DNA Research **12**, 157–166 (2005) doi:10.1093/dnares/dsi002

Complete Genome Sequence of the Facultative Anaerobic Magnetotactic Bacterium *Magnetospirillum* sp. strain AMB-1

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(Received 14 March 2005)

Abstract

Magnetospirillum sp. strain AMB-1 is a Gram-negative α-proteobacterium that synthesizes nano-sized magnetites, referred to as magnetosomes, aligned intracellularly in a chain. The potential of this nano-sized material is growing and will be applicable to broad research areas. It has been expected that genome analysis would elucidate the mechanism of magnetosome formation by magnetic bacteria. Here we describe the genome of *Magnetospirillum* sp. AMB-1 wild type, which consists of a single circular chromosome of 4 967 148 bp. For identification of genes required for magnetosome formation, transposon mutagenesis and determination of magnetosome membrane proteins were performed. Analysis of a non-magnetic transposon mutant library focused on three unknown genes from 2752 unknown genes and three genes from 205 signal transduction genes. Partial proteome analysis of the magnetosome membrane revealed that the membrane contains numerous oxidation/reduction proteins and a signal response regulator that may function in magnetotaxis. Thus, oxidation/reduction proteins and elaborate multidomain signaling proteins were analyzed. This comprehensive genome analysis will enable resolution of the mechanisms of magnetosome formation and provide a template to determine how magnetic bacteria maintain a species-specific, nano-sized, magnetic single domain and paramagnetic morphology.

Key words: magnetotactic bacteria; biomineralization; magnetosome

1. Introduction

Magnetic bacteria contribute to the global iron cycle by acquiring iron and converting it into magnetite (Fe_3O_4)[1] or greigite (Fe_3S_4),[2] which accumulates in intracellular structures known as magnetosomes. Biominerals possess highly ordered, elaborate morphologies since many biological factors strictly control the nucleation and the assembly of single crystals into complex structures.[3] The most significant physical feature of a bacterial magnetic particle is its magnetic properties. Each magnetic nanoparticle synthesized by magnetic bacteria possesses a magnetic dipole moment with a single magnetic domain.[4] The magnetite crystal growth and magnetic anisotropy energy must be strictly controlled by biological factors in magnetic bacteria. Contrary to artificial magnetic particles, magnetosomes can be easily dispersed in aqueous solutions because of their enclosing membrane.[5] Therefore, magnetosomes have vast potential for various technological applications, and the molecular mechanism of their formation is of particular interest. Magnetic particles from *Magnetospirillum* sp. AMB-1 have been utilized as immunoassay platforms for various environmental pollutants including endocrine disruptors,[6–9] as a means of recovering mRNA[10] and DNA[11,12] and as a carrier for DNA[13] in our previous reports. A variety of functional proteins, such as enzymes and antibodies, can be displayed on the bacterial magnetic particles through recombination in *Magnetospirillum* sp. AMB-1.[7,14,15] Clarification of magnetite biomineralization pathways would contribute to further biotechnological application studies in *Magnetospirillum* sp. AMB-1 and the potential of this material is growing and will be applicable to broad research areas. In this paper, the entire genome of *Magnetospirillum* sp. AMB-1 was sequenced, annotated and analyzed.

Communicated by Masahiro Sugiura

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2. Materials and Methods

2.1. Construction, isolation and sequencing of small-insert and large-insert libraries

Magnetospirillum sp. AMB-1, isolated from fresh water in Tokyo, Japan,[16] is available from ATCCATCC #700264. Genomic DNA of *Magnetospirillum* sp. AMB-1 was isolated according to a standard protocol.[17] Isolated DNA from *Magnetospirillum* sp. AMB-1 was sequenced using a conventional whole genome shotgun strategy.[18] Briefly, random 2-kb DNA fragments were isolated after mechanical shearing. These gel-extracted fragments were concentrated, end-repaired and cloned into pUC18 at the *Sma*I site. Double-ended plasmid sequencing reactions were performed using DYEnamic ET terminator chemistry (Americium Bioscience), and sequencing ladders were resolved on MegaBACE1000 and MegaBACE4000 (Amasham Bioscience) automated DNA sequencers. One round (115 200 reads) of small-insert library sequencing generated roughly a 10-fold redundancy.

A large-insert (∼15 kb) Charomid library was also constructed by *Mbo*I partial digestion of genomic DNA followed by cloning into the Charomid9-28 vector[19] at the *Bam*HI site. The Charomids provided a minimal scaffold to order and orient sequences across assembly gaps.

