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Ecology, Diversity, and Evolution of Magnetotactic Bacteria

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SUMMARY 497
INTRODUCTION 498
ECOLOGY AND BIOGEOGRAPHIC DISTRIBUTION OF MAGNETOTACTIC BACTERIA 498
COLLECTION AND DETECTION OF MAGNETOTACTIC BACTERIA 499
Sampling 499
Detection of MTB 499
Magnetic Purification 501
CULTIVATION OF MAGNETOTACTIC BACTERIA 501
TAXIS IN MAGNETOTACTIC BACTERIA 503
Magneto-Aerotaxis and Function of Magnetosomes 503
Phototaxis 504
DIVERSITY AND PHYSIOLOGY OF MAGNETOTACTIC BACTERIA 504
Alphaproteobacteria 504
The genus *Magnetospirillum* 505
Magnetotactic cocci 507
Magnetovibrio blakemorei 508
Magnetospira thiophila strain MMS-1 and strain QH-2 508
Deltaproteobacteria 508
MMPs 509
Desulfovibrio magneticus 511
Alkaliphilic magnetotactic bacteria 511
Large rod-shaped bacteria 511
Symbiotic MTB 511
Gammaproteobacteria 511
Nitrospirae 512
"*Ca.* Magnetobacterium bavaricum" 512
Strain MHB-1 513
Thermophilic MTB 514
Large ovoid *Nitrospirae* 514
Other Phyla 514
Magnetotactic Eukaryotes 514
Is the Known Diversity of Magnetotactic Bacteria an Underestimation? 515
MAGNETOSOME FORMATION 515
Steps Involved in Magnetosome Chain Formation 515
Genetic Determinants of Magnetosomes 517
EVOLUTION OF MAGNETOTAXIS 518
Origin of Magnetotaxis 518
Evidence for HGT of Magnetosome Genes in MTB 518
Evidence for Vertical Transfer of Magnetosome Genes 519
The First Magnetosomes 520
CONCLUDING REMARKS 520
ACKNOWLEDGMENTS 521
REFERENCES 521
AUTHOR BIOS 526

SUMMARY

Magnetotactic bacteria (MTB) are widespread, motile, diverse prokaryotes that biomineralize a unique organelle called the magnetosome. Magnetosomes consist of a nano-sized crystal of a magnetic iron mineral that is enveloped by a lipid bilayer membrane. In cells of almost all MTB, magnetosomes are organized as a well-ordered chain. The magnetosome chain causes the cell to behave like a motile, miniature compass needle where the cell aligns and swims parallel to magnetic field lines. MTB are found in almost all types of aquatic environments, where they can account for an important part of the bacterial biomass. The genes responsible for magnetosome biomineralization are organized as clusters in the genomes of MTB, in some as a magnetosome genomic island. The functions of a number of magnetosome genes and their associated proteins in magnetosome synthesis and construction of the mag-

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netosome chain have now been elucidated. The origin of magnetotaxis appears to be monophyletic; that is, it developed in a common ancestor to all MTB, although horizontal gene transfer of magnetosome genes also appears to play a role in their distribution. The purpose of this review, based on recent progress in this field, is focused on the diversity and the ecology of the MTB and also the evolution and transfer of the molecular determinants involved in magnetosome formation.

INTRODUCTION

Magnetotactic bacteria (MTB) are aquatic prokaryotes whose direction of motility is directed by the Earth's geomagnetic and externally applied magnetic fields (1). These ubiquitous microorganisms represent a morphologically, phylogenetically, and physiologically diverse group of Gram-negative bacteria that biomineralize unique organelles called magnetosomes, which are responsible for the cells' magnetotactic behavior, which is referred to as magnetotaxis (2). Magnetosomes consist of magnetic mineral crystals, either magnetite (Fe_3O_4) or greigite (Fe_3S_4), enveloped by a bilayer membrane composed mostly of phospholipids, called the magnetosome membrane, that contains a number of proteins not present in the cytoplasmic and outer membranes (OMs) and are unique to MTB (3, 4). Although magnetosome magnetite and greigite crystals can have different morphologies, mature crystals of both minerals generally lie within the single-magnetic-domain size range, about 35 to 120 nm, in which they have the highest possible magnetic moment per unit volume (1). Magnetosomes are usually arranged as a chain within the cell, thereby maximizing the magnetic dipole moment of the cell and causing the cell to passively align along magnetic field lines as it swims. Magnetotaxis is thought to function in conjunction with chemotaxis in aiding MTB in locating and maintaining an optimal position in vertical chemical concentration gradients common in stationary aquatic biotopes, by reducing a three-dimensional search problem to one of a single dimension (5).

MTB were first described by Salvatore Bellini in 1963 from water collected from different freshwater environments near Pavia, Italy (6, 7). He observed large numbers of bacteria swimming in a consistent, single, northward direction and speculated that the magnetic behavior of the cells was due to an internal "magnetic compass." Richard P. Blakemore independently rediscovered MTB in 1974 and was the first to demonstrate Bellini's "magnetic compass," the magnetosomes, within cells of MTB (2).

Magnetotactic bacteria thrive in sediments or chemically stratified water columns, where they occur predominantly at the oxic-anoxic interface (OAI), the anoxic regions of the habitat, or both (8). Although the detection of MTB in samples collected from natural environments is relatively simple to do (9), MTB are a fastidious group of prokaryotes, and special culture conditions are necessary for their isolation and cultivation. Most known cultured and uncultured MTB are associated with the *Alpha-*, *Gamma-*, and *Deltaproteobacteria* classes of the *Proteobacteria* phylum and with the *Nitrospirae* phylum (10). All cultured species are either microaerophiles, 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 chemoorganoheterotrophically using organic acids as electron and carbon sources (11). Those organisms in the *Deltaproteobacteria* are sulfate-reducing anaerobes that grow chemoorganoheterotrophically. Almost all cultured species exhibit nitrogenase activity and thus fix atmospheric nitrogen, and many denitrify (8). MTB thus show a great potential for iron, nitrogen, sulfur, and carbon cycling in natural environments (12).

Magnetosome membrane proteins are encoded by the magnetosome genes, which are present as clusters within the genomes of all MTB thus far examined (13). These clusters are in relatively close proximity to each other within the genomes and are surrounded or interrupted by certain types of genomic structures, which suggests that in some MTB, the magnetosome genes are organized as a magnetosome genomic island that might be transmitted to other different bacteria through horizontal gene transfer (HGT). Through recent progress and improvements in genetic systems in some MTB, 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 (14). How the genes involved in magnetotaxis common to all MTB originated and were transferred during evolution is still a matter of debate, although there is evidence that magnetotaxis originated only once, regardless of the composition of the magnetosome crystal, and was then transferred by descent to all groups containing MTB and also through HGT between closely related bacteria (15).

In the last decade, numerous papers have been published involving studies regarding the roles of specific magnetosome proteins and genes, descriptions of new uncultured and cultured MTB, and the evolution of magnetotaxis. The purpose of this paper is to review this new information and to put it in a context together with our thoughts as to how and why MTB biomineralize magnetosomes and how magnetotaxis evolved.

ECOLOGY AND BIOGEOGRAPHIC DISTRIBUTION OF MAGNETOTACTIC BACTERIA

MTB are distributed worldwide, having been found on all continents, and are ubiquitous in sediments of freshwater, brackish, marine, and hypersaline habitats as well as in chemically stratified water columns of these environments (1). The occurrence of MTB, surprisingly, appears to not be dependent on particularly high concentrations of iron in the environment but on the presence of an OAI that represents, in most environments, opposing gradients of oxygen from the surface and reduced compounds (usually reduced sulfur species) in sediments or water columns (5). The largest numbers of MTB are typically found at or slightly below the OAI of sediments or chemically stratified water columns (16). Moreover, within the OAI itself, different species of MTB occupy different positions that represent different specific chemical conditions at that depth. Biogeographic studies indicate that some environmental parameters such as salinity, temperature, nitrate, or sulfur compounds could explain MTB abundance or community differences (17–21). One study reported that despite the fact that the largest proportion of MTB appears to be detected within the suboxic zone, a strict correlation between the distribution of MTB and individual geochemical parameters has never been shown (22). MTB are known to biomineralize two magnetic minerals: the iron oxide magnetite (Fe_3O_4) (23) and the iron sulfide greigite (Fe_3S_4) (24, 25). In general, magnetite-producing MTB are found at or very close to the OAI, while greigite producers are present in reducing biotopes, below the OAI, in the sulfidic anoxic zone (16, 26). MTB are thus excellent examples of gradient (e.g., oxygen concentration and redox)-loving organisms.

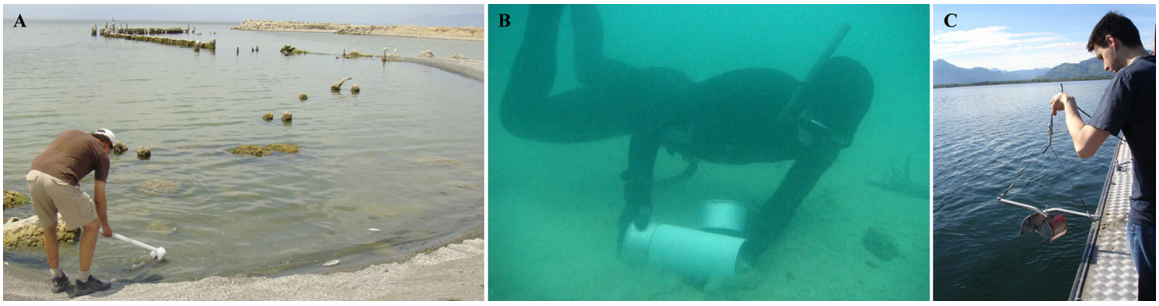

FIG 1 Sampling for magnetotactic bacteria using different strategies: from the shore of the Salton Sea with a scooper (A), underwater in the Mediterranean Sea by free diving (B), and with a bottom sampler in Lake Chiemsee, Bavaria (C). (Panel C courtesy of S. Kolinko and D. Schüler, reproduced with permission.)

Based on numerous environmental studies and characteristics of known axenic strains, MTB were thought to be mesophiles restricted to habitats with pH values near neutral. Recently, however, some MTB have been found to be extremophilic. Lefèvre et al. (27) described an uncultured, moderately thermophilic, magnetotactic bacterium present in hot springs in northern Nevada with a probable upper growth/survival limit temperature of about 63°C. In addition, this same group isolated several strains of obligately alkaliphilic MTB from different aquatic habitats in California, including the hypersaline and extremely alkaline Mono Lake (28). The latter strains had an optimal growth pH of ~9.0. Based on the fact that MTB are gradient-loving microorganisms found primarily at or just below the OAI in natural aquatic habitats, in theory, all chemically stratified, aquatic environments having gradients with the appropriate physical-chemical conditions (e.g., a suitable redox potential and enough soluble iron) could support populations of MTB. Thus, there appears to be no reason why other extremophiles, including acidophilic, piezophilic, halophilic, or psychrophilic bacteria, could not have acquired the ability to biomineralize magnetosomes (29).

Finally, the known biogeographic distribution of MTB is somewhat biased, as there are relatively few laboratories where the research focus is on the ecology and diversity of MTB.

COLLECTION AND DETECTION OF MAGNETOTACTIC BACTERIA

Sampling

Sampling for MTB is based on the collection of the sediment layer or water depth that includes and surrounds the OAI of aquatic environments. When the OAI is located in sediments, this interface is generally in the first centimeter of the sediments, depending mainly on the grain size of the sediment particles. Sampling could occur from shore (Fig. 1A), by free diving (Fig. 1B), or by using a bottom sampler (Fig. 1C) (30). In general, we use 1-liter bottles filled to about 20% to 30% of their volume with sediment and then fill the remainder of the bottle to capacity with water that overlays the sediment. Air bubbles are excluded from the sample bottles. It is not always necessary to take a core, as we have found that the maximum number of MTB is obtained when the top ~5 cm of sediment is collected. When the OAI is located in the water column (e.g., salt pond [31]), sampling at discrete depths is done from a boat, using a depth profiler and an oxygen probe fixed to a peristaltic pump for simultaneous accurate water sampling and oxygen profiling.

Once in the laboratory, samples are stored under dim light at room temperature (~25°C) in order to avoid the proliferation of phototrophic organisms that often leads to a significant decrease or elimination of MTB. Depending on the sample type (e.g., freshwater versus marine habitats), MTB can last from weeks to years, even without the addition of nutrients. In several studies, successions of different magnetotactic bacterial morphotypes have been observed during the enrichment process (e.g., see references 22 and 32). For instance, characterization of the large ovoid *Nitrospirae* organism "*Candidatus* Magnetoovum mohavensis" was possible only due to its enrichment in samples incubated for several months after collection (32).

Detection of MTB

The detection of MTB in environmental water and sediment samples is relatively easy due to their magnetotactic behavior, which is in turn due to their permanent magnetic dipole moment. A simple method is the so-called hanging-drop technique, in which a drop of water/sediment is placed onto a coverslip and then inverted and placed onto a small rubber O ring on an optic microscope slide (9). A bar magnet is placed onto the microscope stage near the drop, with the axis of the magnet parallel to the plane of the slide and passing through the center of the drop. The magnet should be oriented so that the south magnetic pole is nearest the drop, and the magnetic field at the drop should be at least a few gauss. This will cause bacteria collected in the Northern Hemisphere to swim to the edge of the drop nearest the magnet, 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. This technique works well if there are large numbers of MTB in the samples. To ensure visualization of cells if the concentration of MTB is low, MTB can be enriched magnetically by placing a bar magnet adjacent to the outer wall of a bottle filled with sediment and water. If MTB are abundant in the sample, a brownish or grayish-to-white spot consisting mainly of MTB will form next to the inside of the glass wall closest to the bar magnet (8). Cells can be easily removed from the bottle with a Pasteur pipette and examined as described above. When MTB represent an important proportion of the total microorganisms in a sample, the spot against the bar magnet can be larger than 5 mm and easily observed by eye (8). In cases where the overall population of bacteria is very small (including MTB), the sample can be centrifuged to concentrate all bacteria, thereby facilitating the detection of MTB in the sample (8).

Extension and scale-up of the magnetic collection method were recently described (33). By using larger "magnetic traps" that hold up to several liters of sediment slurry, large numbers of diverse,

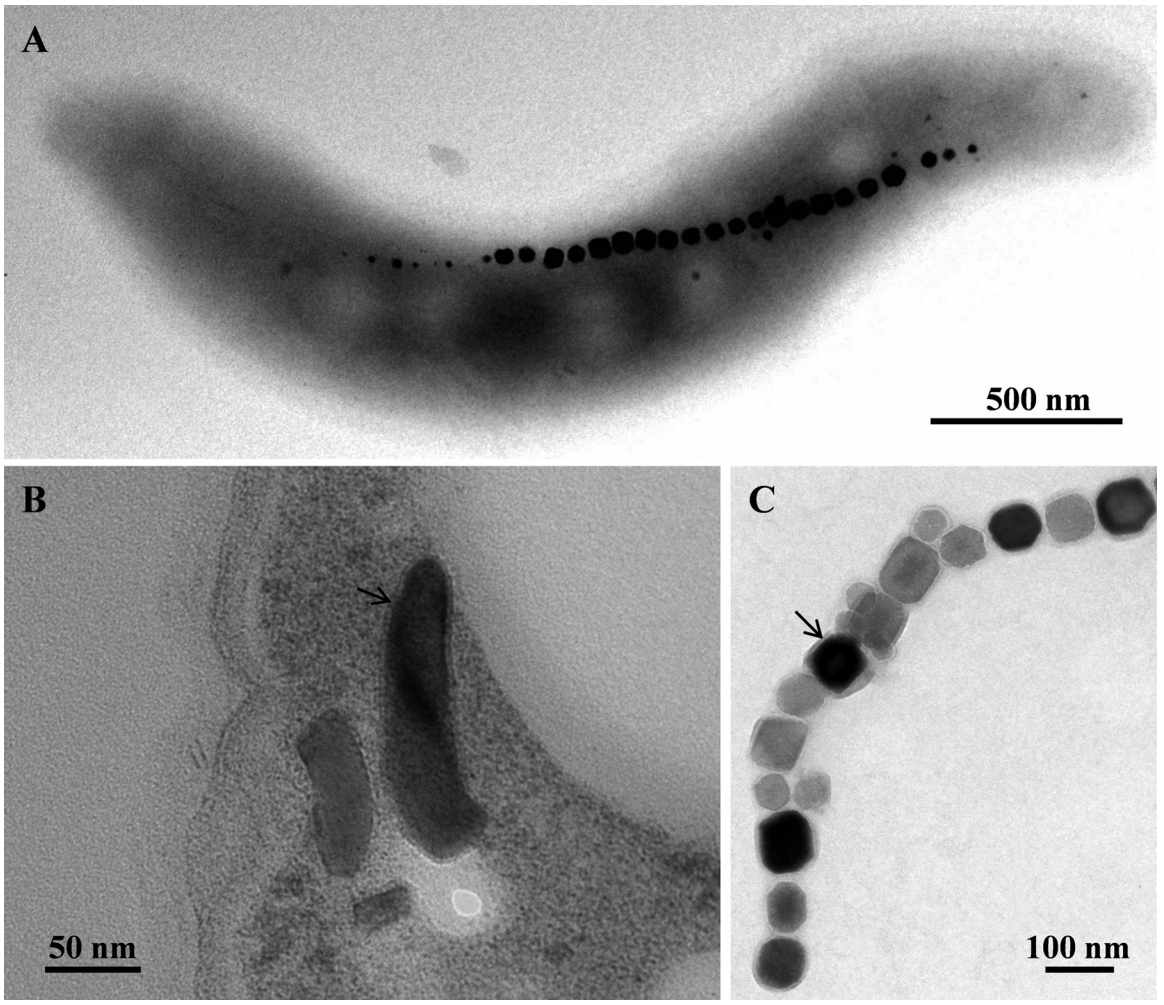

FIG 2 Transmission electron microscope (TEM) images of magnetosomes and the magnetosome membrane. (A) TEM micrograph of a cell of *Magnetospirillum magneticum* strain AMB-1 deposited onto a Formvar-coated electron microscope grid showing a chain of cuboctahedral magnetosomes. (B) TEM micrograph of an ultrathin section of a cell of "*Ca.* Magnetoovum mohavensis" showing the magnetosome membrane (arrow) surrounding bullet-shaped magnetite crystals. (C) TEM micrograph of an extracted and purified magnetosome chain from a *Magnetococcus marinus* MC-1 cell showing prismatic magnetite crystals surrounded by the magnetosome membrane (arrow).

uncultivated MTB can be selectively harvested from large volumes of sediment samples. With this method, MTB are magnetically directed toward the tips of collection tubes, from which they can be conveniently collected for further analyses.

It is important to note that all methods commonly used for the detection and collection of uncultivated MTB are inherently selective for cells that are highly motile and abundant and at least temporarily tolerate exposure to atmospheric concentrations of oxygen. Thus, modifications of these techniques to detect, collect, and cultivate environmental MTB that are found at very low concentrations in the sample, that swim very slowly, or that are poisoned quickly by oxygen may potentially reveal a greater diversity of MTB than is currently known. For example, the development of a single-cell-sorting device coupled with a whole-genome amplification technique allowed for the targeted phylogenetic and ultrastructural analysis of a magnetotactic bacterium in low abundance in sediments of Lake Chiemsee, designated SKK-01, belonging to the candidate division OP3, part of the *Planctomycetes-Verrucomicrobia-Chlamydiae* (PVC) bacterial superphylum (30).

The concentration of MTB in the environment is very variable, and different species exhibit different preferences with regard to depth within vertical gradients, even over a few millimeters (22). When detected, the concentration can be from a few cells to 10^5 cells per milliliter (our unpublished data). There is only a single study reporting the relative biovolume of a magnetotactic bacterium in the environment: the large rod-shaped MTB "*Candidatus* Magnetobacterium bavaricum" appears to account for approximately 30% of the microbial biovolume in Lake Chiemsee surface sediments in Bavaria, Germany, and may therefore constitute a dominant fraction of the microbial community in this sediment layer (34).

To observe the presence, organization, and morphology of the magnetosomes in cells of MTB, it is necessary to use a transmission electron microscope (TEM) or a scanning transmission electron microscope (STEM). Due to their high density, magnetosome crystals of magnetite or greigite are thus easy to observe (Fig. 2A). A drop of water containing MTB is generally deposited onto Formvar-coated electron microscope grids, which are then washed and dried in air. The identification of the composition of

magnetosome minerals is more difficult, but a common method is to use selected-area electron diffraction (SAED) with the electron microscope together with energy-dispersive X-ray analysis (35). To observe the magnetosome membrane, it is necessary to obtain ultrathin sections of cells embedded in resin by using a microtome (Fig. 2B). Alternatively, when a strain of a magnetotactic bacterium is in pure culture and can be grown to a high yield, magnetosomes can be extracted and purified from cells and then negatively stained (e.g., uranyl acetate) on an electron microscope grid for TEM observation of the magnetosome membrane (Fig. 2C).

Magnetic Purification

The general purification of MTB from samples for PCR experiments is again relatively easy due to the cells' magnetotactic behavior. For example, it is possible to obtain small suspensions of MTB completely or mostly free of nonmagnetotactic contaminants by magnetically separating cells using the magnetic capillary "racetrack" described by Wolfe et al. (36). For this technique (modified slightly from the original), a Pasteur pipette is sealed at its thin end in a flame, and a cotton plug is set where the wide-mouthed end of the pipette tapers to the thin portion (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 MTB is placed on top of the sterile, wetted cotton plug in the wide-mouthed end of the pipette. The south end of a bar magnet is placed near the sealed tip of the capillary furthest from the reservoir in order to direct north-seeking MTB toward the sealed end of the capillary. The opposite pole of an additional bar magnet is set near the entrance of the wide-mouthed end of the pipette, again in order to direct cells to the sealed end (see "Magneto-Aerotaxis and Function of Magnetosomes" for the significance of the magnetic poles). Generally, most fast-swimming cells of MTB (e.g., magnetotactic cocci) will reach the sealed tip in about 20 to 30 min and accumulate there. When enough cells have accumulated for study, the tip of the pipette is broken off, and the cells are removed aseptically by using a thin syringe needle. The purified sample obtained can serve as inocula for the cultivation of MTB, for DNA extraction for metagenomics studies, or for microscopic observation (36).

Although the magnetic capillary racetrack method is quite useful for the separation of larger, faster-swimming MTB, such as some large spirilla and the ubiquitous magnetotactic cocci, it can take much longer periods of time for slower-swimming organisms (e.g., cells of *Magnetovibrio blakemorei*) to reach the sealed end of the pipette. After about 30 min, it is not uncommon for motile nonmagnetotactic contaminants, including protozoa, to appear in the previously sterile portion of the capillary, sometimes at the sealed end. In general, the longer the period of time the capillary racetrack is run, the higher the probability of introducing nonmagnetotactic contaminants, which means that the separation and purification of MTB that swim very slowly are somewhat problematic. Another drawback with this technique is that although it has proven effective in a large number of studies, it does not guarantee a homogenous population of MTB unless only one type of MTB is present in the original sample, which is sometimes difficult to determine. Whether cells purified by this technique reflect the diversity of MTB in the original environmental samples is an important question that has been raised (37), although this may not be important depending on what the cells are to be used for. However, in general, this representation of diversity should not be assumed when using various magnetic separation techniques, considering the very diverse swimming speeds of different MTB. Lastly, we have also used the magnetic capillary racetrack technique to separate and purify MTB from enrichment cultures containing nonmagnetotactic contaminants or contaminated cultures of known MTB (38). Limitations of the magnetic capillary racetrack can be circumvented by the application of single-cell-sorting techniques by which any conspicuous morphotype of MTB can be targeted and separated from mixed environmental communities of MTB (30, 39, 40).

CULTIVATION OF MAGNETOTACTIC BACTERIA

MTB are fastidious with respect to growth, and the inability to isolate new strains of MTB due to their long cell-dividing times and the lack of specific enrichment and isolation media for them have frustrated potential and current researchers in this area for many years. This frustration is due in part to the ubiquity of MTB in aquatic habitats and the relative ease of collecting and separating them for observation. In addition, numerous different cell morphotypes can sometimes be present in relatively large numbers in a single environmental sample, and some MTB increase to significant numbers in samples of mud and water collected in bottles or in aquaria that are simply left in dim light at room temperature without special treatments such as the addition of nutrients (22, 41) yet still do not grow in most media! Lastly, based on their ecology and those species already in culture, and as stated above, MTB are clearly gradient-requiring organisms. Oxygen and/or redox gradients appear to be very important and are at best very difficult to replicate in growth medium in the laboratory.

