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ORIGINAL ARTICLE

Genomic insights into the uncultured genus '*Candidatus* Magnetobacterium' in the phylum *Nitrospirae*

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Magnetotactic bacteria (MTB) of the genus '*Candidatus* Magnetobacterium' in phylum *Nitrospirae* are of great interest because of the formation of hundreds of bullet-shaped magnetite magnetosomes in multiple bundles of chains per cell. These bacteria are worldwide distributed in aquatic environments and have important roles in the biogeochemical cycles of iron and sulfur. However, except for a few short genomic fragments, no genome data are available for this ecologically important genus, and little is known about their metabolic capacity owing to the lack of pure cultures. Here we report the first draft genome sequence of 3.42 Mb from an uncultivated strain tentatively named '*Ca.* Magnetobacterium casensis' isolated from Lake Miyun, China. The genome sequence indicates an autotrophic lifestyle using the Wood–Ljungdahl pathway for CO_2 fixation, which has not been described in any previously known MTB or *Nitrospirae* organisms. Pathways involved in the denitrification, sulfur oxidation and sulfate reduction have been predicted, indicating its considerable capacity for adaptation to variable geochemical conditions and roles in local biogeochemical cycles. Moreover, we have identified a complete magnetosome gene island containing *mam*, *mad* and a set of novel genes (named as *man* genes) putatively responsible for the formation of bullet-shaped magnetite magnetosomes and the arrangement of multiple magnetosome chains. This first comprehensive genomic analysis sheds light on the physiology, ecology and biomineralization of the poorly understood '*Ca.* Magnetobacterium' genus.
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Introduction

The discovery of magnetotactic bacteria (MTB) has significantly changed our perception of microbial biomineralization. MTB are a phylogenetically diverse group of microbes that mineralize intracellular nano-sized magnetite (Fe_3O_4) and/or greigite (Fe_3S_4) into magnetosome chain(s) (Bazylinski and Frankel, 2004; Bazylinski *et al.*, 2013). The chain structure in each cell acts like a magnetic compass to facilitate the orientation and navigation of MTB by using the Earth's magnetic field (Frankel *et al.*, 1997; Pan *et al.*, 2009). The magnetosomes in MTB thus represent an ideal system for studying the intracellular biomineralization and bacterial organelle formation (Schüler, 2008; Komeili, 2012). The high chemical purity, strong magnetism and uniform nanometer scale of magnetosome particles also make them potentially applicable in advanced biotechnological and biomedical nano materials (Matsunaga *et al.*, 2007).

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Over the past decades, numerous studies on MTB in the *Proteobacteria* phylum have well demonstrated that the entire process of magnetosome formation is strictly controlled by a group of genes clustered within a coherent genomic fragment described as the magnetosome island (MAI) (Komeili, 2012; Schüler, 2008). However, recent cultivation-independent analyses have revealed an unexpected diversity of MTB outside *Proteobacteria* (Kolinko *et al.*, 2012; Lefèvre and Bazylinski, 2013; Lin *et al.*, 2014). Among them, those belonging to the genus '*Candidatus* Magnetobacterium' in the phylum *Nitrospirae* are of great interest (Garrity and Holt, 2001). Members of this genus, such as '*Candidatus* M. bavaricum' (henceforth referred to as Mbav), have giant cell sizes (6–10 μm) and are capable of forming as many as 1000 bullet-shaped magnetite magnetosomes arranged into multiple bundles of chains in a single cell, which is different from *Proteobacteria* MTB (Spring *et al.*, 1993). Originally discovered through electron microscope observation and ribosomal RNA gene sequencing (Vali *et al.*, 1987; Spring *et al.*, 1993), the *Nitrospirae* MTB are found to be widespread in aquatic environments and sometimes even account for a significant proportion (up to 30%) of microbial biomass in the microhabitats (Spring *et al.*, 1993; Lin *et al.*, 2014). Recent studies have suggested that these organisms may be capable of oxidizing sulfur (Spring and Bazylinski, 2006; Jogler *et al.*, 2010), thus linking this genus to the key steps of iron and sulfur cycles in nature. However, except for a few short genomic fragments (Jogler *et al.*, 2010; Jogler *et al.*, 2011; Lin *et al.*, 2011), very little genetic data of this genus are available owing to their unculturability. Consequently, knowledge of their trophic strategy, physiology and intracellular biomineralization remains very limited.

Here we used a targeted metagenomic approach to assemble the first draft genome sequence of 3.42 Mb from a large rod-shaped MTB strain belonging to the '*Ca.* Magnetobacterium' genus, which has recently been identified from Lake Miyun near Beijing, China (Lin *et al.*, 2009; Li *et al.*, 2010; Lin *et al.*, 2011). This bacterium is morphologically similar to Mbav and contains hundreds bullet-shaped magnetite magnetosomes that are arranged into multiple bundles of chains (Li *et al.*, 2010; Lin *et al.*, 2011; Figure 1). The draft genome sequence retrieved here has allowed us to gain novel insights into the metabolism and physiological ecology of this poorly understood genus. The genomic information will also lay a foundation for understanding the biomineralization mechanism of *Nitrospirae* MTB in general.

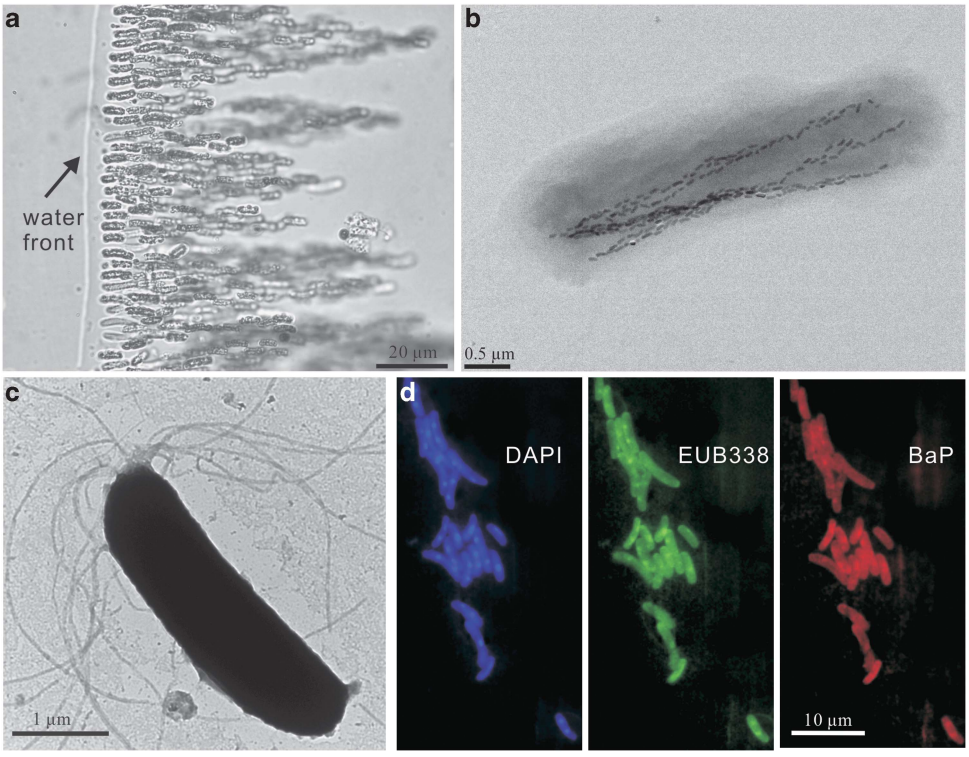

Figure 1 Images of 'Mcas'. (**a**) Light microscopy image of 'Mcas' enrichment. Note that the applied field direction is from right to left, and bacteria were concentrated at the left edge of the water droplet. Transmission electron micrographs of 'Mcas' showing multiple bundles of chains with bullet-shaped magnetosomes (**b**) and flagella (**c**). (**d**) Specific detection of 'Mcas' through fluorescence *in situ* hybridization analysis. Fluorescence microscopy images of the same microorganisms stained with 4,6-diamidino-2-phenylindole (DAPI), with bacterial universal probe EUB338, and with a '*Ca.* Magnetobacterium' genus-specific probe BaP.

Materials and methods

Magnetic enrichment of MTB and genomic DNA extraction

Surface sediments (depth 5–10 cm) were collected from Lake Miyun located in front of the Yanshan Mountain, about 80 km northeast of Beijing. The physical–chemical characteristics of sediments have been described previously (Lin and Pan, 2010). MTB cells were magnetically collected and purified using the double-ended open separation apparatus (MTB trap) as previously described (Jogler *et al.*, 2009). For transmission electron microscopy observation, 10 μl of MTB enrichments were deposited on Formvar-carbon-coated copper grids and were imaged using a JEM-1400 microscope operating at 80 kV (JEOL Corporation, Tokyo, Japan). Genomic DNA was extracted from the enriched MTB cells using the TIANamp Bacteria DNA Kit (Tiangen, Beijing, China) following the manufacturer's instructions.

PCR and fluorescence in situ hybridization

16S rRNA gene was amplified using the universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCT CAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′; Lin *et al.*, 2012). After gel purification, the PCR product was cloned into the pMD19-T vector to construct two clone libraries. Clones were randomly selected and sequenced using an ABI 3730 genetic analyzer (Beijing Genomics Institute, Beijing, China).

Fluorescence *in situ* hybridization was carried out as described previously (Lin *et al.*, 2012). A Cy3-labeled-specific probe for the genus '*Ca.* Magnetobacterium' (5′-GCCATCCCCTCGCTTACT-3′; named BaP in this study) (Spring *et al.*, 1993; Lin *et al.*, 2011) and the 6-carboxyfluorescein-labeled bacterial universal probe EUB338 (5′-GCTGCCTCCCGTAGGAGT-3′; Amann *et al.*, 1990) were used for hybridization.

