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A putative greigite-type magnetosome gene cluster from the candidate phylum *Latescibacteria*

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Summary

The intracellular biomineralization of magnetite and/or greigite magnetosomes in magnetotactic bacteria (MTB) is strictly controlled by a group of conserved genes, termed magnetosome genes, which are organized as clusters (or islands) in MTB genomes. So far, all reported MTB are affiliated within the *Proteobacteria* phylum, the *Nitrospirae* phylum and the candidate division OP3. Here, we report the discovery of a putative magnetosome gene cluster structure from the draft genome of an uncultivated bacterium belonging to the candidate phylum *Latescibacteria* (formerly candidate division WS3) recently recovered by Rinke and colleagues, which contains 10 genes with homology to magnetosome *mam* genes of magnetotactic *Proteobacteria* and *Nitrospirae*. Moreover, these genes are phylogenetically closely related to greigite-type magnetosome genes that were only found from the *Deltaproteobacteria* MTB before, suggesting that the greigite genes may originate earlier than previously imagined. These findings indicate that some members of *Latescibacteria* may be capable of forming greigite magnetosomes, and thus may play previously unrecognized roles in environmental iron and sulfur cycles. The conserved genomic structure of magnetosome gene cluster in *Latescibacteria* phylum supports the hypothesis of horizontal transfer of these genes among distantly related bacterial groups in nature.

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Introduction

Magnetotactic bacteria (MTB) are a group of microorganisms united by the ability to synthesize special intracellular organelles, called magnetosomes, which are nano-sized, membrane-enveloped magnetic crystals of magnetite (Fe_3O_4) and/or greigite (Fe_3S_4) usually arranged into one or multiple chain-like structures (Bazylinski *et al.*, 2013). These magnetic inclusions render MTB able to sense and swim along the Earth's magnetic field, which facilitates the location and orientation of MTB to their favourable low-oxygen positions in vertically chemically stratified sediments or water columns (Frankel *et al.*, 1997; Pan *et al.*, 2009). MTB occur ubiquitously in a wide range of different ecosystems, including lakes, rivers, estuaries, lagoons, mangrove swamps, intertidal zones, deep-sea sediments and even in extreme environments (Lin *et al.*, 2014a). Due to their high intracellular iron content, MTB are believed to play important roles in the biogeochemical cycle of iron. It was estimated that the annual yield of magnetite by global MTB may be more than 10^8 kg (Lin *et al.*, 2014a). In some habitats, such as chemically stratified marine environments, the contribution of MTB to iron cycling could reach 1–10% (Faivre and Schüler, 2008).

So far, MTB have been found within the *Alphaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria* classes of the *Proteobacteria* phylum, the *Nitrospirae* phylum, and the candidate division OP3 (Lefèvre and Bazylinski, 2013). The biomineralization of magnetosomes in MTB is under strictly genetic control by a group of conserved magnetosome genes organized as clusters (Grünberg *et al.*, 2001), which have been identified in all genome-available MTB strains (e.g. Abreu *et al.*, 2011; Jogler *et al.*, 2011; Lefèvre *et al.*, 2013b; Lin *et al.*, 2014b). Two related yet different sets of magnetosome genes have been found responsible for either magnetite or greigite biomineralization (Lefèvre *et al.*, 2011; Kolinko *et al.*, 2014b). Both sets contain nine core *mam* genes (*mamABEIKMOPQ*), which play essential roles in magnetosome formation and biomineralization (Lefèvre and Wu, 2013). Other types of magnetosome genes, such as *mms* and *mamGFCD* genes in *Magnetospirillum* (Scheffel *et al.*, 2008; Murat *et al.*, 2010; Tanaka *et al.*, 2011), *mad* genes in *Deltaproteobacteria* MTB (Lefèvre

et al., 2013b) and *man* genes in magnetotactic *Nitrospirae* (Lin *et al*., 2014b), may perform important accessory functions in magnetosome synthesis among different MTB populations.

