., 1998). It remains unclear if the crystals in these bacteria are still of single-domain size or are multi-domain particles and why such unusually large crystals are formed by certain bacteria, but, interestingly, it seems that the crystal-size corresponds with the size and/or the growth phase of these bacteria, i.e., large cells possess larger crystals than smaller cells of the same type.

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).

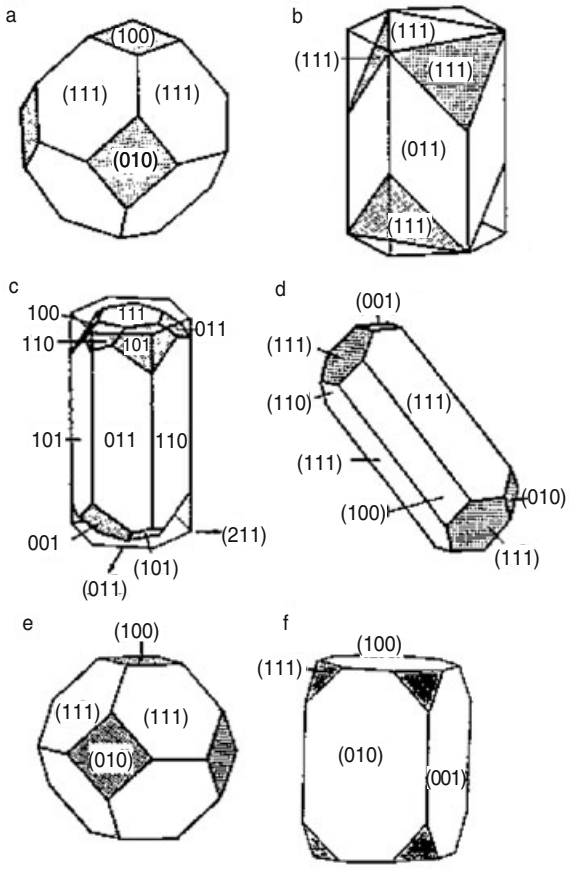

Fig. 12. Idealized magnetite (a–d) and greigite (e–f) crystal morphologies derived from high resolution TEM studies of magnetosome crystals from magnetotactic bacteria: (a) and (e) cubo-octahedrons; (b), (c), and (f) variations of pseudo-hexagonal prisms; (d) elongated cubo-octahedron. Numbers within 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).

The consistent narrow size range (Devouard et al., 1998) and morphologies of the intracellular magnetosome particles represent typical features 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.

MAGNETITE-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 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 (TEM; Blakemore et al., 1989; Mann et al.,

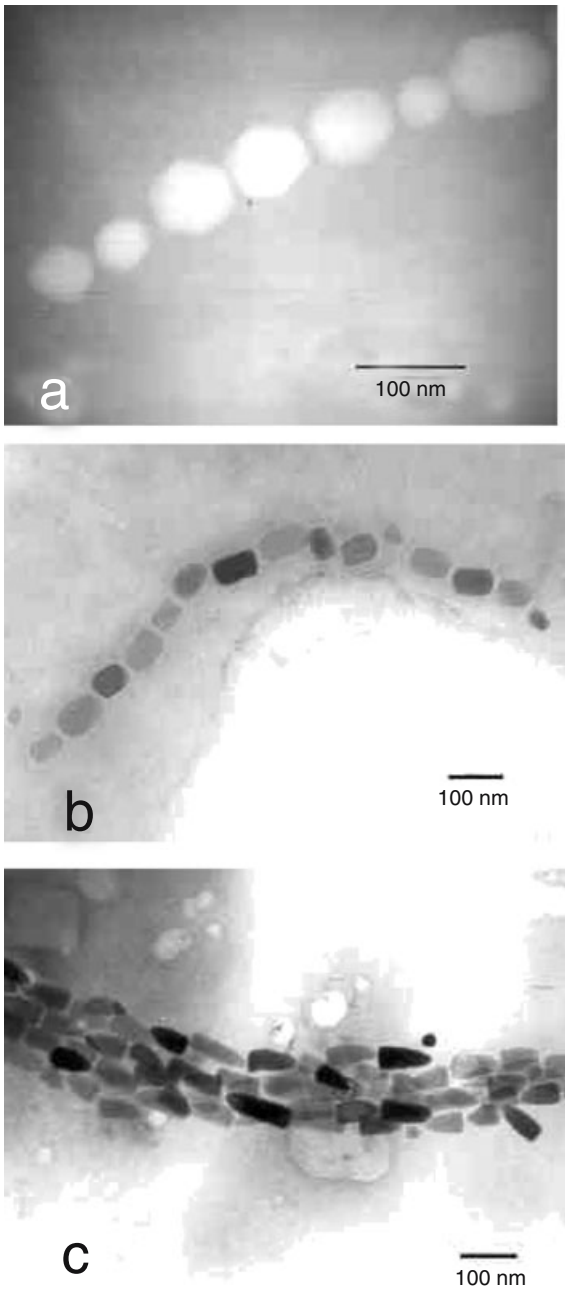

Fig. 13. Morphologies of intracellular magnetite (Fe_3O_4) particles produced by magnetotactic bacteria. (a) Darkfield scanning-transmission electron microscope (STEM) image of a chain of cubo-octahedra in cells of an unidentified rod-shaped bacterium collected from the Pettaquamscutt Estuary, Rhode Island, USA, viewed along a [111] zone axis for which the particle projections appear hexagonal. (b) Brightfield TEM image of a chain of prismatic crystals within a cell of strain MV-2, a marine vibrio, with parallelepipedal projections. (c) Brightfield TEM image of tooth-shaped (anisotropic) magnetosomes from an unidentified rod-shaped bacterium collected from the Pettaquamscutt Estuary.