The identification of the phylogenetic position of specific morphotypes of MTB can sometimes provide clues as to their physiology, which might be helpful in their isolation and cultivation. For example, the phylogeny of the magnetotactic multicellular prokaryotes (MMPs) strongly suggests that these organisms are anaerobic, dissimilatory, sulfate-reducing bacteria, although this information alone did not lead to their isolation and cultivation (42–45). This rationale has been used successfully, however, as several alkaliphilic strains of MTB, including ML-1, ZZ-1, and AV-1, were isolated in culture after their phylogeny was determined in an environmental study. These strains were found to be phylogenetically very closely related to the known nonmagnetotactic dissimilatory sulfate-reducing bacterium *Desulfonatronum thiodismutans* strain MLF-1 (46). By slightly modifying the growth medium for the latter organism, the magnetotactic strains ML-1, ZZ-1, and AV-1 (28) were grown and isolated in axenic culture. The greigite- and magnetite-producing organism "*Candidatus* Desulfamplus magnetomortis" was also isolated in axenic culture after it was found that its closest phylogenetic relative in culture was *Desulfobacterium vacuolatum* (47); "*Ca*. Desulfamplus magnetomortis" grew in and was isolated using a medium similar to that used for *D. vacuolatum* (26). In contrast, the freshwater magnetotactic cocci are among the most abundant MTB known, yet none have been isolated in axenic culture, despite the great amount of phylogenetic information on them. We know that their marine counterparts, including *Magnetococcus marinus* and strain MO-1, are obligate microaerophiles that oxidize reduced sulfur compounds such as thiosulfate or sulfide as electron donors (48, 49). Despite their relatively close phylogenetic relatedness, it is possible that magnetic cocci from freshwater and marine environ-

ments do not have the same metabolic capabilities or differ in the types of oxygen or redox gradients that they require.

Genomic analysis can also help in the cultivation of microorganisms; recently, Abreu et al. (F. Abreu, V. Morillo, F. Ferreira do Nascimento, C. Werneck, M. Egidio Cantão, L. Prioli Ciapina, L. G. P. de Almeida, C. T. Lefèvre, D. A. Bazylinski, A. T. R. de Vasconcelos, and U. Lins, unpublished data) were successful in obtaining an enrichment culture of the MMP "*Ca*. Magnetoglobus multicellularis" after bioinformatic studies on its genome revealed the presence of genes in metabolic pathways involved in the reduction of sulfate and the oxidation of organic compounds such as succinate, acetate, formate, and malate.

Since all known magnetite-producing MTB are microaerophiles, anaerobes, or facultatively anaerobic microaerophiles, most media used for the growth of these organisms are semisolid oxygen concentration gradients or anaerobic liquid media. In general, relatively low concentrations of nutrients appear more favorable for the initial enrichment and isolation of MTB than richer media containing higher concentrations of carbon and nitrogen sources. Although some cultivated species, including all the magnetotactic *Deltaproteobacteria*, are obligate anaerobes (8, 11, 50), most MTB tolerate short exposures to oxygen during magnetic purification and inoculation, making the strict exclusion of oxygen during cell manipulations unnecessary (8). However, it is not clear if this is true for most uncultivated species, and the strict exclusion of atmospheric oxygen from all sampling, enrichment, and cultivation steps wherever possible might increase the success of isolation.

Many magnetite-producing MTB are chemoorganoheterotrophic but facultatively chemolithoautotrophic (38, 48, 51, 52) or are obligately chemolithoautotrophic (53). One species exhibits chemoorganoautotrophic growth, oxidizing formate microaerobically as an electron donor and fixing the product, CO_2, by using the Calvin-Benson-Bassham (CBB) cycle (38). Semisolid oxygen concentration gradient medium can be used for both chemolithoautotrophic and chemoorganoheterotrophic growth. For the former, bicarbonate must be included in the medium, and organic compounds should be omitted, with the possible exception of some reducing agents (e.g., cysteine) and vitamins, if required. The best-known electron donors for chemolithoautotrophic growth of MTB in this medium are sulfide and thiosulfate (11). For chemoorganoheterotrophic growth, the most effective choices appear to be organic acids (e.g., succinate and acetate) and some amino acids, as no MTB have been shown to utilize any other type of organic compound (e.g., carbohydrates) as a carbon source (38, 48, 52, 54, 55).

Only recently has a greigite-producing magnetotactic bacterium been grown in axenic culture. "*Candidatus* Desulfamplus magnetomortis" was isolated from a saline spring at Badwater Basin in Death Valley National Park, CA (26). "*Ca*. Desulfamplus magnetomortis" appears to be an obligate, sulfate-reducing, chemoorganoheterotrophic anaerobe. Interestingly, "*Ca*. Desulfamplus magnetomortis" 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, on the concentration of sulfide (26).

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 by the presence of either chelating agents [particularly if the iron is supplied as Fe(III)] or reducing agents that reduce Fe(III) to the much more soluble Fe(II) form. Ferrous or ferric salts at concentrations of between 20 and 50 μM are generally sufficient to allow for both growth and magnetosome formation (56, 57), concentrations which have been shown to be typical of the free soluble iron found in environmental sediments where MTB are most abundant (22). Remarkably, the growth of cultivated *Magnetospirillum* species is inhibited at iron concentrations of >200 μM (57), suggesting that intracellular magnetite biomineralization is not an adaptation specific to iron-rich environments. Ferric citrate, ferric quinate, ferric malate, and ferrous sulfate are the iron sources most often used for growth and magnetite or greigite biomineralization, as they can be prepared easily and autoclaved together with other medium components, usually without precipitation (28, 58–60). It is important to understand that Fe(II) and Fe(III) inverse concentration gradients form in the oxygen concentration gradient medium described in the paragraph above due to the presence of chemical reducing agents. Both Fe(II) and Fe(III) have been shown to be taken up by cells of some MTB for magnetite synthesis although not necessarily simultaneously (57, 61, 62).

The formation of sulfide in anaerobic cultures of sulfate-reducing MTB can interfere with iron availability for magnetosome formation (26, 28). The strains of obligately alkaliphilic, sulfate-reducing MTB, discussed above, initially displayed weak to no magnetotactic responses when first isolated, 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 was purged every other day with oxygen-free argon gas in order to decrease the concentration of hydrogen sulfide in the cultures (28). This issue of iron availability may be true for other sulfate-reducing MTB such as *Desulfovibrio magneticus*, since this organism produces very few magnetosomes when grown anaerobically with sulfate compared to growth with fumarate (63). When a culture of the magnetite- and greigite-producing organism "*Ca*. Desulfamplus magnetomortis" is flushed with argon every other day, keeping anaerobic conditions with high potential redox, only magnetite is biomineralized (26).

For marine strains and those from other saline habitats, the composition and concentration of salts in the growth medium are important. Salinity of samples can be determined with a handheld refractometer. The medium should be diluted to the salinity of the sample in order to avoid osmotic stress when attempting to isolate MTB from saline environments. Alternatively, filtered water from the sample could be used to make the medium (58).

Once a magnetotactic bacterium is growing in medium, it is essential to isolate it in axenic culture; in other words, it is necessary to isolate a single clone from this culture. Two general methods have been used to isolate MTB in pure culture. The first involves the formation of individual colonies. This has been achieved by using agar plates of appropriate media such as activated charcoal agar (ACA) (64, 65). This technique has proven effective in growing *Magnetospirillum* and related freshwater MTB on solid medium. Activated charcoal is known to scavenge and decompose toxic free oxygen radicals and peroxides thought to inhibit the growth of many microaerophiles (66, 67). Once inoculated, ACA plates are incubated under microaerobic or anaerobic conditions in special gas mixtures (e.g., 1% oxygen in nitro-

gen) or oxygen-free gases, depending upon the organism (65, 68). A second method for obtaining individual colonies is through the use of solid medium in shake tubes (8, 69). This is useful for those organisms that will not form colonies on plates. Both oxygen concentration gradient and anaerobic shake tubes can be made by using air or oxygen-free gas in the headspace, respectively. Using either agar plates or shake tubes, colonies of MTB are usually brown or black due to the formation of magnetite (65, 68). For those organisms that do not form colonies either on plates or in shake tubes, pure cultures can be obtained by a repeated series of dilutions to extinction in media as long as the dominant bacterium present in the original culture is the one targeted for isolation (26, 28, 49, 53).

TAXIS IN MAGNETOTACTIC BACTERIA

Magneto-Aerotaxis and Function of Magnetosomes

The magnetosome chain imparts a permanent magnetic dipole moment to the cell, causing it to behave like a compass needle that aligns along the Earth's geomagnetic field lines (70). The overall direction of the Earth's geomagnetic field lines at any given location is the vectorial sum of the horizontal and vertical components of the geomagnetic field. At the equator, there is no vertical component, and the geomagnetic field lines are flat due to only the horizontal component. As one moves from the equator toward either pole, the geomagnetic field lines deviate from the horizontal at an angle (referred to as the angle of dip), which increases to 90° at the poles where the horizontal component is absent. Thus, geomagnetic field lines on most of Earth are inclined.

MTB were originally thought to have one of two magnetic polarities, north- or south-seeking polarity (71), based on the preferred swimming direction of the cells under oxic conditions. Because of the inclination in the Earth's geomagnetic field lines, north-seeking cells swim downward in the Northern Hemisphere, and south-seeking cells swim downward in the Southern Hemisphere. South-seeking cells would presumably swim upward in the Northern Hemisphere and die from exposure to high concentrations of oxygen and vice versa. Therefore, the Earth's geomagnetic field appeared to select for a dominant cell polarity in each hemisphere by favoring those cells whose polarity caused them to swim downward along the inclined geomagnetic field lines toward microaerobic/anaerobic sediments and away from potentially high, toxic concentrations of oxygen in surface waters. This hypothesis appeared to be supported by results that suggested that north-seeking MTB predominate in the Northern Hemisphere while south-seeking cells predominate in the Southern Hemisphere (71). At the equator, south-seeking and north-seeking MTB appear to be present in about equal concentrations (72). This observation is also consistent with the hypothesis that the vertical component of the geomagnetic field selects the predominant polarity type among MTB in natural environments, as in this case, neither north- or south-seeking cells are selected for or against (72, 73). However, there are some important aspects of magnetotaxis that are still not understood, as significant numbers of some species of MTB at some locations in the Northern Hemisphere have been found to be south seeking (74, 75). In addition, the first isolation and behavior of a polar magneto-aerotactic bacterium, *Magnetococcus marinus*, are not consistent with this hypothesis. North-seeking cells in cultures of *Mc. marinus* incubated in the Northern Hemisphere do not grow at the bottom of culture tubes as expected but grow as microaerophilic bands of cells at the OAI located a centimeter or two below the meniscus (5). Magnetotaxis was found to act in conjunction with aerotaxis (magneto-aerotaxis) in this marine microaerophile and in *Magnetospirillum magnetotacticum* (5, 76). Although these bacteria differ in their mechanism of aerotactic response and in the way in which they use the magnetic field, with *Mc. marinus* using the field as a sense of direction (polar magneto-aerotaxis) and *Ms. magnetotacticum* using the field as an axis (axial magneto-aerotaxis), they both prefer to be located at the OAI, and in this way, magneto-aerotaxis works similarly for both organisms (5, 76). However, it should be noted that *Magnetospirillum* species freshly isolated from the environment have a preferred swimming direction (display polar magnetotaxis), but this polarity is lost after several transfers in media containing a homogenous concentration of oxygen, where there is no selective pressure to retain polar magnetotactic behavior (60). According to the magneto-aerotaxis hypothesis, the direction of migration along the magnetic field is determined by the direction of flagellar rotation (clockwise or counterclockwise), which in turn is determined by the aerotactic response of the cell (5, 76). The presumed function of magneto-aerotaxis for *Mc. marinus* and *Ms. magnetotacticum* is increased efficiently in locating and maintaining a position at a preferred oxygen concentration (and perhaps redox potential) at the OAI in vertical oxygen concentration gradients in aquatic habitats by reducing a three-dimensional search problem (such as for nonmagnetotactic cells of *Escherichia coli*) to one of a single dimension where MTB passively align along geomagnetic field lines and swim up and down (5, 76).

Since their discovery, magnetosomes have also been thought to play other, perhaps physiological, roles because some MTB seem to have more magnetosomes than necessary for magnetotaxis (34). For example, it has been suggested that magnetosomes play a role in iron storage or in the elimination of reactive oxygen species (41). There is no evidence for magnetosomes as an iron storage product; in fact, there is evidence to the contrary (38). Indeed, cells of *Magnetovibrio blakemorei* were shown to produce magnetosomes even when the major source of iron is omitted from the growth medium, thereby starving themselves of iron and limiting their growth yield. It was recently shown that magnetite magnetosomes scavenge reactive oxygen species in *Magnetospirillum gryphiswaldense* and exhibit peroxidase-like activities (77). In terms of evolution, cells likely took up a great deal of iron for some unknown reason before magnetosomes had developed, perhaps for energy conservation (e.g., formation of ATP) during iron reduction where Fe^{3+} serves as the electron acceptor, for oxidation where Fe^{2+} serves as the electron donor, or for both. Magnetosomes may have formed originally as a result of the toxicity of free iron in the cell, as this would lead to the production of toxic radicals due to the Fenton reaction (78), and magnetite is known to be relatively inert and thus is a relatively safe choice when eliminating/precipitating free iron radicals. In any case, magnetosomes may have developed for purposes other than magnetotaxis, and with time and changing environmental conditions (e.g., atmospheric oxygen), magnetosomes became effective in magnetotaxis. However, the most currently accepted hypothesis regarding the function of magnetosomes remains the increase of efficiency in finding their preferred biotope.

Phototaxis

Some MMPs and nonmagnetotactic multicellular prokaryotes (nMMPs) show a strong negative phototactic response to white light and wavelengths of light of ≤480 nm (74, 79, 80). Because shorter wavelengths of light of ≤480 nm (blue to violet) are those that generally penetrate the water column the deepest (81), 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 downward, then, like magnetotaxis (5), 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 (5). Alternatively, light might simply drive MMPs and nMMPs downward toward anoxic conditions, which are likely favorable to them, as they appear to be sulfate-reducing bacteria (43–45).

Magnetococcus marinus also displays a negative phototactic response to light with short wavelengths of ≤500 nm (white, blue, or yellow light), which causes the cells to swim persistently parallel to the magnetic field (downward), similar to when the oxygen concentration is increased (5). Cells of strain QH-2 were also shown to be affected by light with wavelengths ranging from 350 to 550 nm (82). By using a light microscope, it was observed that most cells swam to the north side and accumulated at the edge of the hanging drop under normal conditions. When illuminating with wavelengths ranging from 330 to 550 nm, the emitted energy triggered the QH-2 cells to swim away from the edge to the interior of the drop (82).

DIVERSITY AND PHYSIOLOGY OF MAGNETOTACTIC BACTERIA

Even before the routine use of molecular phylogenetic techniques, the great diversity of MTB was obvious to most investigators who study them because of the large number of different, sometimes unique, morphotypes observed in environmental samples of water and sediment. The cell morphotypes most commonly observed include coccoid-to-ovoid cells, rods, vibrios, and spirilla of various dimensions. Two unique morphotypes include a group of multicellular bacteria, the MMPs, and a very large rod provisionally named "*Ca*. Magnetobacterium bavaricum."

Regardless of their morphology, all cultured and uncultured MTB studied thus far are motile by means of flagella and have a cell wall structure characteristic of typical Gram-negative bacteria, with one exception: some uncultured, freshwater MTB belonging to the *Nitrospirae* phylum appear to have a more complex cell wall structure (32, 39). The arrangement of flagella differs among MTB and can be either polar, bipolar, or in tufts. Another trait that shows considerable diversity is the arrangement of magnetosomes within the cell. In the majority of MTB, magnetosomes are aligned in one or more chains parallel to the long axis of the cell, which is the most magnetically efficient arrangement. However, dispersed aggregates or clusters of magnetosomes occur in some MTB, usually at one side of the cell, which often corresponds to the site of flagellar insertion (83–86). Besides magnetosomes, large inclusion bodies containing elemental sulfur, polyphosphate, or poly-β-hydroxybutyrate (PHB) are common in MTB collected from natural environments and in pure culture (87, 88). A study of MTB from the Seine River indicated that cells of some uncultured MTB contain Ba-rich and CaO inclusions (89). Cells of "*Ca*. Magnetoovum mohavensis" contain numerous sulfur globules and other smaller inclusions of unknown composition that have an electron-dense periphery with a less dense center (32). In some MTB, certain cell inclusions are easily observed by using light microscopy due to their highly refractive nature (e.g., sulfur globules) and provide a clear indication of the physiology of the bacterium (e.g., sulfide oxidizer).

Based on the sequences of their 16S rRNA genes, the phylogenetic diversity of MTB, including both those in axenic culture and those collected from natural environments, is also considerable (90). To date, representatives of the magnetotactic prokaryotes are phylogenetically associated with five major lineages within the domain *Bacteria*, three within the *Proteobacteria*. No magnetotactic bacterium phylogenetically associated with the *Archaea* has yet been discovered. Although most known cultured and uncultured MTB belong to the *Alpha*-, *Gamma*-, and *Deltaproteobacteria* classes of the *Proteobacteria* phylum, several uncultured species are affiliated with the *Nitrospirae* phylum, and one, strain SKK-01, was assigned to the candidate division OP3, part of the *Planctomycetes-Verrucomicrobia-Chlamydiae* (PVC) bacterial superphylum (30) (Fig. 3).

The physiology of known MTB, including that determined experimentally with cultured strains and that inferred from uncultured types, is also quite diverse. In general, however, the physiology of MTB 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

MTB are present in two orders of the *Alphaproteobacteria* class, the *Rhodospirillales* (e.g., *Magnetospirillum*, *Magnetovibrio*, and *Magnetospira*) (38, 52, 55) and the *Magnetococcales* (e.g., *Magnetococcus*) (48) (Fig. 4 and 5). In the *Alphaproteobacteria*, MTB are known only to biomineralize cuboctahedral and elongated prismatic magnetite crystals and include all cultured species of the freshwater genus *Magnetospirillum* (60, 91); all of the bilophotrichous magnetotactic cocci, including the cultured organisms *Magnetococcus marinus* (48) and strain MO-1 (49) and numerous uncultured types (83, 86, 92–94); the marine vibrio *Magnetovibrio blakemorei* strains MV-1 and MV-2 (38, 95); and the marine spirilla *Magnetospira thiophila* and strain QH-2 (52, 82) (Fig. 4 and 5). By using *in situ* hybridization with fluorescently labeled oligonucleotide probes, it has been shown that members of the *Alphaproteobacteria* class represent the dominant proportion of uncultured MTB in many freshwater and marine environments (93, 94, 96), with the magnetotactic cocci being the dominant type of alphaproteobacterial MTB in these habitats (92–94, 96, 97) (Fig. 5B to D). Because many uncultured magnetotactic *Alphaproteobacteria* contain intracellular sulfur globules (83, 84), autotrophy and/or mixotrophy based on the oxidation of reduced sulfur compounds is thought to be a common feature of these organisms (11). The ability to fix atmospheric nitrogen was found in all those organisms tested (11).

All cultured magnetotactic *Alphaproteobacteria* are obligate microaerophiles, anaerobes, or both (11). 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 at temperatures much higher than 30°C.

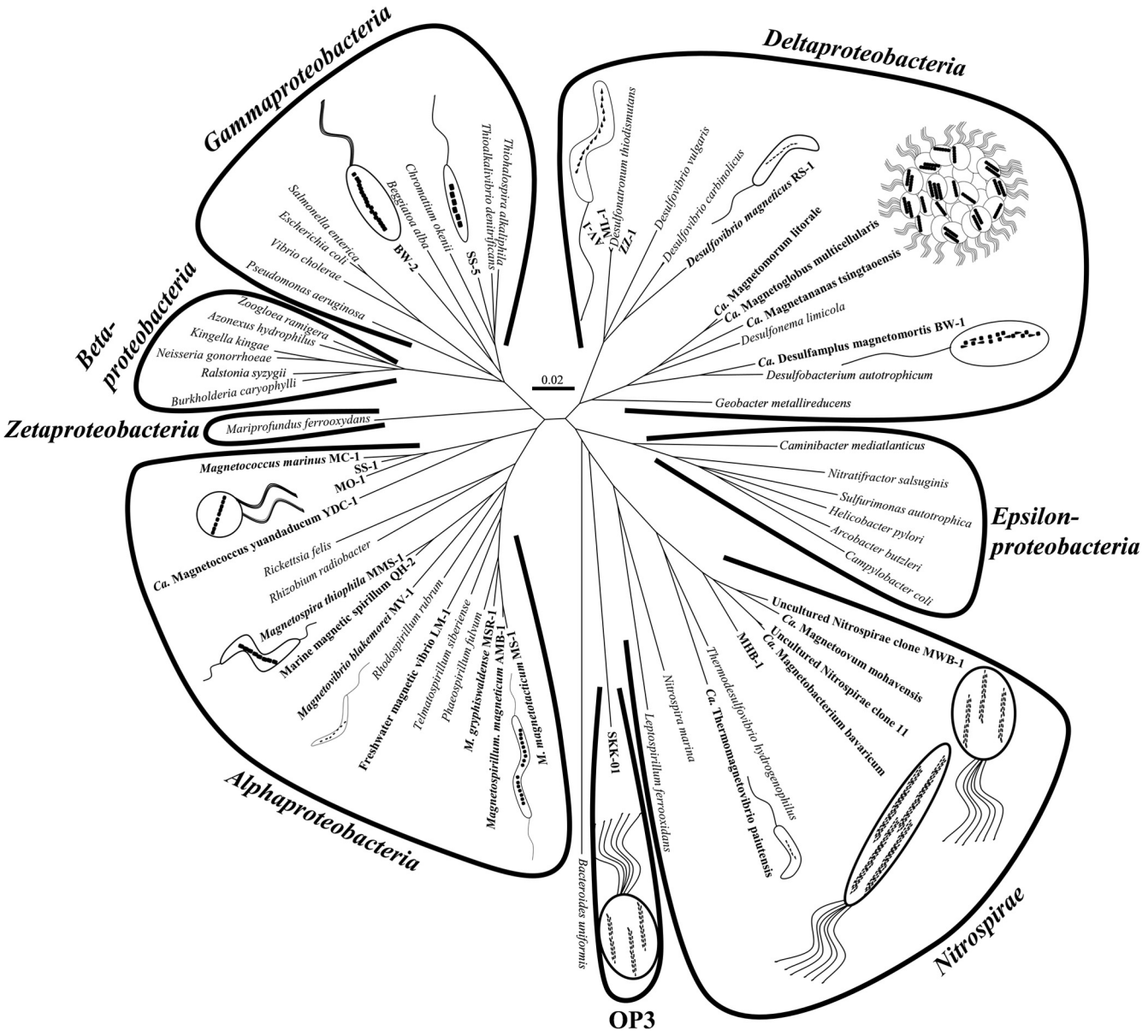

FIG 3 Phylogenetic distribution of cultured and uncultured magnetotactic bacteria in the *Alpha-*, *Gamma-*, and *Deltaproteobacteria* classes of the *Proteobacteria* phylum, the *Nitrospirae* phylum, and the candidate division OP3. Magnetotactic bacteria are in boldface type. The tree is based on neighbor-joining analyses. The bar represents 2% sequence divergence.

The genus *Magnetospirillum*. *Magnetospirillum* species have a respiratory form of metabolism and are chemoorganoheterotrophic, using organic acids as a source of carbon and electrons (55). *Ms. gryphiswaldense* is also capable of autotrophic and mixotrophic growth using reduced sulfur compounds as a source of electrons (51). Although the pathway of autotrophy was not determined, it seems likely that carbon dioxide fixation occurs through the Calvin-Benson-Bassham (CBB) cycle, since a form II ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) gene was found in the genomes of *Ms. magnetotacticum* (87) and other *Magnetospirillum*-related strains (64). While most species are facultative anaerobes that utilize nitrate as an alternative terminal electron acceptor to oxygen, *Ms. magnetotacticum* appears to be an obligate microaerophile that requires oxygen even when growing with nitrate (98, 99). In *Magnetospirillum* species, magnetite synthesis occurs only at very low levels of oxygen or under anaerobic conditions when nitrate is the alternative terminal electron acceptor to oxygen (56, 98–100). In *Ms. gryphiswaldense*, it was shown that in addition to its essential role in anaerobic respiration, the periplasmic nitrate reductase Nap has a further key function by participating in redox reactions required for magnetite biomineralization (101). All three described species of *Magnetospirillum* show dinitrogen-dependent growth and nitrogenase activity, demonstrating their ability to fix atmospheric nitrogen (102, 103). In further support of this, a full series of *nif* genes is present in the genomes of *Ms. magnetotacticum* and *Ms. magneticum*.

