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Complete Genome Sequence of the Chemolithoautotrophic Marine Magnetotactic Coccus Strain MC-1[▽]†

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The marine bacterium strain MC-1 is a member of the alpha subgroup of the proteobacteria that contains the magnetotactic cocci and was the first member of this group to be cultured axenically. The magnetotactic cocci are not closely related to any other known alphaproteobacteria and are only distantly related to other magnetotactic bacteria. The genome of MC-1 contains an extensive (102 kb) magnetosome island that includes numerous genes that are conserved among all known magnetotactic bacteria, as well as some genes that are unique. Interestingly, certain genes that encode proteins considered to be important in magnetosome assembly (*mamJ* and *mamW*) are absent from the genome of MC-1. Magnetotactic cocci exhibit polar magneto-aerotaxis, and the MC-1 genome contains a relatively large number of identified chemotaxis genes. Although MC-1 is capable of both autotrophic and heterotrophic growth, it does not appear to be metabolically versatile, with heterotrophic growth confined to the utilization of acetate. Central carbon metabolism is encoded by genes for the citric acid cycle (oxidative and reductive), glycolysis, and gluconeogenesis. The genome also reveals the presence or absence of specific genes involved in the nitrogen, sulfur, iron, and phosphate metabolism of MC-1, allowing us to infer the presence or absence of specific biochemical pathways in strain MC-1. The pathways inferred from the MC-1 genome provide important information regarding central metabolism in this strain that could provide insights useful for the isolation and cultivation of new magnetotactic bacterial strains, in particular strains of other magnetotactic cocci.

Almost all magnetotactic bacteria (MTB) are microaerophiles that are most abundant at the oxic-anoxic interface (OAI) of natural aquatic environments, where magnetotactic cocci are often ubiquitous and the most dominant morphotype of MTB (43). The original discovery of MTB was based on the observation of polar magnetotaxis (44) in the magnetotactic cocci (19). In addition, phylogenetic studies of the magnetotactic cocci revealed a surprising degree of diversity (104, 105) in spite of their virtually identical cell morphology of coccoid to ovoid cells that are bilophotrichously flagellated on one side of the cell (27, 45, 71). These organisms are phylogenetically distinct from other MTB and form a clade at the base of the alphaproteobacterial branch on the tree of life, whereas other MTB of the *Alphaproteobacteria* (e.g., *Magnetospirillum*) are nested deep within the group (106). Despite their ubiquity in natural aquatic systems, for many years only one strain of magnetotactic cocci, designated strain MC-1, was isolated and grown in pure culture (44). Recently, another related strain was isolated, but it has not yet been characterized by molecular taxonomy (70). Strain MC-1 is a marine species that was isolated from water collected from the OAI of the Pettaquamscutt Estuary (Narragansett, RI) (34). It is presently in the process of valid description as "*Magnetococcus marinus*."

Cells of strain MC-1 are not metabolically versatile, based on previous growth experiments (14, 113) and genomic information presented here. Cells of this species grow chemolithoautotrophically, with thiosulfate or sulfide as an electron donor, and chemoheterotrophically, with acetate as the electron donor and carbon source (14). Unlike some other autotrophic marine MTB, such as vibrio strain MV-1, which represents a new genus of bacteria in the *Alphaproteobacteria* ("*Candidatus* Magnetovibrio blakemorei" [D. A. Bazylinski, unpublished data]), cells of strain MC-1 do not utilize the Calvin-Benson-Bassham cycle for CO_2 fixation but rely on the reverse tricarboxylic acid (rTCA) cycle (113).

The salient feature of MTB is their ability to biomineralize magnetosomes. These intracellular structures consist of well-ordered crystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4) surrounded by a lipid bilayer membrane (5, 42, 50, 76). Different strains of MTB synthesize magnetosome crystals not only of a specific composition but also of a specific morphology and size

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(8, 13). These consistent, species-specific crystal morphologies and sizes indicate that biomineralization of magnetite is genetically controlled in MTB, and it is likely that a common set of genes is responsible for magnetosome biomineralization, although there are presumably some species-specific genes that might be involved in some aspects of biomineralization, such as the control of crystal shape and/or size.

In the last decade, genomic and genetic studies have confirmed that many cultured MTB contain a common set of genes that appear to be involved in magnetite biomineralization (89). It has been shown that the genes encoding magnetosome (membrane) proteins are localized on a 130-kb magnetosome island (MAI) in *Magnetospirillum gryphiswaldense* (94, 111). In addition, this genomic island is conserved among other *Magnetospirillum* strains, including *Magnetospirillum magneticum* strain AMB-1 and *Magnetospirillum magnetotacticum* strain MS-1 (48, 61). This may not be surprising since these strains are closely related. Recent studies show that the magnetotactic marine vibrio strain MV-1 contains an MAI as well. Comparisons to other MTB strains revealed that strain MV-1 contains a highly conserved *mamAB* cluster, but other magnetosome gene clusters are arranged differently or are absent (60). A comparative genomic analysis between these *Magnetospirillum* species and the distantly related strain MC-1 revealed some significant differences in the set of magnetosome genes. Richter et al. (89) compared the gene contents based on reciprocal best matches and showed that strain MC-1 shares only about one-half the number of magnetosome genes with *M. gryphiswaldense* and *M. magneticum* that the *Magnetospirillum* strains share with each other. This study also revealed a "*Magnetospirillum*-specific" set of genes as well as some MTB-specific genes that are also present in strain MC-1 (89). There is evidence that the MC-1 genome is mosaic to the highest degree found within the alphaproteobacteria. Only 33% of the genes showed a best BLAST hit to those in a collection of 18 alphaproteobacterial sequences (39). Earlier studies showed that strain MC-1 is one of the more divergent alphaproteobacteria (117).

In this study, we analyzed the complete genome sequence of the marine magnetotactic coccus strain MC-1. This analysis provided us with considerable new insights into the organization of the magnetosome genes in MC-1 and the MAI in which they reside, as well as allowing us to compare the magnetosome genes to those of other MTB strains to determine whether novel magnetosome genes are present in MC-1 and not in other MTB strains. In addition, we sought to examine whether the genome sequence (through the presence and absence of specific genes) supports the known physiological and metabolic aspects of MC-1 as well as its lack of metabolic versatility and if the sequence could reveal or suggest novel metabolic or physiological features of this organism. It has clearly been shown that growth and environmental conditions that affect physiology also greatly influence magnetosome production in some species (21, 97). The results from this study might prove invaluable in the understanding of environmental control of and physiological effects on magnetosome synthesis as well as for the isolation of other MTB, in particular the ubiquitous magnetotactic cocci, in terms of their use of electron donors and acceptors.

MATERIALS AND METHODS

Growth conditions and isolation of genomic DNA. Cells of strain MC-1 were grown microaerobically under chemolithoautotrophic conditions in liquid medium consisting of an artificial seawater base with thiosulfate as the electron donor, as described previously (113). Cultures were incubated statically under an $[O_2]$ (O_2 concentration) gradient at 25°C in the dark.

Genomic DNA from strain MC-1 was isolated as previously described by Kimble et al. (64). The quality of the DNA was determined by agarose gel electrophoresis.

Genome sequencing and assembly. The MC-1 genome was sequenced using a combination of 3-kb, 8-kb, and 40-kb (fosmid) DNA libraries. The number of clones made for these libraries was enough for about 15× coverage of the genome, using the three libraries. All general aspects of library construction and sequencing were performed at the Joint Genome Institute (JGI), and details can be found at http://www.jgi.doe.gov/.

Draft assemblies were based on 126,624 total reads. The Phred/Phrap/Consed software package was used for sequence assembly and quality assessment (40, 41, 51). After the shotgun stage, the 126,624 reads were assembled with parallel Phrap (High Performance Software, LLC). Possible misassemblies were corrected with Dupfinisher (55) or transposon bombing of bridging clones (Epicentre Biotechnologies, Madison, WI). Gaps between contigs were closed by editing in Consed, custom primer walking, or PCR amplification (Roche Applied Science, Indianapolis, IN). A total of 27,823 additional sequencing reactions were necessary to close gaps and to raise the quality of the finished sequence. The final assembly of the genome of the magnetic coccus strain MC-1 contained 107,421 reads after removal of the majority of the draft reads that landed in duplicated regions during the finishing process. The error rate of the final genome sequence is less than 1 in 100,000 bp.

Automated gene prediction was performed by using the output of Critica (4) complemented with the output of Generation and Glimmer (32). The tRNAScanSE tool (72) was used to find tRNA genes, whereas rRNAs were found by using BLASTN searches of 16S and 23S rRNA databases. Other "standard" structural RNAs (e.g., 5S rRNA, *rnpB*, transfer messenger RNA, and signal recognition particle RNA) were found by using covariance models with the internal search tool (37). The assignment of product descriptions was made by using search results for the following curated databases, in the indicated order: TIGRFam, PRIAM (e^{-30} cutoff), Pfam, Smart, COGs, Swissprot/TrEMBL (SPTR), and KEGG. If there was no significant similarity to any protein in another organism, it was described as a "hypothetical protein." The term "conserved hypothetical protein" was used if at least one match was found to a hypothetical protein in another organism. EC numbering was based on searches in PRIAM with a cutoff of e^{-10}; COG and KEGG functional classifications were based on homology searches in the respective databases.

Comparative analysis of strain MC-1 and related organisms was performed by using a set of tools available at the JGI Integrated Microbial Genomes (IMG) website (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi). Unique and orthologous MC-1 genes were identified by using BLASTP (using cutoffs of E scores of $<10^{-2}$ and 20% identity and reciprocal hits with cutoffs of E scores of $<10^{-5}$ and 30% identity, respectively). Signal peptides and transmembrane helices were predicted using SignalP 3.0 (16) and TMHMM 2.0 (68), set at default values. Protein localizations were predicted with PSORTb (49), and twin-arginine translocation systems were identified using the TatP program (17). Insertion sequence elements were identified by using the ISFinder database (102). Metabolic pathways were constructed using MetaCyc as a reference data set (25). Other details regarding genome properties and genome annotation can be obtained from the JGI's IMG website (77).

Prophage prediction. The prediction of potential prophage loci was done with Prophage Finder under default conditions, using an E value of 0.5, five hits per prophage, and 5,500-bp hit spacing (22; http://bioinformatics.uwp.edu/~phage/ProphageFinder.php).

Nucleotide sequence accession number. The sequence of strain MC-1 can be accessed using GenBank accession number CP000471.

RESULTS AND DISCUSSION

General genome features. The genome of the marine magnetotactic coccus strain MC-1 consists of a single circular chromosome of 4,719,583 bp, which correlates well with size estimates obtained by pulsed-field gel electrophoresis (31). There is no evidence for the presence of extrachromosomal elements such as plasmids. The GC skew shifts near the gene encoding

TABLE 1. General genome features of strain MC-1[a]

Genome feature	Value
Size (bp)	4,719,581
G+C content (mol%)	
Genome	54.17
ORFs	54.79
No. of rRNAs	3 (5S, 16S, and 23S)
No. of tRNAs	45
No. of predicted ORFs	3,815
No. of predicted protein-encoding genes	3,758
No. of coding bases	4,117,746
% of coding bases	87.25
Avg length of ORF (bp)	1,098
No. (%) of genes with function prediction	2,505 (65.66)
No. (%) of genes without known function but with similarity to genes with known function	1,145 (30.01)
No. (%) of genes without known function and without similarity to genes with known function	108 (2.83)
No. (%) of pseudogenes	42 (1.10)

[a] Derived from the DOE JGI IMG server (http://img.jgi.doe.gov).

the DnaA protein (located at "noon" on the circular map; Mmc10001), and thus the origin of replication is likely located nearby. The genome has an average G+C content of 54% and shows no deviation from the average that might be due to horizontal gene transfer. It contains 3,815 predicted open reading frames (ORFs), including those encoding 45 tRNAs and three sets of rRNAs (5S, 16S, and 23S), which corresponds to 87.25% of the genome being coding sequences (Table 1). The average ORF length is 1,098 bp (Table 1). A circular representation of the genome is shown in Fig. 1.