1990a; Stolz, 1993; Bazylinski et al., 1994). They include: 1) roughly cuboidal (Balkwill et al., 1980; Mann et al., 1984); 2) parallelepipedal (rectangular in the horizontal plane of projection; Moench and Konetzka, 1978; Towe and Moench, 1981; Moench, 1988; Bazylinski et al., 1988); and 3) tooth-, bullet-, or arrowhead-shaped (anisotropic; Mann et al., 1987a; Mann et al., 1987b; Thornhill et al., 1994).

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 morphologies (Matsuda et al., 1983; Mann et al., 1984a; 1984b; 1987a; 1987b; Meldrum et al., 1993a; Meldrum et al., 1993b). These morphologies are all derived from combinations of {111}, {110} and {100} forms (a form refers to the equivalent symmetry related lattice planes of the crystal structure) with suitable distortions (Devouard et al., 1998). The roughly cuboidal particles are cubo-octahedra ([100] + [111]), and the parallelepipedal particles are either pseudohexahedral or pseudooctahedral prisms. Examples are shown in Fig. 12a–d. The cubo-octahedral crystal morphology preserves the symmetry of the face-centered cubic spinel structure, i.e., all equivalent crystal faces develop equally. The pseudohexahedral and pseudo-octahedral prismatic particles represent anisotropic growth in which equivalent faces develop unequally (Mann and Frankel, 1989; Devouard et al., 1998). The synthesis of the tooth-, bullet- and arrowhead-shaped magnetite particles (Figs. 10b, 13c) appears to be more complex than that of the other forms. They have been examined by high resolution TEM in one uncultured organism (Mann et al., 1987a; Mann et al., 1987b) and their idealized morphology suggests that growth of these particles occurs in two stages. The nascent crystals are cubo-octahedra which subsequently elongate along the [111] axis parallel to the chain direction.

Whereas the cubo-octahedral form of magnetite can occur in inorganically-formed magnetites (Palache, 1944), the prevalence of elongated pseudohexahedral or pseudo-octahedral habits in magnetosome crystals imply anisotropic growth conditions, e.g., 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, 1989a; Chang et al., 1989b; Stolz et al., 1986; Stolz et al., 1990; Stolz, 1993).

Iron-Sulfide Type Magnetosomes Virtually all freshwater magnetotactic bacteria have been found to synthesize magnetite as the mineral phase of their magnetosomes. In contrast, many marine, estuarine, and salt marsh species produce iron sulfide-type magnetosomes consisting primarily of the magnetic iron sulfide, greigite, Fe_3S_4 (Heywood et al., 1990; Heywood et al., 1991; Mann et al., 1990b; Pósfai et al., 1998a; 1998b). Reports of non-magnetic iron pyrite (FeS_2; Mann et al., 1990b) and magnetic pyrrhotite (Fe_7S_8; Farina et al., 1990) have not been confirmed and may represent misidentifications of additional iron sulfide species occasionally observed with greigite in cells (Pósfai et al., 1998a; Pósfai et al., 1998b). Currently recognized greigite-producing magnetotactic bacteria includes the MMP (Farina et al., 1983; Rodgers et al., 1990a; 1990b; DeLong et al., 1993) and a variety of relatively large, rod-shaped bacteria (Bazylinski et al., 1990; Bazylinski et al., 1993a; Heywood et al., 1990; Heywood et al., 1991; Bazylinski and Frankel, 1992).

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

The de novo synthesis of non-magnetic 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 would be expected to transform into pyrite (Berner, 1967; Berner, 1970) which has not been unequivocally identified in magnetotactic bacteria. It is not known if and how cells prevent this transformation.

As with magnetite, three particle morphologies of greigite have been observed in magnetotactic bacteria (Fig. 14): 1) cubo-octahedral (the equilibrium form of face-centered cubic greigite) (Heywood et al., 1990; Heywood et al., 1991); 2) pseudo-rectangular prismatic as shown in Fig. 14 and 12e–f (Heywood et al., 1990; Heywood et al.,