Recently, *Ms. aberrantis* was isolated from sediment of the

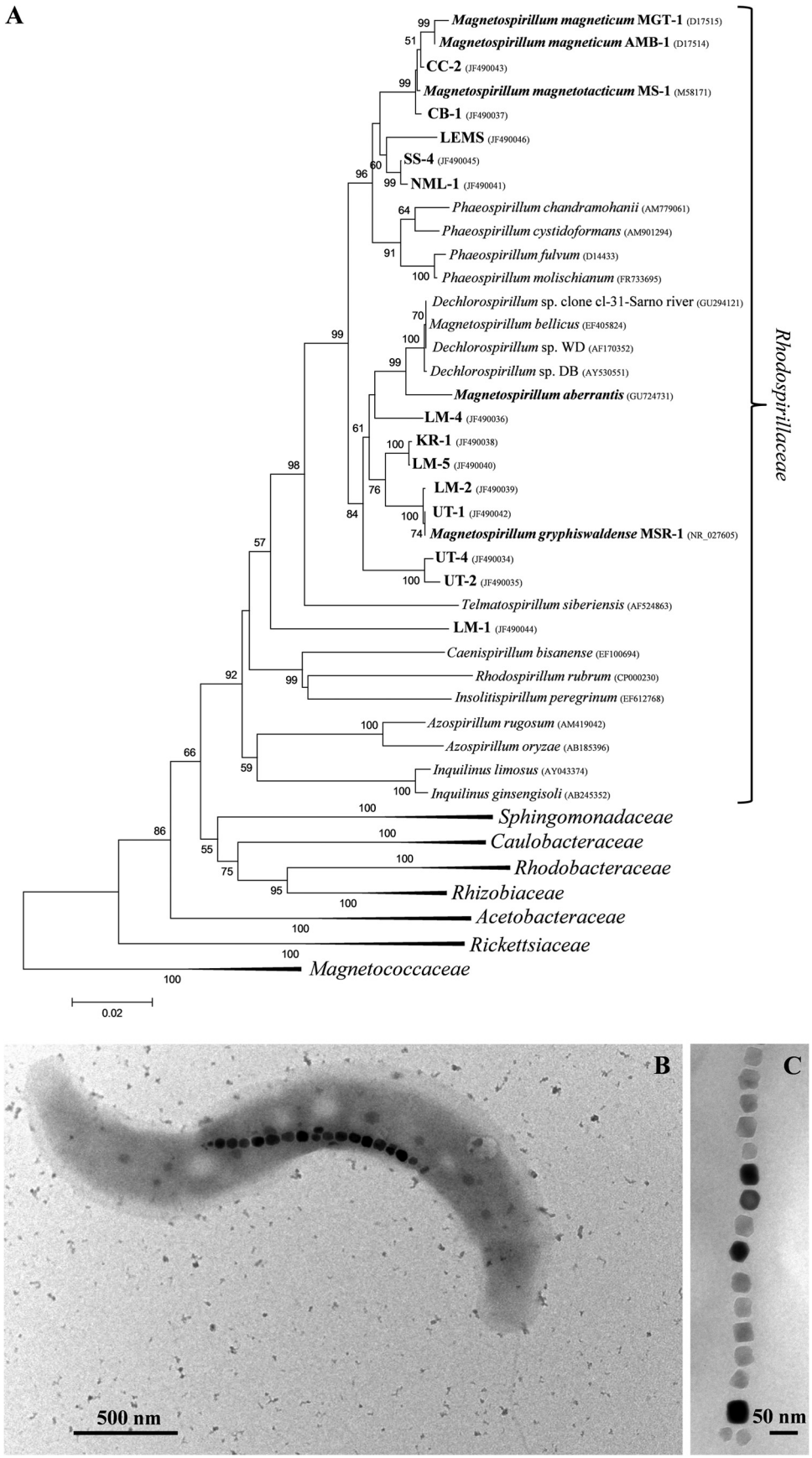

FIG 4 (A) Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic position of MTB closely related to the genus *Magnetospirillum* (in boldface type) in the family *Rhodospirillaceae* of the *Alphaproteobacteria* class. GenBank accession numbers are in parentheses. (B) TEM image of a cell of the cultured vibrioid strain LM-1 isolated from Lake Mead, NV, whose phylogenetic position is basal to the *Magnetospirillum*. (C) TEM image of a chain of cuboctahedral magnetite magnetosomes within a cell of the cultured strain CB-1 that belongs to the genus *Magnetospirillum*.

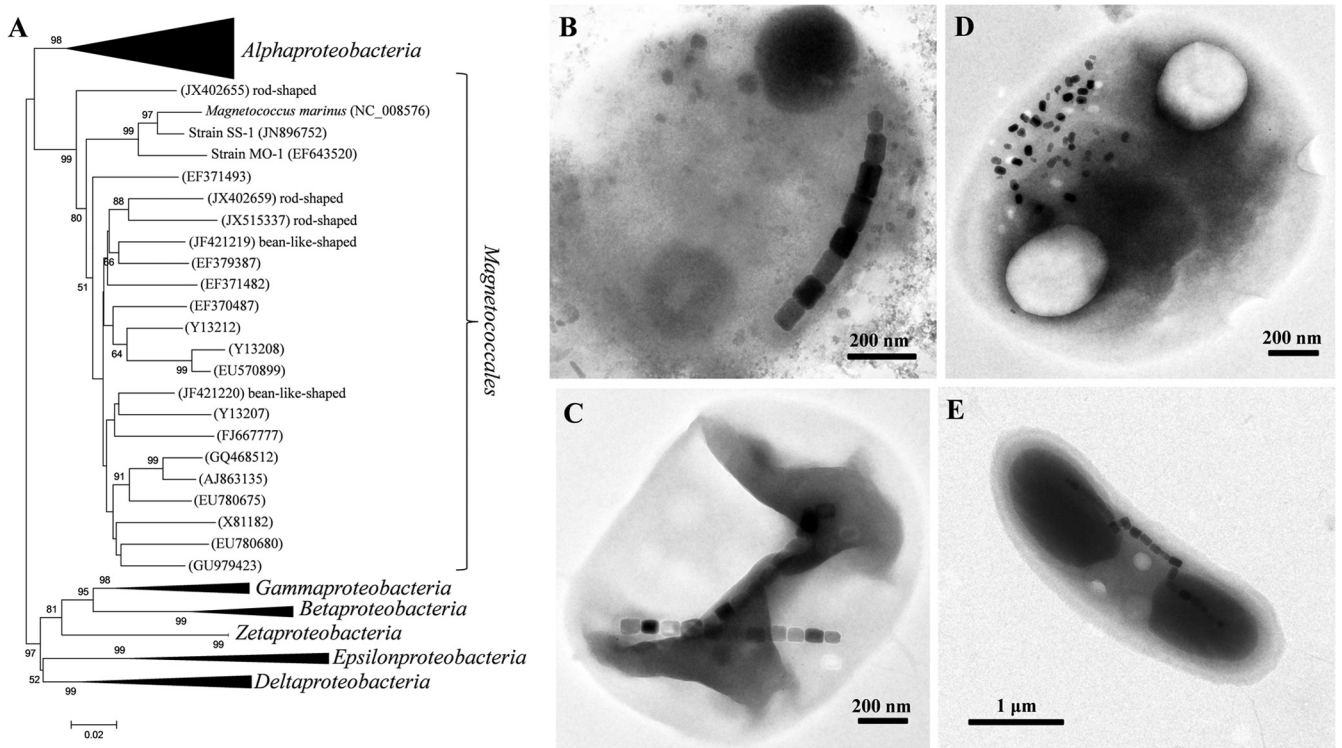

FIG 5 (A) Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic positions of MTB of the *Magnetococcales* order (in boldface type) in the phylum *Proteobacteria*. Bootstrap values (higher than 50) at nodes are percentages of 1,000 replicates. The bar represents 2% sequence divergence. GenBank accession numbers are in parentheses. (B to E) TEM images of different types of uncultured MTB of the order *Magnetococcales*. (B) Cell of a magnetotactic coccus that biomineralizes a single magnetite magnetosome chain. (C) Cell of a magnetotactic coccus that biomineralizes two magnetite magnetosome chains. (D) Cell of a magnetotactic coccus that biomineralizes a clump of magnetite magnetosomes rather than a chain. (E) Rod-shaped cell that biomineralizes a single chain of magnetite. (Panel E courtesy of E. Katzmann, S. Kolinko, and D. Schüler, reproduced with permission.)

Ol'khovka River near Kislovodsk (Caucasus, Russia) (104), while other species or strains of *Magnetospirillum* and related bacteria have been isolated in pure culture from sediment collected from McFarland Pond in Ames, IA (60), and freshwater and brackish environments in Nevada (e.g., Lake Mead), Utah (e.g., Kolob Reservoir), and California (Alamo River near the Salton Sea) (64) (Fig. 4).

Ms. magnetotacticum, the first magnetotactic bacterium to be isolated in culture, was initially classified into the genus *Aquaspirillum* (i.e., *Aquaspirillum magnetotacticum*) based mainly on physiological and morphological features (58), although following the development of molecular phylogenetics, *A. magnetotacticum* was found to represent a new genus, *Magnetospirillum* (55). It now seems clear that the freshwater magnetotactic spirilla represent a large group that appears to phylogenetically span a number of genera. Considering the very close phylogenetic relationship between *Phaeospirillum*, *Dechlorospirillum*, and *Magnetospirillum*, it would be necessary to modify the classification of the branch grouping those genera by including the *Phaeospirillum* and *Dechlorospirillum* species in the genus *Magnetospirillum* or by dividing members of the genus *Magnetospirillum* into several different genera (Fig. 4A) (60, 64). Indeed, the genus *Phaeospirillum* contains spiral-shaped, phototrophic, purple nonsulfur bacterial species (105), a physiological trait that is not shared with species of the genus *Magnetospirillum*, although the presence of intracellular membranes is common to both *Phaeospirillum* (105) and magnetosome-forming *Magnetospirillum* species.

Magnetotactic cocci. The most commonly observed types of MTB present in natural environments are coccoid-to-ovoid cells (Fig. 5B to D), the so-called magnetococci, that possess two flagellar bundles on one somewhat flattened side. This bilophotrichous type of flagellation resulted in the creation of the provisional genus "*Bilophococcus*" for these bacteria (84). Many uncultured magnetotactic cocci contain sulfur globules, even when sulfide is not apparent or measureable in the sample from which they were collected (83, 84), suggesting an autotrophic or mixotrophic metabolism based on the oxidation of reduced sulfur compounds. The two cultured magnetococci, *Magnetococcus marinus* and strain MO-1, are obligately microaerophilic and grow autotrophically on sulfide and thiosulfate (49, 106). *Mc. marinus* utilizes the reverse (or reductive) tricarboxylic acid (rTCA) cycle for carbon dioxide fixation and autotrophy (106). It also grows 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 (11, 107).

The known cultured and uncultured magnetotactic cocci are not closely related to other *Alphaproteobacteria* and form their own clade within the *Alphaproteobacteria* (i.e., the *Magnetococcales* order) that is basal to the rest of the group (Fig. 5A) (48). Previous 16S rRNA phylogenetic analyses and phylogenomic analyses have also recovered *Mc. marinus* as representing the earliest-diverging branch of the *Alphaproteobacteria* (108–110). *Mc. marinus* was regarded by one study as being most closely related to the class *Zetaproteobacteria* (represented by *Mariprofundus fer-*

rooxydans) and was assigned to a novel *Proteobacteria* subdivision that was informally termed magnetococci (110). However, *Mc. marinus* and *Mp. ferrooxydans* do not form a unique clade to the exclusion of other members of the *Proteobacteria* (110). Although certain studies have excluded *Mc. marinus* from the *Alphaproteobacteria* (108, 110), the overall topologies of these trees do not differ with regard to the position of *Mc. marinus*. The secondary structure of the 16S rRNA molecule of *Mc. marinus* is also consistent with its inclusion in the *Alphaproteobacteria* (43, 48). Thus, most investigators regard the magnetotactic cocci as representing the most basal lineage within the *Alphaproteobacteria* rather than as a separate class outside the *Alphaproteobacteria* (48) (Fig. 5A). *Mc. marinus* uses the rTCA cycle for autotrophic carbon assimilation; to date, this metabolic pathway is unique among the *Alphaproteobacteria*, with other autotrophic members of this class employing the CBB cycle (106, 111). Also atypical of the *Alphaproteobacteria* and more characteristic of the *Gammaproteobacteria*, $C_{16:1}$, not $C_{18:1}$, is the dominant cellular fatty acid in *Mc. marinus*. Finally, *Mc. marinus* and other magnetotactic cocci comprise a clade that clearly cannot be assigned to any known order within the *Alphaproteobacteria* (Fig. 5A) (48). Based on 16S rRNA gene sequence divergence between all magnetotactic cocci known to date, it seems likely that this clade consists of several genera.

Uncultured rod-shaped MTB, phylogenetically related to the magnetotactic cocci, have also been identified (40, 93, 112) (Fig. 5A and E). Thus, it seems likely that the *Magnetococcales* order consists not only of several genera of cultured and uncultured magnetotactic cocci but also of rod-shaped MTB, thus representing a larger phylogenetic group than previously thought (Fig. 5A).

Another feature unique to the magnetotactic cocci from marine environments is their very fast swimming speeds (up to 300 μm/s) and the presence of a sheath that surrounds each of their two flagellar bundles (49, 113–115). The marine magnetotactic ovoid bacterium MO-1 has a flagellar propeller with a complex spatial organization and flagellin composition (116). Each flagellar bundle in cells of MO-1 consists of 7 individual flagella, 6 of whose cellular origins appear to be organized as a hexagon, with a seventh in the middle (115, 116). The flagella in bundles of both strain MO-1 and *Mc. marinus* originate from a depressed area or pitlike structure on the cell (48, 115). Fourteen transcribed flagellin or putative flagellin genes have been identified in strain MO-1, and some of these respective proteins are glycosylated (116).

***Magnetovibrio blakemorei*.** The marine vibrio *Magnetovibrio blakemorei* strain MV-1 was isolated from sulfide-rich sediments in a salt marsh near Boston, MA (69). It also has a respiratory metabolism, using oxygen, nitrate, and nitrous oxide (N_2O) as terminal electron acceptors (69). It grows chemoorganoheterotrophically with organic and some amino acids as carbon and electron sources (11, 38, 69) and also grows chemolithoautotrophically using reduced sulfur compounds as an electron source (87). This strain utilizes the CBB cycle for autotrophy: cell extracts display RubisCO activity, and the strain possesses a form II RubisCO gene (87). *Mv. blakemorei* also grows chemoorganoautotrophically with formate as the electron donor (87). This strain shows nitrogenase activity under both heterotrophic and autotrophic conditions (11, 38). Among characterized MTB of the *Alphaproteobacteria*, *Mv. blakemorei* shows the greatest metabolic versatility in the compounds that can be used as potential electron donors and carbon sources for growth during microaerobic and anaerobic growth (38). Strain MV-2, which shares 100% similarity of its 16S rRNA gene sequence with *Mv. blakemorei* (43), was isolated from water collected from the oxic-anoxic interface of the Pettaquamscutt River Estuary, RI (43), and recently, several similar closely related strains have been isolated from other coastal habitats. Strain MV-2 has the same metabolic capacities as *Mv. blakemorei* (87). Cells of *Mv. blakemorei* are vibrioid to helicoid in morphology. Cells are motile by means of a single polar flagellum and possess a single chain of magnetosomes containing truncated hexoctahedral crystals of magnetite, positioned along the long axis of the cell (38) (Fig. 6B).

Phylogenetically, *Magnetovibrio blakemorei* is a member of the *Rhodospirillaceae* within the *Alphaproteobacteria*. Its exact position in the *Rhodospirillaceae* is difficult to resolve; however, its closest nonmagnetotactic relatives appear to be *Terasakiella pusilla*, *Thalassospira lucentensis*, and "*Candidatus* Kopriimonas byunsanensis" (38). Among MTB, the 16S rRNA gene sequence of *Mv. blakemorei* has the highest level of identity with those of *Magnetospira thiophila* (89.2%) and strain QH-2 (89.2%) (Fig. 6A).

***Magnetospira thiophila* strain MMS-1 and strain QH-2.** *Ma. thiophila* was isolated from a salt marsh in Woods Hole, MA (95). *Ma. thiophila* was previously studied under the name "MV-4" (magnetic vibrio number 4) (95), but the morphology is best characterized as a spirillum rather than a vibrio, since cells are bipolarly flagellated, although the cell morphology is somewhat variable (52) (Fig. 6C). Strain QH-2 is an uncharacterized marine magnetotactic spirillum isolated from an intertidal zone of the China Sea (82). These cultured marine spirilla possess a single chain of magnetosomes containing elongated octahedral crystals of magnetite positioned along the long axis of the cell (Fig. 6C and D); they appear to be obligate microaerophiles that grow with organic acids as carbon and electron sources (52, 82). Chemolithoautotrophic growth is also supported in *Ma. thiophila* by thiosulfate but not sulfide (11). The latter species also displays nitrogenase activity under heterotrophic and autotrophic conditions (11, 52). In contrast to their closest characterized magnetotactic relative, *Mv. blakemorei*, *Ma. thiophila* and strain QH-2 can use only a relatively small number of organic acids as carbon and energy sources (38, 52).

Ma. thiophila and strain QH-2 share a 16S rRNA gene sequence similarity of 97% and thus are considered congeneric species (52). Despite low bootstrap values in internal nodes, the inferred tree clearly suggest that these MTB form a clade within the *Rhodospirillaceae* family in the *Alphaproteobacteria* along with *Thalassospira lucentensis*, *Terasakiella pusilla*, "*Ca*. Kopriimonas byunsanensis," and *Mv. blakemorei* (Fig. 6A).

Deltaproteobacteria

Known MTB of the *Deltaproteobacteria* class are present in two orders, the *Desulfovibrionales* (e.g., *Desulfovibrio* and *Desulfonatronum*) (54) and the *Desulfobacterales* (e.g., "*Ca*. Magnetoglobus" and "*Ca*. Desulfamplus") (26, 42) (Fig. 7A). The *Deltaproteobacteria* class contains both magnetite- and greigite-producing MTB and includes the magnetite-producing, rod-shaped sulfate reducers *Desulfovibrio magneticus* strain RS-1 (54, 117) (Fig. 7B) and strain FH-1 (50); several similar strains of obligately alkaliphilic, sulfate-reducing, magnetite-producing vibrios isolated from extremely alkaliphilic habitats in California (28) (Fig. 7C); a group of uncultured and two cultured (strains BW-1 and SS-2) large rod-shaped bacteria that biomineralize either or both minerals

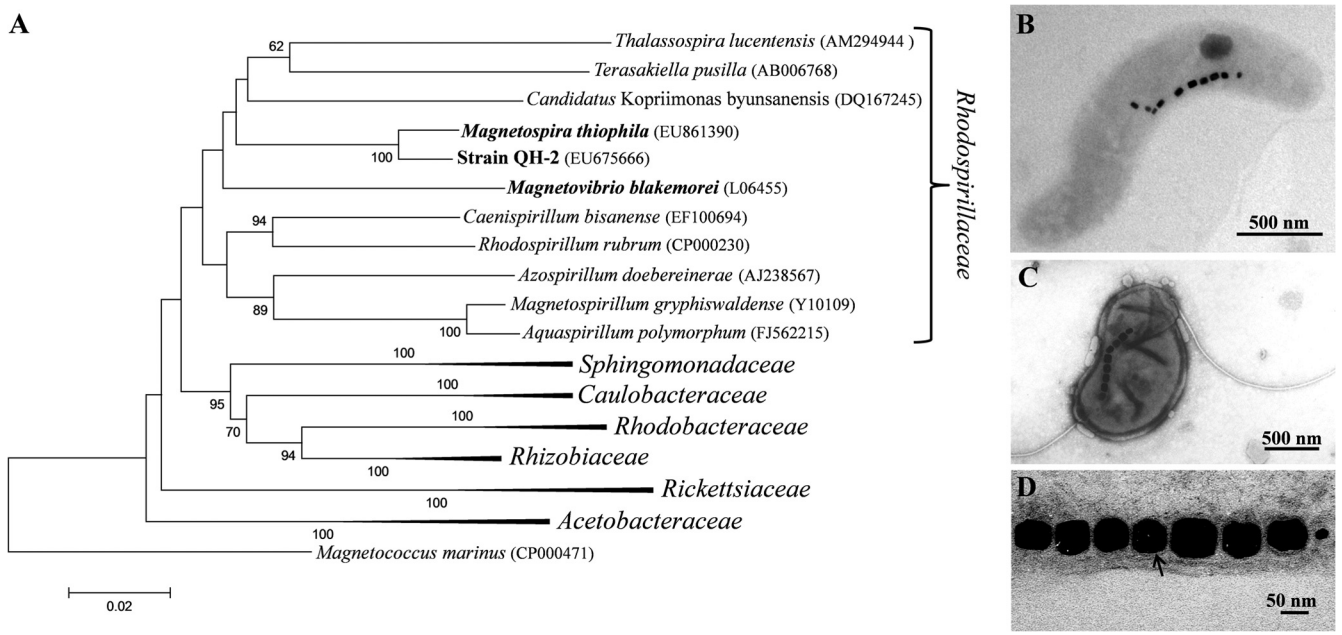

FIG 6 (A) Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic positions of the magnetotactic marine spirilla *Magnetospira thiophila* and strain QH-2 and the magnetotactic marine vibrio *Magnetovibrio blakemorei* in the family *Rhodospirillaceae* (in boldface type). Bootstrap values (higher than 50) at nodes are percentages of 1,000 replicates. The bar represents 2% sequence divergence. GenBank accession numbers are in parentheses. (B to D) TEM images of a cell of *Magnetovibrio blakemorei* (B), a cell of *Magnetospira thiophila* (C), and a thin-sectioned cell of *Magnetospira thiophila* showing the magnetosome chain consisting of elongated octahedral crystals of magnetite surrounded by the magnetosome membrane (arrow) (D).

(26, 118–120) (Fig. 7D); and various forms of the uncultured MMPs which biomineralize either or both minerals (42–45, 121) (Fig. 7E). All magnetotactic *Deltaproteobacteria* are mesophilic based on their growth temperature or the temperature of their habitats.

MMPs. 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 magnetotactic multicellular aggregates [MMAs] [122, 123], magnetotactic multicellular organisms [MMOs] [124], and magnetotactic multicellular bacteria [MMB] [74]). The acronym MMP originally represented many-celled magnetotactic prokaryotes (125), because it was difficult to prove that the organism was truly multicellular. Based on 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., see reference 45). Three MMPs have been tentatively named: "*Candidatus* Magnetoglobus multicellularis" (42), "*Ca*. Magnetomorum litorale" (45), and "*Ca*. Magnetananas tsingtaoensis" (121) (Fig. 7A).

MMPs are relatively large for prokaryotic microorganisms and range from about 3 to 12 μm in diameter (125, 126) (Fig. 7E). They are best described as an aggregation of about 10 to 60 Gram-negative, genetically similar cells that swim only as an intact unit and not as individual cells (44, 124–126). Cells that become separated from the intact unit die quickly (127). Cells are asymmetrically flagellated, with the surface of the cell exposed to the surrounding environment and covered with numerous flagella (125, 128). Most described MMPs are spherical (42, 45, 124–126, 129), although some are ovoid or pineapple shaped in morphology (121, 130), and they all appear to possess a central, acellular compartment (124, 129, 131). Wall structures between cells similar to eukaryotic gap junctions have been described for one MMP (125). The MMP divides as aggregates without an individual-cell stage (121, 129, 132).

MMPs are cosmopolitan in distribution, being found in numerous saline aquatic environments ranging from brackish to hypersaline (19, 42, 80, 124, 132). In all cases, the salinity is due to the input of seawater, and many have considered these organisms indigenous to marine environments only (44). Recently, nonmagnetotactic forms of MMPs (referred to as nMMPs) were found in springs and lakes with relatively low salinities (~5 to 11 ppt) and no marine input (80). Little is known regarding their physiology, but it seems very likely that MMPs and nMMPs are sulfate-reducing bacteria based on the facts that their closest phylogenetic relatives are sulfate reducers (43, 44) (Fig. 7A) and that the genes for dissimilatory sulfite reductase (*dsrAB*) and dissimilatory adenosine-5′-phosphate reductase (*aprA*) have been detected in purified samples of MMPs collected from the environment (45). Recently, Abreu et al. (unpublished) became the first researchers to successfully culture an MMP, "*Ca*. Magnetoglobus multicellularis," by exploiting genomic data that suggested that this MMP respires sulfate and oxidizes succinate and acetate.