Pyrosequencing and sequence annotation

The genome was sequenced by shotgun pyrosequencing using 454 GS-FLX system by the Genomic Core Facility at University of Virginia, and was assembled using MIRA (version 3.4.0; Chevreux *et al.*, 1999). Gene annotations of assembled contigs were done through the RAST annotation server (Aziz *et al.*, 2008). An expectation value E of $<1e-05$ was used as the standard cutoff to define putative protein functions. The entire annotations of genome were subsequently manually checked. Annotated assemblies and predicted proteins (Genome ID 798577.3) are available on the RAST guest account (using login and password 'guest') at the web addresses http://rast.nmpdr.org. Metabolic pathways were reconstructed using the KAAS pathway tool (Moriya *et al.*, 2007). Comparison of the draft genome to complete genomes of magnetotactic *Proteobacteria* was performed by bidirectional best hit analysis at a 30% level of protein identity using the SEED viewer (Overbeek *et al.*, 2014).

Phylogenetic and phylogenomic analyses

16S rRNA gene sequence retrieved from the draft genome was aligned with related MTB and non-MTB sequences from *Proteobacteria*, *Nitrospirae* and the candidate division OP3 using MUSCLE (Edgar, 2004). Gblocks was performed to eliminate poorly aligned portions of the alignment (Castresana, 2000). Maximum-likelihood phylogenetic tree was subsequently constructed through PhyML (Guindon *et al.*, 2010).

For phylogenomic analysis, protein sequences of 31 housekeeping genes (*dnaG, frr, infC, nusA, pgk, pyrG, rplA, rplB, rplC, rplD, rplE, rplF, rplK, rplL, rplM, rplN, rplP, rplS, rplT, rpmA, rpoB, rpsB, rpsC, rpsE, rpsI, rpsJ, rpsK, rpsM, rpsS, smpB* and *tsf*) from the draft genome in this study, complete genomes of magnetotactic *Proteobacteria* strains *Magnetospirillum magneticum* AMB-1, *Magnetococcus marinus* MC-1 and *Desulfovibrio magneticus* RS-1, and other non-MTB genomes of interest were identified, aligned, trimmed and concatenated by species using the software AMPHORA2 (Wu and Scott, 2012). A maximum-likelihood tree with 100 bootstrap replicates was constructed using RAxML with the LG and gamma models engaged.

Homology modeling analysis

The three-dimensional structures of complete MamK proteins from '*Ca.* M. casensis', '*Ca.* Magnetoglobus multicellularis BW-1', *D. magneticus* RS-1, *M. marinus* MC-1, *M. magneticum* AMB-1 and *M. gryphiswaldense* MSR-1 were modeled by Swiss-Model workspace (Arnold *et al.*, 2006) according to the structure of MreB from *Thermotoga maritime* (PDB entry: 1JCF). The quality of final model was checked through QMEANclust (Benkert *et al.*, 2009). Phylogenetic analysis of MamK proteins was conducted through the ClustalW (Thompson *et al.*, 1994). Structure-based sequences alignment was performed using ESPript (Gouet *et al.*, 1999), and the result was created through the UCSF Chimera (Pettersen *et al.*, 2004).

Accession number

This Whole Genome Shotgun project of '*Ca.* M. casensis' has been deposited at DDBJ/EMBL/GenBank under the accession JMFO00000000. The version described in this paper is version JMFO01000000.

Results

General genomic features

A group of giant rod-shaped MTB dominated the MTB community in one microcosm as observed by the Bacteriodrome analysis (Supplementary Movie S1). These MTB were magnetically collected and purified through the 'MTB trap' method (Jogler *et al.*, 2009), which yielded $\sim 10^8$ cells. About 6.2 μg of genomic DNA was extracted from the

enriched cells and was pyrosequenced, yielding around 126 Mb in total with a coverage depth of 37.0×. Sequence reads were assembled to a total length of ~3.42 Mb, which consisted of 70 contigs ranging in size from 1224 to 252 526 bp (with N50 = 90 253 bp; Table 1). The GC content of the assembled genome is 48.9%, within the range of 45.1–50.2% as determined from the metagenomic fosmids of the *Nitrospirae* MTB (Jogler *et al.*, 2011; Lin *et al.*, 2011). The draft genome contains 3140 predicted genes, 40 tRNAs and one complete rRNA gene operon (Table 1) that provides a wealth of genomic information valuable for understanding the biology of this poorly understood magnetotactic lineage.

Several tests were conducted to determine whether there was any potential contamination to the genome assembly. (i) Two 16S rRNA gene clone libraries were constructed from the extracted genomic DNA, and 36 clones were randomly chosen for Sanger sequencing. All 31 successfully sequenced genes showed high similarities (>99%) and were mostly similar to Mbav (Spring *et al.*, 1993; Jogler *et al.*, 2011). (ii) The fluorescence *in situ* hybridization analysis (Figure 1d) indicates that the enriched culture was pure. (iii) A survey of 111 conserved single-copy genes has revealed that 102 out of 104 detected genes were found only once in the genome assembly (Supplementary Table S1). (iv) Only a single copy of rRNA operon was identified in the assembled genome (Table 1). All these results strongly indicated the presence of a single genome in the assembly that was derived from the giant rod-shaped MTB cells belonging to the genus '*Ca.* Magnetobacterium'. This MTB population was tentatively named as '*Ca.* Magnetobacterium casensis' (hereafter referred to as Mcas).

Phylogeny

Cells of Mcas are 1–3 μm in width and 6–8 μm in length, and contain 200–500 magnetosomes organized into 3–5 bundles of chains (Figure 1). According to the phylogenetic analysis of 16S rRNA gene sequences, Mcas is affiliated with the *Nitrospirae* phylum and is most similar to Mbav with 98.2% identity (Figure 2a). Although similar, cells of Mcas are apparently smaller than that of Mbav and contain lower number of magnetosomes per cell (Jogler *et al.*, 2011; Spring *et al.*, 1993), which is consistent with Mcas being a novel magnetotactic population belonging to the genus '*Ca.* Magnetobacterium'. Mcas and Mbav together form a monophyletic group and show >4% phylogenetic divergence compared with other reported magnetotactic *Nitrospirae* (Figure 2a). The closest relative of Mcas with complete genome sequence is *Thermodesulfovibrio yellowstonii*, a thermophilic sulfate reducer isolated from thermal vent in Yellowstone Lake, USA (Henry *et al.*, 1994). The divergence in the 16S rRNA sequences between Mcas and *T. yellowstonii* is up to 14% (Figure 2a).

In addition to 16S rRNA genes, 31 universally conserved single-copy genes were used to conduct a genome-level phylogenetic analysis, which confirmed the affiliation of Mcas within the *Nitrospirae* phylum (Figure 2b and Supplementary Figure S1). Interestingly, the phylum *Nitrospirae* as a group is closely related to the *Deltaproteobacteria*, although the bootstrap support for this relationship is not very strong (Figure 2b). Previous studies using 16S rRNA gene sequences and gene order data suggested that members in the *Nitrospirae* phylum, such as *Nitrospira marina* and *T. yellowstonii*, have a close evolutionary relationship with the *Deltaproteobacteria* (Kunisawa, 2010; Teske *et al.*, 1994).

Table 1 General genomic features of the draft genome of '*Candidatus* Magnetobacterium casensis'

Parameter	*'Ca. M. casensis'*
Total genome size (Mb)	3.415676
GC content	48.90%
Number of contigs	70
N50 (kb)	90.253
Maximum contig length (kb)	252.526
Number of coding sequences (CDS)	3140
Number of hypothetical proteins	1150
Number of tRNAs	40
Number of copies of rRNA operon	1

Carbon fixation

Genes involved in the synthesis of cofactors, vitamins, prosthetic groups and pigments (for example, biotin, coenzyme A, riboflavin, pyridoxine and so on) have been identified in the genome (Figure 3). The salient feature of this genome is its capacity in fixation of CO_2 via the reductive acetyl-CoA (Wood–Ljungdahl or WL) pathway not observed for other *Nitrospirae* members and MTB, in which two CO_2 molecules are fixed through two distinct paths named as the western (or carbonyl) and eastern (or methyl) branches, respectively (Berg, 2011; Figure 3 and Supplementary Figure S2). Genome of Mcas encodes several key enzymes of both branches. The genes encoding the western branch are localized in one gene cluster, including the carbon monoxide dehydrogenase (Peg.0034), the CO dehydrogenase/acetyl-CoA synthase (Peg.0033), two subunits of the acetyl-CoA synthase corrinoid iron–sulfur protein (Peg.0032 and Peg.0031) and the corrinoid iron–sulfur protein methyltransferase (Peg.0030). These enzymes incorporate CO_2 into the carboxylic group of an acetate molecule. Two eastern branch genes, one encoding a formate-tetrahydrofolate ligase (Peg.3045) and one encoding a methylenetetrahydrofolate dehydrogenase (NADP+)/methenyltetrahydrofolate cyclohydrolase (Peg.2652), are likely involved in reducing CO_2 to a methyl group. It should be noted that some sulfate-reducing organisms and aceticlastic methanogens could run the WL pathway in reverse