An interesting feature of the genome of strain MC-1 is that it contains two unusually large ORFs ("giant" genes) (87), which are 44.733 kb (Mmc12179) and 45.738 kb (Mmc12196) long. The gene Mmc12179 encodes a putative outer membrane adhesin-like protein with the closest BLAST hit to a similar protein in *Desulfococcus oleovorans* (8e−120). This protein also shows homology to a protein of the type V secretory pathway of *M. magneticum* (1e−82) and *M. magnetotacticum* (2e−78) and to a large exoprotein (5,299 amino acids [aa]) of *M. magneticum* (4e−81). Mmc12196 encodes a hemolysin-type calcium-binding region that has no similarity to any protein in other magnetotactic bacteria. The putative functions of these proteins are consistent with those coded for by other such giant genes in other bacteria: 90% of bacterial giant genes encode either surface proteins, such as adhesins, hemolysins, or membrane proteins, or polyketide/nonribosomal peptide synthetases (87).

Magnetosome genes. The genomes of three strains of MTB, all in the genus *Magnetospirillum* (*M. magnetotacticum* strain MS-1, *M. gryphiswaldense* strain MSR-1, and *M. magneticum* strain AMB-1), have been sequenced at least partially, revealing an MAI containing the regulatory and structural genes coding for magnetosome (membrane) proteins. The genome of MC-1 reveals a putative MAI of about 102 kb, located on the chromosome between 2.80 and 2.93 Mb (Fig. 1). Strong evidence from tetranucleotide usage patterns in *Magnetospirillum* species and strain MV-1 showing that the MAIs in these MTB are transferred by horizontal gene transfer was recently described (60). However, the same analysis showed that the putative MAI in strain MC-1 lacks a conspicuous tetranucleotide signal (60). The GC bias of the MAI in strain MC-1 shows the same random distribution (Fig. 1). These observations might result from a very early transfer of the island to MC-1-like organisms or from additional horizontal gene transfer events after the transfer of the MAI. These possibilities might also explain the mosaic structure of the MC-1 genome (39). Nonetheless, the presence of an MAI in MC-1 is based on the very close proximity of the majority of the magnetosome genes in a relatively small genomic region and the presence of two integrase encoding genes, IS*4* and IS*3*, that border this genomic region (Fig. 2).Within the putative MAI, the genes are arranged in four clusters (*mamAB*, *mamCEIH*, *mamDXZ*, and *mms*), with a fifth magnetosome-related cluster (*mtx*) situated outside the MAI. In contrast to many hypervariable genomic islands (18, 52, 94), the MC-1 putative MAI contains no transposon genes within the island, and thus the position of the MAI within the genome is likely to be more stable than those in other MTB. However, some characteristic features of genomic islands are in close proximity to the island; for example, two genes with homology to insertion sequence elements of the IS*4* family (35) and three tRNA genes that often serve as attachment sites for phages are located upstream of the MAI. In addition, 14 prophages were identified distributed throughout the genome. This is in contrast to the MAIs in *Magnetospirillum gryphiswaldense* and *M. magneticum*, which contain numerous integrase and transposase genes, presumably involved in the mobility of the MAI, that make up more than 20% of the coding sequence of the MAI. Spontaneous deletions and mutations resulting from these integrase and transposase genes have been shown to cause nonmagnetic mutants to occur at a rate of 10^{-2} in *M. gryphiswaldense* and *M. magneticum* (48, 111). Nonmagnetic mutants have never been observed in strain MC-1. Another key difference in the MC-1 genome compared to the genomes of the *Magnetospirillum* strains is the organization of the magnetosome genes within the MAI (Fig. 2).

(i) *mamAB* cluster. The *mamAB* cluster in strain MC-1 consists of a total of 14 ORFs (Mmc12247 to Mmc12260) that are transcribed from the opposite strand of DNA. Nine of these genes are homologous to genes from the *mamAB* operons of *Magnetospirillum* strains, including *mamA* (Mmc12253), *mamB*/*mamM* (Mmc12250 and Mmc12256), *mamK* (Mmc12259),

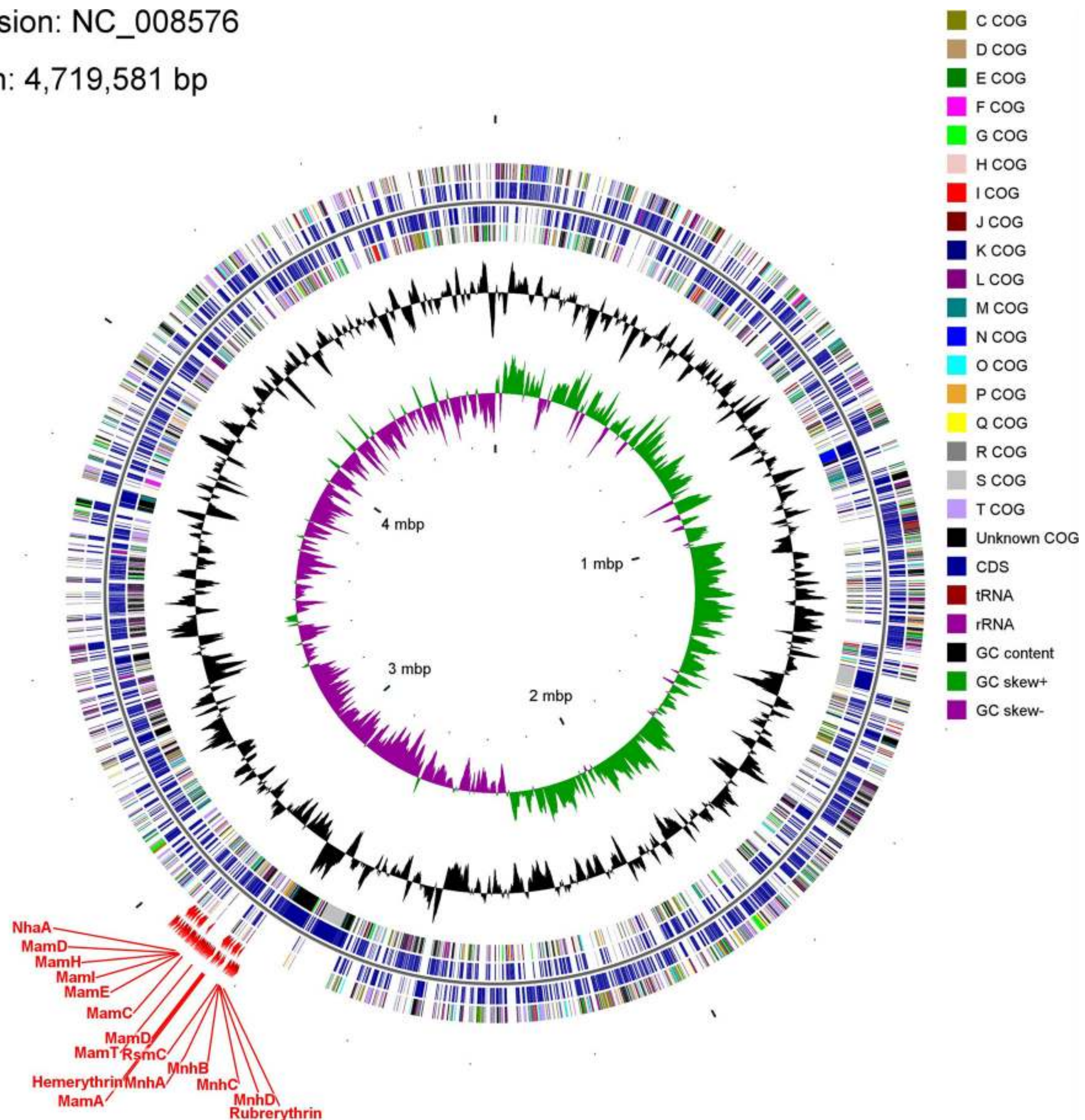

FIG. 1. Circular representation of the genome of the magnetotactic coccus strain MC-1. The location of the MAI on the chromosome is indicated in red outside the circles. The outer two rings (ring 1 and ring 2) depict predicted protein-encoding and structural RNA genes on the plus and minus strands, respectively, and are color coded according to COG category. The black circle (ring 3) indicates the deviation from the average %GC, and the purple and green circle (ring 4) represents the GC skew.

mamO (Mmc12255), *mamP* (Mmc12254), *mamQ* (Mmc12251), *mamS* (Mmc12249), and *mamT* (Mmc12248). The gene order *mamK-mamM-mamOPA-mamQB-mamST* is mostly conserved in MC-1 and *Magnetospirillum* strains, although other genes may be present or absent. The *mamAB* cluster of MC-1 includes three genes (Mmc12247, Mmc12257, and Mmc12260) that have no apparent homologue in *Magnetospirillum* (Table 2). Mmc12247, located at the 3′ end of the *mamAB* cluster, encodes a large protein (1,705 aa) with similarity to a putative chemotaxis sensory transducer; Mmc12257 is located between *mamM* and a *mamF*-like gene and has some similarity to other conserved hypothetical protein genes; and Mmc12260, at the 5′ end of the *mamAB* cluster, shows sequence similarity to a chromosome segregation ATPase. The last of these three genes might play a role in the organization of the magnetosome chain.

On the other hand, several genes in the *Magnetospirillum mamAB* cluster are absent from the entire MC-1 genome, including *mamJ*, *mamL*, *mamN*, *mamR*, and *mamU* as well as *mamV* (also absent from *M. gryphiswaldense*) (96). There are two genes that encode proteins with tetratricopeptide repeat (TPR) motifs adjacent to the MC-1 *mamAB* cluster, namely, the genes encoding MamA (219 aa) and a larger, MamA-like protein (1,022 aa) that has a 222-bp TPR domain in the C terminus. Proteins with TPR domains mediate protein-protein interactions and the assembly of multiprotein complexes (29), and it has been suggested that a TPR-based scaffold may facilitate the stable localization of interacting proteins during