In this study, we report the discovery of a putative magnetosome gene cluster fragment from an uncultivated bacterium belonging to the candidate phylum *Latescibacteria* (formerly candidate division WS3). This fragment contains 10 putative magnetosome genes with homology to greigite-type *mam* genes of *Deltaproteobacteria* MTB, suggesting the potential capability of this *Latescibacteria* bacterium forming greigite magnetosomes.

Results and discussion

In a recent study, Rinke and colleagues (2013) have recovered more than 200 draft genomes from over 20 major uncultivated bacterial and archaeal lineages through single-cell whole genome amplification approach. Among these genomes, we have identified a 17 321-bp contig (GenBank accession ASWY01000093) from the draft genome of an uncultivated *Latescibacteria bacterium* SCGC AAA252-B13 (hereafter referred to as B13) from the brackish Sakinaw Lake in Canada, which contains several putative magnetosome genes. The draft genome of B13 (GenBank accession ASWY00000000) contains 138 contigs with a total length of 1.76 Mb, and has an average GC content of 40.9% (Rinke *et al*., 2013). Both SSU rRNA gene-based and marker genes-based phylogenetic analyses indicate the affiliation of B13 with the candidate phylum *Latescibacteria* of Fibrobacteres-Chlorobi-Bacteroidetes (FCB) superphylum (Rinke *et al*., 2013) (Fig. 1).

In this study, the putative magnetosome gene-containing contig from B13 was annotated through the RAST server (Aziz *et al*., 2008) and was subsequently manually checked. The annotated sequence is available on the RAST guest account at the web address http://rast.nmpdr.org (Genome ID 910048.4, using login and password 'guest'). It contains an average GC content of 39.6% and 20 predicted protein-encoding genes (Fig. 2A and Table 1). Interestingly, 10 of them represent remarkable similarity to magnetosome genes *mamPMIAQBTOEP* in *Proteobacteria* and *Nitrospirae* MTB (Table 2), the products of which have been identified to play vital roles in the biogenesis of magnetosome membrane, magnetosome protein sorting and magnetic crystal biomineralization in cultivated MTB strains (Murat *et al*., 2010; Lohße *et al*., 2011).

BLASTP analyses of the products of these putative *mam* genes have revealed that nine of them are closely related and have shown considerable sequence identity to greigite-type magnetosome proteins of '*Candidatus* Magnetoglobus multicellularis BW-1' (Lefèvre *et al*., 2011) or to those of the greigite-producer '*Ca*. Magnetoglobus