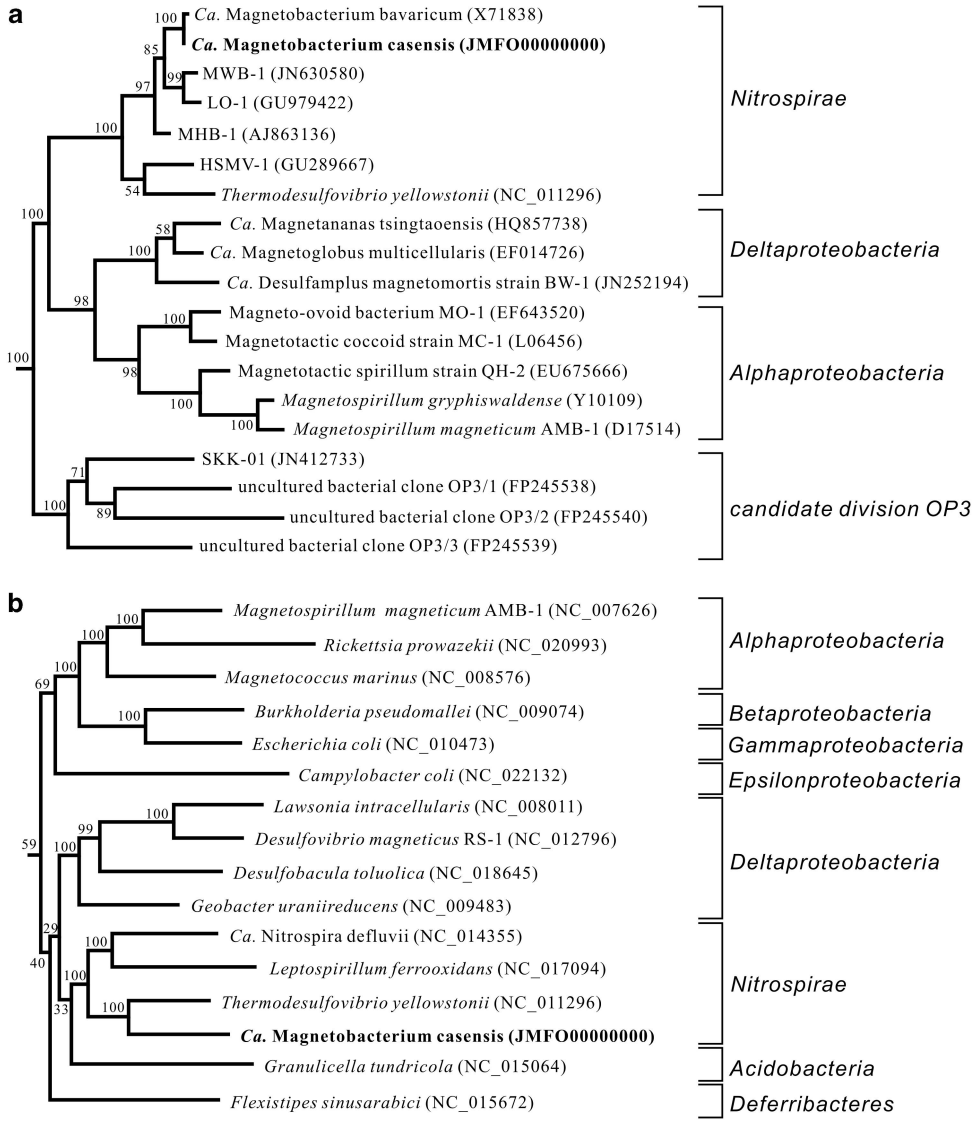

Figure 2 Phylogenetic analyses of 'Mcas'. (**a**) Phylogenetic of 16S rRNA gene sequences based on maximum-likelihood analysis of representative MTB and non-MTB sequences. (**b**) A maximum-likelihood genome tree illustrating the phylogenetic relationship of 'Mcas'. The bootstrap support values (out of 100 replicates) are indicated as percentages.

(Schauder *et al.*, 1988; Hattori *et al.*, 2005). We hypothesized that Mcas may be capable of reversing the WL pathway and generating metabolic energy by oxidizing acetate to CO_2.

The genome also contains several genes encoding enzymes involved in both tricarboxylic acid (TCA) cycle and reductive TCA (rTCA) cycles, including four subunits of 2-oxoglutarate:ferredoxin oxidoreductase (Peg.0666, Peg.0803, Peg.3047 and Peg.3048) and one aconitate hydratase (Peg.0290). Coexistence of different autotrophic pathways has been reported in some microorganisms (Markert *et al.*, 2007), thus it is also possible for Mcas conduct rTCA pathway for CO_2 fixation, although this needs further experimental determination. Similar to Mbav (Jogler *et al.*, 2010), a form IV ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) encoding gene (Peg.1181) was identified in the genome. However, the lack of carboxylase and oxygenase activity of this gene suggests that Calvin–Benson–Bassham pathway for CO_2 fixation should not operate in Mcas.

Nitrogen metabolism

The Mcas contains genes encoding periplasmic nitrate reductases (Nap), membrane-bound nitrate reductases (Nar), nitrite reductases (Nir), nitric oxide (NO) reductases and nitrous oxide reductases, suggesting that it is capable of gaining energy by converting nitrate to N_2 under oxygen-limiting conditions (Figure 3 and Supplementary Figure S2). The nitrate respiration *nap* and *nar* genes form a gene cluster (Peg.1539–Peg.1548), and are predicted to catalyze the reduction of nitrate to nitrite. The *nirS* gene for cytochrome cd_1 nitrite reductase,

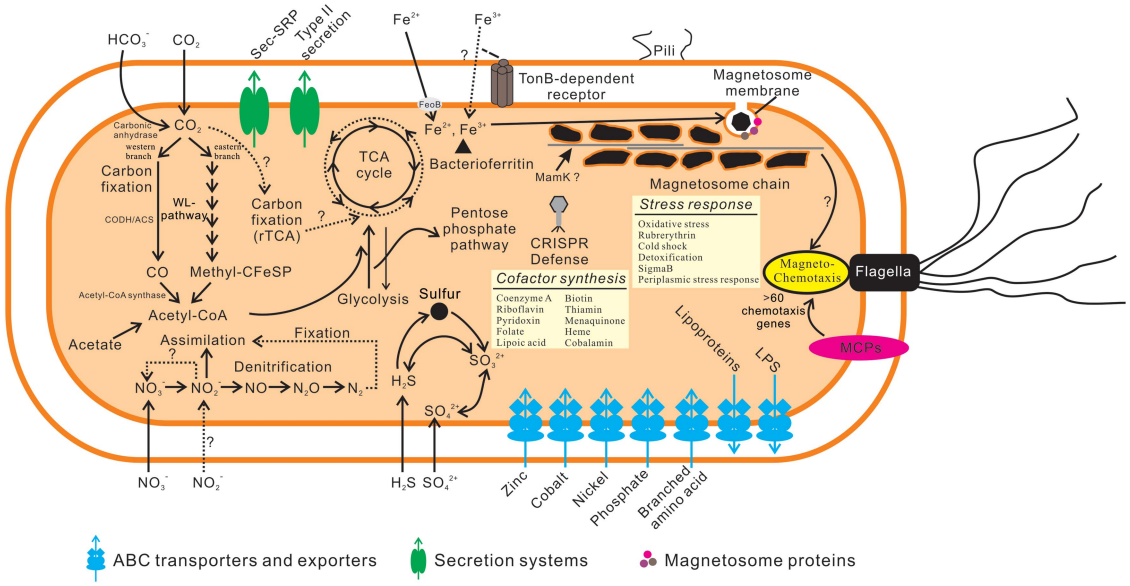

Figure 3 Cell metabolic cartoon constructed from the genome sequence of 'Mcas'. LPS, lipopolysaccharide; rTCA, reductive tricarboxylic acid cycle; TCA cycle, tricarboxylic acid cycle; WL pathway, Wood–Ljungdahl pathway.

together with *nirDFJNJ* genes (Peg.1589–Peg.1594) that are involved in heme d_1 biosynthesis (Kawasaki *et al.*, 1997), may reduce nitrite to NO. NO may be further reduced to nitrous oxide, and finally to N_2 using NO reductases (NorBC) and nitrous oxide reductases (NosZRDFY), respectively. It should be noted that nitrate reductases might operate in the reverse direction and oxidize nitrite to nitrate. In this way, the nitrate reductases serve as nitrite oxidoreductases that shuttle two electrons per oxidized NO_2^- into the electron transport chain to gain energy (Mussmann *et al.*, 2007). NarG is a candidate enzyme involved in this nitrite-oxidizing process (Mussmann *et al.*, 2007) and has been identified in the sequenced genome (Peg.1542).

Mcas encodes a putative nitrite reductase NAD(P)H large subunit (Peg.2700) and a cytochrome *c* nitrite reductase NrfA (Peg.1004) that may catalyze the assimilatory nitrite reduction process (Simon, 2002), indicating that Mcas likely uses nitrate or nitrite as a nitrogen source. The genome lacks the majority of genes involved in nitrogen fixation (*nif* and *fix* genes). Only a few genes encoding NifA (Peg.2180), NifU (Peg.0443), NifS (Peg.1400 and Peg.1786), FixA (Peg.1876) and FixB (Peg.2062) were identified.

Sulfur metabolism

The genome of Mcas contains a group of genes potentially involved in sulfur oxidation (Figure 3 and Supplementary Figure S2). One gene (Peg.0221) encodes a protein with considerable similarity to sulfide:quinone oxidoreductase (*sqr*), which could catalyze the oxidation of sulfide (S^{2-}) to elemental sulfur (S^0) (Pott and Dahl, 1998; Reinartz *et al.*, 1998). Elemental sulfur could be further oxidized to sulfite by the dissimilatory sulfite reductase encoded by *dsrABDEFH* genes that have been found in the sequenced genome (Dahl *et al.*, 2005; Holkenbrink *et al.*, 2011). In addition, genes for adenosine 5′-phosphosulfate reductase (Peg.0013 and Peg.0014, *aprAB*) and adenosine 5′-triphosphate (ATP) sulfurylase (Peg.0012, *sat*) mediating the oxidation of sulfite to sulfate have also been found in the genome. Genes for sulfur oxidation proteins (Sox) were not identified.

It has been suggested that sulfur oxidation enzymes, such as DsrAB, AprAB and Sat, could operate in the reverse direction, mediating a sulfate reduction pathway from sulfate to sulfide (Zhou *et al.*, 2011). We have found additional genes encoding quinone-interacting membrane-bound oxidoreductase (Peg.0016–Peg.0018, *qmoABC*) in the genome that are involved in the respiratory electron transfer chain and act as a link between the membrane menaquinone pool and the cytoplasmic reduction of sulfate (Pires *et al.*, 2003). It has been reported that some bacteria can perform both sulfur oxidation and reduction depending on the conditions (Dilling and Cypionka, 1990; Dannenberg *et al.*, 1992), and therefore can perform sulfur cycling between sulfate and sulfide in response to the redox conditions (Cypionka, 1994). It is thus possible for Mcas to reduce sulfate to sulfide in nature, although this capacity needs further experimental investigations.