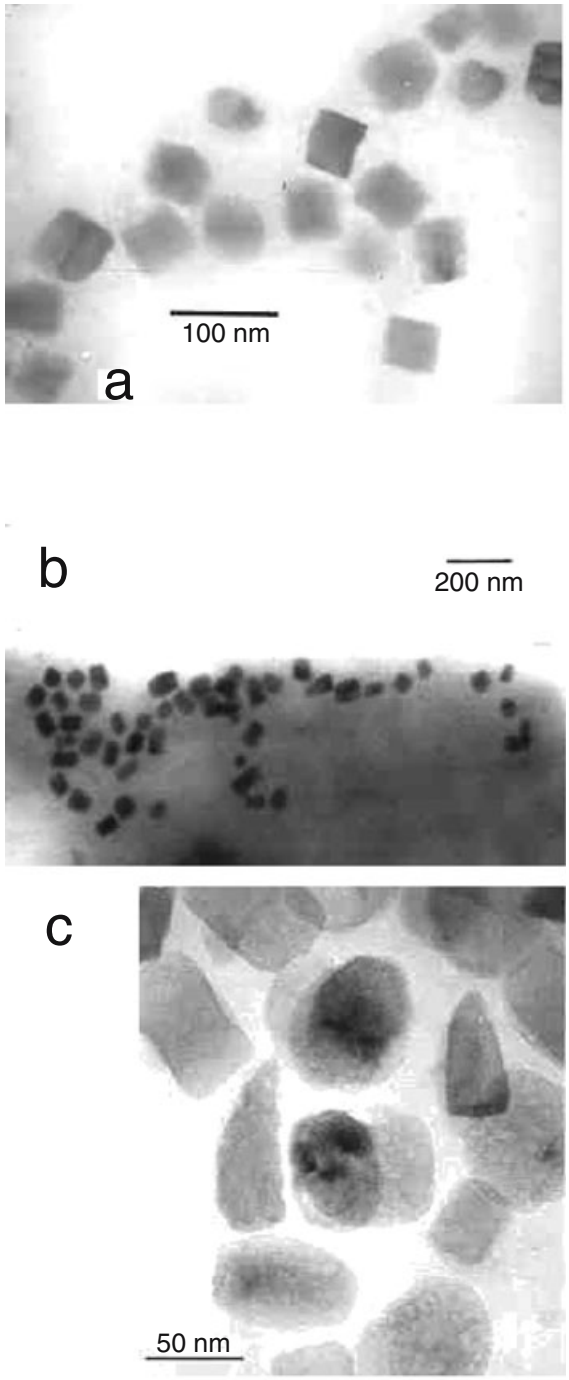

Fig. 14. Morphologies of intracellular greigite (Fe_3S_4) particles produced by magnetotactic bacteria. (a) Brightfield STEM image of cubo-octahedra in an unidentified rod-shaped bacterium collected from the Neponset River estuary, Massachusetts, USA. (b) Brightfield STEM image of rectangular prismatic 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 many-celled magnetotactic prokaryote (MMP), courtesy of M. Pósfai and P. R. Buseck.

1991); and 3) tooth-shaped (Pósfai et al., 1998a; Pósfai et al., 1998b).

Like that of their magnetite counterparts, the morphology of the greigite particles also appears to be species- and/or strain-specific, although confirmation of this observation will require controlled studies of pure cultures of greigite-producing magnetotactic bacteria, none of which is currently available. One clear exception to this rule is the MMP (Farina et al., 1983; Bazylinski et al., 1990; Bazylinski et al., 1993a; Mann et al., 1990b; Rodgers et al., 1990a; 1990b; Bazylinski and Frankel, 1992). This unusual microorganism, found in salt marsh pools all over the world and some deep sea sediments, has been found to contain pleomorphic, pseudorectangular prismatic, tooth-shaped, and cubo-octahedral greigite particles. Some of these particle morphologies are shown in Fig. 7 and 14c. Therefore the biomineralization process(es) appear(s) to be more complicated in this organism than in the rods with greigite-containing magnetosomes or in magnetite-producing, magnetotactic bacteria.

Magnetite and Greigite Crystals in a Single Bacterium One slow-swimming, rod-shaped bacterium, collected from the OATZ from the Pettaquamscutt Estuary, was found to contain arrowhead-shaped crystals of magnetite and rectangular prismatic crystals of greigite co-organized within the same chains of magnetosomes (this organism usually contains two parallel chains of magnetosomes) (Bazylinski et al., 1993b; Bazylinski et al., 1995). In cells of this uncultured organism, the magnetite and greigite crystals occur with different, mineral-specific morphologies and sizes and are positioned with their long axes oriented along the chain direction. Both particle morphologies have been found in organisms with single mineral component chains (Mann et al., 1987a; Mann et al., 1987b; Heywood et al., 1990; Heywood et al., 1991), which suggests that the magnetosome membranes surrounding the magnetite and greigite particles contain different nucleation templates and that there are differences in magnetosome vesicle biosynthesis. Thus, it seems likely that two separate sets of genes control the biomineralization of magnetite and greigite in this organism.

Phylogeny

The phylogeny of many morphotypes of magnetotactic bacteria, including both those in pure culture and those collected from natural environments, has been determined by sequencing their 16S rRNA genes. To date, representatives of the magnetotactic prokaryotes are phylogenetically associated with three major lineages within the Bacteria. Although most are located within the *Proteobacteria*, "*Magnetobacterium bavaricum*" is affiliated with another phylum, the newly designated *Nitrospira* group. Those within the *Proteobacteria* are distributed among the delta- and alpha-subclasses. The uncultured greigite-producing, MMP and the magnetite-producing, sulfate-reducing magnetotactic bacterium RS-1, which is available in pure culture, are located in the delta-subclass, whereas members of the genus *Magnetospirillum* and various vibrios and coccoid magnetotactic bacteria, all of which produce magnetite, belong to the alpha-subclass (Fig. 15). Although these results suggest that the trait of magnetotaxis in bacteria has multiple evolutionary origins (DeLong et al., 1993), it is also possible that the ability of magnetosome

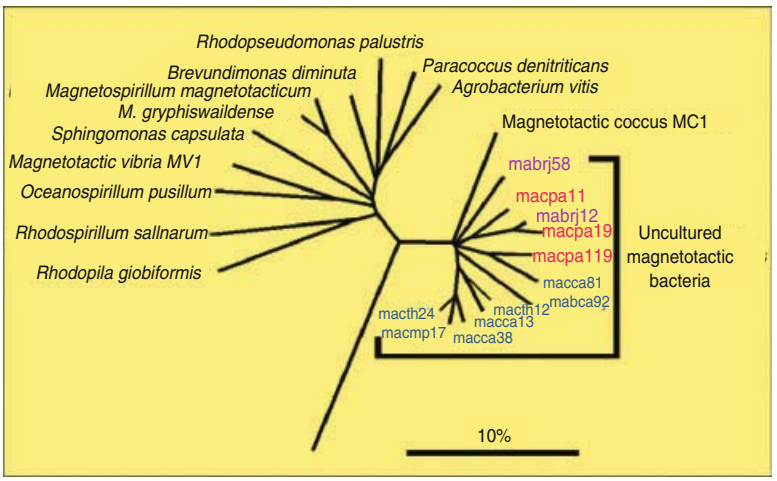

Fig. 15. Phylogenetic tree based on 16S rRNA sequences showing the positions of cultured and uncultured magnetotactic bacteria within the alpha-subclass of *Proteobacteria*. Sequences of uncultured magnetotactic bacteria retrieved from freshwater habitats are in blue, from marine habitats in red, and from a lagoon in pink.

formation was spread among various phylogenetic groups of bacteria and even eukaryotes by lateral gene transfer.