The magnetic mineral greigite in MTB was first discovered in MMPs (24, 25). Since then, they have also been found to contain nonmagnetic precursors to greigite (133, 134), magnetite (121, 135), or both magnetite and greigite magnetosomes (136). The greigite crystals in magnetosomes of MMPs are generally pleomorphic, although cuboctahedral, elongated-prismatic, and bullet-shaped particles have been observed (24, 133, 134). The nonmagnetic precursors to greigite include mackinawite (tetragonal FeS) and a sphalerite-like cubic FeS (133, 134). Only bullet-shaped magnetite crystals have yet been found in magnetosomes of MMPs (121, 135, 136). Magnetosomes are usually loosely ar-

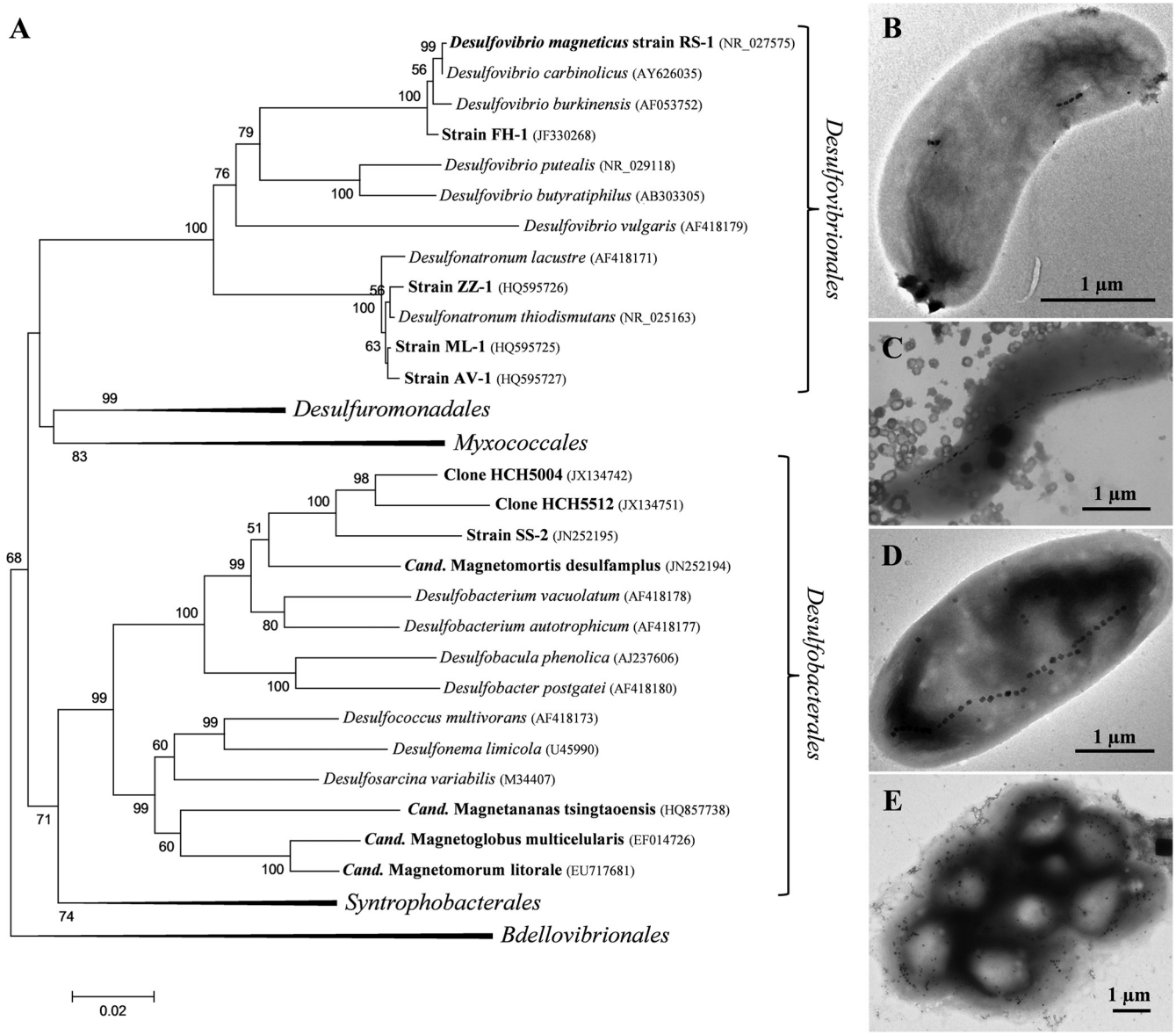

FIG 7 (A) Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic positions of the magnetotactic *Deltaproteobacteria* (in boldface type) of the orders *Desulfovibrionales* and *Desulfobacterales*. Bootstrap values (higher than 50) at nodes are percentages of 1,000 replicates. The bar represents 2% sequence divergence. GenBank accession numbers are in parentheses. (B to E) TEM images of a cell of *Desulfovibrio magneticus* (B); a cell of strain AV-1, an obligately alkaliphilic magnetotactic bacterium isolated from a brackish spring in Armagosa Valley, CA (C); a cell of a greigite-producing, large rod-shaped bacterium collected from a spring at ambient temperature in the Great Boiling Springs geothermal field in Gerlach, NV (D); and a greigite-producing, magnetotactic multicellular prokaryote (MMP) collected from the Salton Sea, CA (E).

ranged in short chains or clusters in individual cells (24, 45, 133, 134, 136), although there is a general-enough consensus in magnetosome arrangement that there is a net magnetic dipole moment to the entire unit (45, 124). It has also been shown that magnetosome chain polarities of individual cells contribute coherently to the total magnetic moment of the MMP in a highly coordinated fashion, suggesting a remarkable degree of magnetic optimization, which is likely inherited by individual cells by a sophisticated reproduction mechanism (131, 137).

The type of magnetotaxis displayed by the MMP appears to be polar (see the section on magneto-aerotaxis). An interesting feature displayed by MMPs in hanging drops viewed by light microscopy is the so-called "ping-pong" motility (125) in magnetic fields at least several times stronger than the Earth's magnetic field (~0.5 G) (138). After reaching the edge of a water drop, individual MMPs spontaneously swim in a direction opposite their initial swimming direction in short excursions of about 100 to 500 μm at twice the speed of the forward motion in the opposite direction, after which they return to the same edge of the drop at a lower speed (125). This unique behavior has also been coined "escape motility" (129), although the significance of this peculiar motion is unclear.

Phylogenetically, the MMPs form a separate clade in the family *Desulfobacteraceae* (Fig. 7A). This clade is composed of several

genera of multicellular organisms having variable sizes and morphologies, biomineralizing either magnetite, greigite, or both (43–45, 121) or, in the case of the nMMPs, known not to biomineralize magnetosomes (80). The closest relatives of the MMPs are species of the genera *Desulfosarcina*, *Desulfonema*, and *Desulfococcus*.

Desulfovibrio magneticus. *Desulfovibrio magneticus* was isolated from a waterway near Kameno River, Wakayama prefecture, in western Japan (117). It is an obligate anaerobe that grows and respires with sulfate or fumarate (54, 117). Like all *Desulfovibrio* species, cells are curved rods that possess a single polar flagellum and show no potential for autotrophic growth (Fig. 7B). Low-molecular-weight organic molecules and some organic acids support chemoorganoheterotrophic growth in this organism. It is the only cultured magnetotactic bacterium known to be capable of fermentation: pyruvate is fermented to acetate and hydrogen (54). It was shown that cells of *Dv. magneticus* produce more magnetosomes when grown under fermentative conditions (59). A similar MTB that likely represents a strain of *Dv. magneticus*, strain FH-1, was isolated in axenic culture from water and mud collected from a fish hatchery in Montana (50) and presents the same metabolic capabilities as *Dv. magneticus* (our unpublished data).

Phylogenetically, *Dv. magneticus* and strain FH-1 belong to the *Desulfovibrionaceae* family along with all *Desulfovibrio* species (Fig. 7A). Their closest characterized relatives based on 16S rRNA gene sequence comparison are *Dv. carbinolicus* (99.5% similarity with the 16S rRNA gene sequence of *Dv. magneticus*) and *Dv. burkinensis* (98.6% similarity with the 16S rRNA gene sequence of *Dv. magneticus*).

Alkaliphilic magnetotactic bacteria. Recently, three strains of obligately alkaliphilic, obligately anaerobic, sulfate-reducing MTB belonging to the *Deltaproteobacteria* with optimal growth pHs of 9.0 to 9.5 were isolated and grown in axenic culture (28). 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 (46), based on the very high sequence identities of their 16S rRNA genes (28) (Fig. 7A). Like *Dn. thiodismutans*, cells are vibrioid to helicoid in morphology and possess a single polar flagellum (Fig. 7C). All strains grow autotrophically and possibly mixotrophically with hydrogen as an electron donor. Formate is also utilized as an electron donor.

Large rod-shaped bacteria. The slow-moving, large rod-shaped MTB were the first organisms described to produce both magnetite and greigite in the same cell (118). It was hypothesized early on that the formation of magnetite and/or greigite by these bacteria was regulated by external environmental conditions such as redox and/or oxygen or hydrogen sulfide concentrations (139). However, it was only recently that this was confirmed, and the phylogenetic position and physiology of these organisms were determined (26). "*Ca*. Desulfamplus magnetomortis," 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 this group of large rod-shaped bacteria that biomineralize greigite and/or magnetite. "*Ca*. Desulfamplus magnetomortis" grows chemoorganoheterotrophically using sulfate as a terminal electron acceptor and produces both minerals, with the dominant mineral present being dependent upon culture conditions (e.g., sulfide concentration). The greigite crystals appear to be pleomorphic, while those of magnetite are bullet shaped, like those of all other magnetotactic *Deltaproteobacteria* (26). Noncultured MTB similar in morphology and phylogenetically related to "*Ca*. Desulfamplus magnetomortis" and strain SS-2 were also reported from different aquatic environments in the United States and in the city moat in Xi'an City, China (26, 120).

"*Ca*. Desulfamplus magnetomortis" and strain SS-2, together with other morphologically related uncultured MTB from other environments, belong to a previously unrecognized group of sulfate-reducing bacteria in the *Desulfobacteraceae* family that does not contain any other known cultured bacteria (26) (Fig. 7A and D). This clade appears to consist of at least two smaller groups, each constituting at least two genera, based on 16S rRNA gene sequence divergence (26, 120). The closest relatives of this group of MTB are species of the genus *Desulfobacterium*.

Symbiotic MTB. The anaerobic oxidation of methane is thought to be mediated by syntrophic consortia of methanotrophic *Archaea* and dissimilatory sulfate-reducing bacteria (140). Vibrioid, dissimilatory, sulfate-reducing MTB have been implicated as symbiotic partners in consortia responsible for the anaerobic oxidation of methane in black microbial mats associated with cold methane seep concretionary carbonate buildups in the anoxic sediment of the Black Sea (141, 142). Immunogold labeling of these cells using an antibody specific to the dissimilatory adenosine-5′-phosphosulfate reductase (a key enzyme in dissimilatory sulfate reduction) β-subunit resulted in a strong positive signal (142). The magnetosome crystals, arranged in a chain within the cells, were shown to consist of an iron sulfide (141). Although the mineral was not identified and the specific phylogenetic affiliation of these MTB was not determined, it seems very likely that these MTB biomineralize greigite and belong to the *Deltaproteobacteria*. The role of magnetosome formation must be questioned here, as these organisms are embedded in an extracellular polymeric substance (EPS) matrix in the biofilm, which likely prevents magnetotaxis (142).

Gammaproteobacteria

Only two cultured MTB, designated strains BW-2 and SS-5, together with two uncultured related magnetotactic *Gammaproteobacteria* collected from the city moat in Xi'an City, China, have been reported to unequivocally belong to the *Gammaproteobacteria* class (53, 120). Thus, there is little information regarding the extent of the diversity of MTB in this group. Strain BW-2 was isolated from sediment and water collected from a brackish, sulfidic spring at Badwater Basin in Death Valley, CA, in which the dominant MTB were greigite-producing rods, as discussed above (26, 53). Cells are motile by a single polar, unsheathed bundle of seven flagella (53). This strain is known only to grow chemolithoautotrophically using sulfide and thiosulfate as electron donors. Cells produce intracellular sulfur globules, and thiosulfate is oxidized completely to sulfate (53). Cells show nitrogenase activity. Strain SS-5 was isolated from sediment and water collected from the southeastern shore of the hypersaline Salton Sea, CA (53). Cells possess a single polar flagellum. Like those of BW-2, cells grow chemolithoautotrophically with sulfide and thiosulfate (which is oxidized completely to sulfate) but also show potential for heterotrophic growth on succinate. Although they do not produce discernible intracellular sulfur globules, they synthesize large deposits of phosphate-rich inclusions. Unlike all MTB tested, SS-5 did not show nitrogenase activity. Both organisms are mesophilic,

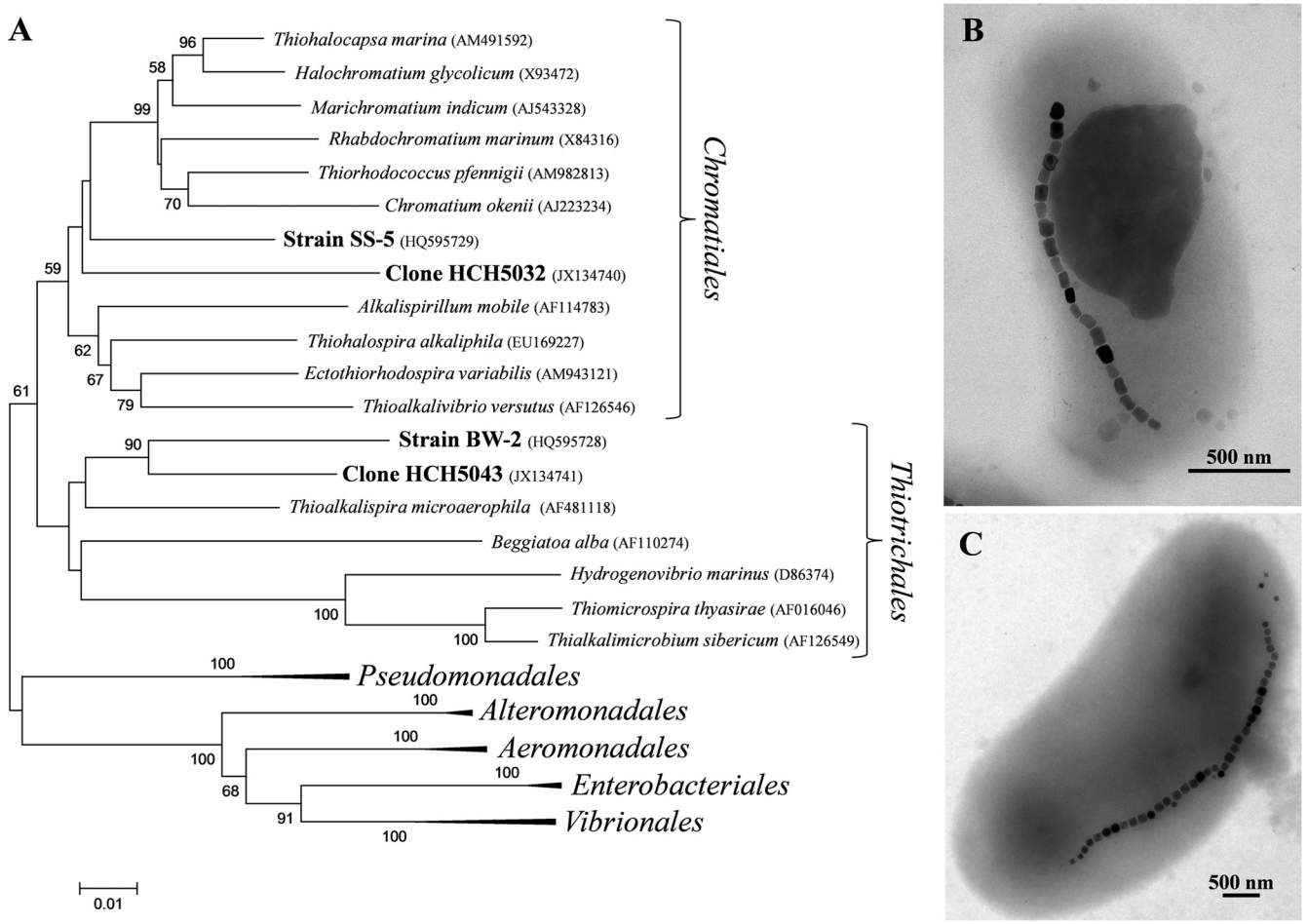

FIG 8 (A) Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing phylogenetic positions of the magnetotactic *Gammaproteobacteria* (in boldface type) of the orders *Chromatiales* and *Thiotrichales*. Bootstrap values (higher than 50) at nodes are percentages of 1,000 replicates. The bar represents 1% sequence divergence. GenBank accession numbers are in parentheses. (B and C) TEM images of a cell of strain SS-5 (B) and a cell of strain BW-2 (C). Cells of strains SS-5 and BW-2 biomineralize prismatic elongated and cuboctahedral magnetite magnetosomes, respectively.

microaerophilic rods and biomineralize either cuboctahedral or elongated prismatic crystals of magnetite in their magnetosomes, like the alphaproteobacterial MTB (53) (Fig. 8B and C).

Strains BW-2 and SS-5 are not closely related: phylogenetically, strain BW-2 belongs to the *Thiotrichales* order, whereas SS-5 belongs to the *Chromatiales* (53) (Fig. 8A). Based on the divergence of their 16S rRNA gene sequences (they have a 16S rRNA gene sequence identity between them of 87.9%), BW-2 and SS-5 clearly represent new genera in the *Gammaproteobacteria* (53). The organisms in culture with the highest 16S rRNA gene sequence identities to SS-5 belong to the *Chromatiales*, including species of the genera *Thiohalocapsa* and *Thiorhodococcus*. The organisms in culture with the highest 16S rRNA sequence identities to BW-2 are species of the genera *Thiohalospira*, *Thioalkalispira*, and *Thioalkalivibrio*.

Nitrospirae

Thus far, no magnetotactic *Nitrospirae* have been isolated in axenic culture. However, four different types of uncultured MTB phylogenetically associated with this phylum have been described in reasonably good detail. The large rod "*Ca*. Magnetobacterium bavaricum" is the most studied and was first discovered in sediment samples from Lake Chiemsee and Lake Ammersee in southern Germany (143, 144). Another magnetotactic member of the *Nitrospirae*, designated strain MHB-1, is a small rod-shaped bacterium collected from sediment of the Waller See, Germany (145). Recently, two new *Nitrospirae* have been described: a moderately thermophilic species tentatively named "*Ca*. Thermomagnetovibrio paiutensis" strain HSMV-1, found in brackish hot springs within the Great Boiling Springs geothermal field in Gerlach, NV (27), and a large ovoid-shaped organism tentatively named "*Ca*. Magnetoovum mohavensis" strain LO-1 from freshwater sediments of Lake Mead, NV (32). Uncultured organisms closely related to "*Ca*. Magnetobacterium bavaricum" and "*Ca*. Magnetoovum mohavensis," isolated from freshwater lakes in Beijing, China, were also recently described (146, 147). All known magnetotactic *Nitrospirae* biomineralize bullet-shaped crystals of magnetite (Fig. 2B and 9B to D).

"*Ca*. Magnetobacterium bavaricum." The cell morphotype of "*Ca*. Magnetobacterium bavaricum" was first observed in samples of littoral sediments collected from Lake Chiemsee and Lake Ammersee in southern Germany (143, 144) (Fig. 9C). Since then, "*Ca*. Magnetobacterium bavaricum"-like cells have also been found in

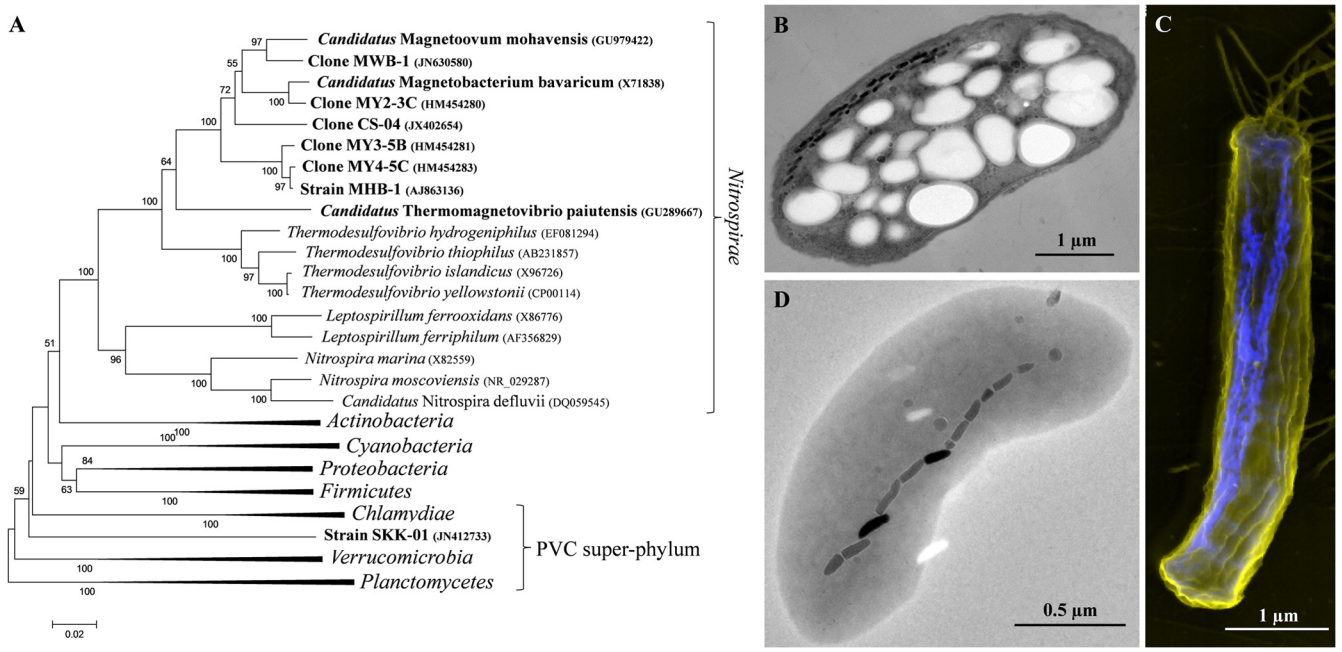

FIG 9 (A) Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing phylogenetic positions of the magnetotactic *Nitrospirae* and strain SKK-01, the only known magnetotactic bacterium belonging to the OP3 division of the PVC (*Planctomycetes-Verrucomicrobia-Chlamydiae*) superphylum. Bootstrap values (higher than 50) at nodes are percentages of 1,000 replicates. The bar represents 2% sequence divergence. GenBank accession numbers are in parentheses. (B) TEM image of a cell of "*Candidatus* Magnetoovum mohavensis." (C) False-color scanning TEM image of a cell of "*Ca.* Magnetobacterium bavaricum." Magnetosome crystals consisting of magnetite are visualized through back-scattered electrons (magenta) by material contrast. (D) TEM image of a cell of the uncultured "*Ca.* Thermomagnetovibrio paiutensis." All magnetotactic *Nitrospirae* biomineralize bullet-shaped crystals of magnetite in their magnetosomes. (Panel C courtesy of G. Wanner and D. Schüler, reproduced with permission.)

Brazil (148), France (89), and China (92, 146, 149). Because of its large size, volume, and relative abundance, "*Ca.* Magnetobacterium 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 (34). In addition, 16S rRNA sequences very similar to that of "*Ca.* Magnetobacterium bavaricum" (>99% identity) have been retrieved from a number of freshwater and marine habitats and biological reactor columns (150).

Cells of "*Ca.* Magnetobacterium bavaricum" are large rods having dimensions of 1 to 1.5 μm by 6 to 9 μm and are motile by a single polar tuft of flagella (Fig. 9C). Cells contain between 600 and 1,000 magnetosomes, which contain bullet-shaped crystals of magnetite that range from 110 to 150 nm in length and are organized in three to six braid-like bundles (generally five per cell) of multiple chains (39, 150–153). Magnetosome bundles are arranged around a central core and form a regular rosette-like bundle, which is situated just beneath the cytoplasmic membrane (CM) and appears to be distributed preferentially within a roughly semicircular segment along the periphery of cells (39). Many of the crystals display a kink or hooklike feature. The average total magnetic moment per cell was experimentally determined to be approximately an order of magnitude higher than that for most other MTB. Large amounts of bullet-shaped magnetite crystals have been found in some sediments where "*Ca.* Magnetobacterium 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 (143, 154).

"*Ca.* Magnetobacterium bavaricum" displays polar magnetotaxis, and in an 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 swimming in the opposite direction for a short time (34).

Because "*Ca.* Magnetobacterium bavaricum" is found mainly in the microaerobic zone (OAI) of sediments and contains sulfur-rich globules, it is thought to be a microaerophilic, sulfide-oxidizing bacterium (34, 150). 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.* Magnetobacterium bavaricum," and although these RubisCO-like proteins do not exhibit RubisCO enzymatic activity (155), it may be linked to sulfur metabolism in this organism (150).

Phylogenetically, "*Ca.* Magnetobacterium bavaricum" and "*Ca.* Magnetobacterium bavaricum"-like MTB form a separate clade in the *Nitrospirae* and are not related to the other nonmagnetotactic members of this phylum (Fig. 9A). The closest 16S rRNA gene sequence from a nonmagnetotactic, cultured bacterium belongs to the *Nitrospirae* organism *Thermodesulfovibrio hydrogeniphilus*, with 86% gene sequence similarity.