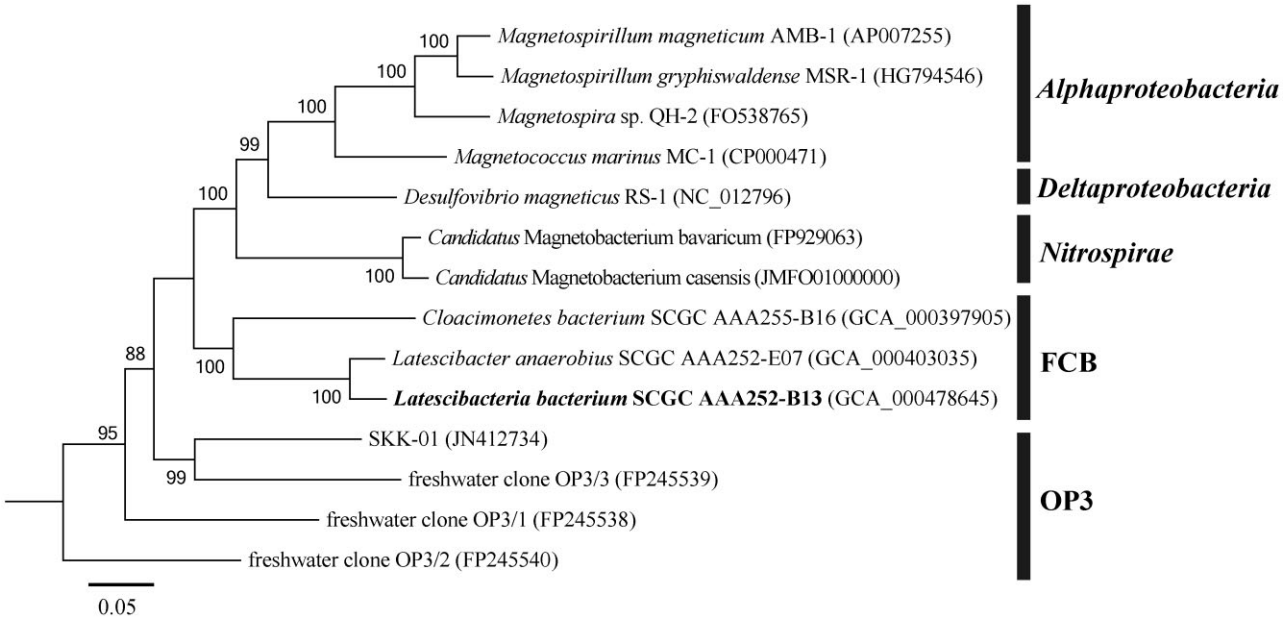

Fig. 1. Phylogenetic tree of 23S rRNA gene sequences showing the phylogenetic affiliation of *Latescibacteria bacterium* SCGC AAA252-B13 and other selected MTB populations. The sequences were aligned using SINA (Pruesse *et al*., 2012), and the phylogenetic relationships were reconstructed using QIIME (Caporaso *et al*., 2010). GenBank accession numbers are given in parentheses. The 23S rRNA gene sequence of archaea *Sulfolobus acidocaldarius* was used to root the tree. FCB, Fibrobacteres-Chlorobi-Bacteroidetes superphylum; OP3, candidate division OP3.