2.2. Sequence assembly and gap closure

Sequence data were converted to ESD data with Cimarron 1.53 Slim Phredify and Cimarron 3.12 Slim Phredify present on the automated DNA sequencer. Data were processed with Phred for base calling, and data quality was assessed before assembly using CAP4 (Paracel, Pasadena, CA, USA). Gaps were closed by primer walking on gap-spanning library clones (identified using linking information from forward and reverse reads). Alternatively, remaining physical gaps were closed by shotgun sequencing of PCR products using primers designed from terminal sequences of scaffolds arranged in order.

2.3. Sequence analysis and annotation

Gene modeling was performed using XanaGen (Kawasaki, Kanagawa, Japan) software. The results were compiled, and searches of the basic local alignment search tool (BLAST) for proteins and GenBank's non-redundant database were compared. Gene models that overlapped by >10% of their length were flagged, giving preference to genes with a BLAST match. The revised gene/protein set was searched against the XanaGenome (incorporating COGs, SWISS-PROT, PROSITE, PRINTS and Pfam), KEGG GENES and TC-DB (http://tcdb.ucsd.edu/tcdb/database.php). From these results, categorizations were developed using the COGs hierarchies. Initial criteria for automated functional assignment required >80% of the length of the match for BLASTP alignments with an E value $< 1 \times 10^{-1}$. All completed and draft genome sequences (accession numbers in Supplementary Table 1 is available at www.dnares.oxfordjournals.org) were reannotated according to this manner as well.

2.4. Nucleotide sequence accession number

The sequence of the complete genome of *Magnetospirillum* sp. AMB-1 is available under DDBJ accession number AP007255.

2.5. Analysis of transposon mutant library

Mutants were generated by using Tn5 mini-transposon.[20] The target sequence of mini-Tn5 in the genome was 5′-GGC CAG GGC-3′. The DNA sequences flanking the transposon-interrupted region were obtained by inverse PCR[20] using primers (R): 5′-ACA CTG ATG AAT GTT CCG TTG-3′ and (F): 5′-ACC TGC AGG CAT GCA AGC TTC-3′. The resulting PCR product was cloned into the vector pGEM-T-easy (pGEM-T-easy Vector System, PROMEGA, WI, USA) and sequenced. The sequences were then aligned against the whole genome database of *Magnetospirillum* sp. AMB-1.

2.6. Two-dimensional polyacrylamide gel electrophoresis and N-terminal amino acid sequence

Magnetosome membranes were dissolved in solubilizing buffer (40 mM Tris base, 7 M urea, 2 M thiourea and 4% CHAPS). Magnetosome membrane proteins were separated in an immobilized dry strip gel (pH 3–10; 130 mm) using IPGphore (Amersham Bioscience). After rehydration at 20°C for 12 h, the strips were run with a previously described program.[21] The strips were subjected to 2D- electrophoresis on a homogeneous SDS–polyacrylamide gel (12.5%). After gel electrophoresis, the gel was electroblotted onto PDVF membrane, Immobilon™-PSQ (Millipore Corp.). The membrane was stained with Coomassie brilliant blue R 250 and visible protein spots were excised. N-terminal amino acid sequencing was performed by automated Edman degradation using a PPSQ-1 amino acid sequencing system (Shimadzu, Kyoto, Japan). Resulting sequences were aligned against the whole genome database of *Magnetospirillum* sp. AMB-1.

3. Results and Discussion

3.1. General features of the genome of Magnetospirillum *sp. strain AMB-1*

The genome of *Magnetospirillum* sp. AMB-1 consists of a single circular chromosome of 4 967 148 bp. Table 1 shows the general features of the genome. The physical map is shown in Figure 1. The GC content of *Magnetospirillum* sp. AMB-1 genome is 65.1%. The GC skew analysis indicated two equal replichores containing five spikes (Fig. 1, the third circle). Several genes encoding bacteriophage core protein, Mu-like protein

Table 1. General features of the *Magnetospirillum* sp. AMB-1 genome.