Iron uptake

Iron is an essential micronutrient for almost all prokaryotes. This is particularly true for MTB in genus '*Ca.* Magnetobacterium' that need a large amount of iron for synthesis of hundreds magnetite magnetosomes. One gene (Peg.1239) encodes a protein with a ferrous iron transport protein A

(FeoA) domain associated with an iron-dependent repressor (TroR) in the genome. In addition, we found two copies of a gene (Peg.0908 and Peg.0909) that encodes protein containing a FeoB domain. Both FeoA and FeoB proteins identified here are most similar to the MAI-containing magnetosome proteins Mad17 and Mad30 of *Deltaproteobacteria* MTB, respectively, (Lefèvre *et al.*, 2013b), although they are outside of the MAI in Mcas. One gene belonging to the general metal 2+ transporter NRAMP family was also found in the genome, which may provide an alternative mechanism for Fe^{2+} uptake (Forbes and Gros, 2001; Hopkinson and Barbeau, 2012). Although genes for Sfu/Fbp/Fut family of ferric iron transporters and siderophore biosynthesis were not found, we identified several putative genes for TonB-dependent transporters (catecholate and heme) and Fe^{3+} ABC ATPase, indicating that Mcas has the potential to acquire Fe^{3+} from the environments as well.

Magnetosome biomineralization

A 67 580-bp contig of Mcas contains many orthologous genes encoding magnetosome proteins (Figure 4 and Supplementary Table S2). This contig contains 78 open reading frames (ORFs) and 2 tRNA genes; nearly half of them encode hypothetical proteins. The average GC content of the MAI-containing contig is 49.3%, slightly higher than that of the whole assembled genome (48.5%). A *mamAB*-like operon containing orthologs of *mamPMQBAIEQ* shows ~85% nucleotide similarity to those found in Mbav (Jogler *et al.*, 2011) (Figure 4). The homologs of these proteins in *Alphaproteobacteria* MTB have been identified to have vital roles in magnetosome membrane biogenesis, magnetosome protein sorting, and magnetosome crystal initiation and maturation (Schüler, 2008; Komeili, 2012).

We have identified one gene (Peg.0733) encoding a protein with high homology to MamK from *Proteobacteria* MTB. MamK protein assembles into actin-like filaments that are involved in the formation of magnetosome chains in *Proteobacteria* MTB (Komeili *et al.*, 2006; Katzmann *et al.*, 2010; Draper *et al.*, 2011; Ozyamak *et al.*, 2013). An acidic protein, MamJ, interacts with MamK and has important role in chain forming and dynamic turnover of MamK filaments (Scheffel *et al.*, 2006; Scheffel and Schüler, 2007; Draper *et al.*, 2011). Intriguingly, Mcas has only one copy of *mamK* and no *mamJ* despite that the numerous magnetosome chains assembled inside cells (Figure 4). A partial MamK protein sequence from Mbav has been retrieved recently (Lefèvre *et al.*, 2013b) and is 95% identical (33% coverage) to MamK from Mcas. Phylogenetic analysis of complete MamK protein sequences has revealed that MamK of Mcas is phylogenetically close to that of *Deltaproteobacteria* MTB (Figure 5a).

Three-dimensional structrue modeling of MamK from Mcas shows that it contains two domains that are each further divided into two subdomains (Figure 5b). Comparison of these structures reveals highly conserved residues of G_{14}, D_{169}, G_{171}, G_{173}, G_{296} and G_{297} that are located near the ATP-binding sites in the interdomain cleft (Figure 5b). Alignment of secondary structures of MamK, MreB and actin has revealed four of five motifs involved in the ATP binding, including two phosphate-binding sites (xxxxGxx and xxxDxGxGxx), one adenosine-binding site (xxxxGGxx) and one connecting region 2 (Gx) that are essentially identical in MamK, MreB and actin proteins (Figure 5c). These highly conserved active motifs indicate that MamK protein might have a general evolutionary origin of ATPase. In addition to these conserved regions, the connecting region 1 motif of MamK and MreB, 'xEPx', is different from that of actin protein (Figure 5c). Although the secondary structure elements are generally similar, some differences exist in the β4, β5, β6, β7, α1 and α2 among different MamKs (Supplementary Figure S3). MamK proteins from Mcas and MTB in the *Deltaproteobacteria* contain two additional randomly coiled loops between β6 and β7 and between α2 and β8, which are absent in MamKs from the *Alphaproteobacteria* MTB strains (Supplementary Figure S3). The additional elements result in the long antiparallel strands β6 and β7 being located to different positions in MamKs of Mcas, '*Ca*. M. multicellularis', '*Ca*. Desulfamplus magnetomortis' and *D. magneticus* RS-1 (Figure 5d and Supplementary Figure S3). Furthermore, a notably conformational change can be seen in the MamK from Mcas, which results from an additional subdomain behind the center of domains I and II (Figures 5b and d).

One gene (Peg.0715) shows remote similarity (*E*-value: 1e − 20) to the *mamEO* gene identified in BW-1, a fusion of the N terminus of MamE and the C terminus of MamO (Lefèvre *et al.*, 2013b). Because it has higher similarity to the C terminus of MamO (TauE domain), we annotated this gene as *mamO-Cter* gene (Figure 4). Both MamO and MamE are predicted as serine proteases, and are likely involved in magnetosome protein localization, biomineralization activation and crystal maturation as demonstrated in *Magnetospirillum* strains of the *Alphaproteobacteria* (Murat *et al.*, 2010; Yang *et al.*, 2010; Komeili, 2012).

Eight genes show remarkable similarities to *mad* genes that appear to be specific to the magnetotactic *Deltaproteobacteria* and/or Mbav (Lefèvre *et al.*, 2013b). Five genes (Peg.0710–Peg.0714) located downstream of *mam* genes show considerable similarities to *mad23* (2 genes), *mad24*, *mad25* and *mad26*, and form a small gene cluster (Figure 4). Mad23-I bears a high similarity (*E*-value: 4e − 64) to a PBS lyase HEAT domain protein repeat-containing protein (DMR_40890) in *D. magneticus* RS-1, which is predicted to be involved in iron storage for magnetosome formation (Matsunaga *et al.*, 2009). Mad25 and Mad26 exhibit remote similarities to

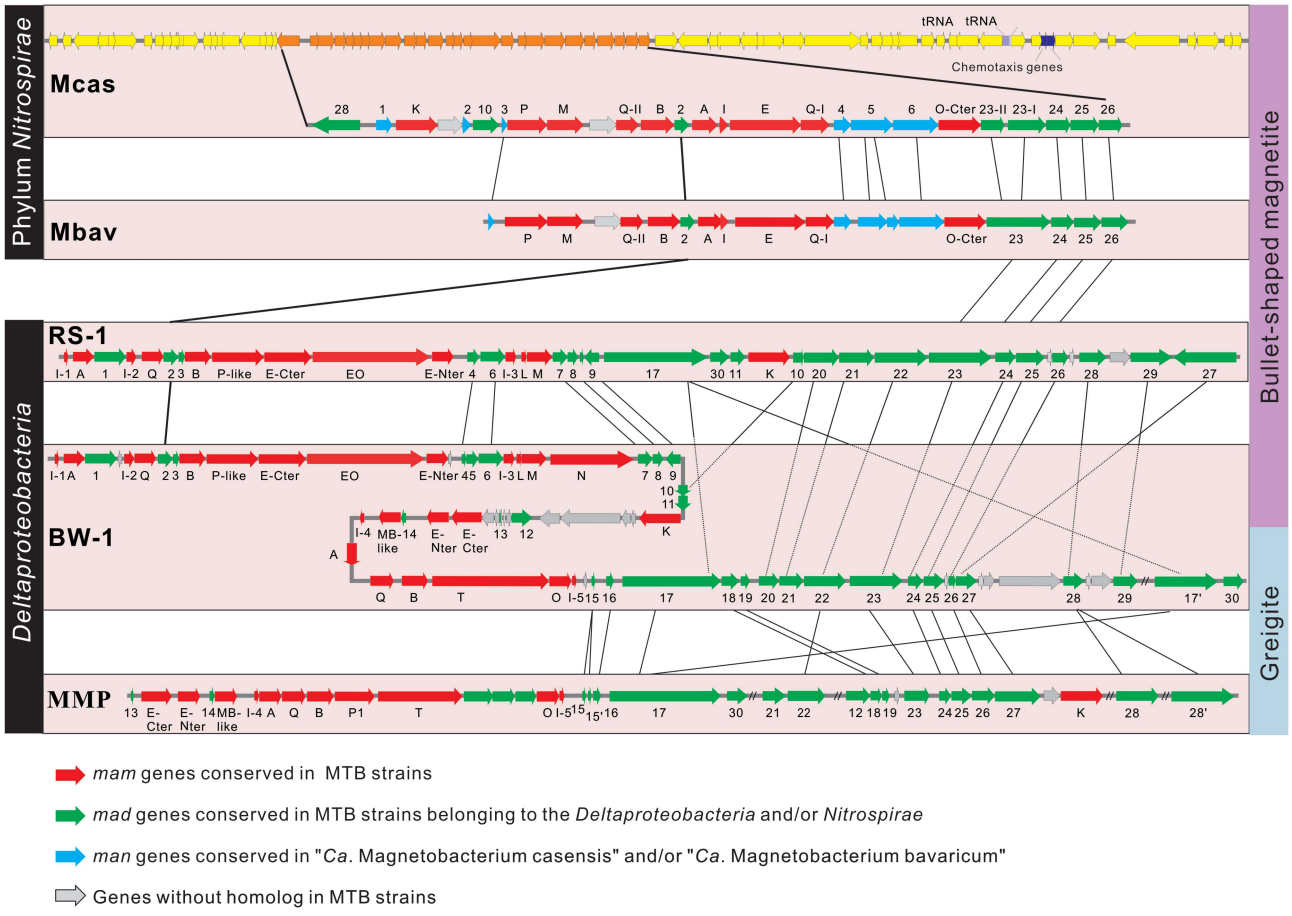

Figure 4 Schematic comparison of the MAI regions in 'Mcas', 'Mbav' and *Deltaproteobacteria* MTB strains. Mcas, '*Ca.* M. casensis'; Mbav, '*Ca.* M. bavaricum'; RS-1, *D. magneticus* RS-1; BW-1, '*Ca.* D. magnetomortis'; MMP, '*Ca.* M. multicellularis'.

chromosome segregation ATPases. In addition, three MAI genes (Peg.0723, Peg.0730 and Peg.0735) scattered in the contig are most similar to the *mad2*, *mad10* and *mad28*, respectively (Figure 4). Only the *mad2* gene appeared to be specifically conserved in all known bullet-shaped magnetite-producing MTB (Figure 4). The functions of Mad2 and Mad10 are so far unknown, while the actin-like Mad28 has been assumed to be involved in the arrangement and/or segregation of magnetosome chain(s) in *Deltaproteobacteria* and *Nitrospirae* MTB (Lefèvre *et al.*, 2013b).