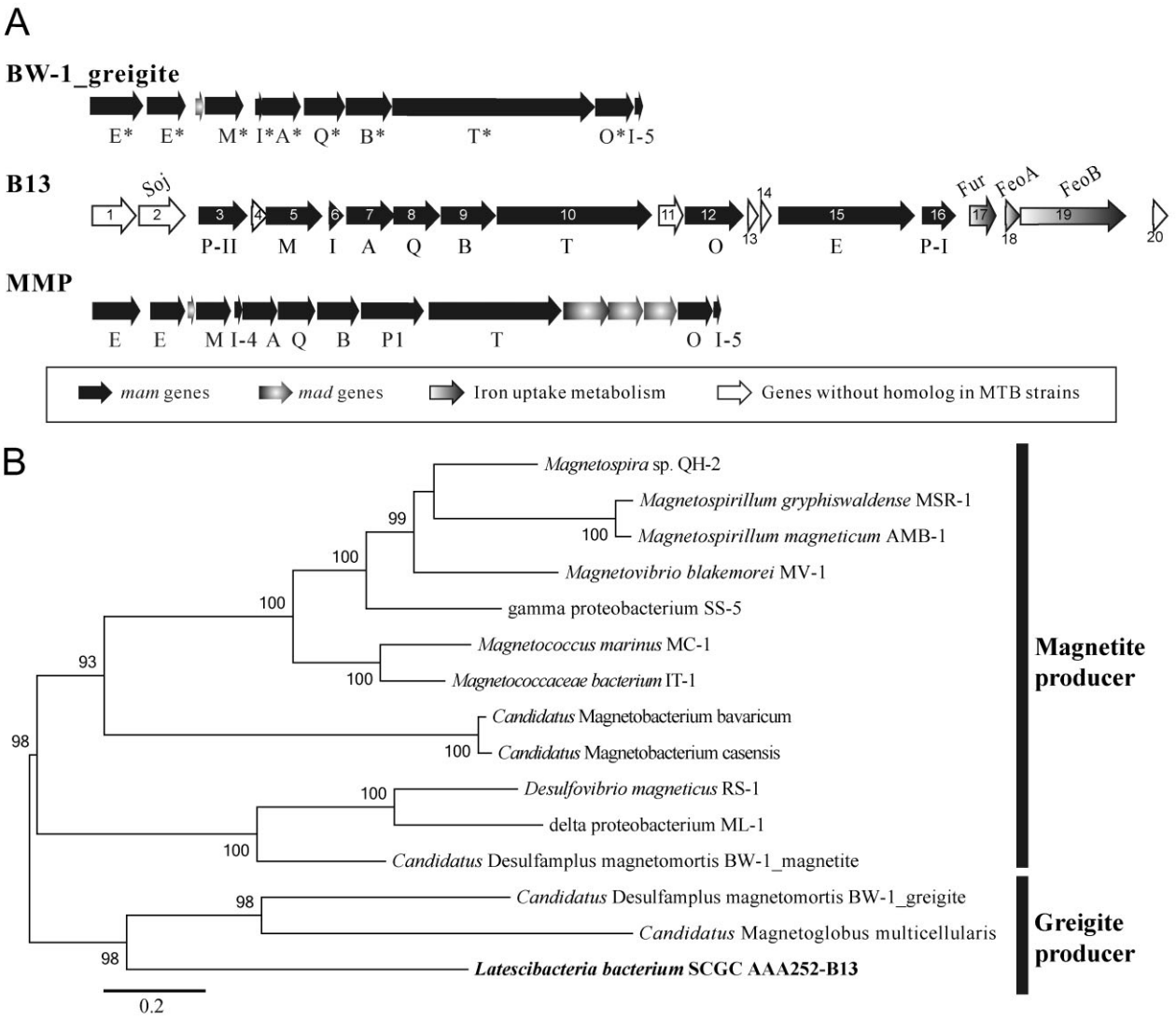

Fig. 2. (A) Schematic comparison of the putative magnetosome gene cluster region identified in *Latescibacteria bacterium* SCGC AAA252-B13 (B13) with those of greigite-producing '*Ca*. Magnetoglobus multicellularis' (MMP) and both magnetite- and greigite-producing '*Ca*. Magnetoglobus multicellularis BW-1' (BW-1). Note that only greigite-type Mam proteins of BW-1 are included here. The 20 putative open reading frames (ORFs) of B13 are numbered, and their detailed information is shown in Table 1. (B) Maximum-likelihood tree based on a concatenation of five Mam protein sequences (MamABEMQ) reconstructed by PHYML v3.0 (Guindon *et al*., 2010), using the Whelan and Golding (WAG) amino acid substitution matrix. Branch support was calculated using the approximate likelihood ratio test (aLRT) with SH-like interpretation.

multicellularis' (Abreu *et al*., 2011) (Table 2). The phylogeny based on concatenated magnetosome protein sequences further supports the close relationship of these proteins with those of greigite-producing MTB (Fig. 2B).

The typical regions harbouring magnetosome gene clusters in MTB populations, such as *Magnetospirillum* strains, contain tRNA and transposase-like genes, and their GC content is distinct from that of the rest of genomes (Ullrich *et al*., 2005). These features are similar to those structures described for genome islands in other bacteria; therefore, the regions containing magnetosome gene clusters in these MTB were described as magnetosome gene island or MAI (Schüler, 2008; Komeili, 2012). The GC content of magnetosome gene cluster identified in this study is 1.3% below the average GC content of B13 draft genome; however, we do not find any tRNA genes or transposase encoding genes in this contig (Fig. 2A). Because the transposase-like genes normally locate at the boundaries of MAIs in other MTB strains, the absence of these genes in this contig is probably due to the incomplete nature of genomic sequence. Alternatively, it is possible that the region containing magnetosome gene cluster in B13 is similar to the MAI

Table 1. Predicted protein coding genes (CDS) in *Latescibacteria bacterium* SCGC AAA252-B13 contig A252B13DRAFT_contig_45_0.46 (accession number ASWY01000093).