Genome length (bp)	4 967 148
Plasmids	none
Protein coding sequences (bp)	4 384 030 (88.26%)
Average length of ORFs (bp)	961
GC contents (%)	
genome	65.09
ORFs	65.55
tRNA	49
16s rRNA	2
23s rRNA	2
Total Number of ORFs	4559
COG functional category*	
Categorized number of genes	2290
Information storage and processing	
Translation, ribosomal structure and biogenesis	142
Transcription	148
DNA replication, recombination and repair	139
Cellular processes	
Cell division and chromosome partitioning	27
Cell envelope biogenesis, outer membrane	186
Cell motility and secretion	83
Posttranslational modification, protein turnover, chaperones	117
Inorganic ion transport and metabolism	171
Signal transduction	205
Metabolism	
Energy production and conversion	226
Amino acid transport and metabolism	234
Nucleotide transport and metabolism	51
Carbohydrate transport and metabolism	123
Coenzyme metabolism	109
Lipid metabolism	113
Secondary metabolites biosynthesis, transport and catabolism	66
Poorly characterized	
General function prediction only	315
Function unknown	168
Uncategorized number of genes	2269

* COG, Clusters of orthologous groups of proteins.

and several transposases were found in Spike 4. The GC content (Fig. 1, the fourth circle) further suggests horizontal gene transfer (HGT) because regions with below average GC content correspond to insertion sequence (IS) elements (Fig. 1, the fifth circle) or phage regions.

3.2. Repeat sequences

The genome contains 33 ISs, which consist of four multicopy and seven single-copy elements. IS elements are intensively localized in three specific regions of the genome (Fig. 1, the fifth circle, positions nt 429 860–444 817; nt 691 208–692 355; and nt 3 526 739–3 608 663). The IS-concentrated regions have a lower GC content than the average of the whole genome, suggestive of gene transfer from other bacteria or phage. The inversions of GC content, or spikes, are observed mainly in IS insertion positions.

There are nine regions that encode phage-related proteins, two of which lack a capsid gene. The GC content of these regions was as low as that of IS elements. Twenty-three proteins encoded within these regions were identified as integrase XerC or functional homologs. XerC is known to promote several DNA deletion reactions. Interestingly, one XerC homolog (*amb0926*) is located 2 kb and 102 kb upstream (positions nt 997 403–998 535 and nt 1 095 895–1 097 027, respectively) of two identical 1132-bp sequences. Both 1132-bp repetitive sequences include truncated incomplete IS elements. The 100-kb region between the two repetitive sequences encodes magnetosome-specific proteins (see below). It is likely that this genomic island would be deleted via the XerC integrase and the two 1132-bp direct repeat, not via an IS element.

An 80-kb cluster encoding magnetosome-specific proteins, such as Mms6 and mamAB, was deficient in a spontaneous non-magnetic mutant of *M. gryphyswaldens* MSR-1.[22] Schubbe et al. determined that 35 kb of the sequence of this 80-kb cluster contains one of a pair of IS 66 elements and suggested that the region was removed because of IS element.[22] We obtained spontaneous non-magnetic mutant lacking the 100-kb region. The complete sequence in this study can explain that integrase recognized the 1132-bp direct repeats and deleted the DNA segment between the two sites.

3.3. Disrupted genes by transposon mutagenesis in non-magnetic mutants

To date, genome sequence analyses have tended toward prediction only. To annotate genes with high reliability, the analysis was performed with 1×10^{-1} of *E*-value in this study and half of the total genes were not annotated. Through usual analysis, only annotated genes would be subject to prediction. Transposon (Tn) mutagenesis can spotlight those of unknown genes function for magnetosome formation. An H^+/Fe(II) antiporter, *magA*, was isolated from a magnetosome depleted Tn5 mutant.[23] Isolation and characterization of the genes that mediate magnetite formation in bacteria are prerequisites for determining the mechanisms of magnetic particle biosynthesis. To identify specific genes involved in magnetite synthesis, transposon mutagenesis was conducted in