Intriguingly, a set of novel MAI genes seems to be conserved in Mcas and/or Mbav (Figure 4). We named these genes as *man* genes (putative magnetosome genes in *Nitrospirae*), and proposed that they may be involved in some special processes of magnetosome synthesis or chain arrangement and segregation in magnetotactic *Nitrospirae*. We did not find *mamHJORSTU* genes that are conserved in sequenced *Magnetospirillum* species (Komeili, 2012; Schüler, 2008). With the exception of the *mamAB* operon, other magnetosome gene operons of *Magnetospirillum*, such as *mamGFDC*, *mamXY* and *mms6*, are missing. Although the genome sequence is incomplete, it is likely that MAI in Mcas only contains genes belonging to the *mamAB* cluster, similar to its counterparts in the *Deltaproteobacteria* (Lefèvre *et al.*, 2013b; Nakazawa *et al.*, 2009).

Genomic comparison with Proteobacteria MTB

In addition to the magnetosome genes, we have compared the draft genome of Mcas with four complete genomes of *Proteobacteria* MTB strains, including *M. magneticum* AMB-1 (Matsunaga *et al.*, 2005), *M. gryphiswaldense* MSR-1 (Wang *et al.*, 2014) and *M. marinus* MC-1 (Schübbe *et al.*, 2009) belonging to the *Alphaproteobacteria* and *D. magneticus* RS-1 (Nakazawa *et al.*, 2009) in the *Deltaproteobacteria*. A total of 458 orthologous genes outside MAI have been identified to be shared among Mcas and the four *Proteobacteria* MTB strains, which were classified into different clusters of orthologous groups categories (Supplementary Table S3). Although this is a conservative estimate because the genome of Mcas is not complete, the products of these orthologous genes seem to be common in *Nitrospirae* and *Proteobacteria* MTB, and hence some of them may have roles in magnetotaxis or magnetosome formation. It is

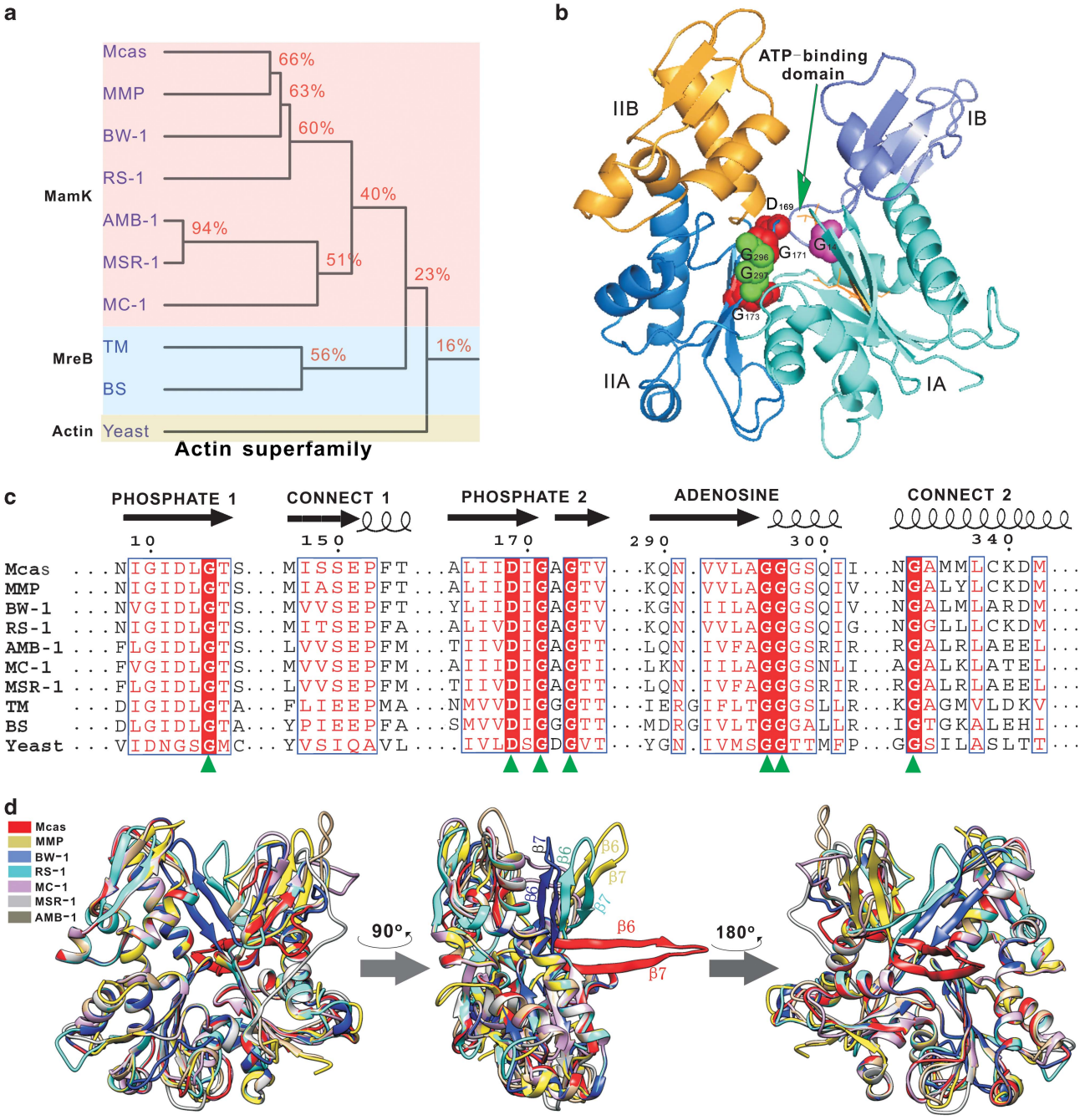

Figure 5 Comparison of MamK from 'Mcas' with its homologs in other bacterial strains. (**a**) A tree showing the phylogenetic relationship of the homologs. (**b**) Three-dimensional (3D) structure model of MamK from 'Mcas'. The image was prepared with PyMol (http://www.pymol.org). (**c**) Conserved sequence alignment. Conserved residues are indicated as '▲'. (**d**) 3D-structure alignment of various MamK proteins in three views by rotation along the *y* axis. Mcas, '*Ca.* M. casensis'; MMP, '*Ca.* M. multicellularis'; BW-1, '*Ca.* D. magnetomortis'; RS-1, *D. magneticus* RS-1; AMB-1, *M. magneticum*; MSR-1, *M. gryphiswaldense*; MC-1, *M. marinus*; TM, *T. maritime*; BS, *Bacillus subtilis*.

widely accepted that chemotaxis and motility have important roles in the magnetotactic swimming behaviors of MTB (Matsunaga *et al.*, 2005; Schübbe *et al.*, 2009), and genes involved in these processes are among the shared orthologous genes identified (Supplementary Table S3).

We further identified 144 genes of Mcas whose best BLAST hits were genes of known MTB strains (Supplementary Table S4). Approximately 79% (114) of them were most similar to genes from the *Nitrospirae* MTB, further confirming the affiliation of this genome to the phylum *Nitrospirae*. The remaining 21% had best BLAST hits to MTB strains of *Alphaproteobacteria* (22 open reading frames) and *Deltaproteobacteria* (8 open reading frames). Although ~54% of these MTB-related genes encoded hypothetical

proteins with unknown or only predicted functions, we identified 27 genes categorized in 'Cell motility and secretion' and 'Signal transduction', indicating their important roles in magnetotaxis behavior of this bacterium.

Motility and chemotaxis

Mcas has multiple flagella located at one pole of the rod-shaped cell body (Figure 1c). They form a flagellar bundle propelling the cells toward the magnetic north. Most flagellar genes are located in four gene clusters (Peg.1652–Peg.1665, Peg.2138–Peg.2144, Peg.2461–Peg.2472 and Peg.0626–Peg.0636) in the genome. Two copies of the *motAB* genes of flagellar motor rotation are found, indicating that flagella in Mcas are powered by the flux of proton (Brown *et al.*, 2011). These two sets of flagellar motor genes may increase the synthesis efficiency of Mcas and provide necessary power to propel cells of unusual size (up to 8 μm) and mass (possessing hundreds magnetite magnetosomes per cell). In addition, Mcas encodes several type IV pilin proteins that may be involved in adherence, motility, DNA uptake and exchange, electron transfer and protein secretion (Giltner *et al.*, 2012).

There is a substantial expansion of chemotaxis genes in Mcas genome, including 26 genes for methyl-accepting chemotaxis proteins and genes for CheABCDRVWXYZ. Although many chemotaxis genes are scattered in the genome, *cheRYZABW* genes (Peg.0639–Peg.0645) form a single cluster next to the flagellar genes (Peg.0626–Peg.0636). Interestingly, two chemotaxis genes, one encoding a methyl-accepting chemotaxis protein methylation inhibitor CheC (Peg.0687) and another encoding a response regulator receiver protein (Peg.0688), are located near MAI genes, suggesting their potential roles in the magneto-chemotaxis signaling system (Figure 4). The average number of chemotaxis genes per Mb is 17.5, which is more than that in the genome of *M. marinus* MC-1 (13.8; Schübbe *et al.*, 2009). Besides the *cheA* genes, we have found more than 100 genes encoding histidine kinases, including genes for osmosensitive K^+ channel and genes for PAS/PAC sensor, and genes encoding predicted signal transduction proteins. The substantial expansion of chemotaxis genes suggests that chemotaxis has an important role in Mcas and allows it to quickly respond to changing environmental conditions.