To date, most of the 16S rRNA sequences of magnetotactic bacteria retrieved from environmental samples form a deep-branching group within the alpha-subclass (Fig. 15). This phylogenetic assemblage consists (up to now) exclusively of bacteria displaying magnetotaxis. Similarity values of 16S rRNA sequences within this monophyletic group of magnetotactic bacteria range from 88.0 to 99.3%. Using in situ hybridization with fluorescently-labeled oligonucleotide probes, it was demonstrated that members of this coherent phylogenetic cluster represent the dominant fraction of magnetotactic bacteria in many environments like lagoons, marine and freshwater sediments (Spring et al., 1992; Spring et al., 1994; Spring et al., 1998). Magnetotactic bacterial morphotypes in this group, as evidenced by in situ hybridization, are mainly represented by coccoid to ovoid bacteria, but also include one rod- to vibrio-shaped bacterium (mabcs92; Fig. 15). Despite continuous effort in several laboratories, most members of this group have resisted attempts (in several laboratories) at isolation to axenic culture. One major reason may be their adaptation to and requirement for gradient systems not easily replicated in synthetic growth media. The only exception is the marine magnetotactic coccus strain MC-1, which can be cultivated in a synthetic oxygen gradient medium.

Cultivation and Physiology

The Genus *Magnetospirillum*

Taxonomy Magnetospirilla are found in freshwater habitats where they usually occur in low numbers as, for example, compared with the magnetotactic cocci. These clockwise spirilla have dimensions of 0.2–0.7 by 1–20 μm and display an axial magneto-aerotaxis, at least when grown in liquid culture. The genus *Magnetospirillum* currently comprises the two validly described species, *M. magnetotacticum* and *M. gryphiswaldense*, and several partially characterized strains. *M. magnetotacticum* was the first magnetotactic bacterium isolated and grown in pure culture and was originally assigned to the genus *Aquaspirillum* based on a number of phenotypic characteristics (Maratea and Blakemore, 1981). At that time, this genus contained a large number of phylogenetically diverse, nonphototrophic, freshwater spirilla with the type species *A. serpens* phylogenetically located among the beta-subclass of the *Proteobacteria*. Phylogenetic analyses of *Magnetospirillum* strains later revealed that they all belong to a phylogenetic branch within the alpha-subclass of the *Proteobacteria* and are closely related to phototrophic spirilla of the genus Phaeospirillum (Fig. 15; Burgess et al., 1993; Schüler et al., 1999). Therefore, it was justified to propose the new genus *Magnetospirillum* for these strains (Schleifer et al., 1991). Members of this genus can be distinguished from other freshwater spirilla by their ability to produce membrane-enveloped cubooctahedral magnetite crystals, averaging about 42 nm in diameter (Balkwill et al., 1980; Schleifer et al., 1991), arranged in a single chain within the cell. Other characteristic traits of members of this genus include bipolar monotrichous flagellation and a preference for microoxic growth conditions. Several strains of magnetospirilla can grow also anaerobically with nitrate as terminal electron acceptor or aerobically with atmospheric concentrations of oxygen. Magnetite synthesis appears to only occur under microaerobic conditions in most species while *Magnetospirillum* strain AMB-1 appears to synthesize magnetite under anaerobic conditions as well (Matsunaga and Tsujimura, 1993). Preferred substrates are intermediates of the tricarboxylic acid cycle and acetate. Carbohydrates are not utilized. Catalase and oxidase may be present or not. The guanine-plus-cytosine content of DNA ranges from 64 to 71 mol% (Burgess et al., 1993).

Biochemistry and Molecular Biology of Magnetosome Formation There has been much interest in the elucidation of magnetosome formation because the crystals synthesized by magnetotactic bacteria are of great structural perfection, have consistent particle morphologies and narrow size distributions, possible indications that the particles may have novel magnetic, physical and/or electrical properties. Understanding the factors controlling the biomineralization of iron in magnetosome synthesis within bacteria could also be helpful for the elucidation of similar processes in animals and man or for the artificial synthesis of biominerals. Despite the dedicated and elaborate efforts in studying magnetosome synthesis in bacteria, published results are rather sparse. This is partly due to the lack of a significant number of magnetotactic bacteria strains and the difficulty in culturing them reproducibly in the laboratory, which would be a prerequisite for the establishing of biochemical or genetic model systems. Nevertheless, some interesting results have been obtained using the few available, but fastidious *Magnetospirillum* strains.

In general, the bacterial magnetite synthesis can be divided into three steps. Initially, extracellular iron has to be transported across the cell wall to the inside of the cell. Once within the cell, iron must accumulate in specialized compart-