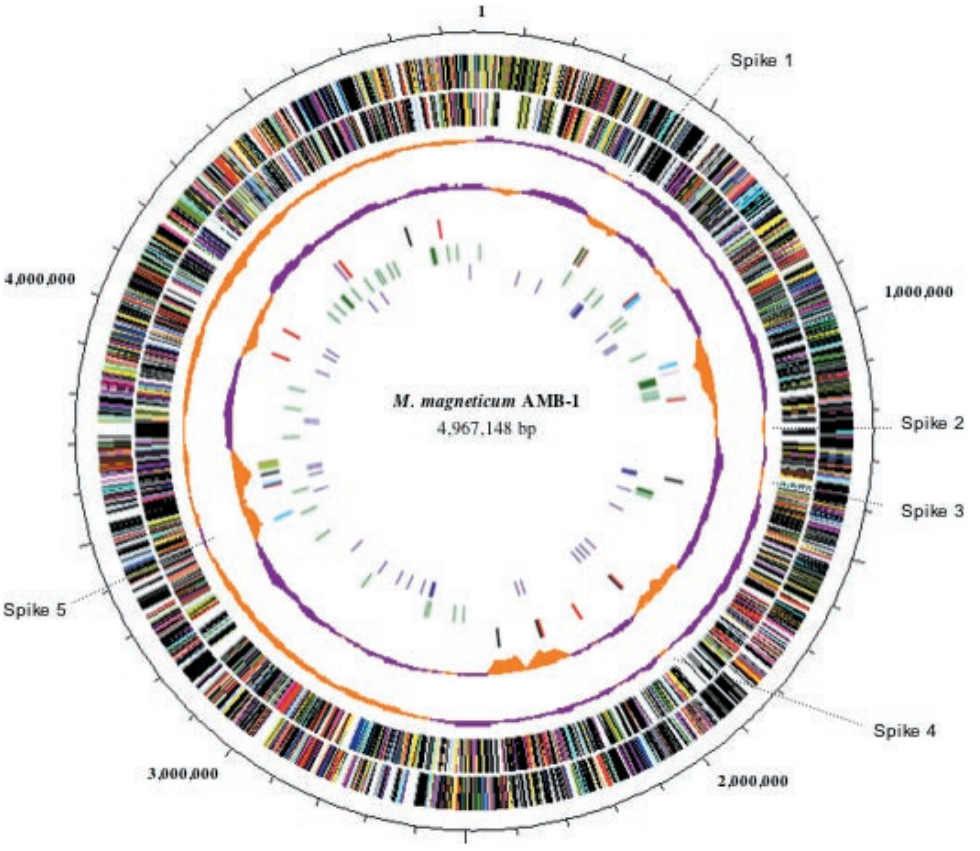

Figure 1. Circular representation of the 4 967 148-bp genome of *Magnetospirillum* sp. AMB-1. The outer and second circles represent predicted ORFs on the plus and minus strands, respectively (salmon: translation, ribosomal structure and biogenesis; light blue: transcription; cyan: DNA replication, recombination and repair; turquoise: cell division; deep pink: post-translational modification, protein turnover and chaperones; olive drab: cell envelope biogenesis; purple: cell motility and secretion; forest green: inorganic ion transport and metabolism; magenta: signal transduction; red: energy production; sienna: carbohydrate transport and metabolism; yellow: amino acid transport; orange: nucleotide transport and metabolism; gold: co-enzyme transport and metabolism; dark blue: lipid metabolism; blue: secondary metabolites, transport and catabolism; gray: general function prediction only; black: function unclassified or unknown). The third circle represents GC skew: purple indicates >0, orange indicates <0. The fourth circle further represents GC content: purple indicates higher than average, orange indicates less than average. The fifth circle represents insertion sequence (IS) elements (black: ISmag1; orange: ISmag2; pink: ISmag3; purple: ISmag4; blue: ISmag5; red: ISmag6; light blue: ISmag7; light green: ISmag8; green: ISmag9; brown: ISmag10; yellow: ISmag11). The sixth and seventh circles indicate the genes encoding magnetosome membrane proteins and loci of genes disrupted by Tn, respectively.

strain AMB-1.[20] Of 5762 Tn mutants, 69 were found to be defective for magnetosomes. The list of mutants and disrupted genes is shown in Table 2. Mapping results (Fig. 1, seventh circle) suggest that the genes required for magnetosome formation are distributed throughout the genome. Based on the COG database, protein functions encoded by disrupted genes were categorized as signal transduction (six mutants), energy production and conversion (four mutants), cell envelope biogenesis and outer membrane (three mutants) or cell motility and secretion (three mutants). However, unknown genes or genes of unknown function were disrupted in most mutants. Therefore, these genes should be focused among 2752 unknown genes. Interestingly, *amb2554* (acetate kinase), *amb2765* (ABC-type transport system) and *amb3450* (signal transduction histidine kinase) were disrupted individually in three mutants. Moreover, among 205 genes categorized as having signal transduction functions (Table 1), *amb0759* (two mutants), *amb2660* (two mutants) and *amb3450* (three mutants) genes were disrupted in two or three mutants. Therefore, these genes are likely to be magnetosome-related signal transduction genes.