Strain MHB-1. Strain MHB-1 is a small, rod-shaped bacterium collected from sediment of the Waller See, Germany (145). 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. Strain MHB-1 falls into the *Nitrospirae* phylum, with 91% 16S rRNA gene sequence similarity to "*Ca.* Magnetobacterium bavaricum," with which it shares the same branch in this phylum (Fig. 9A).

Thermophilic MTB. Uncultured "*Ca.* 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, NV (27). Cells are small vibrios with a single polar flagellum (Fig. 9D). The upper limit of growth of this bacterium is probably a temperature of around 63°C, as it was not present in springs with higher temperatures.

The 16S rRNA gene sequence of strain HSMV-1 places the organism in the phylum *Nitrospirae*, with its closest relative in culture, based on 16S rRNA gene sequence identity, being *Thermodesulfovibrio hydrogeniphilus* (87% identity of their 16S rRNA gene sequences) (156). Strain HSMV-1 has 87% 16S rRNA gene sequence similarity with the unnamed rod-shaped bacterium strain MHB-1 and 86% similarity with "*Ca.* Magnetobacterium bavaricum." Phylogenetically, "*Ca.* Thermomagnetovibrio paiutensis" comprises a separate branch between that of *Thermodesulfovibrio* species and that of the group formed by all other magnetotactic *Nitrospirae* (27) (Fig. 9A).

Large ovoid *Nitrospirae*. "*Ca.* Magnetoovum mohavensis" strain LO-1 was discovered in samples of freshwater sediments collected from Lake Mead, NV, left at room temperature in the dark for about 5 months of storage (32). This bacterium is relatively large and 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 (Fig. 2B and 9B). Although the organism is Gram negative, it appears to have an unusual three-layer cell wall. This organism may be widely distributed, as similar organisms have been observed in and collected from freshwater and estuarine environments including the Exeter River, NH (157, 158); the Pettaquamscutt Estuary, RI (159); several sites in Germany (10, 22); and freshwater lagoons (Jacarepiá Lagoon, Saquarema) and brackish waters (Lagoa de Cima, Rio de Janeiro) in southeastern Brazil (148). Like those of "*Ca.* 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" in that the majority of cells were found at the OAI and the upper layer of the anaerobic zone (32, 150). In semisolid oxygen gradient medium, however, cells immediately migrated to the anoxic zone and remained viable for several days. These results indicate that "*Ca.* Magnetoovum mohavensis" might be an anaerobe that tolerates low concentrations of oxygen (32). 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" (147). The watermelon-shaped strain MWB-1 appears to account for >10% of the natural remanent magnetization of the surface sediment of Lake Beihai (147). Phylogenetically, "*Ca.* Magnetoovum mohavensis" and strain MWB-1 form a separate clade in the group formed by all magnetotactic *Nitrospirae* (Fig. 9A).

Another MTB morphologically similar to "*Ca.* Magnetoovum mohavensis," designated CS-04, was recently collected from sediments of Lake Chiemsee (40). Although CS-04 is affiliated with the *Nitrospirae* phylum, it is not very closely related to "*Ca.* Magnetoovum mohavensis" or to strain MWB-1, suggesting that the phylogenetic diversity of the large ovoid magnetotactic *Nitrospirae* is greater than what is currently recognized (40).

Other Phyla

Recently, by using single-cell-based techniques, a new MTB, designated strain SKK-01, was discovered in low abundance at the OAI in sediments of Lake Chiemsee and was found to belong to the candidate OP3 division of bacteria, which thus far lacks any cultured representatives (160) based on 16S and 23S rRNA gene sequences (30) (Fig. 9A). Strain SKK-01 is a large ovoid bacterium with spherical intracellular sulfur inclusions that occupy a major portion of the cell volume. Cells of SKK-01 possess two polar filaments or tufts of flagella and contain bullet-shaped magnetosomes organized in multiple bundles of 5 to 7 magnetosome chains, traversing the cell along its length. The morphology of strain SKK-01 is similar to those of some *Nitrospirae*, including "*Ca.* Magnetoovum mohavensis" and strain MWB-1 (30). This discovery indicates that the diversity and phylogenetic distribution of MTB are underestimated and may extend to other phylogenetic groups currently not known to contain MTB.

Magnetotactic Eukaryotes

A euglenoid alga, discovered in brackish mud and water samples collected from a coastal mangrove swamp near Fortaleza, Brazil, was reported to be magnetotactic some years ago (161). This organism was tentatively identified as *Anisonema platysomum* and contained numerous, well-organized chains of bullet-shaped magnetite crystals. Since this report, other protists have been discovered to be magnetotactic and contain magnetosomes (162). The origin of these putative "magnetosomes" in magnetotactic protists became an important question (163). Two scenarios are possible: (i) the protists biomineralize the magnetite crystals themselves, or (ii) the protists ingest MTB and/or bacterial magnetosomes from lysed cells and incorporate them either temporarily or permanently in the cell. Both possibilities seem to occur in nature. Because the arrangement of magnetosomes appears to be so precisely structured in the euglenoid alga described by Torres de Araujo et al. (161), it seems unlikely that this arrangement could occur after the ingestion of what would have to be significant numbers of MTB. Instead, it seems more likely that this organism biomineralizes and arranges endogenous magnetite crystals in a highly controlled fashion within the cell, where intracellular structural filaments play a significant role in the synthesis of the magnetosome chain, as has been shown for magnetotactic prokaryotes (164, 165). Moreover, some magnetotactic protists, including dinoflagellates, biflagellates, and ciliates, contain magnetosomes that are not well organized in the cell and thus probably ingest MTB and contain the bacterial magnetosomes for an undetermined amount of time (162, 163, 166).

Magnetotaxis has also been compared to magnetoreception, the ability of some higher vertebrates (e.g., salmons, pigeons, turtles, or bats) to use magnetic fields for orientation, navigation, and homing (167). Indeed, in some of these organisms, magnetoreception appears to be due to the presence of a linear chain(s) of biologically produced nano-sized single-domain magnetite crystals (167). An early publication raised the possibility that a magnetotactic bacterium could be the ancestral eukaryotic host cell (168).

Is the Known Diversity of Magnetotactic Bacteria an Underestimation?

MTB are ubiquitous in freshwater, brackish, and marine habitats, and many different cell morphotypes can be present in relatively large numbers in collected samples of water and sediment. Until recently, only MTB in relatively large numbers in samples (e.g., "*Ca*. Magnetobacterium bavaricum" [150]) or those isolated in axenic culture (e.g., *Magnetospirillum* spp. [64]) could be identified and characterized. With the use of single-cell separation techniques coupled with whole-genome amplification and fluorescent *in situ* hybridization for the authentication of a particular magnetotactic bacterium extracted from the environment, it is now possible to have a more accurate estimation of the biodiversity of MTB. For example, the discovery of a magnetotactic bacterium that belongs to the OP3 phylum would probably never occur without the use of these techniques, considering its low concentration in samples collected from Lake Chiemsee (30). By using this approach, it was also possible to identify and partially characterize eight different phylotypes of MTB of the *Alphaproteobacteria* and the *Nitrospirae* from freshwater (Lake Chiemsee, Germany) and marine (Wadden Sea near Cuxhaven, Germany) sediment samples (40).

Another interesting, important question to consider regarding the diversity of MTB is whether and how many magnetotactic prokaryotes have been isolated and deposited in culture collections but have never been recognized as magnetotactic for various reasons. This may be most applicable to the sulfate-reducing bacteria, as all magnetotactic sulfate-reducing bacteria appear to have difficulty in biomineralizing magnetite magnetosomes in culture and display only a weak magnetotactic response (26, 28, 63). Magnetotactic sulfate-reducing bacteria seem to require a higher concentration of iron for magnetite biomineralization than other non-sulfate-reducing MTB (e.g., *Magnetospirillum* species) (~20 μM) and a low sulfide concentration, if sulfide is used as the reducing agent in the growth medium to prevent scavenging of iron from the medium. Thus, the systematic use of high concentrations of sulfide (e.g., 0.4 g/liter $Na_2S \cdot 9H_2O$ [46]), as a commonly used reducing agent in growth medium for sulfate-reducing bacteria (169), might preclude the formation of magnetite by magnetotactic sulfate reducers. A specific example is whether *Desulfonatronum thiodismutans*, isolated from Mono Lake, where one of the alkaliphilic MTB was also isolated, was magnetotactic when originally isolated. The same question could apply to *Desulfovibrio burkinensis* and *Dv. carbinolicus* based on their high phylogenetic relatedness to *Dv. magneticus* and strain FH-1. Many cultivated magnetite-producing MTB are known to lose their ability to produce magnetosomes relatively easily in culture (13, 170). It is thus possible that bacteria not described as magnetotactic were in fact magnetotactic when isolated from the environment or are still able to produce magnetosomes but lost or do not display this trait due to the use of growth media not appropriate for magnetosome formation.

Although some species, including *Desulfovibrio magneticus* and some greigite-producing species (e.g., "*Ca*. Desulfamplus magnetomortis"), are obligate anaerobes, most MTB 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 of MTB that are strictly anaerobes.

Another factor is that many studies involving the diversity of MTB have been performed by using samples collected in the immediate vicinity of the laboratories of researchers who work in this field. Although this may not seem significant, there are relative few groups or investigators studying the diversity of MTB, although this number is now increasing. As stated above, the diversity of MTB has been studied mainly in several locations in North America (2, 26–28, 31, 32, 44, 53, 75, 171), Brazil (19, 42, 86, 148, 166), China (17–21, 31–33), Germany (22, 34, 45, 93, 94, 96, 145, 150), and France (21, 49, 89). Clearly, then, little is known regarding MTB of the Southern Hemisphere. Thus, we believe that the diversity of MTB is greatly underestimated. In addition, this also demonstrates the need for biogeographical studies of MTB, particularly because their presence has been reported in all continents. By investigating new geographical locations and types of habitats where the diversity of MTB has not been examined, we hope to discover new MTB possibly belonging to taxa (e.g., *Archaea*) that have no known magnetotactic representatives, having types of metabolism (e.g., phototrophy) not now known to be associated with them, or living in unusual habitats (e.g., acidic lakes) not known to support their presence.

MAGNETOSOME FORMATION

Little is known regarding the biomineralization of greigite magnetosomes at the molecular level except that genes and proteins orthologous to those involved in magnetite formation have been identified in two genomes of greigite-producing MTB (26, 172); thus, this section is focused mainly on magnetite magnetosome synthesis. Virtually all the information regarding magnetite synthesis in MTB is based on studies involving two species of *Magnetospirillum*, *Ms. gryphiswaldense* and *Ms. magneticum*, that biomineralize cuboctahedral magnetite magnetosomes. The reason for this is that these organisms are relatively easy to grow, there are tractable genetic systems for these organisms, and their genome sequences are available. In both species, biomineralization of the bacterial magnetosome appears to be a complex process that involves several steps that temporally overlap during the lifetime of the cell.

Steps Involved in Magnetosome Chain Formation

The first step is the invagination of the cytoplasmic membrane (CM), which could result in either the formation of a membrane vesicle truly detached from the CM or a permanent invagination at the CM, an important question that remains unresolved. By using electron cryotomography, it has clearly been shown that the magnetosome membrane in *Magnetospirillum* species originated as an invagination of the CM and that magnetite precipitation occurs after the invagination is formed (164, 173). Presumably, there is some sorting of magnetosome membrane proteins during the invagination and/or membrane vesicle formation process (174), as it is clear that magnetosome membranes contains proteins that are not present in the CM. Different stages of magnetite precipitation have been observed within magnetosome membrane invaginations/vesicles. In *Magnetospirillum magneticum*, cells grown under conditions of iron limitation contain empty magnetosome invaginations/vesicles arranged in a chain attached to the CM (164). 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 detached membrane vesicles. Alternatively, this may be a result of an artifactual problem involving the technique (175, 176). It is also not known if this is a common characteristic of magnetite magnetosomes in all MTB. Results from parallel experiments with *Ms. gryphiswaldense* showed that empty magnetosome membrane vesicles are present in cells grown under conditions of iron limitation and also that magnetic cells contain, in addition to magnetite-filled magnetosome vesicles, many empty vesicles inside the cell (165). Vesicles in *Ms. gryphiswaldense* were also shown by cryo-electron tomography to result from invagination of the CM (173). However, most mature vesicles appeared to no longer be connected to the CM, and it was therefore hypothesized that nascent magnetosome particles become detached during maturation of magnetite crystals in this organism (177). Mature magnetosome membrane invaginations/vesicles probably become aligned in the chain motif during their formation.

Iron uptake by the cell is absolutely required for magnetosome synthesis and is likely occurring continually as long as it is available. Cells of cultured MTB are extremely proficient at iron uptake, as they have been shown to consist of >3% iron on a dry weight basis, a value several orders of magnitude higher than those for nonmagnetotactic bacterial species (41, 100). In addition, iron uptake for magnetite synthesis appears to occur relatively quickly (56, 100), while the rate of iron uptake appears to have an effect on the morphology of the magnetite magnetosome crystal in *Magnetospirillum* (228). It appears that both Fe(II) and Fe(III) can be taken up by cells of MTB for magnetite synthesis (57, 61, 62). How iron is taken up by MTB is unknown, but it would seem that there would be multiple mechanisms for this in a single bacterium, as has been found for other nonmagnetotactic bacteria. Thus far, siderophores, low-molecular-weight ligands produced by the cell that chelate and solubilize Fe(III) (178, 179), have been implicated in iron uptake by MTB (68, 180, 181) as well as in a putative copper-dependent iron uptake system similar to that found in the yeast *Saccharomyces cerevisiae* (68).

Iron must then have to enter the magnetosome invagination/vesicle. If magnetite crystals are truly formed in permanent invaginations of the CM, 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 be only 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 below). Based on Mößbauer spectroscopic analysis of *Magnetospirillum gryphiswaldense*, a mechanism was proposed by which iron required for magnetite biomineralization is processed through the CM directly to the magnetosome membrane without iron transport through the cytoplasm, suggesting that pathways for magnetite formation and biochemical iron uptake and assimilation are distinct (177). Magnetite formation is suggested to occur via the nucleation of membrane-associated crystallites at the CM, whereas the final step of magnetite crystal growth appears to be in mature magnetosome vesicles spatially separated from the CM. Evidence for the presence of distinct pathways for magnetite biomineralization and biochemical iron uptake and assimilation was further supported by using a mutant strain of *Ms. gryphiswaldense* in which the gene for a Fur-like iron uptake regulator was deleted (182). Results from this work revealed that Fur is involved in global iron homeostasis, probably by balancing the competing demands for biochemical iron supply and magnetite biomineralization. It was also shown that Fur in *Ms. gryphiswaldense* directly regulates genes involved in iron and oxygen metabolism, thereby influencing magnetosome biomineralization (182, 183).

Once iron enters the magnetosome invagination/vesicle, there is nucleation and controlled maturation of the magnetite crystal. Magnetite precipitation might occur through the reduction of hydrated ferric oxide(s) (23, 56, 184). However, when cells of *Ms. gryphiswaldense* were shifted from iron-limited to iron-sufficient conditions, they showed no delay in magnetite production (100), suggesting that no mineral precursors to magnetite are formed during biomineralization or that they are unstable and convert to magnetite extremely quickly. A time period of 15 min is sufficient for full-sized, mature magnetosomes after the addition of iron to iron-limited cells (185). In one study, immature magnetite magnetosome crystals were shown to contain a surface layer of nonmagnetic iron oxide-phase hematite (185).

The specificity for iron in the magnetosome mineral crystal appears to be very high. However, there are a number of reports of the presence of other transition metal ions in magnetite and greigite magnetosome crystals in both cultured and uncultured MTB. Trace amounts of titanium were found in magnetite particles of an uncultured freshwater magnetotactic coccus collected from a wastewater treatment pond (186). The incorporation of small amounts of cobalt in surface layers of magnetosome magnetite crystals was demonstrated for three *Magnetospirillum* species (187). Cells grown in cobalt-containing media showed very small changes in their magnetic properties, including the Verwey transition, compared to those of a control culture (188). These results indicate that cobalt was not incorporated into the lattice structure of the magnetite crystals (187). Uncultured MTB exposed to $MnCl_2$ in microcosms took up to 2.8% atomic manganese in ultrathin-sectioned magnetosomes, as detected via localized energy-dispersive X-ray analysis (189). 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 (190). The concentration of copper was extremely variable and ranged from about 0.1 to 10 atomic % relative to iron (190). Again, copper appeared to be concentrated mostly on the surface of the crystals.

Magnetosomes are organized as single or multiple chains in cells of almost all known MTB. In this arrangement, if the magnetosomes are organized head to tail magnetically, the maximal magnetic dipole moment of the cell is achieved. However, a string of magnetic dipoles has a tendency of collapsing to lower its magnetostatic energy, thereby creating a more magnetically stable situation (191). Thus, MTB have developed a dedicated cellular structure allowing for the assembly and maintenance of magnetosome chains. Indeed, it has been shown that the magnetosomes are aligned along an actin-like filament to form a chain (164, 165). Cryo-electron tomography of cells of *Ms. gryphiswaldense* and *Ms. magneticum* revealed the presence of a cytoskeletal network of

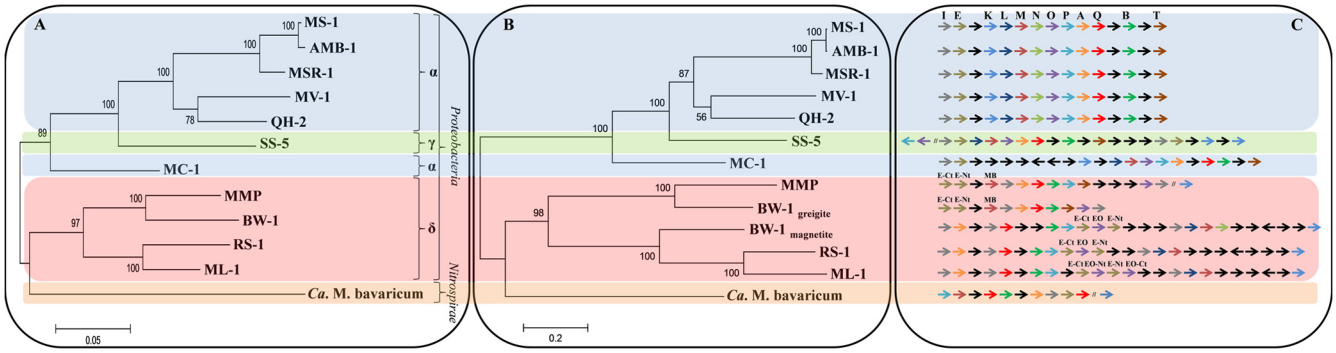

FIG 10 (A and B) Congruency of the phylogenetic trees based on 16S rRNA gene sequences that reflect the evolution of MTB (A) and on concatenated magnetosome protein sequences (MamABIKQ) that reflect the evolution of magnetotaxis (B). (C) Gene synteny (organization) of the conserved magnetosome genes of *Magnetospirillum magnetotacticum* (MS-1), *Ms. magneticum* (AMB-1), *Ms. gryphiswaldense* (MSR-1), *Magnetovibrio blakemorei* (MV-1), *Magnetospira* sp. strain QH-2, *Magnetococcus marinus* (MC-1), strain SS-5, the magnetotactic multicellular prokaryote "*Candidatus* Magnetoglobus multicellularis" (MMP), "*Ca*. Desulfamplus magnetomortis" (BW-1), *Desulfovibrio magneticus* (RS-1), strain ML-1, and "*Ca*. Magnetobacterium bavaricum."

filaments, 3 to 4 nm in diameter, which traverse the cells along their long axis. Magnetosomes are closely arranged along this magnetosome-associated cytoskeleton, which has been tentatively referred to as the magnetosome filament (192). The magnetosome chain thus represents one of the highest biological structural levels found in a prokaryotic cell and is considered by many to be a masterpiece of microbial mechanical engineering.

Genetic Determinants of Magnetosomes

The first genome sequence information from MTB regarding magnetosome synthesis was published at the turn of the 21st century and was based on partial genome sequences of *Magnetospirillum gryphiswaldense* (193). MTB-specific genes were identified and named the *mam* (magnetosome membrane) and *mms* (magnetic particle membrane-specific) genes. Later, similar genes were found in the genomes of other MTB of the *Alphaproteobacteria*, including *Magnetospirillum magneticum* (194), *Magnetovibrio blakemorei* (195), *Magnetococcus marinus* (107), and *Magnetospira* sp. strain QH-2 (229). Recently, it has been shown that *mam* genes, homologous to those of the magnetotactic *Alphaproteobacteria*, are also present in the genomes of the magnetotactic *Deltaproteobacteria*, including *Desulfovibrio magneticus* (196), the greigite-producing organisms "*Ca*. Magnetoglobus multicellularis" and "*Ca*. Desulfamplus magnetomortis" (26, 172) and the alkaliphilic strain ML-1 (50); the gammaproteobacterium strain SS-5; and the uncultured organism "*Ca*. Magnetobacterium bavaricum" of the *Nitrospirae* phylum (39) (Fig. 10C).

In the genomes of all MTB examined, magnetosome genes are present as clusters that are in relatively close proximity to one another. In *Magnetospirillum* species, *Magnetovibrio blakemorei*, and *Desulfovibrio magneticus*, magnetosome genes are organized as a genomic island called the magnetosome island (MAI) (170, 194–196), which shows evidence for an origin via horizontal transfer (see below). In *Magnetospirillum*, magnetosome genes form 3 operons, including the *mamAB*, *mamGFDC*, and *mms* operons (197). These operons appear to be conserved in all magnetotactic *Alphaproteobacteria* (198), but only the *mamAB* cluster is present in other groups of MTB (50). Recent deletion studies of *Ms. magneticum* and *Ms. gryphiswaldense* demonstrated that the *mamAB* cluster is the only operon containing genes that are absolutely essential for magnetite magnetosome biomineralization (174, 199, 200). Other operons or genes found in MTB, depending on their phylogenetic position or the type of magnetosome that they biomineralize, seem to have important accessory functions in controlling the size and morphology of magnetite magnetosome crystals (174, 199, 200). The *mamGFDC* and *mms* operons appear to be specific to the magnetotactic *Alphaproteobacteria* (201), while the so-called, recently described *mad* (magnetosome-associated deltaproteobacterial) genes seem to be specific to magnetotactic *Deltaproteobacteria* and *Nitrospirae* (50).

The genetic determinants responsible for the minimal set of universal functions required for magnetosome chain formation in all MTB lie within the *mamAB* operon. This operon contains 10 genes (*mamABEIKLMOPQ*) that are conserved in all magnetite-producing MTB, while 9 of these genes (*mamABEIKMOPQ*) are also conserved in greigite-producing MTB (50, 174, 199). Identifying the function of the proteins encoded by these conserved genes appears to be the key to understanding magnetosome biomineralization. Putative functions of these proteins, based on comparisons of similar proteins through BLAST searches and through mutagenesis experiments, have been predicted. The *mamI* and *mamL* genes encode proteins that are MTB specific, with no known homologues in other nonmagnetotactic bacteria (202), and do not contain known domains or recognizable sequence patterns. Experimental evidence, however, suggests that MamI and MamL might be involved in the invagination of the magnetosome membrane, the first step in magnetosome formation (174), although the specific mechanism by which this is mediated remains unclear. The functional domains that have been identified in the remainder of this core set of proteins through *in silico* and/or experimental evidence include (i) one tetratricopeptide repeat (TPR) (e.g., MamA) that participates in assembly of the magnetosome membrane through protein-protein interactions (203, 204), (ii) at least one cation diffusion facilitator (CDF) necessary for iron transport and magnetosome membrane assembly (e.g., MamB and MamM) (205), (iii) PDZ domains that mediate protein-protein interactions (e.g., two in MamE and one in MamP) (174, 199, 206), (iv) a LemA domain (MamQ) whose function is uncertain (174, 199), (v) at least four magnetochrome domains (two in MamP and two in MamE and/or MamT) that putatively ensure redox control and Fe^{2+}/Fe^{3+} stoichiometry

(207), and (vi) one or two actin-like domains (MamK) involved in magnetosome chain assembly and its positioning inside the cell (164, 173). There are also proteins with different multiple domains, such as the protease MamE, which contains trypsin, magnetochrome, and PDZ domains, or MamO, which contains one trypsin domain and one TauE domain (174, 199). These proteins and conserved domains appear to constitute the minimum set of genes required for the formation of magnetite and greigite magnetosomes in MTB (Fig. 10C).

These recent advances in genomics, proteomics, and genetics in MTB, as well as phylogenetic studies, provide great insight in how magnetotaxis evolved. Indeed, the presence of specific magnetosome genes and their organization within the genome in different phylogenetic groups of MTB that are not closely related can be used as molecular markers to study the origin and the evolution of this unique prokaryotic organelle responsible for magnetotaxis.