ments, the magnetosome vesicles. There, the iron presumably precipitates and transforms or grows into a single-magnetic-domain magnetite crystal with a specific morphology. It is assumed that the membrane vesicle is synthesized prior to the precipitation of iron but since there is currently little evidence to support this idea, it is possible that the precipitation of iron and crystal nucleation occurs first and the magnetosome membrane then forms around the growing crystal. The uptake of iron from the surrounding environment by cells of *Magnetospirillum* strains has been analyzed by several groups (Paoletti and Blakemore, 1986; Nakamura et al., 1993b; Schüler and Baeuerlein, 1996; Schüler and Baeuerlein, 1998). Generally, the results suggest that iron is taken up by the cell in the ferric form and transported across the membrane by an energy-dependent reductive process. Iron-binding siderophores were thought to be involved in iron uptake by *M. magnetotacticum* (Paoletti and Blakemore, 1986), which appeared to produce a hydroxamate siderophore under high, but not low, iron conditions. However, this finding was never confirmed by other laboratories. Spent culture fluid stimulates the uptake of ferric iron in *M. gryphiswaldense* although there was no evidence for the production of a siderophore by this species. This stimulation may be due to the production of unknown compounds, produced by cells during growth, which mediate iron uptake by an unrecognized novel mechanism (Schüler and Baeuerlein, 1996). In this respect, it is noteworthy that most magnetotactic bacteria are adapted to microenvironments, like the oxic-anoxic transition zone of sediments, where soluble iron is available to the cell in sufficient quantities for magnetite synthesis (generally about 10–20 μM iron; Blakemore et al., 1979). Thus, magnetotactic bacteria probably have no need for high-affinity transport systems like many other aerobic bacteria growing under iron deficient conditions. This is consistent with experiments performed with cells of *M. gryphiswaldense*. Under iron deficient conditions, cells of *M. gryphiswaldense* do not or cannot distinguish between the use of incorporated iron either as a cofactor for cellular proteins or for magnetite synthesis and store this essential element as an inorganic mineral, magnetite, at the expense of their own growth (Schüler and Baeuerlein, 1996). The marine magnetotactic vibrio, strain MV-1, behaves similarly.

The fate of iron taken up by cells was studied by Frankel et al. (1983) in *M. magnetotacticum* using Fe^{57} Moessbauer spectroscopy. It was proposed that the ferrous iron taken up by cells is immediately reoxidized to form a low-density hydrous Fe(III) oxide. It is not yet clear if this step takes place in the cytoplasm or in the magnetosome vesicles. How iron is transported from the cell membrane into the magnetosome vesicle is also not known. Iron is precipitated within the magnetosome vesicle presumably through a dehydration step as ferrihydrite (a high-density Fe(III) hydroxide). Finally, magnetite (Fe_3O_4) is produced by the reduction of one-third of the Fe(III) ions in ferrihydrite and further dehydration steps. The crystallization process(es) involved in magnetite formation is apparently linked closely to the magnetosome membrane and may be controlled by specific proteins present in this membrane. The chemical transformation of amorphous Fe(III) precursors to crystalline magnetite is sensitive to environmental conditions like ion concentration, pH and redox potential (Mann et al., 1990c) which have to be therefore precisely regulated by the magnetosome membrane or by conditions within the magnetosome membrane vesicle. Growth of the magnetite crystal, i.e., its orientation, shape and size, must also be under strict control because these characteristics are specific for one strain and/or species of bacteria and to a great extent independent from the growth conditions (Bazylinski et al., 1994).

Because the magnetosome membrane seems to play a key role in the synthesis of magnetite crystals, its structure and composition has been analyzed in several studies. By analyzing the magnetosome membrane in this way, some clues relating to how magnetite biomineralization occurs within the cell may be found. Gorby et al. (1988) showed that the magnetosome membrane in *M. magnetotacticum* has an architecture similar to that of the cytoplasmic membrane and consists of a lipid bilayer and numerous proteins, some of which appear to be unique to the magnetosome membrane. Okuda et al. (1996) found three proteins with molecular weights of 12, 22 and 28 kDa, specifically associated with the magnetosome membrane in *M. magnetotacticum*. They successfully identified and sequenced the gene encoding for the 22 kDa protein, which was found to belong to a family of protein import receptors common in mitochondria and peroxisomes. The role of this protein in magnetosome synthesis remains unclear however. A gene likely involved in magnetite synthesis was identified and characterized by Matsunaga et al. (1992). They used a genetic approach using the microorganism *Magnetospirillum* strain AMB-1, which forms colonies of magnetite-forming cells on agar surfaces, thereby facilitating the screening for non-magnetic mutants. The gene, designated *magA*, encodes for a membrane protein showing sequence similarities to some cation efflux proteins. Based on experiments with the recombinant protein, it was proposed that the MagA

protein plays a role in the energy-dependent transport of iron across membranes.

ENRICHMENT AND ISOLATION Magnetotactic spirilla have been repeatedly isolated from various freshwater habitats, so that it is possible to give some guidelines for their succesful enrichment and isolation.

Several morphotypes of magnetotactic bacteria can be enriched in the laboratory by putting mud and overlying water from a sampling site into aquaria or jars, which are loosely covered and stored in dim light. After several days to weeks, the number of magnetotactic bacterial cells generally increases significantly. Magnetotactic spirilla, however, are in most cases not among the dominating morphotypes and therefore are only rarely detected using light microscopy. Consequently, the usefulness of this method for the enrichment of representatives of the genus *Magnetospirillum* remains questionable. To date, no selective growth media are known for the cultivation of magnetotactic spirilla, so that a successful isolation procedure will in most cases depend on the purity of the inoculum. Because of the magnetic dipole moment of these bacteria, physical separation from nonmagnetotactic contaminants is possible. A commonly used method for the separation of magnetotactic bacteria from sediment samples was described by Moench and Konetzka (1978). They concentrated magnetotactic bacterial cells using a bar magnet (e.g., stirring bar) fixed to the outer wall of a jar filled with sediment and water. Directed magnetotactic bacteria eventually accumulate at the side of the jar and become concentrated enough to form (opposite to the magnet) a brownish spot from where they can be transferred into a sterile cap using a Pasteur pipette. The sample containing the concentrated magnetotactic cells still cotains non-magnetotactic bacteria, so that a further purification step is advisable before it is used as an inoculum for growth media. The "capillary racetrack" devised by Spormann and Wolfe (Wolfe et al., 1987) has been successfully used for this purpose (Schüler et al., 1999).