3.4. Magnetosome membrane proteins

Magnetosome membrane proteins were identified because a number of proteins expressed *in situ* were expected to play a direct role in magnetite formation. Protein fractions prepared from magnetosome membranes were separated by 2D-electrophoresis and >100 protein spots were analyzed by amino acid sequencing. Based on the protein database of *Magnetospirillum* sp. AMB-1, the genes and annotations were identified from determined sequences. Table 3 provides a list of these genes, and Figure 1 indicates the distribution of

Table 2. Disrupted genes by Transposon mutagenesis in *Magnetospirillum* sp. AMB-1.

Gene ID	Frequency*	Product
Amb0192	1	Hypothetical protein
Amb0291	2	Permeases of the major facilitator superfamily
Amb0503	1	Flagellar biosynthesis/type III secretory pathway lipoprotein
Amb0521	1	Glutamate synthase [NADPH] large chain precursor
Amb0676	1	Acetyl-CoA carboxylase, carboxyltransferase component
Amb0741	1	Predicted membrane protein
Amb0759	1	FOG: GGDEF domain
Amb1309	1	Uncharacterized membrane protein
Amb1394	2	Prokaryotic membrane lipoprotein lipid attachment site
Amb1482	1	Hypothetical protein
Amb1692	1	Hypothetical protein
Amb1722	1	EF-hand calcium-binding domain
Amb1790	1	Hypothetical protein
Amb2051	1	Phosphatidylserine/ phosphatidylglycerophosphate/ cardioli pin synthases
Amb2087	1	Hypothetical protein
Amb2504	2	Predicted *O*-linked *N*-acetylglucosamine transferase, SPINDLY family
Amb2554	3	Acetate kinase
Amb2611	1	Membrane-associated lipoprotein involved in thiamine biosynthesis Note
Amb2660	2	Methyl-accepting chemotaxis protein
Amb2765	3	ABC-type transport system, involved in lipoprotein release, permease component
Amb2922	1	Tungsten-containing aldehyde ferredoxin oxidoreductase (EC 1.2.7.-).20**
Amb3184	3	Predicted transcriptional regulator
Amb3268	1	Tyrosine kinase phosphorylation site
Amb3279	2	Hypothetical protein
Amb3295	2	Hypothetical protein
Amb3450	3	Signal transduction histidine kinase
Amb3458	1	Uncharacterized protein conserved in bacteria
Amb3672	2	Tyrosine recombinase xerD
Amb3734	1	Dienelactone hydrolase and related enzymes
Amb3742	1	Type IV pili component
Amb3766	1	Hypothetical 133.7 kDa protein Y4CA
Amb4107	1	Leucyl aminopeptidase
Amb4111	1	Putative periplasmic protein kinase ArgK and related GTPases of G3E family
Amb4543	2	Uncharacterized protein conserved in bacteria

* Frequency is the disrupting number.
** Indicates reference number.
indicates the gene classified in signal transduction.

magnetosome membrane (MM) proteins throughout the entire genome.

To date, eight proteins specific to the magnetosome membrane in *Magnetospirillum* sp. AMB-1 have been identified and reported.[21,24–27] A 24-kDa protein, designated Mms24[24,25] [corresponding to MamA in (26)], and four proteins tightly bound to the magnetite crystal, designated Mms5, Mms6, Mms7 and Mms13,[21] were encoded within the 100-kb region between the two repetitive sequences. Other magnetosome-specific proteins, such as MpsA,[24] a 67-kDa protein[24] designated Mms67 and Mms16,[27] are located at different loci.

Forty-eight proteins were identified as individual magnetosome membrane proteins. Proteins related to oxidation/reduction were particularly prominent in this group, comprising ∼33% of the total. These proteins were similar to respiratory chain components. They might be a part of the respiratory electron transfer chain, because the magnetosome membrane would be derived from the cytoplasmic membrane. Otherwise, an alternative electron transfer involved in iron oxidation/reduction would exist in the magnetosome membrane. Other unknown genes were also recognized as candidate functional factors.

A signal response regulator (*amb3006*) was identified on the magnetosome membrane in this study. This protein might receive sensor signals related to magnetotaxis originating from the interaction between magnetosomes and the magnetic field. This response regulator will be described in the following section.