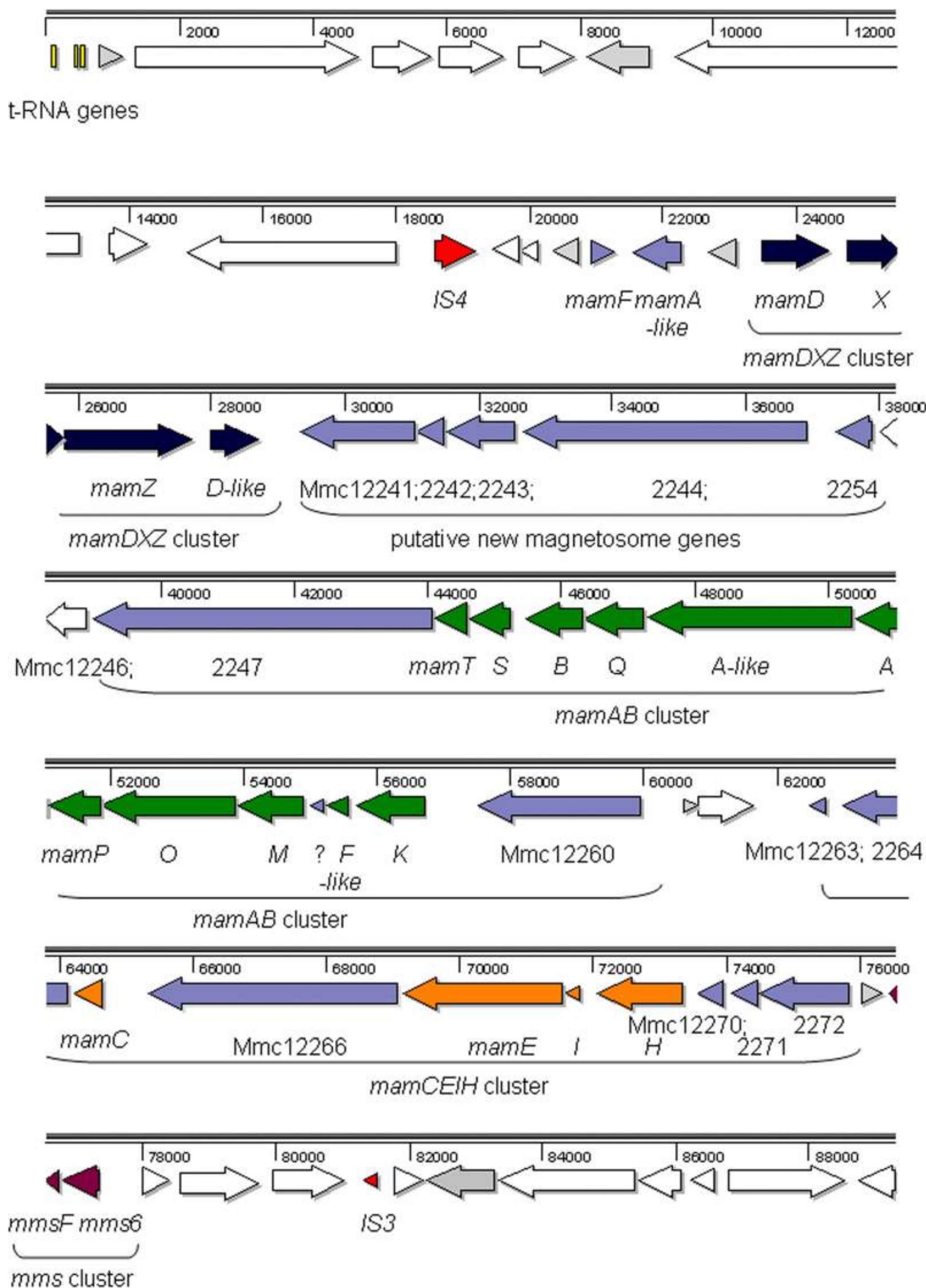

FIG. 2. Genomic organization of the magnetosome genes in MC-1. The magnetosome genes are organized into five different clusters localized on a putative ~102-kb MAI. Green, genes of the *mamAB* cluster; purple, genes of the *mms* cluster; dark blue, genes of the *mamDXZ* cluster; orange, genes of the *mamCEIH* cluster; light blue, putative new magnetosome genes; yellow, tRNA genes; red, mobility genes; gray, hypothetical genes; white, housekeeping genes.

magnetosome assembly (96). Studies with *M. magneticum* showed that MamA displays a dynamic subcellular localization throughout the growth cycle. It is thought that the magnetosome membrane-associated protein MamA is required for the formation of functional magnetosome vesicles (67). Other genes encoding TPR proteins are present outside the *mamAB* cluster, within the MAI (Mmc12235; another *mamA*-like gene) and in the *mtx* cluster (Mmc13695; see below). The precise functions of the majority of *mam*-encoded proteins have yet to be elucidated, although putative functions have been proposed

TABLE 2. Features of deduced genes in the ~102-kb MAI in strain MC-1

Gene no.	Length of protein (aa)	Presence of BLASTP homologues in MTB MS-1/MSR-1/AMB-1 (E value)[a]	BLASTP homologues in other organisms (E value)	Characteristics and/or function
Mmc12203	632	—/—/—	*Nitrococcus mobilis* (0.0)	Transposase IS*4*
Mmc12204	167	—/—/—	*Endoriftia persephone* (7e−42)	Rubrerythrin
Mmc12205	598	3e−41/—/—	*Beggiatoa* sp. glutamate synthase (4e−170)	FAD-dependent pyridine nucleotide-disulfide oxidoreductases
Mmc12206	391	3e−50/—/5e−51	*Beggiatoa* sp. NADH ubiquinone:oxidoreductase (7e−92)	NADH dehydrogenase subunit D
Mmc12207	178	1e−04/—/5e−05	*Fervidobacterium nodosum* (1e−23)	NADH dehydrogenase subunit C
Mmc12208	213	2e−29/—/2e−29	*Pyrococcus furiosus* (3e−50)	NADH dehydrogenase subunit B
Mmc12209	301	—/—/—	*Thermococcus kodakarensis* membrane-bound hydrogenase (4e−43)	NADH dehydrogenase subunit H
Mmc12210	1,047	2e−24/—/7e−25	*Petrotoga mobilis* (6e−61)	NADH dehydrogenase subunit M
Mmc12211	119	7e−04/—/—	*Pyrococcus furiosus* (7e−22)	Putative monovalent cation/H^+ antiporter subunit C
Mmc12212	233	2e−08/—/—	*Petrotoga mobilis* MnhB (2e−38)	Putative monovalent cation/H^+ antiporter subunit B
Mmc12213	84	—/—/—	*Pyrococcus horikoshii* hypothetical protein (6e−06)	Putative monovalent cation/H^+ antiporter subunit A
Mmc12214	112	—/—/—	*Thermotoga maritima* (5e−22)	Putative monovalent cation/H^+ antiporter subunit G
Mmc12215	86	0.98/—/—	*Thermotoga maritima* (9e−13)	Putative monovalent cation/H^+ antiporter subunit F
Mmc12216	172	0.45/—/—	*Thermosipho melanesiensis* (8e−26)	Putative monovalent cation/H^+ antiporter subunit E
Mmc12217	369	1e−10/—/—	*Alteromonadales bacterium* (3e−70)	rRNA small-subunit methyltransferase
Mmc1R0031	91bp	—/—/2e−06	*Alkalilimnicola ehrlichei* unknown protein (1e−26)	tRNA Ser
Mmc12218	150	3e−12/—/3e−10	*Caulobacter* sp. strain K31 (4e−18)	Nucleoside deaminase
Mmc12219	168	—/—/—	*Geobacter uraniireducens* (3e−04)	Hypothetical protein
Mmc12220	971	8e−144/5e−155/8e−153	*Thiomicrospira crunogena* (4e−153)	Diguanylate cyclase/phosphodiesterase with PAC/PAS and GAF sensor
Mmc1R0032	90bp	—/—/—	*Hyphomonas neptunium* (8e−14)	tRNA Ser
Mmc1R0033	77bp	—/—/—	*Desulfotalea psychrophila* (2e−32)	tRNA Ile
Mmc1R0034	76bp	—/—/—	*Nitrosomonas* sp. strain OZK11 (2e−29)	tRNA Ala
Mmc12221	118	—/—/—	*Trypanosoma brucei* (1.6)	Hypothetical protein
Mmc12222	1,120	—/2e−77/1e−75	*Chromobacterium violaceum* hypothetical protein (3e−99)	PAS/PAC sensor hybrid histidine kinase
Mmc12223	293	—/—/—	*Rhodoferax ferrireducens* (8e−71)	Methyltransferase type 11
Mmc12224	327	—/—/—	*Mariprofundus ferrooxydans* (8e−105)	Histone deacetylase
Mmc12225	279	3e−63/—/1e−58	*Ochrobactrum anthropi* (4e−68)	Uroporphyrinogen III methylase
Mmc12226	322	1.2/—/—	*Coxiella burnetii* methyltransferase (0.045)	Hypothetical protein
Mmc12227	1,275	4e−145/2e−155/2e−156	*Azoarcus* sp. strain BH72 (1e−174)	Diguanylate cyclase/phosphodiesterase with PAC/PAS and GAF sensor
Mmc12228	190	—/—/—	*Methanococcus vannielii* (3e−14)	Transcriptional regulator TetR
Mmc12229	1,049	2e−81/1e−94/1e−85	*Shewanella* sp. strain MR7 (7e−94)	PAS/PAC sensor hybrid histidine kinase
Mmc12230	198	—/—/—	*Nitrosomonas eutropha* (3e−54)	Integrase
Mmc12231	134	6e−07/5e−10/1e−06	*Geobacter bemidjiensis* (1e−11)	Hemerythrin protein
Mmc12232	101	0.097/1.3/—	*Marinomonas* sp. (MWYL1) (4e−04)	Putative anti-sigma factor antagonist
Mmc12233	130	—/—/—	None found	Hypothetical protein
Mmc12234	109	2e−09/1e−12/2e−13	*Eubacterium siraeum* hypothetical protein (7e−05)	MamF-like magnetosome protein
Mmc12235	247	2e−13/3e−14/1e−13	*Thermotoga petrophila* TPR protein (2e−04)	MamA-like magnetosome protein, TPR repeat-containing protein
Mmc12236	139		None found	Hypothetical protein
Mmc12237	340	2e−17/3e−14/4e−16	*Magnaporthe grisea* hypothetical protein (0.001)	MamD magnetosome protein
Mmc12238	345	1e−35/3e−38/1e−35	*Monosiga brevicollis* hypothetical protein (2e−04)	MamX magnetosome protein
Mmc12239	642	4e−178/2e−173/5e−179	*Polaromonas* sp. strain JS666 ferric reductase-like transmembrane protein (8e−34)	MamZ magnetosome protein
Mmc12240 (Phi BLAST)	239	1e−13/2e−10/4e−13	*Drosophila ananassae* (0.002)	MamD-like magnetosome protein
Mmc12241	574	—/—/—	*Methanosarcina barkeri* (5e−04)	Hypothetical protein
Mmc12242	147	5.2/—/—	*Methanocaldococcus jannaschi* cobalamin biosynthesis protein B (0.059)	Hypothetical protein
Mmc12243	343	—/—/—	None found	Hypothetical protein
Mmc12244	1,416	4.7/—/—	*Monodelphis domestica* Almstrom syndrome protein (8e−06)	Hypothetical protein similar to type IV pilus assembly protein PilZ
Mmc12245	194	—/—/—	*Theileria annulata* (0.3)	Hypothetical protein
Mmc12246	286	1.2/4.2/—	*Legionella pneumophila* (1e−12)	Hypothetical protein similar to flagellar motor protein OmpA/MotB
Mmc12247	1,705	—/—/—	*Sinorhizobium medicae* (2e−05)	Putative chemotaxis sensory transducer
Mmc12248	167	2e−18/6e−18/1e−18	*Percos grandicollis* NADH dehydrogenase (0.044)	MamT magnetosome protein
Mmc12249	210	2e−13/4e−12/2e−13	*Cyanothece* sp. strain PCC7424 hypothetical protein (0.026)	MamS magnetosome protein
Mmc12250	294	8e−78/3e−78/8e−78	*Natranaerobius thermophilus* CDF transporter protein (2e−48)	MamB magnetosome protein, CDF transporter
Mmc12251	308	4e−36/3e−38/1e−36	*Bacillus* sp. strain NRRL LemA protein (1e−26)	MamQ magnetosome protein, LemA protein
Mmc12252	1,022	—/—/—	*Culex quinquefasciatus* hypothetical protein (6e−18)	MamA-like magnetosome protein, TPR repeat-containing protein
Mmc12253	219	7e−37/5e−36/7e−37	*Methanosarcina acetivorans* O-linked GlcNAc transferase (1e−13)	MamA magnetosome protein, TPR repeat-containing protein
Mmc12254	261	9e−37/5e−39/4e−36	*Synechococcus* sp. peptidase (7e−04)	MamP magnetosome protein, PDZ domain of tryspin-like serine proteases
Mmc12255	671	8e−84/4e−83/7e−88	*Flavobacteriales bacterium* putative heat shock-related protease (3e−13)	MamO magnetosome protein, permease
Mmc12256	332	1e−68/8e−76/3e−77	*Syntrophus aciditrophicus* Co-Zi-Cd-resistant protein (2e−41)	MamM magnetosome protein, CDF transporter