All isolated strains of magnetospirilla, with the possible exception of *Magnetospirillum* strain AMB-1, appear to prefer low oxygen tensions for growth and magnetite synthesis. Thus the creation and maintenance of microoxic conditions in growth media is especially important for the isolation of these organisms starting from small inocula. The growth medium should contain 10–20% of sterilized mud or water from the respective habitat and low concentrations of agar to allow the establishment of a semisolid oxygen gradient. Suitable carbon sources are intermediates of the tricarboxylic acid cycle, e.g., malate or succinate. An oxygen-sulfide gradient medium was successfully used by Schüler et al. (1999) for the effective isolation of magnetotactic spirilla from a freshwater pond. Screw-capped culture tubes are filled with 1 ml of solid sulfide agar (4 mM Na_2S, 1.5% agar, pH 7.4) and overlaid with 10 ml of slush-agar. The slush-agar consists of (per 800 ml deionized water): 200 ml of filtered pond water, 1 ml of vitamin elixir, 2 ml of mineral elixir (Wolin et al., 1963), 0.05 g, sodium succinate, 0.05 g, yeast extract, 0.05 g, NH_4Cl, 0.05 g, $MgSO_4$, 0.5 mM potassium phosphate buffer (pH 7.0); 2 mg of resazurin and 2 g of agar. After adjusting the pH to 7.0 and autoclaving, sterile solutions of ferric citrate and neutralized cysteine·HCl are added (final concentrations 10 μM and 0.01%, respectively). The culture tubes can be inoculated after the establishment of sulfide and oxygen gradients within the medium, which takes about 24 hours. Several days to weeks of incubation at room temperature in the dark may be required until growth becomes apparent, usually as fluffy pinpoint colonies.

Although the original inoculum always contains various types of magnetotactic bacteria, in most cases only magnetotactic spirilla grow and are eventually isolated in pure culture. Modifications of this medium may eventually prove useful for the isolation of hitherto uncultured types of magnetotactic bacteria.

Following isolation, most strains of magnetospirilla can be cultured in liquid media without added water or mud from the sampling site. However, the gas composition of the headspace of the cultures is crucial for good growth and magnetite synthesis by most species. The maximum concentration of oxygen allowing growth and/or magnetite synthesis differs among the described *Magnetospirillum* strains. *M. magnetotacticum* grows optimally and produces the highest number of magnetosomes at an oxygen tension of 1% in the headspace and tolerates higher initial oxygen concentrations only if a large number of cells is inoculated into the growth medium. In contrast, *Magnetospirillum* strain AMB-1 grows (but does not produce magnetosomes) aerobically under atmospheric concentrations of oxygen (approximately 21% O_2; Matsunaga et al., 1991b). Magnetite synthesis is inhibited by all *Magnetospirillum* strains when cells are cultured under oxygen concentrations above 2 to 6% (Blakemore et al., 1985; Schüler and Baeuerlein, 1998).

Strain MV-1, a Facultatively Anaerobic Magnetotactic Vibrio

A marine magnetotactic vibrioid to helicoid bacterium, strain MV-1, was isolated by Bazylinski et al. (1988). Cells of strain MV-1 are small, rang-

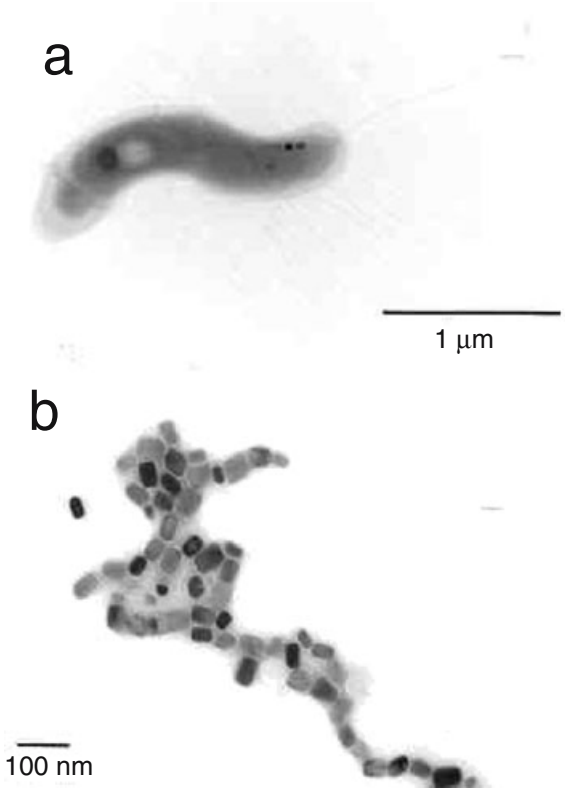

Fig. 16. Brightfield TEM image of negatively stained cell and magnetosomes of strain MV-1. (a) Cell stained with uranyl acetate showing a single polar flagellum and a chain of magnetite-containing magnetosomes. (b) Preparation of purified magnetosomes from strain MV-1 stained with 2% aqueous sodium phosphotungstate, pH 7.0. The "magnetosome membrane" is visualized as an electron-lucent area surrounding each individual crystal and is easily removed with detergents such as sodium deodecyl sulfate.

ing from 1–5 μm by 0.2–0.5 μm, and possess a single, unsheathed, polar flagellum (Fig. 16a). Cells grow and synthesize pseudohexahedral prismatic crystals of magnetite, averaging 53 by 35 nm in size (Fig. 16b; Sparks et al., 1990), in their magnetosomes microaerobically and anaerobically, with nitrous oxide as the terminal electron acceptor. Cells appear to produce more magnetite under anaerobic conditions than under microaerobic conditions (Bazylinski et al., 1988) and, like *M. magnetotacticum*, synthesize a number of magnetosome membrane proteins that are not present in other cellular fractions (Dubbels et al., 1998). A stable, spontaneous nonmagnetotactic mutant strain of MV-1 that does not produce magnetosomes has recently been isolated and partially characterized (Dubbels and Bazylinski, 1998).