CDS	No. of amino acids (aa)	Predicted function
1	236	Hypothetical protein
2	259	Sporulation initiation inhibitor Soj
3	266	Putative magnetosome protein MamP-II
4	77	Hypothetical protein
5	298	Putative magnetosome protein MamM
6	55	Putative magnetosome protein MamI
7	256	Putative magnetosome protein MamA
8	251	Putative magnetosome protein MamQ
9	288	Putative magnetosome protein MamB
10	842	Putative magnetosome protein MamT
11	134	Hypothetical protein
12	313	Putative magnetosome protein MamO
13	44	Hypothetical protein
14	39	Hypothetical protein
15	734	Putative magnetosome protein MamE
16	176	Putative magnetosome protein MamP-I
17	148	Ferric uptake regulation protein Fur
18	80	Ferrous iron transport protein A (FeoA)
19	570	Ferrous iron transport protein B (FeoB)
20	75	Hypothetical protein

structure in *Magnetospira* sp. QH-2 that lacks transposase encoding genes (Ji *et al*., 2014).

Three genes in the contig are found to encode proteins for iron uptake metabolism (Fig. 2A). The colocalization of them with magnetosome genes suggests their potential roles in magnetosome formation. Among them, genes encoding FeoAB may be responsible for acquiring the ferrous form of iron from the environment. It was reported that FeoAB in *Magnetospirillum gryphiswaldense* also plays accessory roles in magnetosome formation (Rong *et al*., 2008; Kolinko *et al*., 2014a). A ferric uptake regulator Fur protein-encoding gene locates next to *feoA* gene (Fig. 2A), the homologue of which has been found to be involved in the iron homeostasis as well as the regulation of magnetite biomineralization in *Magnetospirillum* (Uebe *et al*., 2010; Qi *et al*., 2012).

So far, magnetite-producing bacteria are found from all known taxa of MTB, while greigite-producing members have exclusively been detected in the *Deltaproteobacteria* (Lefèvre and Bazylinski, 2013). It was thus hypothesized that magnetite, specifically bullet-shaped magnetite, may be the ancient form of magnetosomes because it has been found in the deep-branching MTB lineages of *Nitrospirae* and the candidate division OP3 (Lefèvre *et al*., 2013a). In other words, the biomineralization of greigite magnetosomes that was only detected in *Deltaproteobacteria* may originate later (Lefèvre *et al*., 2013a). This study represents the first evidence of putative greigite-type magnetosome gene cluster in *Latescibacteria*, a more deeply divergent phylum than *Nitrospirae* (Hugenholtz, 2002), which suggests that greigite

Table 2. Comparison of putative Mam proteins identified from the contig of draft genome of *Latescibacteria bacterium* SCGC AAA252-B13 with those of all MTB.

Putative magnetosome protein	Peptide length (aa)	MTB with magnetosome protein with lowest *E-value*	Accession no.	*E-value*	Coverage (%)	Identity (%)	MTB with magnetosome protein with second lowest *E-value*	Accession no.	*E-value*	Coverage (%)	Identity (%)
MamP-I	176	BW-1 (greigite)	AET24922	5e-12	46	37	BW-1 (greigite)	AET24924	7e-11	66	32
MamE	734	ML-1 (magnetite)	AFZ77017	3e-16	30	28	ML-1 (magnetite)	AFX88981	7e-16	33	28
MamO	313	MMP (greigite)	ADV17386	2e-66	76	48	BW-1 (greigite)	AET24915	2e-66	75	50
MamT	842	MMP (greigite)	ADV17387	3e-34	17	43	BW-1 (greigite)	AET24924	4e-30	96	31
MamB	288	MMP (greigite)	ADV17392	8e-60	93	40	IT-1 (magnetite)	AHG23879	2e-57	99	37
MamQ	251	BW-1 (greigite)	AET24917	4e-23	66	35	Mcas (magnetite)	AIM41314	1e-20	73	31
MamA	256	BW-1 (greigite)	AET24906	2e-37	80	36	MMP (greigite)	ADV17394	5e-36	78	36
MamI	55	BW-1 (greigite)	AFX88993	2e-06	94	45	Mcas (magnetite)	AIM41316	1e-04	94	47
MamM	298	BW-1 (greigite)	AET24909	6e-32	92	31	ML-1 (magnetite)	AFZ77026	2e-30	94	27
MamP-II	266	MMP (greigite)	ADV17397	5e-38	97	32	BW-1 (greigite)	AET24922	3e-37	90	34