EVOLUTION OF MAGNETOTAXIS

Origin of Magnetotaxis

Magnetosome biomineralization is responsible for magnetotaxis in MTB. Thus, here we assume that the evolution of the genes involved in magnetosome formation reflects the evolution of magnetotaxis. The initial discovery that greigite- and magnetite-producing MTB were affiliated with two different major phyla, the *Deltaproteobacteria* and the *Alphaproteobacteria*, respectively, which represent two distinct evolutionary lines of descent, led DeLong et al. (43) to suggest that magnetotaxis based on iron sulfide and iron oxide magnetosomes had independent evolutionary origins. In other words, magnetite- and greigite-producing MTB belong to polyphyletic groups that include bacteria that have the trait of magnetotaxis in common, although the trait was not inherited from a common ancestor. At present, however, considering the now considerable amount of new genomic and phylogenetic information, it seems more likely that the magnetotactic trait is monophyletic and that it emerged once evolutionarily from a single common ancestor, regardless of magnetosome mineral composition (39, 172). Much of the evidence for this monophyletic origin of magnetotaxis is the result of the discovery of magnetosome genes in an uncultivated MMP from the *Deltaproteobacteria* (172) and in "*Ca.* Magnetobacterium bavaricum," belonging to the deeply branching *Nitrospirae* phylum (39), that are homologous to those genes previously found only in the remotely related magnetotactic *Alphaproteobacteria*.

At the genetic level, however, the monophyletic origin of magnetosome genes appears to be true only for those that are conserved in all MTB, i.e., the *mamAB* operon. At present, data suggest that the *mamAB* operon is the only carrier of genetic information that was transferred by the common ancestor of all MTB (50). Indeed, the genes present in the *mamGFDC* and *mms* operons specific to the magnetotactic *Alphaproteobacteria* appear to have been acquired independently by members of this group of MTB (50). This also seems true for the *mad* genes that appear to have emerged independently, as they are specific to the magnetotactic *Deltaproteobacteria* and *Nitrospirae* (50). If the same functions are encompassed by genes of the *mamGFDC* and *mms* operons and the *mad* genes (e.g., control of the size and shape of magnetosomes), this would mean that even if magnetotaxis evolved monophyletically, the latter genetic determinants involved in magnetosome formation evolved polyphyletically.

It was recently shown that all MTB have in common an *feoAB*-like gene cluster that is specific to MTB and generally in close proximity to the *mamAB* operon (50). The proteins encoded by this cluster, FeoA-like and FeoB-like proteins, are paralogous (they diverged after a duplication event and have similar but not identical functions) to the FeoA and FeoB proteins involved in iron transport and found in all bacteria (50, 208). Phylogenetically, FeoA-like and FeoB-like genes found in the genome of the magnetotactic *Alphaproteobacteria* appear to have a different evolutionary origin than that of the genes present in the genomes of the magnetotactic *Deltaproteobacteria* and *Nitrospirae* (50). In *Ms. gryphiswaldense*, the FeoB-like protein is involved in iron transport in magnetosomes (209). Thus, if the *feoAB*-like gene clusters in the *Alphaproteobacteria* and in the *Deltaproteobacteria* and *Nitrospirae* have the same function, it would imply that they have a polyphyletic origin.

Although results from recent studies indicate that magnetotaxis emerged only once during evolution, there remains the question of how the common ancestor of all MTB transferred its *mam* genes to the different phylogenetic groups of MTB.

Evidence for HGT of Magnetosome Genes in MTB

One of the most intriguing features of the MTB is their biodiversity and their wide phylogenetic distribution throughout the *Proteobacteria* and the *Nitrospirae* phyla as well as in the candidate OP3 division (Fig. 3). Even before recent progress in the genomics of MTB, it seemed to many that the most obvious hypothesis to explain the great diversity of MTB was HGT. Much of the support for this was the finding of a putative genomic island that encloses the genes involved in magnetosome formation described previously (197). For example, the genomic region that harbors the magnetosome genes in *Magnetospirillum gryphiswaldense* contains 42 mobile elements as transposases of the insertion sequence type and integrases (197). These mobile elements are common, important features in genomic islands (210, 211). Other characteristics of genomic islands include the presence of tRNA genes that act as insertion sites for integrases (212, 213) and a different guanine-plus-cytosine (G+C) content compared to that of the rest of the genome (214). In *Ms. gryphiswaldense*, the magnetosome gene region is about 130 kb, 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 (170, 197) that apparently have no function, as their deletions had no obvious effect on either growth or magnetosome formation (199). Therefore, it seems very likely that this genomic region represents a large MAI that appears to be present with variations in other cultured and uncultured MTB (195, 196, 202, 215). More evidence comes from the loss of magnetosome formation and, thus, magnetotaxis by some cultivated MTB relatively easily in culture following the loss of the MAI (68, 170).

Genes and genomic islands are reported to be distributed to different bacteria through HGT and thus may be a major pathway for the evolution of bacterial genomes (216). In addition, genomic islands are thought to undergo frequent gene rearrangements (216). Gene rearrangements, gene deletions, and duplications may be the reason for the frequent development of spontaneous nonmagnetotactic mutants of various strains (68, 170). Spontaneous deletions that lead to a loss of the magnetotactic phenotype with a frequency of 10^{-2} were observed under starvation condi-

tions in late-stationary-phase cultures of *Ms. gryphiswaldense* and most likely were caused by RecA-dependent homologous recombination between numerous repeats present in the MAI (197, 217). Frequent nonmagnetic mutants that do not synthesize magnetosomes were also observed in cultures of *Magnetovibrio blakemorei* (68) and *Ms. magneticum* (164, 215).

Rioux et al. (218) identified a separate group of *mam*-like genes, which includes *mamKDLJEFQ*-like genes, in the genome of *Ms. magneticum*. These genes are clustered as a genomic islet distinct and distant from the known MAI. In this study, *mamK*-like and *mamE*-like genes were shown to be transcribed. Several genetic features indicate that this magnetotaxis islet was acquired by HGT, rather than by simple genetic rearrangements of the MAI. Besides having a lower G+C content, numerous transposable elements and bacteriophage-related genes within or in the vicinity of the islet were identified. Moreover, phylogenetic analyses indicate that the origin of the genes in the magnetosome islet is distinct from that of the genes present in the MAI (218). Without completely deleting the islet and examining the phenotype of the strain, it is presently impossible to tell whether the islet has a specific function in magnetosome formation or any other process. It seems likely, however, that because it contains specific magnetosome genes, it might have some redundant functions with some of the magnetosome genes in the MAI.

The evolution of the MamA protein, one of the most conserved magnetosome-associated proteins, has been studied in distantly related MTB by analyzing the tertiary structure of this protein (219). It was shown that the folding of the MamA protein is highly conserved between MTB of the *Nitrospirae* phylum and those of the *Alphaproteobacteria* class. Zeytuni et al. (219) concluded that this result offers additional support for HGT of magnetosome genes by showing that MamA proteins present distinctive structural features unlikely to have evolved as parallel events.

Evidence for Vertical Transfer of Magnetosome Genes

A recent study showed that phylogenies based on 16S rRNA gene sequences and some amino acid sequences of housekeeping proteins of 11 MTB are congruent, that is, the pattern of divergence is similar to that of the phylogeny based on 9 concatenated magnetosome protein sequences (15). This indicates that the evolution and divergence of these proteins and the organisms' 16S rRNA genes occurred similarly and strongly suggests that magnetotaxis evolved vertically by descent. Indeed, if the trait of magnetotaxis was distributed to the many different MTB through recent HGT, this congruence would not be expected and instead would result in a phylogenetic tree of Mam proteins where MTB in the *Proteobacteria* formed a clade that is clearly not observed in the 16S rRNA gene tree (Fig. 10A and B). However, if this hypothesis is accurate, it does not preclude the possibility of ancient HGT of magnetosome genes or even recent HGT of magnetosome genes. A phylogenetic study focused on the evolution of the clade that contains *Magnetospirillum* and similar closely related species indicates that genes involved in magnetotaxis were acquired by a common ancestor of *Magnetospirillum* and transferred by descent to different MTB of this clade (64). However, for one species, there is evidence that magnetosome genes might have been acquired through HGT involving another species of *Magnetospirillum*, because in phylogenies based on the 16S rRNA gene sequence and Mam proteins, the positioning of its 16S rRNA gene sequence is not congruent with the positioning of its Mam proteins (64). Thus, based on currently available data, it seems likely that the genes for magnetotaxis were acquired by the different groups that contain MTB by descent, although HGT occurred and probably still occurs between phylogenetically closely related species.

The fact that the genes responsible for magnetosome formation are present in some MTB as a genomic island is one of the main lines of evidence for HGT between different groups of MTB. However, the presence of a putative magnetosome genomic island, based on genomic features described above, appears to occur only in the genomes of three *Magnetospirillum* species and *Desulfovibrio magneticus*. In the genomes of some other MTB, for example, in *Magnetococcus marinus* (107) and "*Ca*. Desulfamplus magnetomortis" (50), the magnetosome genes are organized as a cluster that does not show features of a genomic island. This might be an indication that the magnetosome genes in the latter organisms and other MTB were acquired at a much earlier time and that magnetosome genes are now stable within the chromosome. The fact that magnetosome genes have been shown to be organized as operons in *Magnetospirillum gryphiswaldense* (220) might explain why these genes continue to be present as clusters, as genes organized as operons have selective pressure to keep these genes as a single unit during evolution. Thus, conservation of gene clusters is not necessarily evidence for HGT (221, 222).

Variations in the gene synteny of the MAI in different MTB may be the result of rearrangements (e.g., duplication) within the MAI occurring over time. The organization of the *mam* genes is relatively well conserved in *Magnetospirillum* strains (198). In addition, there are high similarities for specific Mam proteins and their encoding genes, respectively, in recognized *Magnetospirillum* species (198). The organization and sequence of the magnetosome genes are less conserved in the genomes of other unrelated MTB (39, 50, 107, 195, 196, 202) (Fig. 10C).

If magnetotaxis originated in a common ancestor of all MTB and was then transferred by descent, the current data indicate that the first proteobacterium, the common ancestor of all subgroups of the *Proteobacteria*, was magnetotactic (Fig. 11). If true, this is an important finding because known MTB represent only a minority of organisms in the *Proteobacteria*. This might be explained by the fact that some cultivated MTB appear to lose the magnetotactic trait relatively easily in culture through loss of the MAI (68, 170). Presumably, then, the *Beta-*, *Epsilon-*, and *Zetaproteobacteria* groups that do not appear to contain MTB diverge from such organisms that lost the ability to produce magnetosomes (Fig. 11). It is also possible that magnetotactic members of these groups exist but that they have not yet been discovered. There is also the possibility that the common ancestor of all *Proteobacteria*, *Nitrospirae*, and the candidate division OP3 was magnetotactic (Fig. 11). However, additional magnetosome gene sequences from more MTB from the *Nitrospirae* and OP3 are required to posit such a conclusion.

Alternatively, it is also possible that the common ancestor of all *Proteobacteria* was not magnetotactic but only had orthologous *mam* genes (most likely the *mamAB* operon) similar to those found in extant MTB that served a function other than for magnetotaxis. For example, at the time when the *Proteobacteria* emerged, about 2.5 to 3.0 billion years ago (223), when levels of atmospheric oxygen were low (224) and anaerobic-to-microaerobic environments dominated, magnetosomes may have been important to MTB in scavenging reactive oxygen species (77) and

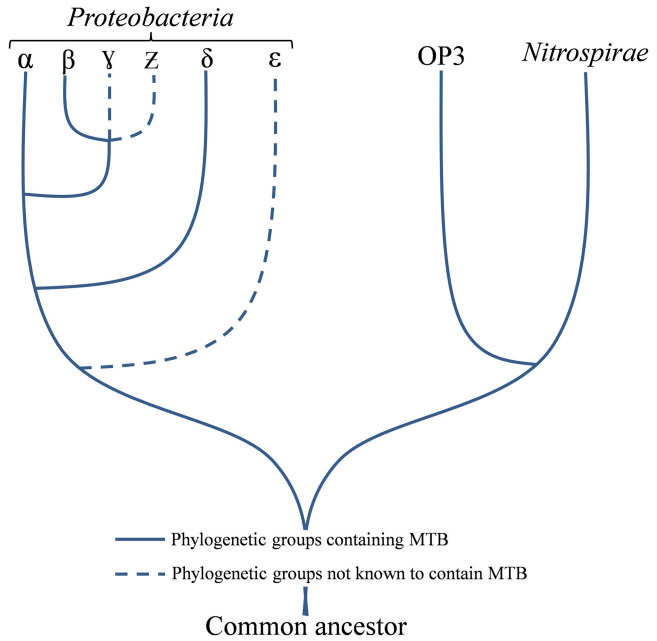

FIG 11 Schematic representation of the evolution of magnetotactic bacteria (MTB) from a common ancestor that transferred the genes involved in magnetosome formation by descent to the groups known to display magnetotaxis. Species that do not have this ability but appear to have an ancestor that had it have likely lost the genes involved in magnetosome formation.

later, when atmospheric oxygen levels increased, served to aid MTB in navigation.

The First Magnetosomes

There is an obvious, strong, and apparently important correlation between the composition and morphology of the magnetosome mineral crystals produced by MTB and their phylogenetic affiliation (15, 53, 172, 225). Magnetotactic *Alpha-* and *Gammaproteobacteria*, the later-diverging classes of the *Proteobacteria*, biomineralize morphologically consistent, well-defined crystals of magnetite that include cuboctahedral and elongated prisms (which appear rectangular in projection in electron micrographs) (53, 226) (Fig. 4 to 6 and 8). In contrast, in the magnetotactic *Deltaproteobacteria*, the most deeply diverging group of the *Proteobacteria*, that biomineralize magnetite, greigite, or both, the magnetite crystals are always bullet shaped and show much more morphological variation and defects (e.g., kinks) than those produced by the *Alphaproteobacteria* (Fig. 7). The magnetotactic *Nitrospirae* and strain SKK-01 of the candidate division OP3, the more deeply branching phylogenetic groups that contain MTB (30, 39), are known to biomineralize only magnetite crystals whose morphologies are very similar, if not identical, to those found in the *Deltaproteobacteria* (225) (Fig. 9). Thus, based on the phylogeny of MTB and the type of magnetosomes that they biomineralize, it has been suggested that bullet-shaped magnetite crystals represent the first magnetosome mineral phase (15).

An interesting question is how the biomineralization of iron-sulfide (greigite) magnetosomes evolved. It appears likely that greigite magnetosome formation originated in the *Deltaproteobacteria*, the only group known to contain greigite-producing MTB (26, 172), and not the most deeply branching of the groups that contain MTB. Both magnetite- and greigite-producing MTB possess a common set of 9 *mam* genes (*mamABEIKMOPQ*), although those for greigite biomineralization are slightly different than those for magnetite biomineralization; they are phylogenetically most closely related to the *mam* genes of magnetotactic *Deltaproteobacteria* (50) (Fig. 10B). "*Ca*. Desulfamplus magnetomortis," the only greigite-producing magnetotactic bacterium currently in pure culture, biomineralizes both greigite and magnetite and contains two sets of magnetosome genes (26). Considering that one set of genes is more similar to that of the magnetite-producing *Deltaproteobacteria* and that the other is more similar to that of the greigite-producing organism "*Ca*. Magnetoglobus multicellularis," it was suggested that the first set is responsible for magnetite biomineralization and that the second set is responsible for greigite production (26, 50) (Fig. 10). 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. Thus, it seems plausible that the genes for greigite biomineralization originated from gene duplication and/or subsequent mutation or other genetic changes that appear to have occurred in the *Desulfobacterales* order of the *Deltaproteobacteria* (Fig. 7 and 10). This adaptation may have resulted in the substitution of oxygen by sulfur in magnetosome crystals of some anaerobic MTB in highly reduced environments (26). Indeed, the magnetite producers are found at the OAI, while the greigite producers are found in reducing biotopes, below the OAI, where the anoxic zone is strongly sulfidic (16, 26). The oxygen in magnetite magnetosome crystals biomineralized by MTB has been shown to come from water based on oxygen isotope experiments (227); it is thus possible that in sulfide-rich niches, environmental conditions are more favorable for the biomineralization of greigite by MTB, and the sulfur in greigite likely comes from sulfur in hydrogen sulfide either in the environment or produced by sulfate reduction.

CONCLUDING REMARKS

By synthesizing single-magnetic-domain magnetic crystals and arranging them in chains within the cell, MTB have optimized the magnetic dipole moments of each individual crystal and the cell itself, respectively. Thus, the process of magnetosome biomineralization, which includes the choice of mineral composition and the control over the size and morphology of the crystals as well as their position within the cell, has been refined and optimized in the course of evolution, especially considering that it probably originated first as cells taking up large amounts of iron. At some point, the genes for magnetosome membrane proteins developed, leading to the biomineralization of the magnetosome mineral phase and the first MTB. The primitive lines of MTB that acquired the original pool of genes necessary for magnetosome formation seem to have evolved differently in different phylogenetic groups that contain MTB, leading to different magnetosome crystal compositions and morphologies and the emergence of new genes involved in magnetosome formation, thus explaining the great diversity of MTB.

Although recent progress in many areas of MTB research has revealed a great deal of information regarding the biodiversity and evolution of MTB as well as the elucidation of many of the functions of specific magnetosome genes, there are many questions that remain unanswered. In fact, much of this progress opened up many new questions. For example, some important questions to answer include the following. (i) How much do we really know

about the phylogenetic diversity of MTB; i.e., are there MTB in other phyla in the domain *Bacteria* or even in the *Archaea* that have not been discovered? (ii) How are the magnetosome genes organized in and how similar are they to those of MTB of the *Gammaproteobacteria* class or the OP3 division (those groups that contain MTB whose genomes have not been studied)? (iii) How did some eukaryotes develop the ability to biomineralize magnetite, and do they have genes for this ability that are similar to those in prokaryotes? (iv) To which phylogenetic lineage did the common ancestor of all MTB belong, and when did it emerge? (v) Are there other unrecognized functions for magnetosomes in MTB that are still applicable today? (There is a good deal of evidence that questions the current function of magnetoreception.) The discovery of new MTB from other evolutionary lineages and the sequencing of their genomes will hopefully help to answer these and other questions.

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REFERENCES

1. **Bazylinski DA, Frankel RB.** 2004. Magnetosome formation in prokaryotes. Nat. Rev. Microbiol. **2:**217–230.
2. **Blakemore R.** 1975. Magnetotactic bacteria. Science **190:**377–379.
3. **Gorby YA, Beveridge TJ, Blakemore RP.** 1988. Characterization of the bacterial magnetosome membrane. J. Bacteriol. **170:**834–841.
4. **Grünberg K, Müller E-C, Otto A, Reszka R, Linder D, Kube M, Reinhardt R, Schüler D.** 2004. Biochemical and proteomic analysis of the magnetosome membrane in *Magnetospirillum gryphiswaldense*. Appl. Environ. Microbiol. **70:**1040–1050.
5. **Frankel RB, Bazylinski DA, Johnson MS, Taylor BL.** 1997. Magneto-aerotaxis in marine coccoid bacteria. Biophys. J. **73:**994–1000.
6. **Bellini S.** 2009. Further studies on "magnetosensitive bacteria." Chin. J. Oceanol. Limnol. **27:**6–12.
7. **Bellini S.** 2009. On a unique behavior of freshwater bacteria. Chin. J. Oceanol. Limnol. **27:**3–5.
8. **Bazylinski DA, Lefèvre CT, Schüler D.** 2013. Magnetotactic bacteria, p 453–494. *In* Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), The prokaryotes. Springer, Berlin, Germany.
9. **Schüler D.** 2002. The biomineralization of magnetosomes in *Magnetospirillum gryphiswaldense*. Int. Microbiol. **5:**209–214.
10. **Amann R, Peplies J, Schüler D.** 2007. Diversity and taxonomy of magnetotactic bacteria, p 25–36. *In* Schüler D (ed), Magnetoreception and magnetosomes in bacteria. Springer, Berlin, Germany.
11. **Bazylinski DA, Williams TJ.** 2007. Ecophysiology of magnetotactic bacteria, p 37–75. *In* Schüler D (ed), Magnetoreception and magnetosomes in bacteria. Springer, Berlin, Germany.
12. **Simmons SL, Edwards KJ.** 2007. Geobiology of magnetotactic bacteria, p 77–102. *In* Schüler D (ed), Magnetoreception and magnetosomes in bacteria. Springer, Berlin, Germany.
13. **Schüler D.** 2008. Genetics and cell biology of magnetosome formation in magnetotactic bacteria. FEMS Microbiol. Rev. **32:**654–672.
14. **Komeili A.** 2012. Molecular mechanisms of compartmentalization and biomineralization in magnetotactic bacteria. FEMS Microbiol. Rev. **36:**232–255.
15. **Lefèvre CT, Trubitsyn D, Abreu F, Kolinko S, Almeida LG, Vasconcelos AT, Lins U, Schüler D, Ginet N, Pignol D, Bazylinski DA.** 29 January 2013. Monophyletic origin of magnetotaxis and the first magnetosomes. Environ. Microbiol. [Epub ahead of print.]. doi:10.1111/1462-2920.12097.
16. **Moskowitz BM, Bazylinski DA, Egli R, Frankel RB, Edwards KJ.** 2008. Magnetic properties of marine magnetotactic bacteria in a seasonally stratified coastal pond (Salt Pond, MA, USA). Geophys. J. Int. **174:**75–92.
17. **Lin W, Wang Y, Gorby Y, Nealson K, Pan Y.** 2013. Integrating niche-based process and spatial process in biogeography of magnetotactic bacteria. Sci. Rep. **3:**1643. doi:10.1038/srep01643.
18. **Lin W, Wang Y, Li B, Pan Y.** 2012. A biogeographic distribution of magnetotactic bacteria influenced by salinity. ISME J. **6:**475–479.
19. **Martins JL, Silveira TS, Silva KT, Lins U.** 2009. Salinity dependence of the distribution of multicellular magnetotactic prokaryotes in a hypersaline lagoon. Int. Microbiol. **12:**193–201.
20. **Martins JL, Silveira TS, Abreu F, de Almeida FP, Rosado AS, Lins U.** 2012. Spatiotemporal distribution of the magnetotactic multicellular prokaryote *Candidatus* Magnetoglobus multicellularis in a Brazilian hypersaline lagoon and in microcosms. Int. Microbiol. **15:**141–149.
21. **Postec A, Tapia N, Bernadac A, Joseph M, Davidson S, Wu L-F, Ollivier B, Pradel N.** 2012. Magnetotactic bacteria in microcosms originating from the French Mediterranean Coast subjected to oil industry activities. Microb. Ecol. **63:**1–11.
22. **Flies CB, Jonkers HM, de Beer D, Bosselmann K, Bottcher ME, Schüler D.** 2005. Diversity and vertical distribution of magnetotactic bacteria along chemical gradients in freshwater microcosms. FEMS Microbiol. Ecol. **52:**185–195.
23. **Frankel RB, Papaefthymiou GC, Blakemore RP, Obrien W.** 1983. Fe_3O_4 precipitation in magnetotactic bacteria. Biochim. Biophys. Acta **763:**147–159.
24. **Mann S, Sparks NHC, Frankel RB, Bazylinski DA, Jannasch HW.** 1990. Biomineralization of ferrimagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a magnetotactic bacterium. Nature **343:**258–261.
25. **Farina M, Esquivel D, Debarros HGP.** 1990. Magnetic iron-sulfur crystals from a magnetotactic microorganism. Nature **343:**256–258.
26. **Lefèvre CT, Menguy N, Abreu F, Lins U, Pósfai M, Prozorov T, Pignol D, Frankel RB, Bazylinski DA.** 2011. A cultured greigite-producing magnetotactic bacterium in a novel group of sulfate-reducing bacteria. Science **334:**1720–1723.
27. **Lefèvre CT, Abreu F, Schmidt ML, Lins U, Frankel RB, Hedlund BP, Bazylinski DA.** 2010. Moderately thermophilic magnetotactic bacteria from hot springs in Nevada. Appl. Environ. Microbiol. **76:**3740–3743.
28. **Lefèvre CT, Frankel RB, Pósfai M, Prozorov T, Bazylinski DA.** 2011. Isolation of obligately alkaliphilic magnetotactic bacteria from extremely alkaline environments. Environ. Microbiol. **13:**2342–2350.
29. **Bazylinski DA, Lefèvre CT.** 2013. Magnetotactic bacteria from extreme environments. Life **3:**295–307.
30. **Kolinko S, Jogler C, Katzmann E, Wanner G, Peplies J, Schüler D.** 2012. Single-cell analysis reveals a novel uncultivated magnetotactic bacterium within the candidate division OP3. Environ. Microbiol. **14:**1709–1721.
31. **Simmons SL, Sievert SM, Frankel RB, Bazylinski DA, Edwards KJ.** 2004. Spatiotemporal distribution of marine magnetotactic bacteria in a seasonally stratified coastal salt pond. Appl. Environ. Microbiol. **70:**6230–6239.
32. **Lefèvre CT, Frankel RB, Abreu F, Lins U, Bazylinski DA.** 2011. Culture-independent characterization of a novel, uncultivated magnetotactic member of the *Nitrospirae* phylum. Environ. Microbiol. **13:**538–549.
33. **Jogler C, Lin W, Meyerdierks A, Kube M, Katzmann E, Flies C, Pan Y, Amann R, Reinhardt R, Schüler D.** 2009. Toward cloning of the magnetotactic metagenome: identification of magnetosome island gene clusters in uncultivated magnetotactic bacteria from different aquatic sediments. Appl. Environ. Microbiol. **75:**3972–3979.
34. **Spring S, Amann R, Ludwig W, Schleifer KH, Vangemerden H, Petersen N.** 1993. Dominating role of an unusual magnetotactic bacterium in the microaerobic zone of a fresh-water sediment. Appl. Environ. Microbiol. **59:**2397–2403.
35. **Bazylinski DA, Garratt-Reed A, Frankel RB.** 1994. Electron-microscopic studies of magnetosomes in magnetotactic bacteria. Microsc. Res. Tech. **27:**389–401.
36. **Wolfe RS, Thauer RK, Pfennig N.** 1987. A "capillary racetrack" method for isolation of magnetotactic bacteria. FEMS Microbiol. Lett. **45:**31–35.
37. **Lin W, Tian L, Li J, Pan Y.** 2008. Does capillary racetrack-based enrichment reflect the diversity of uncultivated magnetotactic cocci in environmental samples? FEMS Microbiol. Lett. **279:**202–206.
38. **Bazylinski DA, Williams TJ, Lefèvre CT, Trubitsyn D, Fang J, Beveridge TJ, Moskowitz BM, Ward B, Schübbe S, Dubbels BL, Simpson B.** 2013. *Magnetovibrio blakemorei*, gen. nov. sp. nov., a new magnetotactic bacterium (*Alphaproteobacteria*: *Rhodospirillaceae*) isolated from a salt marsh. Int. J. Syst. Evol. Microbiol. **63:**1824–1833.
39. **Jogler C, Wanner G, Kolinko S, Niebler M, Amann R, Petersen N, Kube M, Reinhardt R, Schüler D.** 2011. Conservation of proteobacterial