Continued on following page

TABLE 2—*Continued*

Gene no.	Length of protein (aa)	Presence of BLASTP homologues in MTB MS-1/MSR-1/AMB-1 (E value)[a]	BLASTP homologues in other organisms (E value)	Characteristics and/or function
Mmc12257	73	—/—/—	*Dechloromonas aromatica* putative membrane protein (0.15)	Hypothetical protein
Mmc12258	108	3e−19/5e−20/1e−19	*Clostridium scindens* hypothetical protein (4e−04)	Magnetosome protein similar to MamF
Mmc12259	346	2e−94/2e−100/2e−101	Deltaproteobacterium MLMS-1 (2e−98)	MamK magnetosome protein
Mmc12260	814	—/—/—	*Caenorhabditis briggsae* hypothetical protein (5e−06)	Chromosome segregation ATPase
Mmc12261	72	—/—/—	None found	Hypothetical protein
Mmc12262	275	1e−13/—/2e−11	*Pseudoalteromonas atlantica* (2e−32)	Metal-dependent phosphohydrolase
Mmc12263	87	—/—/—	None found	Hypothetical protein
Mmc12264	385	—/—/—	*Beggiatoa* sp. strain PS (8e−38)	Ferritin-like hypothetical protein
Mmc12265	133	8e−19/2e−20/6e−19	*Physcomitrella patens* putative Na^+/H^+ antiporter (0.31)	MamC magnetosome protein
Mmc12266	1,250	—/—/1.1	*Culex quinquefasciatus* (1e−13)	Hypothetical protein
Mmc12267	803	2e−75/4e−35/1e−74	*Rhodopirellula baltica*(5e−42)	MamE magnetosome protein, PDZ domain of tryspin-like serine proteases
Mmc12268	76	4e−07/3e−06/4e−07	None found	MamI magnetosome protein
Mmc12269	427	1e−100/2e−117/3e−120	*Chlorobium tepidum* (4e−34)	MamH magnetosome protein, permease of the major facilitator superfamily
Mmc12270	136	3e−07/4e−06/8e−07	*Lyngbya* sp. strain PCC8106 putative phosphate transport protein (0.004)	MamD-like magnetosome protein
Mmc12271	138	0.002/1e−09/0.036	*Geobacter sulfurreducens* (2e−06)	Putative methyl-accepting chemotaxis protein
Mmc12272	448	—/—/—	*Mannheimia succiniciproducens* (5e−63)	Na^+/Ala symporter
Mmc12273	110	—/—/—	*Geobacter* sp. strain M21 cytochrome *c* family protein (0.25)	Hypothetical protein
Mmc12274	111	3e−23/4e−24/7e−24	*Clostridium scindens* hypothetical protein (7e−04)	MmsF magnetosome protein
Mmc12275	194	5e−15/5e−09/2e−14	*Ostreococcus lucimarinus* (1e−05)	Hypothetical protein similar to Mms6 (mgI461)
Mmc12276	145	0.56/0.75/0.88	*Xanthobacter autotrophicus* (0.003)	Cyclic nucleotide binding protein
Mmc12277	397	7e−96/—/2e−95	*Aeromonas salmonicida* (4e−116)	Na^+/H^+ antiporter NhaA
Mmc12278	357	1e−11/—/1e−12	*Geobacter lovleyi* (7e−38)	Signal transduction histidine kinase
Mmc12279	75	—/—/—	*Marinobacter aquaeolei* (0.022)	Putative transposase IS*3*/IS*911* family protein
Mmc12280	143	—/—/—	*Thermoplasma acidophilum* hypothetical protein (1e−09)	Rhodanase domain protein
Mmc12281	344	—/0.41/—	*Trichodesmium erythraeum* (7e−30)	Conserved hypothetical protein
Mmc12282	685	2e−86/1e−86/2e−82	"*Candidatus* Kuenenia stuttgartiensis" (3e−106)	PAS/PAC sensor hybrid histidine kinase
Mmc12283	219	—/3e−34/7e−34	*Rhodopseudomonas palustris* (1e−35)	CheC, inhibitor of MCP methylation
Mmc12284	121	—/8e−20/4e−22	*Beggiato* sp. strain BS (1e−24)	Response regulator receiver protein
Mmc12285	584	3e−66/6e−65/2e−65	*Acidobacteria bacterium* (1e−95)	CheA signal transduction histidine kinases
Mmc12286	188	—/—/—	*Anaeromyxobacter* sp. strain FW109-5 methyltransferase (0.86)	Putative methyltransferase

[a] MS-1, *Magnetospirillum magnetotacticum* strain MS-1; MSR-1, *M. gryphiswaldense* strain MSR-1; AMB-1, *M. magneticum* strain AMB-1. —, not present in respective strain of MTB.

based on sequence identity: MamB and MamM belong to the cation diffusion facilitator (CDF) family of metal transporters and may be involved in magnetosome-directed iron uptake (54), and MamO, MamP, and MamE are putative proteases (96). The MamJ and MamK proteins in *M. gryphiswaldense* and *M. magneticum* have been demonstrated to be involved in the construction of the magnetosome chain (66, 92, 93). MamK forms actin-like cytoskeletal filaments necessary for organizing magnetosomes into chains (66), and MamJ was accorded an essential role in magnetosome chain assembly in *M. gryphiswaldense* (92). However, *mamJ* is absent from the MC-1 genome. In MC-1, *mamK* (Mmc12259) is flanked by a *mamF*-like gene (Mmc12258) and Mmc12260.

(ii) *mamCEIH* cluster. The genes *mamC*, *mamD*, and *mamF* are unique to MTB and, along with *mamG*, form a single cluster in *Magnetospirillum* strains (89). There is no *mamGFDC* cluster in MC-1; instead, *mamC*, *mamD*, and *mamF* are scattered throughout the MAI, and *mamG* is absent from the genome (this study). In MC-1, *mamC* belongs to a gene cluster 12.4 kb downstream of the *mamAB* cluster, along with *mamE*, *mamI*, and *mamH* as well as six additional ORFs (indicated in light blue in Fig. 2). Thus, strain MC-1 appears to contain a new magnetosome gene cluster, here termed the *mamCEIH* cluster (Mmc12263 to Mmc12272), that is transcribed divergently as well. MamH belongs to the major facilitator superfamily, a family of transporters capable of transporting small solutes (89). MamE contains a PDZ domain and shows sequence similarity to trypsin-like (HtrA-like) serine proteases (53). Also within the MC-1 *mamCEIH* cluster is a gene that encodes a protein with a ferritin-like domain (Mmc12264), a gene (Mmc12263) that encodes a hypothetical protein, and a gene that encodes a Na^+/Ala symporter (Mmc12272); none of these are found in *Magnetospirillum* strains. *mamD* belongs to the *mamDXZ* cluster (see below) and also shows weak similarity to Mmc12270 in the *mamCEIH* cluster. *mamF* is present as three different copies in the MC-1 genome: a *mamF*-like gene (Mmc12258) lies within the *mamAB* cluster, *mamF* (Mmc12234) is adjacent to the *mamDXZ* cluster, and *mmsF* (Mmc12274) is located within the *mms6* cluster (see below).

(iii) *mamDXZ* cluster. The *mamDXZ* cluster (Mmc12237 to Mmc12240), a cluster containing four genes, was formerly designated the *mamXY* cluster, which is conserved in *Magnetospirillum* strains (89). In strain MC-1, *mamY* is replaced by *mamD*, and we could not identify the *mamY* gene or any homologous genes in the MC-1 genome. This cluster is located 15.4 kb upstream of the *mamAB* cluster, transcribed in the

right orientation. The MamD protein appears to be involved in the control of the size of the magnetosome magnetite crystals in *M. gryphiswaldense* (93). This cluster also contains another *mamD*-like gene within 5 kb of *mamD*, *mamX* (similar to *mamE* and *mamS*), and *mamZ* (a *mamH*-like gene) (see above). The genes in the *mamXY* cluster of *Magnetospirillum* strains encode an FtsZ-like protein, a MamH-like protein, MamX, and MamY (89). A gene encoding an FtsZ-like protein was not identified in the MAI of MC-1, though one is present elsewhere in the genome (Mmc10747).

(iv) *mms* genes. The genomes of both *M. gryphiswaldense* and *M. magneticum* contain an *mms* gene cluster consisting of five genes (48, 111). In *M. magneticum*, *mms6* encodes a magnetosome membrane-associated protein implicated in magnetite crystal nucleation (3). In MC-1, the *mms* cluster is located 589 bp downstream of the *mamCEIH* cluster, transcribed in the opposite direction, and contains two genes: one is homologous to *mms6* (Mmc12275), and the other is homologous to *mmsF* (Mmc12274). The remaining three genes of the *mms* cluster in *Magnetospirillum* could not be identified in the genome of strain MC-1.

(v) *mtx* cluster. A gene cluster referred to as the *mtx* cluster was found to be conserved among different MTB strains, including MC-1, but is not located within the putative MAI in any MTB (89). In MC-1, there is an *mtx* cluster (Mmc13695 to Mmc13697) located about 1.7 Mb downstream of the MAI. The gene cluster consists of a TPR protein gene (Mmc13695); the *mtxA* gene (Mmc13696), which encodes a magnetosome membrane protein conserved among different MTB strains; and a gene encoding an adenylate cyclase family protein presumed to be involved in signal transduction.

(vi) Putative new magnetosome genes in strain MC-1. Within the MAI in strain MC-1, 14 previously unrecognized putative genes (ORFs) cluster together with the *mam* and *mms* genes. Because most genes within the MAI encode magnetosome membrane proteins in strain MC-1 and/or other MTB, it seems likely that these new genes encode such proteins and therefore might be involved in magnetosome biomineralization in strain MC-1. Between the *mamAB* and *mamDXZ* cluster is a cluster of five genes (Mmc12241 to Mmc12245) which are transcribed in the opposite direction. The *mamCEIH* cluster contains a total of 10 genes arranged in the same orientation for transcription. Aside from the four genes which give the cluster its name, there are six other genes, including a gene that encodes a protein with a ferritin-like domain (Mmc12264) and a MamD-like protein gene (Mmc12270) (Table 2). The presence of genes in the MAI of MC-1 that are absent from *Magnetospirillum* strains and/or are unique to MC-1 suggests that at least some of these genes are specific to the magnetosome biomineralization process in strain MC-1 and, for example, may be involved in synthesis of elongated magnetite crystals (versus the equidimensional crystals synthesized by *Magnetospirillum* strains) (13). This may be especially true of genes that encode proteins such as TPR proteins and the Mmc12260 protein (see above), which show motifs consistent with a role in multiprotein assembly and/or cytoskeletal structures. Conversely, genes for certain proteins that have been ascribed an important (and even essential) role in magnetosome chain formation and/or are associated with the magnetosome membranes of other MTB could not be identified in the MC-1 genome. These include the gene for MamJ (discussed earlier) and the gene for MamW. The latter was identified as a magnetosome membrane protein in *M. gryphiswaldense* by Ullrich et al. (111) and is encoded by a gene found within the MAI but outside previously identified gene clusters. The absence of such genes from the MC-1 genome further supports the hypothesis that the processes of magnetosome biomineralization and chain assembly in MC-1 may exhibit marked differences from those in *Magnetospirillum* strains, possibly resulting in magnetosome crystal size and morphology differences.