3.5. Iron oxidation/reduction

Although *Magnetospirillum* sp. AMB-1 is a facultative anaerobic bacterium, respiratory nitrate reduction allows the oxidation of a substrate under anaerobic conditions. The terminal electron acceptor may be Fe(III) via membrane-bound ferric reductase (amb3335). This enzyme is encoded in only 18 other eubacterial genomes, including microaerobic magnetic bacterium *M. magnetotacticum* MS-1. Specifically, ferric reductase activity has been measured in strain MS-1.[28] Moreover, iron reduction was coupled with nitrate reduction in both strains.[29,30] It is suggested that electron flow branching from quinone reduced iron. The membrane potential derived from electron transfer can be used in iron oxidoreduction.[29] The number of oxidation/reduction proteins expressed on the magnetosome membranes is remarkable so that the genes encoding ferredoxin and cytochrome were analyzed. Strain AMB-1 possesses the most ferredoxin and related genes compared with 165 other eubacteria (Supplementary Table 2 is available at www.dnaresearch.oxfordjournals.org). Additionally, the number of cytochrome genes is comparable with that found in other bacteria containing multiple cytochrome genes among the 165 strains, with completely sequenced genomes (Supplementary Table 3 is available at www.dnaresearch.oxfordjournals.org). These enrichment genes would

Table 3. List of the proteins and coding genes expressed on the magnetosome membrane.

Gene ID	Product	*e* value	Identical protein in MS-1	Identical protein in MSR-1
Amb0025	Hypothetical PE-PGRS family protein Rv1325c/MT1367 precursor	3e−06		
Amb0400	Hypothetical protein	—		
Amb0512	Glyceraldehyde-3-phosphate dehydrogenase/erythrose-4-phosphate dehydrogenase	1e−152		
Amb0546	Mms16, magnetic particle membrane specific GTPase (experimental data)[27*]	0		Mms16[35*]
Amb0664	Peroxiredoxin	7e−69		
Amb0696	Glutamine synthetase	0		
Amb0842	MpsA, Acetyl-CoA acetyltransferase, alpha subunit	5e−163		
Amb0951	Mms13, tightly bound bacterial magnetic particle protein (experimental data)[21*]	0		MamC[36*]
Amb0952	Mms7, tightly bound bacterial magnetic particle protein (experimental data)[21*]	0		MamD[36*]
Amb0956	Mms6, bacterial magnetic particle specific iron-binding protein (experimental data)[21*]	0		Mms6[35*]
Amb0963	unknown	1e−43		MamE[36*]
Amb0965	Actin-like ATPase involved in cell morphogenesis	7e−16		
Amb0971	Mms24[24, 25*], TPR protein essential for magnetite filling in vesicle (experimental data)[26*]	3e−15	Mam22[34*]	MamA[36*]
Amb0975	Hypothetical protein	—		MamS[35*]
Amb1003	FraH protein	1e−07		
Amb1017	Hypothetical protein	—		
Amb1027	Mms5, tightly bound bacterial magnetic particle protein (experimental data)[21*]	0		
Amb1380	Fructose/tagatose bisphosphate aldolase	1e−155		
Amb1395	Nitrite reductase precursor	0		
Amb2317	Pyruvate dehydrogenase E1 component, beta subunit	1e−166		
Amb2318	Pyruvate/2-oxoglutarate dehydrogenase complex	1e−134		
Amb2321	Pyruvate/2-oxoglutarate dehydrogenase complex	3e−94		
Amb2359	Dihydroorotate dehydrogenase	4e−13		
Amb2497	Translation elongation factor Ts	8e−96		
Amb2511	Superoxide dismutase	4e−76		
Amb2792	Protease subunit of ATP-dependent Clp proteases	2e−88		
Amb2793	FKBP-type peptidyl-prolyl cis-trans isomerase (trigger factor)	1e−119		
Amb3006	Response regulator containing a CheY-like receiver domain and a GGDEF domain	2e−33		
Amb3133	Translation elongation factors (GTPases)	0		EF-Tu[35*]
Amb3211	Periplasmic component of the Tol biopolymer transport system	1e−145		
Amb3421	Hypothetical protein	—		
Amb3492	Mms67[24*], Trypsin-like serineproteases, contain C-terminal PDZ domain	1e−127		
Amb3561	Nucleoside diphosphate kinase	3e−24		
Amb3876	Peroxiredoxin	2e−53		
Amb3903	Electron transfer flavoprotein, alpha subunit	1e−117		
Amb3953	Succinate dehydrogenase/fumarate reductase, Fe-S protein subunit	1e−110		
Amb3957	Malate/lactate dehydrogenases	1e−133		
Amb3958	Succinyl-CoA synthetase, beta subunit	1e−145		
Amb4012	Acetyl-CoA carboxylase beta subunit	4e−91		

Table 3. Continued.