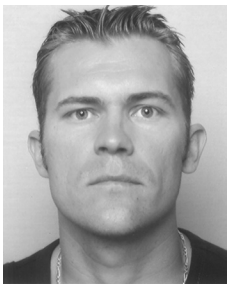
magnetosome genes and structures in an uncultivated member of the deep-branching *Nitrospira* phylum. Proc. Natl. Acad. Sci. U. S. A. **108:** 1134–1139.
40. **Kolinko S, Wanner G, Katzmann E, Kiemer F, Fuchs MB, Schüler D.** 2013. Clone libraries and single cell genome amplification reveal extended diversity of uncultivated magnetotactic bacteria from marine and freshwater environments. Environ. Microbiol. **15:**1290–1301.
41. **Blakemore R.** 1982. Magnetotactic bacteria. Annu. Rev. Microbiol. **36:** 217–238.
42. **Abreu F, Martins JL, Silveira TS, Keim CN, de Barros HGPL, Filho FJG, Lins U.** 2007. "*Candidatus* Magnetoglobus multicellularis," a multicellular, magnetotactic prokaryote from a hypersaline environment. Int. J. Syst. Evol. Microbiol. **57:**1318–1322.
43. **DeLong EF, Frankel RB, Bazylinski DA.** 1993. Multiple evolutionary origins of magnetotaxis in bacteria. Science **259:**803–806.
44. **Simmons SL, Edwards KJ.** 2007. Unexpected diversity in populations of the many-celled magnetotactic prokaryote. Environ. Microbiol. **9:**206–215.
45. **Wenter R, Wanner G, Schüler D, Overmann J.** 2009. Ultrastructure, tactic behaviour and potential for sulfate reduction of a novel multicellular magnetotactic prokaryote from North Sea sediments. Environ. Microbiol. **11:**1493–1505.
46. **Pikuta EV, Hoover RB, Bej AK, Marsic D, Whitman WB, Cleland D, Krader P.** 2003. *Desulfonatronum thiodismutans* sp. nov., a novel alkaliphilic, sulfate-reducing bacterium capable of lithoautotrophic growth. Int. J. Syst. Evol. Microbiol. **53:**1327–1332.
47. **Rees GN, Harfoot CG, Sheehy AJ.** 1998. Amino acid degradation by the mesophilic sulfate-reducing bacterium *Desulfobacterium vacuolatum*. Arch. Microbiol. **169:**76–80.
48. **Bazylinski DA, Williams TJ, Lefèvre CT, Berg RJ, Zhang CL, Bowser SS, Dean AJ, Beveridge TJ.** 2013. *Magnetococcus marinus* gen. nov., sp. nov., a marine, magnetotactic bacterium that represents a novel lineage (*Magnetococcaceae* fam. nov.; *Magnetococcales* ord. nov.) at the base of the *Alphaproteobacteria*. Int. J. Syst. Evol. Microbiol. **63:**801–808.
49. **Lefèvre CT, Bernadac A, Yu-Zhang K, Pradel N, Wu L-F.** 2009. Isolation and characterization of a magnetotactic bacterial culture from the Mediterranean Sea. Environ. Microbiol. **11:**1646–1657.
50 **Lefèvre CT, Trubitsyn D, Abreu F, Kolinko S, Jogler C, de Almeida LGP, de Vasconcelos ATR, Kube M, Reinhardt R, Lins U, Pignol D, Schüler D, Bazylinski DA, Ginet N.** 1 April 2013. Comparative genomic analysis of magnetotactic bacteria from the *Deltaproteobacteria* provides new insights into magnetite and greigite magnetosome genes required for magnetotaxis. Environ. Microbiol. [Epub ahead of print.] doi:10.1111/1462-2920.12128.
51. **Geelhoed JS, Kleerebezem R, Sorokin DY, Stams AJM, van Loosdrecht MCM.** 2010. Reduced inorganic sulfur oxidation supports autotrophic and mixotrophic growth of *Magnetospirillum* strain J10 and *Magnetospirillum gryphiswaldense*. Environ. Microbiol. **12:**1031–1040.
52. **Williams TJ, Lefèvre CT, Zhao W, Beveridge TJ, Bazylinski DA.** 2012. *Magnetospira thiophila* gen. nov., sp. nov., a marine magnetotactic bacterium that represents a novel lineage within the *Rhodospirillaceae* (*Alphaproteobacteria*). Int. J. Syst. Evol. Microbiol. **62:**2443–2450.
53. **Lefèvre CT, Viloria N, Schmidt ML, Pósfai M, Frankel RB, Bazylinski DA.** 2012. Novel magnetite-producing magnetotactic bacteria belonging to the *Gammaproteobacteria*. ISME J. **6:**440–450.
54. **Sakaguchi T, Arakaki A, Matsunaga T.** 2002. *Desulfovibrio magneticus* sp nov., a novel sulfate-reducing bacterium that produces intracellular single-domain-sized magnetite particles. Int. J. Syst. Evol. Microbiol. **52:** 215–221.
55. **Schleifer KH, Schüler D, Spring S, Weizenegger M, Amann R, Ludwig W, Köhler M.** 1991. The genus *Magnetospirillum* gen. nov. Description of *Magnetospirillum gryphiswaldense* sp. nov. and transfer of *Aquaspirillum magnetotacticum* to *Magnetospirillum magnetotacticum* comb. nov. Syst. Appl. Microbiol. **14:**379–385.
56. **Schüler D, Baeuerlein E.** 1998. Dynamics of iron uptake and Fe_3O_4 biomineralization during aerobic and microaerobic growth of *Magnetospirillum gryphiswaldense*. J. Bacteriol. **180:**159–162.
57. **Schüler D, Baeuerlein E.** 1996. Iron-limited growth and kinetics of iron uptake in *Magnetospirillum gryphiswaldense*. Arch. Microbiol. **166:**301–307.
58. **Blakemore RP, Maratea D, Wolfe RS.** 1979. Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium. J. Bacteriol. **140:**720–729.
59. **Byrne ME, Ball DA, Guerquin-Kern J-L, Rouiller I, Wu T-D, Downing KH, Vali H, Komeili A.** 2010. *Desulfovibrio magneticus* RS-1 contains an iron- and phosphorus-rich organelle distinct from its bullet-shaped magnetosomes. Proc. Natl. Acad. Sci. U. S. A. **107:**12263–12268.
60. **Schüler D, Spring S, Bazylinski DA.** 1999. Improved technique for the isolation of magnetotactic spirilla from a freshwater sediment and their phylogenetic characterization. Syst. Appl. Microbiol. **22:**466–471.
61. **Matsunaga T, Arakaki A.** 2007. Molecular bioengineering of bacterial magnetic particles for biotechnological applications, p 227–254. *In* Schüler D (ed), Magnetoreception and magnetosomes in bacteria. Springer, Berlin, Germany.
62. **Suzuki T, Okamura Y, Calugay RJ, Takeyama H, Matsunaga T.** 2006. Global gene expression analysis of iron-inducible genes in *Magnetospirillum magneticum* AMB-1. J. Bacteriol. **188:**2275–2279.
63. **Pósfai M, Moskowitz B, Arató B, Schüler D, Flies C, Bazylinski D, Frankel R.** 2006. Properties of intracellular magnetite crystals produced by *Desulfovibrio magneticus* strain RS-1. Earth Planet. Sci. Lett. **249:**444–455.
64. **Lefèvre CT, Schmidt ML, Viloria N, Trubitsyn D, Schüler D, Bazylinski DA.** 2012. Insight into the evolution of magnetotaxis in *Magnetospirillum* spp., based on *mam* gene phylogeny. Appl. Environ. Microbiol. **78:**7238–7248.
65. **Schultheiss D, Schüler D.** 2003. Development of a genetic system for *Magnetospirillum gryphiswaldense*. Arch. Microbiol. **179:**89–94.
66. **Hoffman PS, Pine L, Bell S.** 1983. Production of superoxide and hydrogen peroxide in medium used to culture *Legionella pneumophila*: catalytic decomposition by charcoal. Appl. Environ. Microbiol. **45:**784–791.
67. **Krieg NR, Hoffman PS.** 1986. Microaerophily and oxygen toxicity. Annu. Rev. Microbiol. **40:**107–130.
68. **Dubbels BL, DiSpirito AA, Morton JD, Semrau JD, Neto JNE, Bazylinski DA.** 2004. Evidence for a copper-dependent iron transport system in the marine, magnetotactic bacterium strain MV-1. Microbiology **150:** 2931–2945.
69. **Bazylinski DA, Frankel RB, Jannasch HW.** 1988. Anaerobic magnetite production by a marine, magnetotactic bacterium. Nature **334:**518–519.
70. **Frankel RB.** 1984. Magnetic guidance of organisms. Annu. Rev. Biophys. Biomol. Struct. **13:**85–103.
71. **Blakemore RP, Frankel RB, Kalmijn AJ.** 1980. South-seeking magnetotactic bacteria in the southern-hemisphere. Nature **286:**384–385.
72. **Frankel RB, Blakemore RP, De Araujo FF, Esquivel DMS, Danon J.** 1981. Magnetotactic bacteria at the geomagnetic equator. Science **212:** 1269–1270.
73. **De Araujo FF, Germano FA, Gonçalves LL, Pires MA, Frankel RB.** 1990. Magnetic polarity fractions in magnetotactic bacterial populations near the geomagnetic equator. Biophys. J. **58:**549–555.
74. **Shapiro OH, Hatzenpichler R, Buckley DH, Zinder SH, Orphan VJ.** 2011. Multicellular photo-magnetotactic bacteria. Environ. Microbiol. Rep. **3:**233–238.
75. **Simmons SL, Bazylinski DA, Edwards KJ.** 2006. South-seeking magnetotactic bacteria in the Northern Hemisphere. Science **311:**371–374.
76. **Frankel RB, Williams TJ, Bazylinski DA.** 2007. Magneto-aerotaxis, p 1–24. *In* Schüler D (ed), Magnetoreception and magnetosomes in bacteria. Springer, Berlin, Germany.
77. **Guo FF, Yang W, Jiang W, Geng S, Peng T, Li JL.** 2012. Magnetosomes eliminate intracellular reactive oxygen species in *Magnetospirillum gryphiswaldense* MSR-1. Environ. Microbiol. **14:**1722–1729.
78. **Stohs SJ, Bagchi D.** 1995. Oxidative mechanisms in the toxicity of metal-ions. Free Radic. Biol. Med. **18:**321–336.
79. **Almeida FP, Viana NB, Lins U, Farina M, Keim CN.** 2013. Swimming behaviour of the multicellular magnetotactic prokaryote "*Candidatus* Magnetoglobus multicellularis" under applied magnetic fields and ultraviolet light. Antonie Van Leeuwenhoek **103:**845–857.
80. **Lefèvre CT, Abreu F, Lins U, Bazylinski DA.** 2010. Nonmagnetotactic multicellular prokaryotes from low-saline, nonmarine aquatic environments and their unusual negative phototactic behavior. Appl. Environ. Microbiol. **76:**3220–3227.
81. **Braatsch S, Moskvin OV, Klug G, Gomelsky M.** 2004. Responses of the *Rhodobacter sphaeroides* transcriptome to blue light under semiaerobic conditions. J. Bacteriol. **186:**7726–7735.
82. **Zhu K, Pan H, Li J, Yu-Zhang K, Zhang S-D, Zhang W-Y, Zhou K, Yue H, Pan Y, Xiao T, Wu L-F.** 2010. Isolation and characterization of a

marine magnetotactic spirillum axenic culture QH-2 from an intertidal zone of the China Sea. Res. Microbiol. **161:**276–283.
83. **Cox BL, Popa R, Bazylinski DA, Lanoil B, Douglas S, Belz A, Engler DL, Nealson KH.** 2002. Organization and elemental analysis of P-, S-, and Fe-rich inclusions in a population of freshwater magnetococci. Geomicrobiol. J. **19:**387–406.
84. **Moench TT.** 1988. *Bilophococcus magnetotacticus* gen. nov. sp. nov, a motile, magnetic coccus. Antonie Van Leeuwenhoek **54:**483–496.
85. **Moench TT, Konetzka WA.** 1978. Novel method for isolation and study of a magnetotactic bacterium. Arch. Microbiol. **119:**203–212.
86. **Freitas F, Keim CN, Kachar B, Farina M, Lins U.** 2003. Envelope ultrastructure of uncultured naturally occurring magnetotactic cocci. FEMS Microbiol. Lett. **219:**33–38.
87. **Bazylinski DA, Dean AJ, Williams TJ, Long LK, Middleton SL, Dubbels BL.** 2004. Chemolithoautotrophy in the marine, magnetotactic bacterial strains MV-1 and MV-2. Arch. Microbiol. **182:**373–387.
88. **Schultheiss D, Handrick R, Jendrossek D, Hanzlik M, Schüler D.** 2005. The presumptive magnetosome protein Mms16 is a poly(3-hydroxybutyrate) granule-bound protein (phasin) in *Magnetospirillum gryphiswaldense*. J. Bacteriol. **187:**2416–2425.
89. **Isambert A, Menguy N, Larquet E, Guyot F, Valet J-P.** 2007. Transmission electron microscopy study of magnetites in a freshwater population of magnetotactic bacteria. Am. Miner. **92:**621–630.
90. **Lin W, Li B, Pan Y.** 2011. DMTB: a comprehensive online resource of 16S rRNA genes, ecological metadata, oligonucleotides, and magnetic properties of magnetotactic bacteria. Chin. Sci. Bull. **56:**476–478.
91. **Burgess JG, Kawaguchi R, Sakaguchi T, Thornhill RH, Matsunaga T.** 1993. Evolutionary relationships among *Magnetospirillum* strains inferred from phylogenetic analysis of 16S rDNA sequences. J. Bacteriol. **175:**6689–6694.
92. **Lin W, Pan Y.** 2009. Uncultivated magnetotactic cocci from Yuandadu Park in Beijing, China. Appl. Environ. Microbiol. **75:**4046–4052.
93. **Spring S, Amann R, Ludwig W, Schleifer KH, Schüler D, Poralla K, Petersen N.** 1994. Phylogenetic analysis of uncultured magnetotactic bacteria from the alpha-subclass of *Proteobacteria*. Syst. Appl. Microbiol. **17:**501–508.
94. **Spring S, Lins U, Amann R, Schleifer KH, Ferreira LCS, Esquivel DMS, Farina M.** 1998. Phylogenetic affiliation and ultrastructure of uncultured magnetic bacteria with unusually large magnetosomes. Arch. Microbiol. **169:**136–147.
95. **Meldrum FC, Mann S, Heywood BR, Frankel RB, Bazylinski DA.** 1993. Electron-microscopy study of magnetosomes in 2 cultured vibrioid magnetotactic bacteria. Proc. R. Soc. Lond. B Biol. Sci. **251:**237–242.
96. **Spring S, Amann R, Ludwig W, Schleifer KH, Petersen N.** 1992. Phylogenetic diversity and identification of nonculturable magnetotactic bacteria. Syst. Appl. Microbiol. **15:**116–122.
97. **Lin W, Li J, Schüler D, Jogler C, Pan Y.** 2009. Diversity analysis of magnetotactic bacteria in Lake Miyun, northern China, by restriction fragment length polymorphism. Syst. Appl. Microbiol. **32:**342–350.
98. **Bazylinski DA, Blakemore RP.** 1983. Denitrification and assimilatory nitrate reduction in *Aquaspirillum magnetotacticum*. Appl. Environ. Microbiol. **46:**1118–1124.
99. **Blakemore RP, Short KA, Bazylinski DA, Rosenblatt C, Frankel RB.** 1985. Microaerobic conditions are required for magnetite formation within *Aquaspirillum magnetotacticum*. Geomicrobiol. J. **4:**53–71.
100. **Heyen U, Schüler D.** 2003. Growth and magnetosome formation by microaerophilic *Magnetospirillum* strains in an oxygen-controlled fermentor. Appl. Microbiol. Biotechnol. **61:**536–544.
101. **Li Y, Katzmann E, Borg S, Schüler D.** 2012. The periplasmic nitrate reductase Nap is required for anaerobic growth and involved in redox control of magnetite biomineralization in *Magnetospirillum gryphiswaldense*. J. Bacteriol. **194:**4847–4856.
102. **Bazylinski DA, Dean AJ, Schüler D, Phillips EJ, Lovley DR.** 2000. N_2-dependent growth and nitrogenase activity in the metal-metabolizing bacteria, *Geobacter* and *Magnetospirillum* species. Environ. Microbiol. **2:**266–273.
103. **Bazylinski DA, Blakemore RP.** 1983. Nitrogen fixation (acetylene-reduction) in *Aquaspirillum magnetotacticum*. Curr. Microbiol. **9:**305–308.
104. **Gorlenko VM, Dziuba MV, Maleeva AN, Panteleeva AN, Kolganova TV, Kuznetsov BB.** 2011. *Magnetospirillum aberrantis* sp. nov., a new freshwater bacterium with magnetic inclusions. Mikrobiologiia **80:**679–690.
105. **Gonçalves RP, Bernadac A, Sturgis JN, Scheuring S.** 2005. Architecture of the native photosynthetic apparatus of *Phaeospirillum molischianum*. J. Struct. Biol. **152:**221–228.
106. **Williams TJ, Zhang CL, Scott JH, Bazylinski DA.** 2006. Evidence for autotrophy via the reverse tricarboxylic acid cycle in the marine magnetotactic coccus strain MC-1. Appl. Environ. Microbiol. **72:**1322–1329.
107. **Schübbe S, Williams TJ, Xie G, Kiss HE, Brettin TS, Martinez D, Ross CA, Schüler D, Cox BL, Nealson KH, Bazylinski DA.** 2009. Complete genome sequence of the chemolithoautotrophic marine magnetotactic coccus strain MC-1. Appl. Environ. Microbiol. **75:**4835–4852.
108. **Esser C, Martin W, Dagan T.** 2007. The origin of mitochondria in light of a fluid prokaryotic chromosome model. Biol. Lett. **3:**180–184.
109. **Georgiades K, Madoui M-A, Le P, Robert C, Raoult D.** 2011. Phylogenomic analysis of *Odyssella thessalonicensis* fortifies the common origin of *Rickettsiales*, *Pelagibacter ubique* and *Reclimonas americana* mitochondrion. PLoS One **6:**e24857. doi:10.1371/journal.pone.0024857.
110. **Singer E, Emerson D, Webb EA, Barco RA, Kuenen JG, Nelson WC, Chan CS, Comolli LR, Ferriera S, Johnson J, Heidelberg JF, Edwards KJ.** 2011. *Mariprofundus ferrooxydans* PV-1 the first genome of a marine Fe(II) oxidizing zetaproteobacterium. PLoS One **6:**e25386. doi:10.1371/journal.pone.0025386.
111. **Hügler M, Sievert SM.** 2011. Beyond the Calvin cycle: autotrophic carbon fixation in the ocean. Annu. Rev. Mar. Sci. **3:**261–289.
112. **Zhang W-Y, Zhou K, Pan H-M, Du H-J, Chen Y-R, Zhang R, Ye W, Lu C, Xiao T, Wu L-F.** 2013. Novel rod-shaped magnetotactic bacteria belonging to *Alphaproteobacteria*. Appl. Environ. Microbiol. **79:**3137–3140.
113. **Lefèvre CT, Santini C-L, Bernadac A, Zhang W-J, Li Y, Wu L-F.** 2010. Calcium ion-mediated assembly and function of glycosylated flagellar sheath of marine magnetotactic bacterium. Mol. Microbiol. **78:**1304–1312.
114. **Lefèvre CT, Song T, Yonnet J-P, Wu L-F.** 2009. Characterization of bacterial magnetotactic behaviors by using a magnetospectrophotometry assay. Appl. Environ. Microbiol. **75:**3835–3841.
115. **Ruan J, Kato T, Santini C-L, Miyata T, Kawamoto A, ZhangW-J, Bernadac A, Wu L-F, Namba K.** 2012. Architecture of a flagellar apparatus in the fast-swimming magnetotactic bacterium MO-1. Proc. Natl. Acad. Sci. U. S. A. **109:**20643–20648.
116. **Zhang W-J, Santini C-L, Bernadac A, Ruan J, Zhang S-D, Kato T, Li Y, Namba K, Wu L-F.** 2012. Complex spatial organization and flagellin composition of flagellar propeller from marine magnetotactic ovoid strain MO-1. J. Mol. Biol. **416:**558–570.
117. **Sakaguchi T, Burgess JG, Matsunaga T.** 1993. Magnetite formation by a sulfate-reducing bacterium. Nature **365:**47–49.
118. **Bazylinski DA, Heywood BR, Mann S, Frankel RB.** 1993. Fe_3O_4 and Fe_3S_4 in a bacterium. Nature **366:**218.
119. **Heywood BR, Bazylinski DA, Garratt-Reed A, Mann S, Frankel RB.** 1990. Controlled biosynthesis of greigite (Fe_3S_4) in magnetotactic bacteria. Naturwissenschaften **77:**536–538.
120. **Wang Y, Lin W, Li J, Pan Y.** 2013. High diversity of magnetotactic *Deltaproteobacteria* in a freshwater niche. Appl. Environ. Microbiol. **79:**2813–2817.
121. **Zhou K, Zhang W-Y, Yu-Zhang K, Pan H-M, Zhang S-D, Zhang W-J, Yue H-D, Li Y, Xiao T, Wu L-F.** 2012. A novel genus of multicellular magnetotactic prokaryotes from the Yellow Sea. Environ. Microbiol. **14:**405–413.
122. **Farina M, Lins de Barros H, Esquivel MS, Danon J.** 1983. Ultrastructure of a magnetotactic bacterium. Biol. Cell **48:**85–88.
123. **Lins U, Farina M.** 1999. Organization of cells in magnetotactic multicellular aggregates. Microbiol. Res. **154:**9–13.
124. **Keim CN, Abreu F, Lins U, de Barros HL, Farina M.** 2004. Cell organization and ultrastructure of a magnetotactic multicellular organism. J. Struct. Biol. **145:**254–262.
125. **Rodgers FG, Blakemore RP, Blakemore NA, Frankel RB, Bazylinski DA, Maratea D, Rodgers C.** 1990. Intercellular structure in a many-celled magnetotactic prokaryote. Arch. Microbiol. **154:**18–22.
126. **Bazylinski DA, Frankel RB, Garratt-Reed AJ, Mann S.** 1991. Biomineralization of iron sulfides in magnetotactic bacteria from sulfidic environments, p 239–255. *In* Frankel RB, Blakemore RP (ed), Iron biominerals. Plenum Press, New York, NY.
127. **Abreu F, Silva KT, Martins JL, Lins U.** 2006. Cell viability in magnetotactic multicellular prokaryotes. Int. Microbiol. **9:**267–272.
128. **Silva KT, Abreu F, Almeida FP, Keim CN, Farina M, Lins U.** 2007.