Electron transport chain and oxygen respiration

Mcas encodes a respiratory chain consisting of complexes I, III, IV and V for energy generation and electron transport. One gene encoding bidirectional [NiFe] hydrogenase diaphorase (Peg.0036) and several genes encoding bacterial F-type ATPase (Peg.2767–Peg.2773) have also been identified in the genome. Members of '*Ca.* Magnetobacterium' genus are known to live near microoxic to anoxic layers (Jogler *et al.*, 2010), suggesting they being occasionally exposed to oxygen. Accordingly, Mcas contains genes encoding aa_3-type cytochrome oxidases that may allow adaptation to microoxic conditions. Genes encoding thioredoxin (Peg.1457) and thioredoxin reductase (Peg.1871) are present in genome as well, which may also allow Mcas to cope with the oxidative stress (Serata *et al.*, 2012).

Cell envelope and cell cytoskeleton

Mcas possesses genes putatively encoding outer- and inner-membrane proteins, such as YidC (Peg.1299), OmpA/MotB (Peg.2197, Peg.1427 and Peg.1479), OmpH (Peg.1841), YaeT (Peg.1840) and lipopolysaccharides-related genes (Peg.0381 and Peg.0970). The presence of a periplasm is suggested by the identification of genes encoding periplasmic proteins. These results indicate that Mcas is a *bona fide* Gram-negative bacterium.

The genome also contains genes for typical bacterial cytoskeletal systems. Three genes encoding the MinCDE system (Peg.1820–Peg.1822) have been identified, which may be responsible for the placement of bacterial septation sites at mid-cell location (Barak and Wilkinson, 2007). Interestingly, one gene for DivIVA (Peg.0652) is identified in the sequenced genome, which is thought to perform topological control function and regulate the position of MinCD complex in those bacterial species without MinE (for example, *Bacillus subtilis*). The coexistence of genes for DivIVA and MinE has been reported in certain Gram-positive bacteria (for example, *Clostridia acetobutylicum* and *C. difficile*), although it is currently unknown whether both are expressed and how they contribute to mid-cell site selection (Stragier, 2001). We assume that this unique system may have a role in the segregation of multiple magnetosome chains and other cellular contents in Mcas.

Discussion

The draft genome sequence of 3.42 Mb from the uncultivated strain Mcas in the present study provides a solid start point for discussing the trophic strategy, physiology and unique intracellular biomineralization of MTB in the *Nitrospirae* phylum. The finding of gene clusters for CO_2 fixation through the WL pathway was unexpected because this pathway has previously been found in *Firmicutes*, *Planctomycetes*, *Spirochaetes*, *Deltaproteobacteria* and *Euryarchaeota*, but not in *Nitrospirae* (Hügler and Sievert, 2011). The WL pathway is the only pathway present in both CO_2-fixing bacteria and archaea, and is considered to be the first autotrophic process on Earth (Hügler and Sievert, 2011). To our knowledge, this is the first report of this pathway in '*Ca.* Magnetobacterium' genus, which is different from all known autotrophic members in *Nitrospirae* as well as MTB in

Proteobacteria that conduct either rTCA cycle or Calvin–Benson–Bassham pathway for CO_2 fixation (Garrity and Holt, 2001; Bazylinski *et al.*, 2004; Matsunaga *et al.*, 2005; Williams *et al.*, 2006; Richter *et al.*, 2007; Levican *et al.*, 2008; Goltsman *et al.*, 2009; Lucker *et al.*, 2010; Ji *et al.*, 2014). Compared with rTCA and Calvin–Benson–Bassham, the WL pathway is biochemically simple and energetically favorable (Peretó *et al.*, 1999), which may lead to a growth advantage of these microorganisms in oxygen-limited environments.

Through magneto–aerotaxis behavior, MTB can efficiently migrate between anoxic and microoxic layers near oxic–anoxic transition zone (Frankel *et al.*, 1997; Bazylinski and Frankel, 2004). In combination with the results of previous microhabitat study (Jogler *et al.*, 2010), the genomic analysis presented here has allowed us to predict the ecology of '*Ca.* Magnetobacterium' genus. Members in this genus might be capable of adapting to the chemical gradients near oxic–anoxic transition zone by adjusting its metabolic strategies. Specifically, in the upper microoxic layer, Mcas may conduct sulfur oxidation with nitrate and/or oxygen as the electron acceptors, and denitrification with reduced sulfur compounds such as sulfur and thiosulfate as potential electron donors. As it moves down to the anoxic lower layer, it may switch from sulfur oxidation to sulfate reduction where sulfate is one of the major terminal electron acceptors (Brune *et al.*, 2000). At this stage, the WL pathway may also operate in the reverse oxidative direction and generate metabolic energy by coupling the sulfate reduction process to the oxidation of acetate to CO_2. Moreover, a large number of histidine kinases response regulators (>150) and chemotaxis genes have been found, which may allow Mcas cells to effectively sense and respond to the microenvironmental conditions. Our results suggest that the metabolic strategies of '*Ca.* Magnetobacterium' genus may be complex, and further studies are necessary to better understand the true nature of these bacteria.

Comparative genomic analyses of MAIs from *Alphaproteobacteria* and *Deltaproteobacteria* MTB and an incomplete MAI from Mbav have suggested a common set of magnetosome genes existed in these MTB (Nakazawa *et al.*, 2009; Jogler *et al.*, 2011; Lefèvre and Wu, 2013; Lefèvre *et al.*, 2013b). The complete MAI of Mcas presented here confirms the presence of these core genes (*mamKPMQBAIEO*) that should be essential for magnetosome biomineralization in both *Nitrospirae* and *Proteobacteria* MTB (Figure 4). Although unlike their *Proteobacteria* counterparts, the hallmarks of MTB in '*Ca.* Magnetobacterium' genus are their ability to forms hundreds to a thousand bullet-shaped magnetite magnetosomes organized into multiple bundles of chains (Spring *et al.*, 1993; Jogler *et al.*, 2011). In this study, the complete sequence of magnetosome protein MamK, a key protein for organizing magnetosomes into chain arrangement in *Proteobacteria* MTB (Komeili *et al.*, 2006; Ozyamak *et al.*, 2013), has been identified, suggesting a similar strategy of magnetosomes chain organization in both *Nitrospirae* and *Proteobacteria* MTB. Further phylogenetic and protein structure analyses have revealed that this MamK protein is generally more similar to that of *Deltaproteobacteria* MTB with bullet-shaped magnetite and/or irregular greigite crystals than that of magnetotactic *Alphaproteobacteria* containing cuboidal and elongated prismatic magnetite magnetosomes (Figure 5). Considering the genomic structures of MAIs in *Nitrospirae* MTB are rather similar to *Deltaproteobacteria* MTB (Figure 4), the magnetosome synthesis and chain arrangement of these two groups may share the same evolutionary history (Jogler *et al.*, 2011; Lefèvre *et al.*, 2013b; Lefèvre and Wu, 2013). However, we do note differences in the conformational structure of MamK protein of Mcas from that of *Alphaproteobacteria* and *Deltaproteobacteria* MTB (Figure 5d), which, together with products of other MAI-containing genes (such as *mad* and *man* genes), may lead to the formation of unique organization of multiple magnetosome chains in '*Ca.* Magnetobacterium' genus.

There is a set of *man* genes that appears to be conserved between Mcas and Mbav. The colocalization of these genes with *mam* and *mad* genes indicates their specific functions in magnetosome synthesis and chain arrangement in '*Ca.* Magnetobacterium' genus (Figure 4). Among them, protein encoded by *man1* has remote similarity (*E*-value: $7e-08$) to a putative magnetosome protein Mad11 with unknown function in a *Deltaproteobacteria* strain ML-1 (Lefèvre *et al.*, 2013b) but does not bear similarity (*E*-value $>1e-05$) to any magnetosome proteins of other *Proteobacteria* MTB. The predicted products of *man2*, *man3* and *man4* lack recognizable homology (*E*-value $>1e-05$) to any proteins in the databases. Both Man5 and Man6 appear to contain a chromosome segregation protein SMC domain that acts in organizing and segregating chromosomes for cell partition (Jensen and Shapiro, 2003). Hence, we speculate that Man5 and Man6 may conduct similar functions in the arrangement and segregation of multiple magnetosomes chains during cell division. Additional sequencing of novel MTB strains related to Mcas and Mbav is necessary to further confirm the conservation of *man* genes in '*Ca.* Magnetobacterium' genus.

The formation of bullet-shaped magnetite magnetosomes has been discovered in the deep-branching MTB lineages such as *Deltaproteobacteria* and *Nitrospirae*, leading to the hypothesis that the first magnetosomes were bullet-shaped magnetite particles (Lefèvre *et al.*, 2013a). Nakazawa *et al.* (2009) assumed that the absence of magnetosome size and morphology controlling genes (such as *mamGFDC* and *mms6*) might lead to the formation of bullet-shaped magnetosomes. Recently, a group of *mad*

genes with unknown functions specific to *Deltaproteobacteria* MTB and/or Mbav has been identified by Lefèvre *et al.* (2013b), which are predicted to be involved in the intracellular biomineralization of these organisms. Orthologues of *mad* genes have also been found in the genome of Mcas (Figure 4). Notably, only the *mad2* gene appears to be conserved in the available genomes of bullet-shaped magnetite-producing MTB but absent from the draft genome of greigite-producing '*Ca.* M. multicellularis' (Figure 4), suggesting its potential role in the formation of bullet-shaped magnetosomes. Magnetite magnetosomes with bullet shape have been identified in a MTB population belonging to the candidate division OP3 recently (Kolinko *et al.*, 2012), while so far no genomic data are available for this novel magnetotactic division. Thus, additional genomes of MTB strains from the candidate division OP3 are required to elucidate the genetic mechanisms of biomineralization of bullet-shaped magnetosomes.