Gene ID	Product	*e* value	Identical protein in MS-1	Identical protein in MSR-1
Amb4088	Ubiquinol-cytochrome C reductase iron-sulfur subunit	3e−56		
Amb4138	ATP synthase epsilon chain	1e−20		
Amb4139	F0F1-type ATP synthase, beta subunit	0		ATP synthase, β[35*]
Amb4141	F0F1-type ATP synthase, alpha subunit	0		ATP synthase, α[35*]
Amb4177	Acyl-coenzyme A synthetases/AMP-(fatty) acid ligases	0		
Amb4204	Acetyl-CoA carboxylase alpha subunit	1e−106		
Amb4391	S-adenosylhomocysteine hydrolase	0		
Amb4440	Molecular chaperone	0		GroEL[35*]
Amb4486	Inorganic pyrophosphatase	1e−62		

indicate oxidation/reduction proteins.
indicates signaling protein.
* indicate reference number.

contribute to their membrane potential and a considerable amount of iron reduction.

3.6. Regulation and signal transduction

In response to several environmental conditions, *Magnetospirillum* sp. AMB-1 alters the respiratory pathway and magnetosome formation. Therefore, it should appropriately regulate gene expression. It must also integrate its metabolism and distribute intracellular iron pools, which can be toxic to the cells. Several signaling genes were identified with relation to magnetosome formation from Tn mutant library.

As shown in Table 4, *Magnetospirillum* sp. AMB-1 contains numerous regulatory and signaling genes that conserve multiple domains of bacterial signal transduction systems. Remarkably, the sensor module histidine kinase, corresponding to HisKA and HATPase, is encoded in 105 genes, 77 of which contain both histidine kinases (Table 4 and Supplementary Table 4 is available at www.dnaresearch.oxfordjournals.org). This redundancy is much higher than that of other bacteria, such as *M. loti*, *Pseudomonas aeruginosa* and *Caulobacter crescentus*.[31] Moreover, GGDEF, EAL and HD-GYP domains were maintained more frequently than in typical free-living bacteria.[31] The response regulator involving the GGDEF domain was characterized as a typical two-component signal transduction system like a CheY domain. Numerous bacterial signaling proteins show multidomain structures involving response domains (not only CheY-like but also GGDEF, EAL and HD-GYP) with ligand-binding sensor domains (PAS and GAF). These multiple domains in the signaling proteins reflect the mechanism of signal transduction, from an N-terminal sensor domain to a C-terminal response domain, and suggest that the novel domains comprise a distinct system that provides an additional output module and a means of feedback control (Supplementary Table 4 is available at www.dnaresearch.oxfordjournals.org).[31] The frequent occurrence of typical regulator receiver domains containing CheY and other modules (GGDEF, EAL and HD-GYP) in *Magnetospirillum* sp. AMB-1 suggests that they provide strict specificity to various environments, especially for switching between magnet/non-magnet synthesis and magnetotaxis. Alexandre et al.[32] hypothesized that large numbers of chemoreceptors in *M. magnetotacticum* MS-1 are related to its energy taxis functions. Therefore, it would be required to monitor changes in the cellular energy genesis and to seek an environment that provides efficient energy generation. Tn-mutants led us to focus on three genes (*amb0759*, *amb2660* and *amb3450*) classified in signal transduction. Histidine kinase (sensor signal) and methyl-accepting chemotaxis proteins are encoded in *amb3450* and *amb2660*, respectively. The *amb0759* gene encodes a conserved GGDEF protein domain. The results provided several genes among hundreds that should be analyzed, but it is still unclear where they function in the signaling cascade. Proteome analysis identified a response regulator containing a CheY-like receiver and a GGDEF domain (*amb3006*) that was expressed on the magnetosome membrane. This protein probably functions in magnetotaxis.[33] A cell capable of magnetotaxis must be able to sense a geomagnetic field line by using a magnetosome chain, to transmit the information to flagella, and to move flagella to propel the organism in the appropriate direction.

3.7. Conclusions

For the process of magnetosome formation, we hypothesized four major stages: (i) invagination of the cytoplasmic membrane and vesicle formation for the magnetosome membrane precursor, (ii) accumulation of ferrous/ferric ions in the cell and the vesicles, (iii) strictly controlled iron oxidation–reduction and (iv) magnetite crystal nucleation and morphology regulation.[25] Moreover, signaling pathways are important for

Table 4. Regulatory and signaling proteins in *Magnetospirillum* sp. AMB-1.