Strain MV-1 is nutritionally versatile being able to grow chemoorganoheterotrophically with organic and some amino acids as carbon and energy sources, and chemolithoautotrophically with thiosulfate or sulfide as energy sources oxidizing them to sulfate, and carbon dioxide as the sole carbon source (Kimble and Bazylinski, 1996). Cells produce intracellular sulfur deposits when grown with sulfide (Kimble and Bazylinski, 1996). As do virtually all aerobic chemolithoautotrophic bacteria, strain MV-1 uses the Calvin-Benson cycle for autotrophic carbon dioxide fixation (McFadden and Shively, 1991). Cell-free extracts from thiosulfate-grown cells of strain MV-1 show ribulose bisphosphate carboxylase/oxygenase (rubisCO) activity (Kimble and Bazylinski, 1996), and recently (Dean and Bazylinski, 1999a) the gene for a form II rubisCO enzyme (*cbbM*) was cloned and sequenced from strain MV-1. There was no evidence for a *cbbL* gene (encodes for form I rubisCO enzymes) in DNA hybridization analyses despite using *cbbL* gene probes from several different organisms. Because many uncultured magnetotactic bacteria collected from natural habitats thrive in oxygen-sulfide inverse gradients, as previously mentioned, and contain internal sulfur deposits (Moench, 1988; Spring et al., 1993; Frankel and Bazylinski, 1994; Iida and Akai, 1996; Kimble and Bazylinski, 1996), it seems many species are likely chemolithoautotrophs that obtain energy from the oxidation of sulfide and perhaps other reduced sulfur sompounds. Using pulsed-field gel electrophoresis (PFGE), the genome of strain MV-1 was found to consist of a single, circular chromosome of approximately 3.7 Mb (Dean and Bazylinski, 1999b). There was no evidence of linear chromosomes or extrachromosomal DNA such as plasmids. The guanine-plus-cytosine content of the DNA of this strain is 52.9 mol% as determined by HPLC and 53.5 mol% by T_m.

A virtually identical strain to strain MV-1, designated MV-2, was isolated from the Pettaquamscutt Estuary (DeLong et al., 1993; Meldrum et al., 1993b). Cells of this strain produce the same morphological type of magnetite crystals as strain MV-1 (Meldrum et al., 1993b) and display many of the same phenotypic traits as strain MV-1 (such as anaerobic growth with nitrous oxide as a terminal electron acceptor, heterotrophic growth with organic and amino acids, and chemolithoautotrophic growth on reduced sulfur compounds). However, strain MV-2 shows slightly different restriction fragment patterns in pulsed-field gels than strain MV-1 using the same restriction enzymes (Dean and Bazylinski, 1999b). As with strain MV-1, the genome of strain MV-2 consists of a single, circular chromosome of a similar size, about 3.6 Mb (Dean and Bazylinski, 1999b). The guanine-plus-cytosine content of the DNA of this strain is 56.2 mol% as determined by HPLC and 56.6% by T_m.

Strain RS-1, a Sulfate-Reducing Magnetotactic Bacterium

It was thought for a long time that all magnetotactic bacteria are obligate or facultative microaerophiles (*Magnetospirillum* strain AMB-1 and the marine vibrio, strain MV-1, grow anaerobically with nitrate and nitrous oxide, respectively, as well as with oxygen) adapted to the microoxic zone of their environment. With the isolation of an obligately anaerobic strain from a sulfidic freshwater habitat by Sakaguchi et al. (1993), this assumption is clearly incorrect. Cells of this organism, designated strain RS-1, are 0.9–1.5 by 3–5 μm with a helicoid to rod-shaped morphology and possess a single polar flagellum. They exhibit an axial magnetotaxis coupled with a strong anaerotaxis reflecting their obligate anaerobic metabolism. According to the revised model of magnetotaxis, they may have developed an axial magnetotaxis because they do not have to oscillate between microoxic and anoxic zones of their habitat, which would select for polar magnetotaxis.

Strain RS-1 is a dissimilatory sulfate-reducing, chemoorganoheterotrophic bacterium that utilizes a variety of organic substrates, e.g., pyruvate, lactate, ethanol and fumarate). Cells can use sulfate or fumarate as electron acceptor but not oxygen. They are catalase positive and oxidase negative. The guanine-plus-cytosine content of DNA was determined by HPLC to be 66 mol%.

Sequencing of the 16S rRNA gene of strain RS-1 showed that it is phylogenetically affiliated to the delta-subclass of the *Proteobacteria* (Kawaguchi et al., 1995). The nearest neighbors in a phylogenetic tree are members of the genus *Desulfovibrio*, typical representatives of the obligately anaerobic, dissimilatory sulfate-reducing bacteria. In contrast to Desulfovibrio sp., cells of strain RS-1 are able to produce intracellular bean-shaped crystals of magnetite, responsible for its magnetotactic response. Consequently, the production of magnetosomes consisting of magnetite is found in bacteria belonging to three different phylogenetic groups, viz. the α- and δ-subclasses of *Proteobacteria* and the *Nitrospira* group ("*Magnetobacterium bavaricum*"), indicating multiple evolutionary origins of intracellular magnetite synthesis or lateral gene transfer between different phylogenetic groups.