In summary, this study represents the first comprehensive genomic analysis of the poorly understood '*Ca.* Magnetobacterium' genus. Our results provide a better understanding of the trophic strategy, physiology, evolution and biomineralization mechanism of *Nitrospirae* MTB. The genome information also provides important clues to guide future cultivation attempts of this fascinating group.

Conflict of Interest

The authors declare no conflict of interest.

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References

Amann R, Krumholz L, Stahl DA. (1990). Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *J Bacteriol* **172**: 762–770.

Arnold K, Bordoli L, Kopp J, Schwede T. (2006). The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* **22**: 195–201.

Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA *et al.* (2008). The RAST server: rapid annotations using subsystems technology. *BMC Genomics* **9**: 75.

Barak I, Wilkinson AJ. (2007). Division site recognition in *Escherichia coli* and *Bacillus subtilis*. *FEMS Microbiol Rev* **31**: 311–326.

Bazylinski D, Lefèvre C, Schüler D. (2013). Magnetotactic bacteria. In: Rosenberg E, DeLong E, Lory S, Stackebrandt E, Thompson F (eds) *The Prokaryotes*. Springer: Berlin Heidelberg, pp 453–494.

Bazylinski DA, Dean AJ, Williams TJ, Long LK, Middleton SL, Dubbels BL. (2004). Chemolithoautotrophy in the marine, magnetotactic bacterial strains MV-1 and MV-2. *Arch Microbiol* **182**: 373–387.

Bazylinski DA, Frankel RB. (2004). Magnetosome formation in prokaryotes. *Nat Rev Microbiol* **2**: 217–230.

Benkert P, Schwede TTosatto SCE (2009). QMEANclust: estimation of protein model quality by combining a composite scoring function with structural density information. *BMC Struct Biol* **9**: 35.

Berg IA. (2011). Ecological aspects of the distribution of different autotrophic CO2 fixation pathways. *Appl Environ Microbiol* **77**: 1925–1936.

Brown MT, Delalez NJ, Armitage JP. (2011). Protein dynamics and mechanisms controlling the rotational behaviour of the bacterial flagellar motor. *Curr Opin Microbiol* **14**: 734–740.

Brune A, Frenzel P, Cypionka H. (2000). Life at the oxic–anoxic interface: microbial activities and adaptations. *FEMS Microbiol Rev* **24**: 691–710.

Castresana J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* **17**: 540–552.

Chevreux B, Wetter T, Suhai S. (1999). Genome sequence assembly using trace signals and additional sequence information. *Comput Sci Biol* **GCB99**: 45–56.

Cypionka H. (1994). Novel metabolic capacities of sulfate-reducing bacteria, and their activities in microbial mats. In: Stal L, Caumette P (eds) *Microbial Mats*. Springer: Berlin Heidelberg, pp 367–376.

Dahl C, Engels S, Pott-Sperling AS, Schulte A, Sander J, Lübbe Y *et al.* (2005). Novel genes of the *dsr* gene cluster and evidence for close interaction of Dsr proteins during sulfur oxidation in the phototrophic sulfur bacterium *Allochromatium vinosum*. *J Bacteriol* **187**: 1392–1404.

Dannenberg S, Kroder M, Dilling W, Cypionka H. (1992). Oxidation of H2, organic compounds and inorganic sulfur compounds coupled to reduction of O2 or nitrate by sulfate-reducing bacteria. *Arch Microbiol* **158**: 93–99.

Dilling W, Cypionka H. (1990). Aerobic respiration in sulfate-reducing bacteria. *FEMS Microbiol Lett* **71**: 123–127.

Draper O, Byrne ME, Li Z, Keyhani S, Barrozo JC, Jensen G *et al.* (2011). MamK, a bacterial actin, forms dynamic filaments *in vivo* that are regulated by the acidic proteins MamJ and LimJ. *Mol Microbiol* **82**: 342–354.

Edgar RC. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**: 1792–1797.

Forbes JR, Gros P. (2001). Divalent-metal transport by NRAMP proteins at the interface of host-pathogen interactions. *Trends Microbiol* **9**: 397–403.

Frankel RB, Bazylinski DA, Johnson MS, Taylor BL. (1997). Magneto-aerotaxis in marine coccoid bacteria. *Biophys J* **73**: 994–1000.

Garrity G, Holt J. (2001). Phylum BVIII. Nitrospirae phy. nov. In: Boone D, Castenholz R (eds) *Bergey's Manual of Systematic Bacteriology*. Springer: New York, pp 451–464.

Giltner CL, Nguyen Y, Burrows LL. (2012). Type IV pilin proteins: versatile molecular modules. *Microbiol Mol Biol Rev* **76**: 740–772.

Goltsman DSA, Denef VJ, Singer SW, VerBerkmoes NC, Lefsrud M, Mueller RS *et al.* (2009). Community genomic and proteomic analyses of chemoautotrophic

iron-oxidizing '*Leptospirillum rubarum*' (Group II) and '*Leptospirillum ferrodiazotrophum*' (Group III) bacteria in acid mine drainage biofilms. *Appl Environ Microbiol* **75**: 4599–4615.

Gouet P, Courcelle E, Stuart DI, Metoz F. (1999). ESPript: analysis of multiple sequence alignments in PostScript. *Bioinformatics* **15**: 305–308.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* **59**: 307–321.

Hattori S, Galushko AS, Kamagata Y, Schink B. (2005). Operation of the CO dehydrogenase/acetyl coenzyme A pathway in both acetate oxidation and acetate formation by the syntrophically acetate-oxidizing bacterium *Thermacetogenium phaeum*. *J Bacteriol* **187**: 3471–3476.

Henry EA, Devereux R, Maki JS, Gilmour CC, Woese CR, Mandelco L *et al.* (1994). Characterization of a new thermophilic sulfate-reducing bacterium. *Arch Microbiol* **161**: 62–69.

Holkenbrink C, Ocón Barbas S, Mellerup A, Otaki H, Frigaard N-U. (2011). Sulfur globule oxidation in green sulfur bacteria is dependent on the dissimilatory sulfite reductase system. *Microbiology* **157**: 1229–1239.

Hopkinson BM, Barbeau KA. (2012). Iron transporters in marine prokaryotic genomes and metagenomes. *Environ Microbiol* **14**: 114–128.

Hügler M, Sievert SM. (2011). Beyond the Calvin cycle: autotrophic carbon fixation in the ocean. *Annu Rev Mar Sci* **3**: 261–289.

Jensen RB, Shapiro L. (2003). Cell-cycle-regulated expression and subcellular localization of the *Caulobacter crescentus* SMC chromosome structural protein. *J Bacteriol* **185**: 3068–3075.

Ji B, Zhang S-D, Arnoux P, Rouy Z, Alberto F, Philippe N *et al.* (2014). Comparative genomic analysis provides insights into the evolution and niche adaptation of marine *Magnetospira* sp. QH-2 strain. *Environ Microbiol* **16**: 525–544.

Jogler C, Lin W, Meyerdierks A, Kube M, Katzmann E, Flies C *et al.* (2009). Towards cloning the magnetotactic metagenome: identification of magnetosome island gene clusters in uncultivated magnetotactic bacteria from different aquatic sediments. *Appl Environ Microbiol* **75**: 3972–3979.

Jogler C, Niebler M, Lin W, Kube M, Wanner G, Kolinko S *et al.* (2010). Cultivation-independent characterization of '*Candidatus* Magnetobacterium bavaricum' *via* ultrastructural, geochemical, ecological and metagenomic methods. *Environ Microbiol* **12**: 2466–2478.

Jogler C, Wanner G, Kolinko S, Niebler M, Amann R, Petersen N *et al.* (2011). Conservation of proteobacterial magnetosome genes and structures in an uncultivated member of the deep-branching *Nitrospira* phylum. *Proc Natl Acad Sci USA* **108**: 1134–1139.

Katzmann E, Scheffel A, Gruska M, Plitzko JM, Schüler D. (2010). Loss of the actin-like protein MamK has pleiotropic effects on magnetosome formation and chain assembly in *Magnetospirillum gryphiswaldense*. *Mol Microbiol* **77**: 208–224.

Kawasaki S, Arai H, Kodama T, Igarashi Y. (1997). Gene cluster for dissimilatory nitrite reductase (nir) from *Pseudomonas aeruginosa*: sequencing and identification of a locus for heme d1 biosynthesis. *J Bacteriol* **179**: 235–242.

Kolinko S, Jogler C, Katzmann E, Wanner G, Peplies J, Schüler D. (2012). Single-cell analysis reveals a novel uncultivated magnetotactic bacterium within the candidate division OP3. *Environ Microbiol* **14**: 1709–1721.

Komeili A, Li Z, Newman DK, Jensen GJ. (2006). Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science* **311**: 242–245.

Komeili A. (2012). Molecular mechanisms of compartmentalization and biomineralization in magnetotactic bacteria. *FEMS Microbiol Rev* **36**: 232–255.

Kunisawa T. (2010). Evaluation of the phylogenetic position of the sulfate-reducing bacterium *Thermodesulfovibrio yellowstonii* (phylum *Nitrospirae*) by means of gene order data from completely sequenced genomes. *Int J Syst Evol Microbiol* **60**: 1090–1102.

Lefèvre CT, Bazylinski DA. (2013). Ecology, diversity, and evolution of magnetotactic bacteria. *Microbiol Mol Biol Rev* **77**: 497–526.

Lefèvre CT, Trubitsyn D, Abreu F, Kolinko S, de Almeida LGP, de Vasconcelos ATR *et al.* (2013a). Monophyletic origin of magnetotaxis and the first magnetosomes. *Environ Microbiol* **15**: 2267–2274.

Lefèvre CT, Trubitsyn D, Abreu F, Kolinko S, Jogler C, de Almeida LGP *et al.* (2013b). Comparative genomic analysis of magnetotactic bacteria from the *Deltaproteobacteria* provides new insights into magnetite and greigite magnetosome genes required for magnetotaxis. *Environ Microbiol* **15**: 2712–2735.