Protein	Number
Regulatory protein	
Bacterial regulatory protein, LuxR family	10
Bacterial regulatory protein, LysR family	11
Bacterial regulatory protein, MarR family	9
Bacterial regulatory protein, ArsR family	4
Bacterial regulatory protein, AsnC family	2
Bacterial regulatory protein, Crp family	7
Bacterial regulatory protein, GntR family	6
Bacterial regulatory protein, MerR family	2
Bacterial regulatory protein, TetR family	8
Transcriptional regulatory protein	13
HTH Fis type	7
HTH CopG family	1
RpoD (Sigma 70/Sigma 32)	2
RpoN (Sigma 54)	1
Rpo32 (Sigma 32)	1
RpoE (Sigma 24)	3
Nitrogen regulatory protein PII	2
Signaling protein	
Signal transduction histidine kinase	
HATPase domain containing	98
HisKA domain containing	83
Methyl-accepting chemotaxis protein	44
Bacterial chemotaxis sensory transducer	26
CheA	1
CheB methylesterase	7
CheR	7
Chew	3
CheY	20
Response regulator receiver domain (CheY-like receiver)	45
Domain	
Hpt	5
HD–GYP or HD	19
EAL domain	25
GGDEF domain	46
GAF domain	15
PAC motif	35
PAS domain	49
Serine/threonine protein kinase	2

maintaining the balance of each process as well as protein or gene expression. The molecular mechanisms of each stage and the linkage of steps are expected to follow. Knowledge from whole genome sequence and gene repertories reveal organismal metabolism and insightful physiology.

The entire sequence of the *Magnetospirillum* sp. AMB-1 genome was determined to learn the mechanism of fine and nano-sized magnet formation, which we have investigated as novel material applicable for recombination. The genes were annotated with an E value 1×10^{-1} however, almost half of the 4559 ORFs were still unknown and useless for functional prediction. Therefore, Tn mutagenesis and magnetosome proteomics were performed to find several candidates for magnetosome formation among 2269 ORFs, and the resulting seven and six genes identified through Tn mutagenesis and magnetosome proteomics, respectively. Moreover, both analyses revealed several genes that were categorized into a signal transduction class. Remarkable numbers of sensor and response domains were found in *Magnetospirillum* sp. AMB-1 in this study, and 65 chemotaxis transducers were also reported in *M. magnetotacticum* MS-1.[31] The magnetosome synthesis pathway in AMB-1 competes with oxygen respiration and couples with respiratory nitrate reduction whereas *M. magnetotacticum* MS-1 magnetosome synthesis is coupled with oxygen respiration. Therefore, each species has its own signal transduction gene sets that respond to different environmental stimuli. Although gene predictions were confined within annotated genes, the predictions were also observed in other bacteria. Perhaps, machinery to provide iron or others for magnetosome formation might be simple, but their controls must be complex and strict. Interestingly, magnetosome-related genes identified by Tn mutagenesis and proteome analysis are scattered throughout the genome, and similar genes have also been found in other bacteria. Therefore, magnetosome synthesis requires some genes encoded in the 100-kb region as well as other housekeeping genes. This genome analysis also suggests that the 100-kb region is a necessary element that is necessary but not sufficient for magnetosome formation. Magnetic bacteria are distributed over a heterogeneous group of Gram-negative bacteria with diverse morphologies and habitats. The wide diversity of these organisms suggests that their magnetic properties have no taxonomic significance. Comparative genomic approaches will reveal common factors for magnetosome formation or magnetotaxis. Unfortunately, the genome sequencing of microaerobe *M. magnetotacticum* MS-1 or *Magnetococcus* sp. MC-1 (JGI Microbial Genomics, http://genome.jgi-psf.org/microbial/) has not been completed, but the draft sequences are comparable. The sequencing data provided lays the foundation for future studies to clarify magnetosome synthesis.

Acknowledgements: This work was funded in part by Grant-in-Aid for Specially Promoted Research, no. 13002005 from the Ministry of Education, Science, Sports and Culture of Japan. TC-DB version 2.0 was kindly provided by Dr Can Tran, University of California at San Diego.

Supplementary Material: Supplementary material is available online at www.dnaresearch.oxfordjournals.org.

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