Other Magnetotactic Strains in Pure Culture

Several other pure cultures of magnetotactic bacteria exist, but they appear to be obligate microaerophiles and grow poorly (D. A. Bazylinski, unpublished results). Hence, very little is known about them. Strain MC-1 (Fig. 17), a marine biliophotrichous coccus, was isolated from water collected from the Pettaquamscutt Estuary, a chemically-stratified semi-anaerobic basin in Rhode Island, USA. Cells of this strain produce pseudohexahedral prisms of magnetite, averaging 72 by 70 nm in size (when grown autotrophically), and grow chemolithoautotrophically with thiosulfate or sulfide as an electron and energy source (Meldrum et al., 1993a; Frankel et al., 1997). Cells may also be able to grow chemoorganoheterotrophically. Like all magnetotactic cocci observed, cells of strain MC-1 show polar magneto-aerotaxis regardless of whether they are grown in liquid or semi-solid oxygen gradient media. This strain has a genome size of approximately 4.5 Mb as determined by pulsed-field gel electrophoresis (Dean and Bazylinski, 1999b). The guanine-plus-cytosine content of the DNA of strain MC-1, as determined by HPLC, is 55.8 mol%. This organism has not been completely characterized and described.

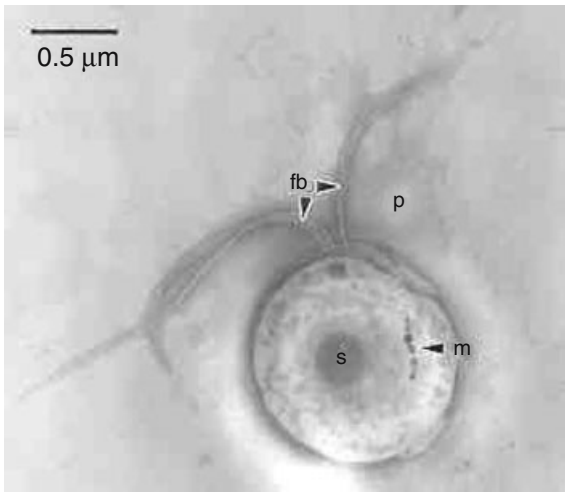

Fig. 17. Brightfield TEM image of a cell of the bilophotrichously flagellated marine coccus strain MC-1 negatively stained with uranyl acetate. Note the two flagellar bundles (fb), the presence of pili (p), sulfur globules(s), and chain of Fe_3O_4-containing magnetosomes (m).

Strain MV-4 (Fig. 18), a small marine spirillum, was isolated from sulfide-rich mud and water collected from School Street Marsh, Woods Hole, Massachusetts, USA. Cells of this strain produce elongated octahedrons of magnetite, averaging 61 by 52 nm in size, and grow chemolithoautotrophically with thiosulfate or chemoorganoheterotrophically with succinate (Meldrum et al., 1993b). Unlike most freshwater magnetotactic spirilla, this strain shows polar magneto-aerotaxis at least when grown in semi-solid oxygen gradient media. Like strain MC-1,

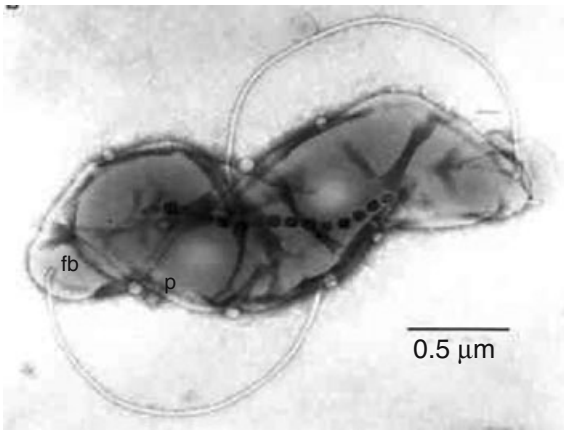

Fig. 18. Brightfield TEM image of a cell of the marine spirillum strain MV-4 negatively stained with uranyl acetate showing bipolar flagellation and a chain of Fe_3O_4-containing magnetosomes.

this strain has not been completely characterized and described.

Biotechnological Applications

It was not long after the discovery of magnetotactic bacteria that publications of physical studies and of commercial and medical applications involving the magnetotactic cells, isolated magnetosomes and/or magnetite crystals began to appear. It is clear that magnetotactic bacterial cells and their magnetic crystals have novel physical, magnetic and possibly electrical properties. In addition, in certain types of applications, bacterial magnetite offers several advantages compared to chemically synthesized magnetite. Bacterial magnetosome particles, unlike those produced chemically, have a consistent shape, a narrow size distribution within the single magnetic domain range, and a membrane coating consisting of lipids and proteins. The magnetosome envelope allows for easy couplings of bioactive substances to its surface, a characteristic important for many applications.

Magnetotactic bacterial cells have been used to determine south magnetic poles in meteorites and rocks containing fine-grained magnetic minerals (Funaki et al., 1989; Funaki et al., 1992) and for the separation of cells after the introduction of magnetotactic bacterial cells into granulocytes and monocytes by phagocytosis (Matsunaga et al., 1989). Magnetotactic bacterial magnetite crystals have been used in studies of magnetic domain analysis (Futschik et al., 1989) and in many commercial applications including: the immobilization of enzymes (Matsunaga and Kamiya, 1987); the formation of magnetic antibodies in various fluoroimmunoassays (Matsunaga et al., 1990) involving the detection of allergens (Nakamura and Matsunaga, 1993a) and squamous cell carcinoma cells (Matsunaga, 1991a), and the quantification of IgG (Nakamura et al., 1991); the detection and removal of *Escherichia coli* cells with a fluorescein isothiocyanate conjugated monoclonal antibody, immobilized on magnetotactic bacterial magnetite particles (Nakamura et al., 1993c); and the introduction of genes into cells, a technology in which magnetosomes are coated with DNA and "shot" using a particle gun into cells that are difficult to transform using more standard methods (Matsunaga, 1991a). Unfortunately, the prerequisite for any large scale commercial application is mass cultivation of magnetotactic bacteria or the introduction and expression of the genes responsible for magnetosome synthesis into a bacterium, e.g., *E. coli*, that can be grown relatively cheaply to extremely large yields. Although some progress has been made, the former has not been achieved with the available pure cultures.

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