Iron metabolism. Although magnetosome biomineralization was discussed in general in a previous section, we focus here on iron uptake and its oxidation and reduction, processes which must be important in magnetosome biomineralization in strain MC-1 and other MTB. In the cell, iron can be oxidized and reduced either chemically (without the use of enzymes) or enzymatically, through respiration and/or metabolism. Important processes involved in the biomineralization of magnetite by MTB include iron uptake by the cell, transport into the magnetosome vesicles (unless the vesicles are permanent invaginations of the cell membrane, as suggested for *M. magneticum* [65, 66]), and the controlled precipitation of magnetite.

(i) Iron uptake into the cell. Iron transport is obviously important in MTB, as it is known that up to 3% of the dry weight of cells of these organisms consists of iron (20), which is several orders of magnitude higher than the level in nonmagnetotactic bacteria (12). Even though MTB have been shown to be especially facile with regard to iron uptake, enabling the synthesis of up to 16.7 mg magnetite liter^{-1} day^{-1} (108), currently recognized iron uptake systems in MTB seem not to be unique. A comparison of the genomes of four different MTB strains revealed a group of MTB-specific genes that included some magnetosome membrane protein genes but no unique iron or metal transporter genes (89). Surprisingly, only a few specific metal ion transporter genes were identified in the MC-1 genome. There are four genes encoding CDF transporters, including *mamB* (Mmc12250), *mamM* (Mmc12256), Mmc13400, and Mmc13150, two ferrous iron transport-encoding genes (*feoA* and *feoB*) (Mmc11730 and Mmc11729), and three genes encoding outer membrane receptors, two for ferrienterochelin (Mmc11596 and Mmc12372) and one for iron (Mmc13560). Two Fe(III) uptake regulator-encoding genes (Mmc10894 and Mmc13182) belonging to the Fur (ferric uptake regulator) family were identified as well. Interestingly, although 52 of the 115 transporter-encoding genes present in the genome of MC-1 are part of putative ATP-binding cassette (ABC) systems, none of them appear to be specific for iron.

The genomes of other MTB contain more iron transporter genes than the genome of strain MC-1. For example, *M. magneticum* contains 10 iron transport-encoding genes. The putative iron transporters are divided into five CDF transporters, two copies of both *feoA* and *feoB*, and an additional Fe(II) transporter. The genome of *M. magnetotacticum* contains 87 genes involved in iron transport, including 27 specific for Fe(III)-siderophore transport systems. In contrast to the MTB, *Nitrosomonas europaea* has over 100 genes involved in iron transport, including genes for receptors for Fe(III), but it is apparently incapable of synthesizing siderophores (26). It is not known whether cells of strain MC-1 are able to synthesize siderophores. Because MTB take up roughly 100 times more

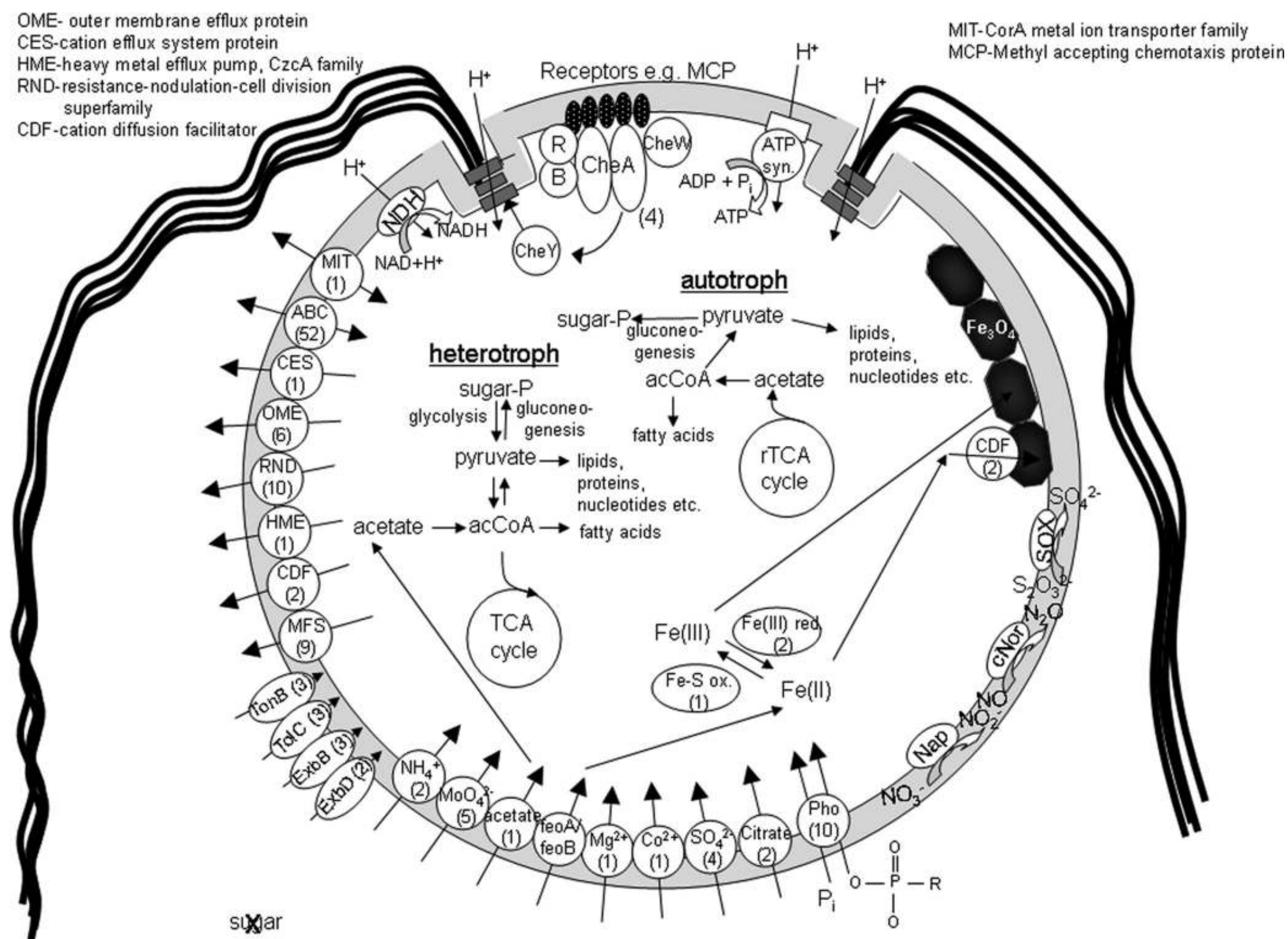

FIG. 3. Cell model of strain MC-1, with an emphasis on ultrastructure, transport, energy, carbon metabolism, and chemotaxis. Genes encoding virtually all of the steps for the synthesis of nucleotides and amino acids by canonical pathways are present in the bacterium but are omitted here for simplicity. Electron transport components include NADH dehydrogenase (NDH), periplasmic nitrate reductase (Nap), nitric oxide reductase (cNor), and the sox system (SOX). MCPs are methyl-accepting chemotaxis proteins. CheABRYW form a chemotaxis protein complex (see text). Influx and efflux transporter families with representatives in this genome are indicated on the figure, with the number of genes encoding each type of transporter shown in parentheses. Abbreviations: ABC, ATP binding cassette superfamily; ATP syn, ATP synthetase; CES, cation efflux system protein; CDF, cation diffusion facilitator; FeoA and -B, ferrous iron uptake family proteins; HME, heavy metal efflux pump CzcA family; MFS, major facilitator superfamily; MIT, CorA metal ion transporter family; OME, outer membrane efflux protein; Pho, regulon for phosphate uptake; RND, resistance-nodulation-cell division superfamily.

iron than other bacteria (12), we expected to identify more metal transporter-encoding genes, especially specific iron transporter-encoding genes, in the genomes of MC-1 and other MTB. Our analysis shows the opposite trend, with nonmagnetotactic bacteria exhibiting an equal number of, and in some cases more, iron transporters.

(ii) Iron redox reactions. We could not identify any Fe(II) oxidase genes in the genome of strain MC-1. The copper-containing, periplasmic Fe(II) oxidase of strain MV-1, which may be partly responsible for Fe(II) uptake and processing in this strain (36), has no similarity to any protein predicted by the genome of MC-1. However, two putative Fe(III) reductase-encoding genes (Mmc12239 and Mmc12484) were identified (Fig. 3). The Mmc12239 protein was earlier referred to as MamZ and may be involved in magnetosome biomineralization, as discussed above. The amino acid sequence of the Mmc12484 protein shows high sequence similarity to a putative Fe(III) reductase of other MTB (e.g., *M. magnetotacticum* [E value, 8e−33], *M. magneticum* [E value, 3e−32], and *M. gryphiswaldense* [E value, 2e−25]). For *M. magnetotacticum*, it was suggested that a cytoplasmic Fe(III) reductase was involved in the biomineralization process (119). Therefore, putative Fe(III) reductase genes, such as that for MamZ (Mmc12239) and Mmc12484, one of which seems to be conserved in these MTB strains, might be important in magnetite biomineralization. We also identified an Fe-S oxidoreductase-encoding gene (Mmc10050) that may also be involved in magnetite biomineralization, as it shows very high similarity to Fe-S oxidoreductases from both *M. magneticum* and *M. gryphiswaldense* (E value, 0.0) and a lower similarity to that of *M. magnetotacticum*, with an E value of 4e−146. For *M. magnetotacticum*, there is evidence suggesting that an Fe(II):nitrite oxidoreductase is involved in cytoplasmic Fe(II) oxidation, which appears to be essential for magnetosome biomineralization (81).

(iii) Iron transport into magnetosomes. As previously stated, the genome of MC-1 contains four genes encoding CDF transporters. Two of them have been assumed to have a role in iron transport into magnetosome vesicles in *Magnetospirillum* species (94; K. Junge, D. Schultheiss, and D. Schüler, unpublished data). The genes that encode these proteins, *mamB* and *mamM*, are also present on the MC-1 chromosome (Mmc12250 and Mmc12256, respectively) and are located within the MAI. These genes are conserved in all MTB (89), and they represent some of the most abundant proteins in the magnetosome membrane (53). Another protein, MagA of *M. magneticum*, may be involved in iron transport into magnetosome vesicles (79). MagA shows homology to the cation-efflux protein KefC (79) and also has homologues in *M. magnetotacticum*, *M. gryphiswaldense*, and strain MC-1 (54; this study). However, the amount of similarity between MagA and these homologues is less than one would expect if its proposed function was required for magnetosome synthesis in MTB. In fact, MagA has greater similarity to proteins of *Francisella tularensis* (E value, 3e−23) or *Dehalococcoides ethenogenes* (E value, 2e−22) than to its homologue in strain MC-1 (E value, 4e−18). Accordingly, the lack of significant sequence identity among these orthologous proteins suggests that MagA (and other Kef-type domain-containing proteins) is involved in other cell processes.

Because we identified very few of the known iron uptake proteins, iron transporters, and iron reductases and no iron oxidases in MC-1, it is possible that MC-1 contains unknown genes that encode novel proteins involved in these processes in the cell.