Flagellar apparatus of south-seeking many-celled magnetotactic prokaryotes. Microsc. Res. Tech. **70:**10–17.
129. **Keim CN, Martins JL, de Barros HL, Lins U, Farina M.** 2007. Structure, behavior, ecology and diversity of multicellular magnetotactic prokaryotes, p 103–132. *In* Schüler D (ed), Magnetoreception and magnetosomes in bacteria. Springer, Berlin, Germany.
130. **Lefèvre CT, Bernadac A, Pradel N, Wu LF, Yu-Zhang K, Xiao T, Yonnet J-P, Lebouc A, Song T, Fukumori Y.** 2007. Characterization of mediterranean magnetotactic bacteria. J. Ocean Univ. China **6:**355–359.
131. **Abreu F, Silva KT, Leão P, Guedes IA, Keim CN, Farina M, Lins U.** 2013. Cell adhesion, multicellular morphology, and magnetosome distribution in the multicellular magnetotactic prokaryote *Candidatus* Magnetoglobus multicellularis. Microsc. Microanal. **19:**535–543.
132. **Keim CN, Martins JL, Abreu F, Rosado AS, de Barros HL, Borojevic R, Lins U, Farina M.** 2004. Multicellular life cycle of magnetotactic prokaryotes. FEMS Microbiol. Lett. **240:**203–208.
133. **Pósfai M, Buseck PR, Bazylinski DA, Frankel RB.** 1998. Iron sulfides from magnetotactic bacteria: structure, composition, and phase transitions. Am. Miner. **83:**1469–1481.
134. **Pósfai M, Buseck PR, Bazylinski DA, Frankel RB.** 1998. Reaction sequence of iron sulfide minerals in bacteria and their use as biomarkers. Science **280:**880–883.
135. **Zhou K, Pan H, Zhang S, de Barros HL, Xiao T, Wu LF.** 2011. Occurrence and microscopic analyses of multicellular magnetotactic prokaryotes from coastal sediments in the Yellow Sea. Chin. J. Oceanol. Limnol. **29:**246–251.
136. **Lins U, Keim CN, Evans FF, Farina M, Buseck PR.** 2007. Magnetite (Fe_3O_4) and greigite (Fe_3S_4) crystals in multicellular magnetotactic prokaryotes. Geomicrobiol. J. **24:**43–50.
137. **Winklhofer M, Abraçado LG, Davila AF, Keim CN, Lins de Barros HGP.** 2007. Magnetic optimization in a multicellular magnetotactic organism. Biophys. J. **92:**661–670.
138. **Greenberg M, Canter K, Mahler I, Tornheim A.** 2005. Observation of magnetoreceptive behavior in a multicellular magnetotactic prokaryote in higher than geomagnetic fields. Biophys. J. **88:**1496–1499.
139. **Bazylinski DA, Frankel RB, Heywood BR, Ahmadi S, King JW, Donaghay PL, Hanson AK.** 1995. Controlled biomineralization of magnetite (Fe_3O_4) and greigite (Fe_3S_4). Appl. Environ. Microbiol. **61:**3232–3239.
140. **Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jorgensen BB, Witte U, Pfannkuche O.** 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature **407:**623–626.
141. **Reitner J, Peckmann J, Blumenberg M, Michaelis W, Reimer A, Thiel V.** 2005. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. Paleogeogr. Paleoclimatol. Paleoecol. **227:**18–30.
142. **Wrede C, Krukenberg V, Dreier A, Reitner J, Heller C, Hoppert M.** 2013. Detection of metabolic key enzymes of methane turnover processes in cold seep microbial biofilms. Geomicrobiol. J. **30:**214–227.
143. **Petersen N, Weiss DG, Vali H.** 1989. Magnetic bacteria in lake sediments, p 231–241. *In* Lowes FJ, Collinson DW, Parry JH, Runcorn SK, Tozer DC, Soward A (ed), Geomagnetism and palaeomagnetism. Springer, Berlin, Germany.
144. **Vali H, Förster O, Amarantidis G, Petersen N.** 1987. Magnetotactic bacteria and their magnetofossils in sediments. Earth Planet. Sci. Lett. **86:**389–400.
145. **Flies CB, Peplies J, Schüler D.** 2005. Combined approach for characterization of uncultivated magnetotactic bacteria from various aquatic environments. Appl. Environ. Microbiol. **71:**2723–2731.
146. **Lin W, Jogler C, Schüler D, Pan Y.** 2011. Metagenomic analysis reveals unexpected subgenomic diversity of magnetotactic bacteria within the phylum *Nitrospirae*. Appl. Environ. Microbiol. **77:**323–326.
147. **Lin W, Li J, Pan Y.** 2012. Newly isolated but uncultivated magnetotactic bacterium of the phylum *Nitrospirae* from Beijing, China. Appl. Environ. Microbiol. **78:**668–675.
148. **Lins U, Freitas F, Keim CN, Farina M.** 2000. Electron spectroscopic imaging of magnetotactic bacteria: magnetosome morphology and diversity. Microsc. Microanal. **6:**463–470.
149. **Lin W, Pan Y.** 2010. Temporal variation of magnetotactic bacterial communities in two freshwater sediment microcosms. FEMS Microbiol. Lett. **302:**85–92.
150. **Jogler C, Niebler M, Lin W, Kube M, Wanner G, Kolinko S, Stief P, Beck AJ, De Beer D, Petersen N, Pan Y, Amann R, Reinhardt R, Schüler D.** 2010. Cultivation-independent characterization of "*Candidatus* Magnetobacterium bavaricum" via ultrastructural, geochemical, ecological and metagenomic methods. Environ. Microbiol. **12:**2466–2478.
151. **Hanzlik M, Winklhofer M, Petersen N.** 2002. Pulsed-field-remanence measurements on individual magnetotactic bacteria. J. Magn. Magn. Mater. **248:**258–267.
152. **Hanzlik M, Winklhofer M, Petersen N.** 1996. Spatial arrangement of chains of magnetosomes in magnetotactic bacteria. Earth Planet. Sci. Lett. **145:**125–134.
153. **Li J, Pan Y, Liu Q, Yu-Zhang K, Menguy N, Che R, Qin H, Lin W, Wu W, Petersen N, Yang X.** 2010. Biomineralization, crystallography and magnetic properties of bullet-shaped magnetite magnetosomes in giant rod magnetotactic bacteria. Earth Planet. Sci. Lett. **293:**368–376.
154. **Pan YX, Petersen N, Davila AF, Zhang LM, Winklhofer M, Liu QS, Hanzlik M, Zhu RX.** 2005. The detection of bacterial magnetite in recent sediments of Lake Chiemsee (southern Germany). Earth Planet. Sci. Lett. **232:**109–123.
155. **Hanson TE, Tabita FR.** 2001. A ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO)-like protein from *Chlorobium tepidum* that is involved with sulfur metabolism and the response to oxidative stress. Proc. Natl. Acad. Sci. U. S. A. **98:**4397–4402.
156. **Haouari O, Fardeau M-L, Cayol J-L, Fauque G, Casiot C, Elbaz-Poulichet F, Hamdi M, Ollivier B.** 2008. *Thermodesulfovibrio hydrogeniphilus* sp. nov., a new thermophilic sulphate-reducing bacterium isolated from a Tunisian hot spring. Syst. Appl. Microbiol. **31:**38–42.
157. **Mann S, Sparks NHC, Blakemore RP.** 1987. Structure, morphology and crystal-growth of anisotropic magnetite crystals in magnetotactic bacteria. Proc. R. Soc. Lond. B Biol. Sci. **231:**477–487.
158. **Mann S, Sparks NHC, Blakemore RP.** 1987. Ultrastructure and characterization of anisotropic magnetic inclusions. Proc. R. Soc. Lond. B Biol. Sci. **231:**469–476.
159. **Bazylinski DA, Frankel RB.** 2003. Biologically controlled mineralization in prokaryotes, p 217–247. *In* Dove PM, DeYoreo JJ, Weiner S (ed), Biomineralization. Mineralogical Society of America, Washington, DC.
160. **Hugenholtz P, Pitulle C, Hershberger KL, Pace NR.** 1998. Novel division level bacterial diversity in a Yellowstone hot spring. J. Bacteriol. **180:**366–376.
161. **Torres de Araujo FF, Pires MA, Frankel RB, Bicudo CEM.** 1986. Magnetite and magnetotaxis in algae. Biophys. J. **50:**375–378.
162. **Bazylinski DA, Schlezinger DR, Howes BH, Frankel RB, Epstein SS.** 2000. Occurrence and distribution of diverse populations of magnetic protists in a chemically stratified coastal salt pond. Chem. Geol. **169:**319–328.
163. **Bazylinski DA, Lefèvre CT, Frankel RB.** 2012. Magnetotactic protists at the oxic-anoxic transition zones of coastal aquatic environments, p 133–143. *In* Altenbach AV, Bernhard JM, Seckbach J (ed), Anoxia: evidence for eukaryote survival and paleontological strategies. Springer, Berlin, Germany.
164. **Komeili A, Li Z, Newman DK, Jensen GJ.** 2006. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. Science **311:**242–245.
165. **Scheffel A, Gruska M, Faivre D, Linaroudis A, Plitzko JM, Schüler D.** 2006. An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. Nature **440:**110–114.
166. **Martins JL, Silveira TS, Abreu F, Silva KT, da Silva-Neto ID, Lins U.** 2007. Grazing protozoa and magnetosome dissolution in magnetotactic bacteria. Environ. Microbiol. **9:**2775–2781.
167. **Kirschvink JL, Walker MM, Diebel CE.** 2001. Magnetite-based magnetoreception. Curr. Opin. Neurobiol. **11:**462–467.
168. **Vali H, Kirschvink JL.** 1991. Observations of magnetosome organization, surface structure, and iron biomineralization of undescribed magnetic bacteria: evolutionary speculations, p 97–115. *In* Frankel RB, Blakemore RP (ed), Iron biominerals. Plenum Press, New York, NY.
169. **Postgate JR.** 1963. Versatile medium for the enumeration of sulfate-reducing bacteria. Appl. Microbiol. **11:**265–267.
170. **Schübbe S, Kube M, Scheffel A, Wawer C, Heyen U, Meyerdierks A, Madkour MH, Mayer F, Reinhardt R, Schüler D.** 2003. Characterization of a spontaneous nonmagnetic mutant of *Magnetospirillum gryphiswaldense* reveals a large deletion comprising a putative magnetosome island. J. Bacteriol. **185:**5779–5790.
171. **Simmons SL, Bazylinski DA, Edwards KJ.** 2007. Population dynamics

of marine magnetotactic bacteria in a meromictic salt pond described with qPCR. Environ. Microbiol. **9:**2162–2174.
172. **Abreu F, Cantão ME, Nicolás MF, Barcellos FG, Morillo V, Almeida LG, do Nascimento FF, Lefèvre CT, Bazylinski DA, de Vasconcelos ATR, Lins U.** 2011. Common ancestry of iron oxide- and iron-sulfide-based biomineralization in magnetotactic bacteria. ISME J. **5:**1634–1640.
173. **Katzmann E, Scheffel A, Gruska M, Plitzko JM, Schüler D.** 2010. Loss of the actin-like protein MamK has pleiotropic effects on magnetosome formation and chain assembly in *Magnetospirillum gryphiswaldense*. Mol. Microbiol. **77:**208–224.
174. **Murat D, Quinlan A, Vali H, Komeili A.** 2010. Comprehensive genetic dissection of the magnetosome gene island reveals the step-wise assembly of a prokaryotic organelle. Proc. Natl. Acad. Sci. U. S. A. **107:**5593–5598.
175. **Komeili A.** 2007. Cell biology of magnetosome formation, p 163–174. *In* Schüler D (ed), Magnetoreception and magnetosomes in bacteria. Springer, Berlin, Germany.
176. **Komeili A.** 2007. Molecular mechanisms of magnetosome formation. Annu. Rev. Biochem. **76:**351–366.
177. **Faivre D, Böttger LH, Matzanke BF, Schüler D.** 2007. Intracellular magnetite biomineralization in bacteria proceeds by a distinct pathway involving membrane-bound ferritin and an iron(II) species. Angew. Chem. Int. Ed. Engl. **46:**8495–8499.
178. **Neilands JB.** 1991. A brief history of iron metabolism. Biol. Met. **4:**1–6.
179. **Neilands JB.** 1995. Siderophores: structure and function of microbial iron transport compounds. J. Biol. Chem. **270:**26723–26726.
180. **Calugay RJ, Miyashita H, Okamura Y, Matsunaga T.** 2003. Siderophore production by the magnetic bacterium *Magnetospirillum magneticum* AMB-1. FEMS Microbiol. Lett. **218:**371–375.
181. **Paoletti LC, Blakemore RP.** 1986. Hydroxamate production by *Aquaspirillum magnetotacticum*. J. Bacteriol. **167:**73–76.
182. **Uebe R, Voigt B, Schweder T, Albrecht D, Katzmann E, Lang C, Böttger L, Matzanke B, Schüler D.** 2010. Deletion of a *fur*-like gene affects iron homeostasis and magnetosome formation in *Magnetospirillum gryphiswaldense*. J. Bacteriol. **192:**4192–4204.
183. **Qi L, Li J, Zhang W, Liu J, Rong C, Li Y, Wu L.** 2012. Fur in *Magnetospirillum gryphiswaldense* influences magnetosomes formation and directly regulates the genes involved in iron and oxygen metabolism. PLoS One **7:**e29572. doi:10.1371/journal.pone.0029572.
184. **Frankel RB, Blakemore RP, Wolfe RS.** 1979. Magnetite in freshwater magnetotactic bacteria. Science **203:**1355–1356.
185. **Staniland S, Ward B, Harrison A, van der Laan G, Telling N.** 2007. Rapid magnetosome formation shown by real-time X-ray magnetic circular dichroism. Proc. Natl. Acad. Sci. U. S. A. **104:**19524–19528.
186. **Towe KM, Moench TT.** 1981. Electron-optical characterization of bacterial magnetite. Earth Planet. Sci. Lett. **52:**213–220.
187. **Staniland S, Williams W, Telling N, Van Der Laan G, Harrison A, Ward B.** 2008. Controlled cobalt doping of magnetosomes in vivo. Nat. Nanotechnol. **3:**158–162.
188. **Verwey EJW.** 1939. Electronic conduction of magnetite (Fe_3O_4) and its transition point at low temperatures. Nature **144:**327–328.
189. **Keim CN, Lins U, Farina M.** 2009. Manganese in biogenic magnetite crystals from magnetotactic bacteria. FEMS Microbiol. Lett. **292:**250–253.
190. **Bazylinski DA, Garratt-Reed AJ, Abedi A, Frankel RB.** 1993. Copper association with iron sulfide magnetosomes in a magnetotactic bacterium. Arch. Microbiol. **160:**35–42.
191. **Kobayashi A, Kirschvink JL, Nash CZ, Kopp RE, Sauer DA, Bertani LE, Voorhout WF, Taguchi T.** 2006. Experimental observation of magnetosome chain collapse in magnetotactic bacteria: sedimentological, paleomagnetic, and evolutionary implications. Earth Planet. Sci. Lett. **245:**538–550.
192. **Frankel RB, Bazylinski DA.** 2006. How magnetotactic bacteria make magnetosomes queue up. Trends Microbiol. **14:**329–331.
193. **Grünberg K, Wawer C, Tebo BM, Schüler D.** 2001. A large gene cluster encoding several magnetosome proteins is conserved in different species of magnetotactic bacteria. Appl. Environ. Microbiol. **67:**4573–4582.
194. **Matsunaga T, Okamura Y, Fukuda Y, Wahyudi AT, Murase Y, Takeyama H.** 2005. Complete genome sequence of the facultative anaerobic magnetotactic bacterium *Magnetospirillum* sp. strain AMB-1. DNA Res. **12:**157–166.
195. **Jogler C, Kube M, Schübbe S, Ullrich S, Teeling H, Bazylinski DA, Reinhardt R, Schüler D.** 2009. Comparative analysis of magnetosome gene clusters in magnetotactic bacteria provides further evidence for horizontal gene transfer. Environ. Microbiol. **11:**1267–1277.
196. **Nakazawa H, Arakaki A, Narita-Yamada S, Yashiro I, Jinno K, Aoki N, Tsuruyama A, Okamura Y, Tanikawa S, Fujita N, Takeyama H, Matsunaga T.** 2009. Whole genome sequence of *Desulfovibrio magneticus* strain RS-1 revealed common gene clusters in magnetotactic bacteria. Genome Res. **19:**1801–1808.
197. **Ullrich S, Kube M, Schübbe S, Reinhardt R, Schüler D.** 2005. A hypervariable 130-kilobase genomic region of *Magnetospirillum gryphiswaldense* comprises a magnetosome island which undergoes frequent rearrangements during stationary growth. J. Bacteriol. **187:**7176–7184.
198. **Jogler C, Schüler D.** 2009. Genomics, genetics, and cell biology of magnetosome formation. Annu. Rev. Microbiol. **63:**501–521.
199. **Lohsse A, Ullrich S, Katzmann E, Borg S, Wanner G, Richter M, Voigt B, Schweder T, Schüler D.** 2011. Functional analysis of the magnetosome island in *Magnetospirillum gryphiswaldense*: the *mamAB* operon is sufficient for magnetite biomineralization. PLoS One **6:**e25561. doi:10.1371/journal.pone.0025561.
200. **Ullrich S, Schüler D.** 2010. Cre-lox-based method for generation of large deletions within the genomic magnetosome island of *Magnetospirillum gryphiswaldense*. Appl. Environ. Microbiol. **76:**2439–2444.
201. **Scheffel A, Gärdes A, Grünberg K, Wanner G, Schüler D.** 2008. The major magnetosome proteins MamGFDC are not essential for magnetite biomineralization in *Magnetospirillum gryphiswaldense* but regulate the size of magnetosome crystals. J. Bacteriol. **190:**377–386.
202. **Richter M, Kube M, Bazylinski DA, Lombardot T, Glöckner FO, Reinhardt R, Schüler D.** 2007. Comparative genome analysis of four magnetotactic bacteria reveals a complex set of group-specific genes implicated in magnetosome biomineralization and function. J. Bacteriol. **189:**4899–4910.
203. **Komeili A, Vali H, Beveridge TJ, Newman DK.** 2004. Magnetosome vesicles are present before magnetite formation, and MamA is required for their activation. Proc. Natl. Acad. Sci. U. S. A. **101:**3839–3844.
204. **Zeytuni N, Ozyamak E, Ben-Harush K, Davidov G, Levin M, Gat Y, Moyal T, Brik A, Komeili A, Zarivach R.** 2011. Self-recognition mechanism of MamA, a magnetosome-associated TPR-containing protein, promotes complex assembly. Proc. Natl. Acad. Sci. U. S. A. **108:**E480–487.
205. **Uebe R, Junge K, Henn V, Poxleitner G, Katzmann E, Plitzko JM, Zarivach R, Kasama T, Wanner G, Pósfai M, Böttger L, Matzanke B, Schüler D.** 2011. The cation diffusion facilitator proteins MamB and MamM of *Magnetospirillum gryphiswaldense* have distinct and complex functions, and are involved in magnetite biomineralization and magnetosome membrane assembly. Mol. Microbiol. **82:**818–835.
206. **Quinlan A, Murat D, Vali H, Komeili A.** 2011. The HtrA/DegP family protease MamE is a bifunctional protein with roles in magnetosome protein localization and magnetite biomineralization. Mol. Microbiol. **80:**1075–1087.
207. **Siponen MI, Adryanczyk G, Ginet N, Arnoux P, Pignol D.** 2012. Magnetochrome: a c-type cytochrome domain specific to magnetotatic bacteria. Biochem. Soc. Trans. **40:**1319–1323.
208. **Cartron ML, Maddocks S, Gillingham P, Craven CJ, Andrews SC.** 2006. Feo—transport of ferrous iron into bacteria. Biometals **19:**143–157.
209. **Rong C, Huang Y, Zhang W, Jiang W, Li Y, Li J.** 2008. Ferrous iron transport protein B gene (*feoB1*) plays an accessory role in magnetosome formation in *Magnetospirillum gryphiswaldense* strain MSR-1. Res. Microbiol. **159:**530–536.
210. **Mahillon J, Chandler M.** 1998. Insertion sequences. Microbiol. Mol. Biol. Rev. **62:**725–774.
211. **Mahillon J, Léonard C, Chandler M.** 1999. IS elements as constituents of bacterial genomes. Res. Microbiol. **150:**675–687.
212. **Blum G, Ott M, Lischewski A, Ritter A, Imrich H, Tschäpe H, Hacker J.** 1994. Excision of large DNA regions termed pathogenicity islands from tRNA-specific loci in the chromosome of an *Escherichia coli* wild-type pathogen. Infect. Immun. **62:**606–614.
213. **Reiter WD, Palm P.** 1990. Identification and characterization of a defective SSV1 genome integrated into a tRNA gene in the archaebacterium *Sulfolobus* sp. B12. Mol. Gen. Genet. **221:**65–71.
214. **Dobrindt U, Hochhut B, Hentschel U, Hacker J.** 2004. Genomic islands in pathogenic and environmental microorganisms. Nat. Rev. Microbiol. **2:**414–424.
215. **Fukuda Y, Okamura Y, Takeyama H, Matsunaga T.** 2006. Dynamic

analysis of a genomic island in *Magnetospirillum* sp. strain AMB-1 reveals how magnetosome synthesis developed. FEBS Lett. **580:**801–812.
216. **Juhas M, van der Meer JR, Gaillard M, Harding RM, Hood DW, Crook DW.** 2009. Genomic islands: tools of bacterial horizontal gene transfer and evolution. FEMS Microbiol. Rev. **33:**376–393.
217. **Kolinko I, Jogler C, Katzmann E, Schüler D.** 2011. Frequent mutations within the genomic magnetosome island of *Magnetospirillum gryphiswaldense* are mediated by RecA. J. Bacteriol. **193:**5328–5334.
218. **Rioux J-B, Philippe N, Pereira S, Pignol D, Wu L-F, Ginet N.** 2010. A second actin-like MamK protein in *Magnetospirillum magneticum* AMB-1 encoded outside the genomic magnetosome island. PLoS One **5:**e9151. doi:10.1371/journal.pone.0009151.
219. **Zeytuni N, Baran D, Davidov G, Zarivach R.** 2012. Inter-phylum structural conservation of the magnetosome-associated TPR-containing protein, MamA. J. Struct. Biol. **180:**479–487.
220. **Schübbe S, Würdemann C, Peplies J, Heyen U, Wawer C, Glöckner FO, Schüler D.** 2006. Transcriptional organization and regulation of magnetosome operons in *Magnetospirillum gryphiswaldense*. Appl. Environ. Microbiol. **72:**5757–5765.
221. **Fang G, Rocha EPC, Danchin A.** 2008. Persistence drives gene clustering in bacterial genomes. BMC Genomics **9:**4. doi:10.1186/1471-2164-9-4.
222. **Tamames J.** 2001. Evolution of gene order conservation in prokaryotes. Genome Biol. **2:**RESEARCH0020. doi:10.1186/gb-2001-2-6-research0020.
223. **Battistuzzi FU, Feijao A, Hedges SB.** 2004. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. BMC Evol. Biol. **4:**44. doi:10.1186/1471-2148-4-44.
224. **Anbar AD, Duan Y, Lyons TW, Arnold GL, Kendall B, Creaser RA, Kaufman AJ, Gordon GW, Scott C, Garvin J, Buick R.** 2007. A whiff of oxygen before the Great Oxidation Event? Science **317:**1903–1906.
225. **Lefèvre CT, Pósfai M, Abreu F, Lins U, Frankel RB, Bazylinski DA.** 2011. Morphological features of elongated-anisotropic magnetosome crystals in magnetotactic bacteria of the *Nitrospirae* phylum and the *Deltaproteobacteria* class. Earth Planet. Sci. Lett. **312:**194–200.
226. **Devouard B, Pósfai M, Hua X, Bazylinski DA, Frankel RB, Buseck PR.** 1998. Magnetite from magnetotactic bacteria: size distributions and twinning. Am. Miner. **83:**1387–1398.
227. **Mandernack KW, Bazylinski DA, Shanks WC, III, Bullen TD.** 1999. Oxygen and iron isotope studies of magnetite produced by magnetotactic bacteria. Science **285:**1892–1896.
228. **Faivre D, Menguy N, Posfai M, Schuler D.** 2008. Environmental parameters affect the physical properties of fast-growing magnetosomes. Am. Mineral. **93:**463–469.
229. **Ji B, Zhang SD, Arnoux P, Rouy Z, Alberto F, Philippe N, Murat D, Zhang WJ, Rioux JB, Ginet N, Sabaty M, Mangenot S, Pradel N, Tian J, Yang J, Zhang L, Zhang W, Pan H, Henrissat B, Coutinho PM, Li Y, Xiao T, Médigue C, Barbe V, Pignol D, Talla E, Wu LF.** 11 June 2013. Comparative genomic analysis provides insights into the evolution and niche adaptation of marine Magnetospira sp. QH-2 strain. Environ. Microbiol. [Epub ahead of print.] doi:10.1111/1462-2920.12180.



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