Lefèvre CT, Wu L-F. (2013). Evolution of the bacterial organelle responsible for magnetotaxis. *Trends Microbiol* **21**: 534–543.

Levican G, Ugalde J, Ehrenfeld N, Maass A, Parada P. (2008). Comparative genomic analysis of carbon and nitrogen assimilation mechanisms in three indigenous bioleaching bacteria: predictions and validations. *BMC Genomics* **9**: 581.

Li J, Pan Y, Liu Q, Yu-Zhang K, Menguy N, Che R *et al.* (2010). Biomineralization, crystallography and magnetic properties of bullet-shaped magnetite magnetosomes in giant rod magnetotactic bacteria. *Earth Planet Sci Lett* **293**: 368–376.

Lin W, Li J, Schüler D, Jogler C, Pan Y. (2009). Diversity analysis of magnetotactic bacteria in Lake Miyun, northern China, by restriction fragment length polymorphism. *Syst Appl Microbiol* **32**: 342–350.

Lin W, Pan Y. (2010). Temporal variation of magnetotactic bacterial communities in two freshwater sediment microcosms. *FEMS Microbiol Lett* **302**: 85–92.

Lin W, Jogler C, Schüler D, Pan Y. (2011). Metagenomic analysis reveals unexpected subgenomic diversity of magnetotactic bacteria within the phylum *Nitrospirae*. *Appl Environ Microbiol* **77**: 323–326.

Lin W, Li J, Pan Y. (2012). Newly isolated but uncultivated magnetotactic bacterium of the phylum *Nitrospirae* from Beijing, China. *Appl Environ Microbiol* **78**: 668–675.

Lin W, Bazylinski DA, Xiao T, Wu L-F, Pan Y. (2014). Life with compass: diversity and biogeography of magnetotactic bacteria. *Environ Microbiol* **16**: doi:10.1111/1462-2920.12313.

Lucker S, Wagner M, Maixner F, Pelletier E, Koch H, Vacherie B *et al.* (2010). A *Nitrospira* metagenome illuminates the physiology and evolution of globally important nitrite-oxidizing bacteria. *Proc Natl Acad Sci USA* **107**: 13479–13484.

Markert S, Arndt C, Felbeck H, Becher D, Sievert SM, Hügler M *et al.* (2007). Physiological proteomics of the uncultured endosymbiont of *Riftia pachyptila*. *Science* **315**: 247–250.

Matsunaga T, Okamura Y, Fukuda Y, Wahyudi AT, Murase Y, Takeyama H. (2005). Complete genome sequence of the facultative anaerobic magnetotactic

bacterium *Magnetospirillum* sp. strain AMB-1. *DNA Res* **12**: 157–166.

Matsunaga T, Suzuki T, Tanaka M, Arakaki A. (2007). Molecular analysis of magnetotactic bacteria and development of functional bacterial magnetic particles for nano-biotechnology. *Trends Biotechnol* **25**: 182–188.

Matsunaga T, Nemoto M, Arakaki A, Tanaka M. (2009). Proteomic analysis of irregular, bullet-shaped magnetosomes in the sulphate-reducing magnetotactic bacterium *Desulfovibrio magneticus* RS-1. *Proteomics* **9**: 3341–3352.

Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. (2007). KAAS: an automatic genome annotation and pathway reconstruction server. *Nucl Acids Res* **35**: W182–W185.

Murat D, Quinlan A, Vali H, Komeili A. (2010). Comprehensive genetic dissection of the magnetosome gene island reveals the step-wise assembly of a prokaryotic organelle. *Proc Natl Acad Sci USA* **107**: 5593–5598.

Mussmann M, Hu FZ, Richter M, de Beer D, Preisler A, Jorgensen BB *et al.* (2007). Insights into the genome of large sulfur bacteria revealed by analysis of single filaments. *PLoS Biol* **5**: 1923–1937.

Nakazawa H, Arakaki A, Narita-Yamada S, Yashiro I, Jinno K, Aoki N *et al.* (2009). Whole genome sequence of *Desulfovibrio magneticus* strain RS-1 revealed common gene clusters in magnetotactic bacteria. *Genome Res* **19**: 1801–1808.

Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T *et al.* (2014). The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* **42**: D206–D214.

Ozyamak E, Kollman J, Agard DA, Komeili A. (2013). The bacterial actin MamK: *in vitro* assembly behavior and filament architecture. *J Biol Chem* **288**: 4265–4277.

Pan Y, Lin W, Li J, Wu W, Tian L, Deng C *et al.* (2009). Reduced efficiency of magnetotaxis in magnetotactic coccoid bacteria in higher than geomagnetic fields. *Biophys J* **97**: 986–991.

Peretó JG, Velasco AM, Becerra A, Lazcano A. (1999). Comparative biochemistry of CO2 fixation and the evolution of autotrophy. *Int Microbiol* **2**: 3–10.

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC *et al.* (2004). UCSF Chimera—A visualization system for exploratory research and analysis. *J Comput Chem* **25**: 1605–1612.

Pires RH, Lourenço AI, Morais F, Teixeira M, Xavier AV, Saraiva LgM *et al.* (2003). A novel membrane-bound respiratory complex from *Desulfovibrio desulfuricans* ATCC 27774. *Biochim Biophys Acta* **1605**: 67–82.

Pott AS, Dahl C. (1998). Sirohaem sulfite reductase and other proteins encoded by genes at the dsr locus of *Chromatium vinosum* are involved in the oxidation of intracellular sulfur. *Microbiology* **144**: 1881–1894.

Reinartz M, Tschäpe J, Brüser T, Trüper HG, Dahl C. (1998). Sulfide oxidation in the phototrophic sulfur bacterium *Chromatium vinosum*. *Arch Microbiol* **170**: 59–68.

Richter M, Kube M, Bazylinski DA, Lombardot T, Glockner FO, Reinhardt R *et al.* (2007). Comparative genome analysis of four magnetotactic bacteria reveals a complex set of group-specific genes implicated in magnetosome biomineralization and function. *J Bacteriol* **189**: 4899–4910.

Schauder R, Preuß A, Jetten M, Fuchs G. (1988). Oxidative and reductive acetyl CoA/carbon monoxide dehydrogenase pathway in *Desulfobacterium autotrophicum*. 2. Demonstration of the enzymes of the pathway and comparison of CO dehydrogenase. *Arch Microbiol* **151**: 84–89.

Scheffel A, Gruska M, Faivre D, Linaroudis A, Plitzko JM, Schüler D. (2006). An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. *Nature* **440**: 110–114.

Scheffel A, Schüler D. (2007). The acidic repetitive domain of the *Magnetospirillum gryphiswaldense* MamJ protein displays hypervariability but is not required for magnetosome chain assembly. *J Bacteriol* **189**: 6437–6446.

Schübbe S, Williams TJ, Xie G, Kiss HE, Brettin TS, Martinez D *et al.* (2009). Complete genome sequence of the chemolithoautotrophic marine magnetotactic coccus strain MC-1. *Appl Environ Microbiol* **75**: 4835–4852.

Schüler D. (2008). Genetics and cell biology of magnetosome formation in magnetotactic bacteria. *FEMS Microbiol Rev* **32**: 654–672.

Serata M, Iino T, Yasuda E, Sako T. (2012). Roles of thioredoxin and thioredoxin reductase in the resistance to oxidative stress in *Lactobacillus casei*. *Microbiology* **158**: 953–962.

Simon J. (2002). Enzymology and bioenergetics of respiratory nitrite ammonification. *FEMS Microbiol Rev* **26**: 285–309.

Spring S, Amann R, Ludwig W, Schleifer KH, van Gemerden H, Petersen N. (1993). Dominating role of an unusual magnetotactic bacterium in the microaerobic zone of a freshwater sediment. *Appl Environ Microbiol* **59**: 2397–2403.

Spring S, Bazylinski DA. (2006). Magnetotactic bacteria. In: Dworkin M (ed) *The prokaryotes: an evolving electronic resource for the microbiological community*. Springer Verlag: New York, USA, pp 842–862.

Stragier P. (2001). A gene odyssey: exploring the genomes of endospore-forming bacteria. In: Sonenshein L, Losick R, Hoch JA (eds) *Bacillus subtilis and its relatives: from genes to cells*. American Society for Microbiology: Washington, USA, pp 519–525.

Teske A, Alm E, Regan JM, Toze S, Rittmann BE, Stahl DA. (1994). Evolutionary relationships among ammonia- and nitrite-oxidizing bacteria. *J Bacteriol* **176**: 6623–6630.

Thompson JD, Higgins DG, Gibson TJ. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673–4680.

Vali H, Forster O, Amarantidis G, Petersen N. (1987). Magnetotactic bacteria and their magnetofossils in sediments. *Earth Planet Sci Lett* **86**: 389–400.

Wang X, Wang Q, Zhang W, Wang Y, Li L, Wen T *et al.* (2014). Complete genome sequence of *Magnetospirillum gryphiswaldense* MSR-1. *Genome Announc* **2**: e00171–00114.

Williams TJ, Zhang CL, Scott JH, Bazylinski DA. (2006). Evidence for autotrophy via the reverse tricarboxylic acid cycle in the marine magnetotactic coccus strain MC-1. *Appl Environ Microbiol* **72**: 1322–1329.

Wu M, Scott AJ. (2012). Phylogenomic analysis of bacterial and archaeal sequences with AMPHORA2. *Bioinformatics* **28**: 1033–1034.

Yang W, Li RG, Peng T, Zhang Y, Jiang W, Li Y *et al.* (2010). *mamO* and *mamE* genes are essential for magnetosome crystal biomineralization in *Magnetospirillum gryphiswaldense* MSR-1. *Res Microbiol* **161**: 701–705.

Zhou J, He Q, Hemme CL, Mukhopadhyay A, Hillesland K, Zhou A *et al.* (2011). How sulphate-reducing microorganisms cope with stress: lessons from systems biology. *Nat Rev Micro* **9**: 452–466.

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