Transport. The genome of strain MC-1 contains 115 genes predicted to encode transporters (Fig. 3). This is a relatively small number compared to those in other bacteria, including other MTB. For example, 259 and 694 transporter-encoding genes were identified in the genomes of *M. magneticum* and *M. magnetotacticum*, respectively (see Table S1 in the supplemental material). The genomes of some nonmagnetotactic bacteria that are not coccoid in morphology appear to contain many more of these genes. For example, the genome of *Escherichia coli* contains 405 transporter-encoding genes, and those of *Rhodospirillum rubrum* and *Roseobacter denitrificans* contain 241 and 395, respectively. Nonmagnetotactic cocci contain fewer transporters; only 50 to 200 transporter-encoding genes have been found in the genomes of these organisms (see Table S1 in the supplemental material). The small number of transporter genes in MC-1 is also comparable with those in other (albeit obligate) autotrophic proteobacteria and cyanobacteria as well as intracellular pathogenic bacteria (82, 86). Heterotrophic bacteria are known to have more transporter-encoding genes than autotrophs, and these likely function in the uptake of multiple organic carbon and energy sources (e.g., sugars, amino acids, and organic acids) (86, 100). Alternatively, it is possible that MC-1 has transporters which have broad specificity and transport a number of different solutes. In general, prokaryotes vary widely with regard to the number of transporters (see Table S1 in the supplemental material), and the small number of transporters in MC-1 seems to be in keeping with its very limited heterotrophic abilities and its autotrophic lifestyle.

The number of transporters in bacteria has been reported to correlate with the genome size (86), but in a comparison of the numbers of transporter-encoding genes in the genomes of a number of different bacteria relative to the genome size (per Mb) (see Table S1 in the supplemental material), we found a large amount of variation. Values ranged from 16.3 (*Mycobacterium leprae*) to 161.4 (*M. magnetotacticum*), and the average for 30 species was 58.0 per Mb. Again, strain MC-1 was at the low end, having 24.5 transporter-encoding genes per Mb.

Genes for metal transporters other than iron transporters (discussed in an earlier section) in the MC-1 genome include those for magnesium (Mmc13074) and zinc (Mmc12530), as well as ABC transport systems for cobalt and molybdate/molybdenum (discussed in the next paragraph). Also notable is the presence of 10 resistance-nodulation-cell division (RND) superfamily genes (Mmc10309, Mmc10980, Mmc11818, Mmc11894, Mmc13248, Mmc12511, Mmc12524, Mmc13162, Mmc13187, and Mmc12361). In *Sulfurimonas denitrificans*, these genes are predicted to encode transporters involved in metal efflux (101). In MC-1, the presence of these genes could be important in magnetite synthesis if they transport iron; they might perhaps act as an efflux system involved in transporting iron from the cytoplasm into magnetosomes. The system may be unique to strain MC-1, as only four RND superfamily genes were found in *M. magnetotacticum* and none were identified in the genomes of the other MTB strains.

Fifty-two of the 115 transporter-encoding genes in the genome of MC-1 are for putative ABC transport systems. ABC systems are ubiquitous in prokaryotes; most of these are transmembrane proteins that function in the transport of a wide variety of substrates, at the cost of ATP hydrolysis (30). The majority of ABC transporter genes in MC-1 appear to be for the export of compounds, with only two components (inner membrane/permease component and ATP-binding cassette [ATPase]). The genome indicates that MC-1 has a relatively small number of ABC transport systems for uptake (inner membrane/permease, ATPase, and periplasmic solute-binding components are all present), including transporters for the uptake of amino acids (two transporters [Mmc10486, Mmc10487, Mmc10488, Mmc10489, and Mmc10490 and Mmc11019, Mmc 11020, Mmc11021, Mmc11022, and Mmc11023]), zinc (Mmc 13180, Mmc13181, and Mmc13183), cobalt (Mmc13138, Mmc13139, and Mmc13720), molybdate/molybdenum (Mmc11183, Mmc11184, Mmc11732, and Mmc12961), and phosphate (Mmc11539, Mmc11540, and Mmc11541). ABC transporters typically have high affinities for solutes and consequently can target compounds at low concentrations in the environment. There is also a symporter available for the uptake of acetate by secondary transport (Mmc11042), which correlates with the ability of MC-1 to use this compound as a substrate (see above). In strain MC-1, several transporters appear to be specific for phosphate/phosphonate transport. Other predicted transporters include transport proteins for ammonium, phosphate, citrate, and sulfate ions.

We also identified several genes encoding proteins that might play a role in transport across the outer membrane; these include three ExbB-, two ExbD-, three TonB family protein-, four TonB-dependent receptor-, and three TolC-encoding genes. Translocation of proteins across the cytoplasmic membrane is apparently mediated by two different systems that can be identified from the MC-1 genome, namely, (i) the preprotein translocase system (SecABDEFGY system; Mmc13327, Mmc13422, Mmc13212, Mmc10834, Mmc13211, Mmc11884,

and Mmc10867) and (ii) the TatABC (twin-arginine translocation) (Mmc11619 to Mmc11617) system. Genes encoding the major components included in type II secretion (Gsp proteins) (Mmc10931, Mmc10932, Mmc10933, and Mmc13531) are also present and presumably involved in protein translocation across the outer membrane.

Signal transduction. The MC-1 genome contains a variety of genes that encode proteins involved in signal transduction processes, including the chemotaxis protein CheC (of which there are 3), 5 chemotaxis protein histidine kinases, 5 chemotaxis response regulators containing a CheY-like receiver domain, 9 chemotaxis signal transduction proteins, 7 CheY-like receivers, and 32 methyl-accepting chemotaxis proteins. The MC-1 genome appears to contain 34 PAS/PAC domain-encoding genes, which may function as redox sensors (120). The genome also harbors 73 genes encoding response regulator proteins that contain a GGDEF domain, an HD-GYP domain, a helix-turn-helix DNA-binding domain, an AAA-type ATPase and DNA-binding domain, or a winged-helix DNA-binding domain. In addition, 15 genes that encode proteins with both EAL and GGDEF domains are present. Proteins with these two domains have been described to be involved in the synthesis (GGDEF domain) and phosphorylation (EAL domains that function as phosphodiesterases) of cyclic di-GMP, a novel bacterial signaling system (91).

It is noteworthy that the genomes of MTB contain a large number of genes that encode signal transduction histidine kinases responsible for signal detection (1). For example, the genome of strain MC-1 contains 87 of this type of genes, while there are 107 signal transduction histidine kinases in *M. magneticum* and 212 in *M. magnetotacticum.* The genomes of non-magnetotactic bacteria such as *S. denitrificans*, *Thiomicrospira crunogena*, and *Nitrosococcus oceani* generally contain much smaller numbers of signal transduction histidine kinases, with these three having 36, 17, and 18, respectively (101). Overall, the genomes of these three species also contain fewer genes encoding signaling proteins (101).

Regulation of gene transcription. As previously discussed, the genome of MC-1 appears to contain many genes that encode receptors involved in the sensing of environmental changes, and therefore it is expected that the genome contains an equal or similar number of response regulator genes. In MTB, oxygen and iron concentrations are known to control magnetite biomineralization, whose level can be determined by measuring both magnetism and iron content of cells (21, 57, 97, 98). In *M. gryphiswaldense*, a high oxygen concentration and iron starvation downregulate *mam* and *mms* gene transcription (95). Characterizing the signal transduction pathways in MC-1 and other MTB is necessary to understand which environmental conditions trigger the specific biochemical pathways resulting in magnetosome magnetite biomineralization. The MC-1 genome contains four genes that encode members of the FNR (fumarate and nitrate reductase) family of transcription regulators. These regulators might function as redox sensors but also as DNA-binding transcription factors (103). An important regulator for iron uptake is Fur (ferric uptake regulator) (69). The MC-1 genome contains two genes that encode proteins that are members of the Fur family (Mmc10894 and Mmc13182). Another known iron uptake regulator is AraC, which is coded for by the Mmc10422 and Mmc10423 genes in MC-1. It has been postulated that AraC transcriptional regulators allow bacteria to fine-tune the expression of siderophore biosynthesis and transport genes in such a manner that maximum expression of these genes occurs only in environments where the corresponding ferrisiderophore complex can serve as an efficient iron source (23). It was also reported that regulators of the AraC family are able to activate the expression of multidrug efflux genes (80). An interesting possibility is that this regulator might be involved in the regulation of iron transport into the cell and then efflux into magnetosomes. Besides the major sigma factors, σ^{70} and σ^{54}, we also identified the genes for σ^{28} (FliA), which controls the transcription of flagellin genes, along with its antagonist, the anti-σ^{28} factor FlgM.

Motility. Like all magnetotactic cocci, cells of strain MC-1 always possess two bundles of flagella. A flagellar bundle consists of seven to nine separate flagella, each with a diameter of about 10 nm (44). Each bundle originates from a pit-like structure (D. A. Bazylinski and T. J. Beveridge, unpublished data). Genes encoding the necessary components of a complete flagellar apparatus (*fli*, *flh*, and *flg* genes) form five distinct clusters within the MC-1 genome (see Table S2 and Fig. S1 in the supplemental material), with the largest being 29.9 kb and containing 29 genes. Genes in these clusters encode proteins that make up the flagellar filament, basal body, and hook and two proteins that make up the motor of the flagellum (see Table S2 in the supplemental material).

Chemotaxis. Like those of other MTB, cells of strain MC-1 passively align and swim along geomagnetic field lines. However, magnetotactic cocci exhibit a polar preference in their swimming direction that is dependent upon the local O_2 (electron acceptor) concentration, the concentration of electron donor (e.g., S^{2-}), and possibly redox conditions (44). Cells use magnetotaxis in conjunction with aerotaxis (i.e., magneto-aerotaxis) to more efficiently position themselves at an optimal local O_2 concentration (44) where their metabolism also appears to be optimal and there is continual energy generation. The ability of cells to navigate to niches that support maximum energy levels has been termed energy taxis (2). In the case of MC-1, the optimal O_2 concentration is very low, and cells form microaerobic bands at the OAI in semisolid O_2-gradient cultures (44). Because of the cells' unidirectional swimming pattern and polar preference in swimming direction in magnetic fields, the term polar magneto-aerotaxis has been used to describe the behavior of cells of strain MC-1. MC-1 has been shown to use the magnetic field for direction as well as for an axis for a presumably more efficient aerotaxis response. This is in contrast to the response of the magnetospirilla, which upon repeated cultivation in liquid medium swim in both directions and appear to use the magnetic field only as an axis (i.e., they display axial magneto-aerotaxis) (44). Cells of strain MC-1 display strong aerotaxis under very low concentrations of oxygen and appear to quickly locate and maintain a position at the OAI even when a magnetic field is completely absent (Bazylinski, unpublished data).

In comparison to many chemotactic bacterial species (see Table S3 in the supplemental material), strain MC-1 contains a relatively large number of known chemotaxis genes, i.e., 65 of them, possibly suggesting an important role for these genes in the ecology of MC-1 (locating and maintaining their optimal position at the OAI), and probably other MTB as well. Indeed,

the genomes of some *Magnetospirillum* species, for example, *M. magneticum* and *M. magnetotacticum*, contain more than twice the number of recognized chemotaxis genes (162 and 326 genes, respectively). Like strain MC-1, these species also show a strong aerotactic response, but they are not polar magneto-aerotactic. Analysis of other motile bacteria, however (see Table S3 in the supplemental material), reveals that the number of chemotaxis genes in MC-1 is not exceptionally high. When the number of chemotaxis genes is normalized to genome size (number of chemotaxis genes per Mb), strain MC-1 has a slightly larger number of these genes than most of the other motile bacteria examined. In a comparison of 29 motile prokaryotes, the number of chemotaxis genes per Mb ranged from 0 (*Pyrococcus furiosus* DSM 3638 and *Dehalococcoides* sp. strain BAV1) to 75.8 (*M. magnetotacticum*), while the average was 12.2 chemotaxis genes per Mb (see Table S3 in the supplemental material). MC-1 contains 13.8 chemotaxis genes per Mb.

The genome of strain MC-1 contains genes for 18 chemotaxis sensory transducers, 12 CheW domain proteins (8 CheW and 4 CheA), and 7 hemerythrin-like metal-binding proteins. Chemotaxis sensory transducers consist of two domains, a methyl-accepting chemotaxis protein and a histidine kinase (HAMP) domain. This protein is thought to transduce the signal to CheA. CheW is an adaptor protein and forms a complex with CheA. In other bacteria, CheA associates with a receptor (CheY) and the phosphate group from CheA is transferred to CheY. This activates the flagellum, where it modulates the motor reaction (63).