MMP, '*Candidatus* Magnetoglobus multicellularis'; BW-1, '*Ca.* Magnetoglobus multicellularis BW-1'; Mcas, '*Ca.* Magnetobacterium casensis'; ML-1, delta proteobacterium ML-1; IT-1, *Magnetofaba australis* strain IT-1.

magnetosome genes may originate earlier than previously thought.

Recent comparative genomic analyses (Jogler *et al*., 2009; Lefèvre *et al*., 2013b) and experimental study (Kolinko *et al*., 2014a) have both suggested that magnetotactic trait of phylogenetically different MTB may be acquired by horizontal gene transfer. Eight of nine core magnetosome genes (*mamABEIMOPQ*) are found in *Latescibacteria* (Fig. 2A). Their conserved gene structures strongly suggest the event of horizontal transfer of magnetosome genes among distantly related organisms. Genes encoding MamK protein that plays key role in organizing magnetosomes into chain arrangement has not been found here (Komeili *et al*., 2006; Katzmann *et al*., 2010). In addition, homologues to magnetosome *mad* genes that were recently discovered from the MAIs of greigite-producing magnetotactic *Deltaproteobacteria* (Lefèvre *et al*., 2013b) are not identified either. Further studies are required to identify whether the absence of these genes in *Latescibacteria* is due to the incomplete nature of the draft genome (57% of estimated completeness of the single-cell genome) (Rinke *et al*., 2013) or the existence of a novel mode of magnetosome formation and/or chain arrangement in this organism.

Latescibacteria is a globally distributed candidate phylum belonging to the FCB superphylum (Dojka *et al*., 1998; Derakshani *et al*., 2001; Rinke *et al*., 2013). Members of *Latescibacteria* appear to be associated with methanogenic environments (Kim *et al*., 2008); however, only limited information is available for this phylum because of the lack of cultivated representatives to date. In the study of Rinke and colleagues (2013), genes encoding for the Ribulose 1,5-bisphosphate carboxylase (RuBisCO) type III, Ni,Fe-hydrogenase large subunit, flavoprotein-quinone oxidoreductase, NADH-quinone oxidoreductase, succinate/fumarate quinone oxidoreductase and cytochrome/quinol oxidase were identified from the draft genomes of B13 and other three uncultivated *Latescibacteria* strains, which provides some insights into the metabolism and biology of this poorly understood candidate division. With the finding of greigite type *mam* genes, *feoAB* genes and *fur* gene in the present study, it appears that some members of *Latescibacteria* (such as B13) could be involved in greigite magnetosome formation. Considering their worldwide distribution in many ecosystems, magnetotactic *Latescibacteria* may play important roles in biogeochemical cycles of iron and sulfur in their living habitats.

In summary, we show here a magnetosome gene cluster containing orthologues of greigite-type *mam* genes from the candidate phylum *Latescibacteria*. This finding suggests that this organism may be capable of forming greigite magnetosomes and is widely involved in environmental iron and sulfur cyclings. This finding also indicates that MTB in nature may be more diverse than previously anticipated.

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