Hemerythrin proteins are oxygen-binding proteins common in invertebrates and a large number of prokaryotes, including members of both the *Bacteria* and the *Archaea* (46). Each has a nonheme di-iron site that reversibly binds oxygen. Two iron ions are bound to the protein via a motif comprised of seven conserved amino acid residues (62). Despite the ubiquity of these proteins in prokaryotes, specific functions of these proteins have not been elucidated, although a number of them have been proposed, including binding of oxygen or iron (or other metals) as a storage or detoxification mechanism (46). The putative methyl-accepting protein DcrH of *Desulfovibrio vulgaris* contains a C-terminal hemerythrin-like domain and has been ascribed a role as an oxygen sensor for aerotaxis in this organism (58, 118). Given the strong aerotactic response by MTB under conditions of low oxygen, it is tempting to speculate on a similar function in the MTB.

Many of the chemotaxis genes are clustered in the genome of strain MC-1 (see Table S2 and Fig. S1 in the supplemental material). There are four clusters of chemotaxis genes in MC-1. Each cluster, except one, contains one CheW, CheA, CheB, CheR, and CheY gene and a chemotaxis sensory transducer-encoding gene, although the genes are not in the same order in the three clusters. The fourth cluster is missing the CheA protein gene (see Fig. S1 in the supplemental material). The clustering of the *che* genes is conserved in many prokaryotes, and the respective encoded proteins represent a pathway common to most chemotactic bacteria and archaea (63, 115). Thus, it is likely that these proteins in MC-1 have similar functions to the proteins in other bacteria.

Metabolic functions. (i) Nitrogen metabolism. We previously found that cells of strain MC-1 show nitrogenase activity (i.e., cells reduce acetylene to ethylene under nitrogen-limited conditions) (14) and thus can fix atmospheric nitrogen. The genome of MC-1 contains a cluster of 19 nitrogenase genes (*nif*) that include *nifZVXNEYTKDHABQ* (Mmc11189 to Mmc 11209) (see Fig. S2 in the supplemental material). In addition to the *nif* genes, the cluster also contains dinitrogenase and dinitrogenase reductase genes, known to be involved in the nitrogen fixation process. The genes *nifR* (Mmc13670) and *nifU* (Mmc10723) are also present in the genome but are not part of this cluster. *M. gryphiswaldense*, *M. magnetotacticum*, and *M. magneticum* display nitrogenase activity as well (10, 11). The organization and presence of the *nif* genes in these strains are different from those in strain MC-1. A cluster of 20 genes encoding nitrogenases, dinitrogenases, and nitrogenase reductases is present in *M. magnetotacticum*. This cluster also contains 13 *nif* genes, including *nifWVQXNEKDHHTZ* and *nifB* (14). The genome of *M. magneticum* contains one cluster of 23 genes encoding enzymes involved in nitrogen fixation, including *nifWQXXNHZ* and *nifB*. Seven additional *nif* genes, such as *nifR* and *nifU*, are distributed throughout the genome in *M. magneticum*. The presence of specific genes within the genome indicates that there are three available pathways for the assimilation of ammonium (either taken up from the environment or synthesized de novo via nitrogen fixation), namely, glutamine synthetase and glutamate synthase (GS-GOGAT cycle); glutamate dehydrogenase; and alanine dehydrogenase.

Analysis of the MC-1 genome revealed two gene clusters encoding enzymes necessary for dissimilatory nitrate reduction and no apparent ORFs that might carry genes involved in assimilatory nitrate reduction. *Magnetospirillum* strains are capable of assimilatory and dissimilatory nitrate reduction and can grow either anaerobically or microaerobically (9) with nitrate as a terminal electron acceptor. All are known to be capable of complete denitrification (converting nitrate to dinitrogen gas). Cells of strain MC-1 do not grow anaerobically with nitrate as a terminal electron acceptor under chemolithoautotrophic (thiosulfate as an electron donor) or chemoorganoheterotrophic (acetate as an electron donor) conditions (14). However, MC-1 is fastidious with regard to growth in general, and it is possible that the culture conditions were not optimal for anaerobic growth with nitrate. Interestingly, nitrate added to the growth medium did not inhibit acetylene reduction by cells of strain MC-1 as it does in other organisms that fix dinitrogen (e.g., *Magnetospirillum* species) (10, 14; Bazylinski, unpublished data), further indicating that MC-1 cannot utilize nitrate as a sole source of nitrogen.

The dissimilatory nitrate reductases have been classified into periplasmic (Nap) enzymes and membrane-bound (Nar) enzymes (88, 107). Strain MC-1 appears to have the ability to synthesize a functional periplasmic nitrate reductase, as seven *nap* genes (*napCBHGADF*) (Mmc11587 to Mmc11593) are present in the genome of MC-1 (see Fig. S2 in the supplemental material). Prokaryotic *nap* gene clusters are complex and diverse. *M. magnetotacticum* and *M. magneticum* each contain a *nap* gene cluster of seven genes (*napFDAGHBC*) similar to that in strain MC-1, but in reversed order. The *napFDAGHBC* arrangement of *nap* genes is also present in *E. coli* (59), in which *napA*, *napB*, *napC*, and *napD* are essential for enzymatic activity, while *napF*, *napG*, and *napH* are not (83). In contrast, the genome of *Bradyrhizobium japonicum* contains a different

arrangement, *napEDABC*, and all genes are essential for nitrate respiration (33).

A gene encoding nitrite reductase, the enzyme that defines denitrification and catalyzes the reduction of nitrite to nitric oxide (NO), appears to be missing from the genome. Two types of NO reductases (Nor), catalyzing the reduction of NO to nitrous oxide (N_2O), have been classified, including (i) cNor, which receives electrons from cytochrome *c*; and (ii) qNor, which receives electrons from quinol (15, 121). A gene cluster is present in the genome of MC-1 that includes *norCBQ*, an Fe-S cluster-binding protein gene, and *norD* (Mmc10121 to Mmc10117). The *norCB* genes encode the small and large subunits of cNor, respectively (15). However, even though the cluster contains all genes required for cNor assembly and activation, activity of this protein has not yet been observed. Nitrous oxide reductase, the enzyme catalyzing the last step for complete denitrification (the reduction of N_2O to N_2), appears to be missing from the genome.

(ii) Sulfur metabolism. Our analysis shows that the genes encoding the first two enzymes of the assimilatory sulfate reduction pathway are present in the genome of MC-1. These are the small and large subunits of sulfate adenylyltransferase, encoded by the *cysN* and *cysD* genes (Mmc11018 and Mmc11017), respectively. This enzyme activates sulfate and catalyzes the synthesis of adenosine-5′-phosphosulfate (APS). APS becomes phosphorylated by the APS kinase, encoded by *cysC* (Mmc12549), and 3′-phosphoadenosine-5′-phosphosulfate (PAPS) is produced. Genes encoding the PAPS reductase, CysH, which catalyzes the conversion of PAPS to sulfite, as well as the sulfite reductase CysI, which reduces sulfite to sulfide, appear to be missing from the genome, and thus MC-1 appears to be incapable of assimilatory sulfate reduction to H_2S. MC-1 should still be able to synthesize cysteine from serine, however, if another source of S^{2-} is present (possibly from thiosulfate in the growth medium) or is derived from sulfate in a pathway different from the recognized pathway of assimilatory sulfate reduction, because the genome contains *cysE* (gene for serine *O*-acetyltransferase; Mmc13065) and *cysK* (gene for cysteine synthase; Mmc13416). Despite MC-1's apparent ability to synthesize cysteine, this amino acid is required for the growth of MC-1 in [O_2]-gradient medium, at least as a reducing agent. It may also utilize cysteine as a general source of cell sulfur, which might partially explain the requirement for the amino acid, based on the presumed inability of the organism to utilize sulfate as a sole source of sulfur (based on the absence of specific genes as described above). However, although it was present in the growth medium, cysteine was not originally added to the medium as a source of sulfur but as a reducing agent, and cysteine may have fortuitously served a dual role in the growth medium. Alternatively, perhaps other novel genes are present in MC-1 that encode currently unrecognized enzymes for the reduction of PAPS to sulfide. In contrast, the genomes of *M. magneticum* and *M. magnetotacticum* contain all genes necessary for assimilatory sulfate reduction, including the genes encoding PAPS reductase (*cysH*) and assimilatory sulfite reductase (*cysI*).

There is no evidence for the presence of any of the genes for dissimilatory sulfate reduction in the MC-1 genome, consistent with the fact that cells of MC-1 do not grow anaerobically with sulfate as a terminal electron acceptor.

Cells of MC-1 are able to use thiosulfate or sulfide as an electron donor when they grow chemolithoautotrophically (14, 113). The MC-1 genome contains 8 of the 15 *sox* genes, which in *Paracoccus pantotrophus* encode enzymes for sulfur oxidation (47). These genes include *soxXYZAB* (Mmc11905 to Mmc 11909), *soxW* (Mmc13719 and Mmc13718), *soxE* (Mmc12995), and *soxF* (Mmc12996). In addition, the MC-1 genome contains another copy of the *soxA* and *soxX* genes (Mmc13714 and Mmc13717) and two additional copies of the *soxZ* and *soxY* genes (Mmc12998, Mmc13715, Mmc12997, and Mmc13716) (see Fig. S3 in the supplemental material). SoxZY are known to interact with SoxAB, and their duplication may facilitate differential regulation of different loci, as proposed for specific homologues in *S. denitrificans* (101). The *sox* genes are organized into three clusters located at three different positions on the chromosome (see Fig. S3 in the supplemental material). A similar organization of different clusters also appears in *S. denitrificans* (101). The *soxXYZAB* genes encode three periplasmic proteins (38, 85), and *soxF* encodes a monomeric flavoprotein that has sulfide dehydrogenase activity (7, 84). *soxW* encodes a periplasmic thioredoxin described as essential for chemotrophic growth with thiosulfate (6). However, the *soxCD* genes that encode the sulfur dehydrogenase, which are important for electron transfer (47), appear to be missing from the genome. This might indicate that MC-1 uses *soxBXAYZ* gene products to oxidize thiosulfate to sulfur, which is either stored inside the cell or excreted (56). In the case of MC-1, sulfur globules accumulate intracellularly. Another possibility is that strain MC-1 accomplishes sulfur oxidation in combination with the *dsr* genes, as described for *Chlorobaculum tepidum* (47). The MC-1 genome contains nine of the *dsr* genes, including *dsrAB* (Mmc12156 and Mmc12157, respectively), *dsrC* (Mmc10048), and *dsrEFHJOP* (Mmc11704 to Mmc11709). The complete *dsr* gene cluster in *Allochromatium vinosum* is comprised of 15 genes that are essential for sulfur oxidation in that species (28). The *M. magneticum* chromosome contains 12 *dsr* genes (*dsrABEFHCMKLOOP*) organized in a single cluster. In contrast, only three *dsr* genes (*dsrABC*) were found in *M. magnetotacticum*. In addition to these sulfide-oxidizing enzyme-encoding genes, we also identified seven genes that encode sulfite oxidases, including *yedY* and *yedZ* (Mmc12485 and Mmc12484), which are also present in *M. magneticum*.

(iii) Phosphonate metabolism. Phosphorus is a growth-limiting factor in many ecosystems, particularly some marine habitats (109, 110, 116). Therefore, it is especially important for marine bacteria to degrade organic phosphates and phosphonates for the release and assimilation of phosphorus. In other characterized systems, the uptake of phosphate is controlled by the *Pho* regulon, a set of genes and operons that are regulated by the extracellular concentration of phosphate (112). The regulation of this response is controlled by the PstSCAB transporter, which "senses" different P_i levels and communicates through PhoU to the two-component system PhoR and PhoB (74, 75). A similar regulation might occur in MC-1, since its genome contains all of the genes for these components. However, the *pst* cluster is interrupted by a rhodanese domain-containing protein gene, as follows: *pstCAB*-*phoU*-RHOD-*pstS* (Mmc11539 to Mmc11544); *phoRB* (Mmc11492 and Mmc 11491) are further upstream. Another operon that might be

regulated by the *Pho* regulon, as described for other characterized systems, contains the *phn* genes that regulate phosphonate uptake. The MC-1 genome contains *phnC* (Mmc10734), *phnA* (Mmc12533), and four additional phosphonate transporter genes (Mmc11819, Mmc11923, Mmc13404, and Mmc13535). The *pstSCAB*, *phoU*, and *phoRB* transporter system is also present in *M. magnetotacticum*, *M. magneticum*, and *M. gryphiswaldense*.

All three *Magnetospirillum* strains also contain genes encoding polyphosphate kinase and exopolyphosphatase. These enzymes are involved in the accumulation of phosphate in polyphosphate granules, which have been shown to be present in these species (99; this study). Cells of strain MC-1 also produce polyphosphate inclusions, and we identified two genes that might contribute to the synthesis of these structures, as they are putative exopolyphosphatase-encoding genes (Mmc11700 and Mmc12960).

(iv) Carbon fixation and central carbon metabolism. Cells of strain MC-1 have been shown to grow chemolithoautotrophically with sulfide or thiosulfate as an electron donor (113). It was initially suspected that strain MC-1 used the Calvin-Benson-Bassham cycle for autotrophy, as shown for nearly all aerobic chemolithoautotrophic bacteria (14). However, genes with significant homology to either form I or form II ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) genes were not identified in the MC-1 genome, and Rubisco activity was not observed during autotrophic growth (113). Recently, it was reported that genes for the enzymes of the rTCA cycle were present in MC-1, and activities of these enzymes were demonstrated (113). Thus, we infer that MC-1 cells use only the rTCA cycle for CO_2 fixation. Our genome analysis shows that the genes for all enzymes necessary for the TCA cycle to operate in the reductive mode (101) are present in strain MC-1 (see Fig. S4 in the supplemental material). These include pyruvate:ferredoxin oxidoreductase (Mmc11741 and Mmc11742), 2-oxoglutarate:ferredoxin oxidoreductase (Mmc11749 and Mmc11750), and ATP-dependent citrate lyase (Mmc13638 and Mmc13639).

Acetyl-coenzyme A (acetyl-CoA) and oxaloacetate produced by the rTCA cycle likely feed into central carbon metabolism. Given that acetate can support heterotrophic growth of MC-1, it is probably converted to acetyl-CoA through the action of acetyl-CoA synthetase (Mmc10598). Neither of the genes associated with operation of the glyoxylate bypass (encoding isocitrate lyase and malate synthase) are present in the MC-1 genome. Acetyl-CoA is likely converted to pyruvate via pyruvate:ferredoxin oxidoreductase (see above). Oxaloacetate could be used to form phosphoenolpyruvate via phosphoenolpyruvate carboxykinase (Mmc10369) or fed into the TCA cycle in the forward (oxidative) direction. Acetyl-CoA is also likely used in the synthesis of fatty acids (acetyl-CoA carboxylase; Mmc12330).

Carbon fixed by the rTCA cycle is likely converted to pyruvate that can then be channeled in different directions for the synthesis of lipids, proteins, nucleotides, and cell wall components. All of the enzymes necessary to direct carbon through gluconeogenesis are encoded by the MC-1 genome. A gene for the key enzyme fructose-1,6-bisphosphatase (EC 3.1.3.11) was not recognized by the automated annotation, but one of the two genes annotated as inositol monophosphatase genes (Mmc10078) appears to be an archaeal protein-type fructose-1,6-bisphosphatase gene. All of the genes necessary for glycolysis (albeit only from phosphorylated sugars) are present, even though MC-1, like other MTB strains investigated thus far, is unable to utilize sugars as sole sources of carbon (14, 113). Of special note in the glycolytic pathway of MC-1 is the presence of a 2,3-bisphosphoglycerate-independent phosphoglycerate mutase. The glycolytic pathway is used to generate metabolic intermediates to replenish levels of cellular reductant that can be used by the rTCA cycle or the citric acid cycle.

The genes encoding glucose permease (EC 2.7.1.69) and components of the phosphotransferase system are not present in the MC-1 genome. This suggests that the reason that MC-1 cannot utilize sugars as a source of energy and carbon is that cells simply are not able to take up sugars from the extracellular environment. Genes encoding the enzymes necessary to direct pyruvate to the citric acid cycle are present. The gene encoding citrate synthase (Mmc10897), a key enzyme of the oxidative citric acid cycle, is present, and therefore the citric acid cycle is complete in strain MC-1.

Other cellular processes. As expected, the MC-1 genome contains all genes necessary for the F_1-F_0 ATP synthase and for oxidative phosphorylation for energy. We identified all genes necessary for the synthesis of the 20 common amino acids. All 20 tRNAs are present as well. MC-1 also contains the genes necessary to synthesize nucleotides, fatty acids, phospholipids, peptidoglycan, and vitamins and cofactors, such as riboflavin, nicotinic acid, pantothenate, biotin, and heme. However, no genes encoding the biosynthesis of enzymes involved in secondary metabolism or enzymes for the biodegradation and metabolism of xenobiotics were identified in the MC-1 genome.

Oxygen as a terminal electron acceptor and oxygen stress. Experimental evidence shows that cells of strain MC-1 use only O_2 as a terminal electron acceptor, not nitrate, nitrite, nitrous oxide, sulfate, sulfite, thiosulfate, dimethyl sulfoxide, or trimethylamine oxide (Bazylinski, unpublished data). Genomic data appear to also support this (with the exception of nitrate reduction), and the Mmc12353, Mmc12354, and Mmc12355 genes appear to represent the three subunits of a cytochrome *c* oxidase which could be the terminal oxidase for O_2 respiration.

Genes encoding superoxide dismutase and catalase, proteins involved in the detoxification of toxic, reactive by-products of oxygen respiration found in almost all aerobic bacteria, were not present in strain MC-1. The only genes involved in the detoxification of reactive oxygen species that were identified were those that encode cytochrome *c* peroxidase (Mmc12947, Mmc11488, and Mmc12527) and alkyl hydroperoxide reductase (Mmc11472 and Mmc11679). These enzymes remove hydrogen peroxide from the cell. The small number of genes to detoxify reactive oxygen species might explain the obligately microaerophilic nature of strain MC-1.

Prophages. Another striking feature of the MC-1 genome is the large number (14) of predicted prophages (22) distributed widely over the entire genome, with lengths of 5,287 to 42,314 bp and G+C contents of 52.14 to 58.34% (see Table S4 in the supplemental material). One example of these, predicted prophage 7, is depicted in Fig. S5 of the supplemental material. Prophage 7 is 42,314 bp in length, contains 54 coding se-

quences, and is transcribed in opposite orientation. This prophage "genome" starts with a lysogeny module containing an integrase gene (Mmc12087) and a cI-like repressor gene (Mmc12084). This type of lysogeny module is characteristic of lambdoid prophages (73). In most cases, the lysogeny module is located between lysis and DNA replication modules (73). However, no genes encoding lysine and holin, representing the lysis module, could be identified. Downstream of the lysogeny module, a DNA replication module was identified. This cluster includes all genes involved in DNA replication and metabolism, such as peptidase (Mmc12081), helicase (Mmc12074), DNA primase (Mmc12070), DNA-binding protein (Mmc12066), and DNase I (Mmc12065) genes. Some genes encoding regulatory proteins that control gene expression are scattered throughout the prophage genome. These early expressed genes encode nonvirion components and have nonprophage bacterial homologues. Some phage virion assembly proteins encoded by the late operons are more conserved, including the large terminase, portal protein, head maturation protein, and tail tape-measure protein (24). Even though the above proteins appear to be more highly conserved, many of the virion assembly proteins are very variable and difficult to identify from sequence information (24, 90). The phage genomes show striking gene clustering with a well-conserved gene order that we were able to identify in prophage 7 as well. The late operons start with the gene for a terminase (Mmc12050) that cleaves virion-length molecules from replicating DNA and is involved in packaging the phage genome into the phage head. This gene is followed by a cluster of genes that encode head structural components and assembly components, including the portal protein (Mmc12048), which forms the hole through which DNA is packaged into the capsid, and head maturation protease (Mmc12046), which controls proteolytic cleavage of the phage head. A cluster of genes encoding tail structure components and assembly components follows the capsid assembly genes. The most significant of these is the tail tape-measure protein (Mmc12034), which determines the length of the tail shaft (24). The high similarity of the ORFs to those of lambdoid temperate phages, which are generally members of the *Siphoviridae*, allows us to assume that MC-1 prophage 7 belongs to this family.

In contrast to that of strain MC-1, the genome of *M. magneticum* AMB-1 contains 12 putative prophages and that of *M. gryphiswaldense* MSR-1 contains 6, with its plasmid containing 1 (this study). To our knowledge, this is the first time that prophages have been reported within the genomes of MTB. The presence of these prophages may prove important in future studies (e.g., genetics) involving MTB. Prophages might also be another cause of horizontal gene transfer of the MAI. During times of stress, phage genes may become activated and cause phages to enter the lytic cycle. During this process, some phages may encapsulate bacterial genes and pass them on to new microbes (114). In addition, the MC-1 chromosome harbors 135 transposase/integrase-related genes that are distributed throughout the chromosome and not localized in hot spots as in *M. magneticum* AMB-1 (78).

Conclusions. The marine magnetotactic coccus strain MC-1 has a number of unique features that clearly differentiate it from other MTB whose genomes have been sequenced. Although the size of the genome of MC-1 is comparable to those of other MTB, the organization of the magnetosome genes is quite different. In addition, there are a number of genes within the MAI of strain MC-1 that may represent magnetosome genes not found in other MTB, and the large number of mobile elements found in and surrounding the MAI in other MTB are not present in MC-1. There are also a large number of predicted prophage sequences in the genome of strain MC-1. Although the lack of mobile elements (e.g., insertion sequences) in and surrounding the MAI might suggest that magnetosome genes in MC-1 did not originate from lateral gene transfer, the presence of these prophages might suggest, in fact, that they did or that they are transferred from MC-1 to other prokaryotes. Lastly, genomic data support the lack of metabolic versatility of strain MC-1 (e.g., in the use of different sources of carbon and electron sources and donors) compared to *Magnetospirillum* species.

On the other hand, there are some significant similarities between MC-1 and *Magnetospirillum* species. For example, there are numerous chemotaxis proteins probably used in energy taxis (2). Some of these have redox sensory domains which likely function to efficiently position cells at their optimal position at the OAI, thereby enabling them to obtain the electron donors (S^{2-}) and acceptors (O_2) required for energy generation and growth (44). Magnetotaxis and the ability to biomineralize magnetosomes are currently thought to make chemotaxis even more efficient for organisms such as MTB, whose survival seems to rely on locating and maintaining an optimal position in vertical chemical gradients in aquatic habitats (44). In sum, the information derived from the genome of MC-1 helps to emphasize the metabolic and physiological diversity of MTB and highlights the unique adaptations of magnetotactic cocci such as MC-1, for example, its exquisitely sensitive, fine-tuned aerotactic response. In addition, genomic information from MC-1 might provide insights leading to the isolation of other magnetotactic cocci.

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