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Ferrous iron transport protein B gene (*feoB1*) plays an accessory role in magnetosome formation in *Magnetospirillum gryphiswaldense* strain MSR-1

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Abstract

To investigate the role of ferrous iron transport (Feo) systems in magnetosome formation, the gene for protein FeoB (*feoB1*), encoding 704 amino acids, was cloned from magnetotactic bacterium *Magnetospirillum gryphiswaldense* strain MSR-1. *feoB1* constitutes a putative operon with *feoA1*, and the interval between the two genes is 36 base pairs. A *feoB1*-deficient mutant (Δ*feoB1*) was constructed, and compared with wild-type in terms of iron uptake, iron content and functional complementation. Ferrous iron and ferric iron uptake in wild-type were respectively 1.8-fold and 1.3-fold higher than in the Δ*feoB1* mutant. Iron content (w/w) of Δ*feoB1* mutant was enhanced only slightly as extracellular iron concentration (either ferrous or ferric citrate) increased, whereas iron content of wild-type increased about 2-fold as extracellular iron concentration rose from 20 to 80 μM. Transmission electron microscopy revealed that Δ*feoB1* cells grown with either ferrous or ferric citrate produced fewer magnetosomes, with smaller diameter, compared to wild-type cells. Assay of *feoAB1* promoter–*lacZ* transcriptional fusions indicated that the *feoAB1* putative operon was downregulated when MSR-1 cells were grown under iron-rich condition. Magnetosome formation was reduced but not abolished in the *feoB1* mutant, indicating that FeoB1 protein plays a significant role in this process. Other iron transport systems are presumed to be involved in iron uptake in MSR-1.

Keywords: *Magnetospirillum gryphiswaldense*; Magnetotactic bacteria; Magnetosome formation; Ferrous iron transport; *feoB1* Gene; Ferric uptake regulator

1. Introduction

The element iron (Fe) acts as a cofactor in a wide range of biological reactions [9], and is required by virtually all organisms. Fe^{3+} (ferric iron) is insoluble at neutral pH, and many aerobic microorganisms secrete ferric chelators called siderophores to obtain an iron supply. At acidic pH or under anaerobic condition, the equilibrium shifts to the soluble Fe^{2+} form (ferrous iron). Many anaerobic or microaerophilic bacteria transport Fe^{2+} via Feo (‘‘ferrous iron transport’’) systems, sometimes accompanied by reduction of Fe^{3+} to Fe^{2+} through ferric reductase [1,22]. Enterobacterial Feo systems are composed of three proteins: FeoA, a small, soluble SH3-domain protein probably located in the cytosol [5]; FeoB, a large protein with a cytosolic N-terminal G-protein domain and a C-terminal integral inner-membrane domain containing two ‘Gate’ motifs, which likely functions as the Fe^{2+} permease [5,15]; and FeoC, a small protein apparently functioning as an [Fe–S]-dependent transcriptional repressor [5]. The *feoABC* genes constitute an operon. FeoB is responsible for ferrous iron transport, but the functions of FeoA and FeoC remain unclear. The *feoA* and *feoC* genes are not always present alongside *feoB*. So far, *feoC* has been found only in γ-proteobacteria. Many pathogenic bacteria show reduced virulence when the *feoB* gene is lost, reflecting its important role in iron uptake [6,26].

Magnetotactic bacteria have intracellular magnetic inclusions termed magnetosomes, which usually consist of nanometer-sized, membrane-coated magnetite (Fe_3O_4), aligned in a chain [2,8]. In order to form magnetosomes, magnetotactic

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bacteria require levels of iron about 100 times those required by non-magnetic bacteria [3,16]. The molecular mechanism whereby magnetotactic bacteria transport large amounts of iron is unclear. Surprisingly, *Magnetospirillum magnetotacticum* strain MS-1 and *Magnetospirillum magneticum* strain AMB-1 secrete siderophores only under iron-rich conditions [4,19]. *Magnetospirillum gryphiswaldense* strain MSR-1 does not form siderophores under either high- or low iron conditions [23], but it has more than five isozymes of ferric reductase, which are located in the cytoplasm, the cytoplasmic membrane, and extracellularly [17]. We hypothesize that ferric iron is reduced to ferrous iron by ferric reductase, and that ferrous iron is transported to the intercellular space via a Feo system.

To investigate the role of the Feo system in magnetosome formation of *M. gryphiswaldense* MSR-1, the *feoB* gene was cloned from MSR-1 and a *feoB* mutant was generated. After generation of the *feoB* mutant, the draft version of *M. gryphiswaldense* MSR-1 was published. A search revealed an open reading frame (ORF) with homology to FeoB, but with only 53% similarity. The cloned gene was renamed *feoB1*, and the second one *feoB2*. Functional analysis revealed that *feoB1* plays a significant role in magnetosome formation.

2. Materials and methods

2.1. Bacteria and growth conditions

Bacterial strains and plasmids used in this study are described in Table 1. *M. gryphiswaldense* MSR-1 was cultured in sodium lactate medium (SLM) at 30 °C as described previously [14]. The medium contained (per liter deionized water) 2.6 g sodium lactate, 0.4 g NH_4Cl, 0.1 g yeast extract, 0.5 g K_2HPO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.05 g sodium thioglycolate, and 5 ml trace element mixture. The iron source, ferric citrate, was added after autoclaving. For conjugation, *M. gryphiswaldense* strains were cultured in selection medium, in which NH_4Cl and yeast extract were substituted by 4 g sodium glutamate [14]. Strains were cultured in 250 ml serum bottles containing 100 ml medium with shaking at 100 rpm. At high cell densities, microaerobic conditions arose in the medium because of oxygen consumption. *Escherichia coli* strains were cultured in Luria broth (LB) at 37 °C. Antibiotics were applied at the following concentrations ($\mu g\ ml^{-1}$): for *E. coli*: ampicillin (Amp), 100; kanamycin (Km), 50; chloramphenicol (Cm), 50; gentamicin (Gm), 5; for MSR-1: nalidixic acid (Nx), 5; Km, 5, Cm, 5; Gm, 5.

2.2. Cloning the feoB1 gene

A 675 bp fragment of *feoB1* was amplified using primers feo6f/feo6r (5′-CCTGTTCCTGACCACCCAG-3′; 5′-CCCG CTTTGATCCAGTCG-3′). The 675 bp fragment was ligated to pGEM®-T Easy (Promega) to produce plasmid pTfeoB6. This sequence was confirmed by custom sequencing (Invitrogen Corp.). The EcoRI-cut 675 bp fragment of pTfeoB6 was subcloned into pUX19, yielding pUXf6e. pUXf6e was integrated into the chromosome of MSR-1 wild-type strain resulting from a single-crossover recombination event, and total DNA was isolated, digested with ApaI, ligated, and transformed into *E. coli* DH5α. Km^r colonies were selected. A plasmid, pAfeo, containing the *feoAB1* gene, was sequenced by pUX19 primer (5′-CCATGATTA CGCCACTAGTCG-3′) and T7 primer.

Table 1
Strains and plasmids used in this study

Strain/plasmid	Description	Source/reference
Strains		
Magnetospirillum gryphiswaldense MSR-1	Wild-type, Nx^r	DSM6361
M. gryphiswaldense MSR-1 Δ*feoB1*	*feoB1*-Deficient mutant, Nx^r, Gm^r	Present study
M. gryphiswaldense MSR-1 cΔ*feoB1*	Complementation of Δ*feoB1*, Nx^r, Gm^r, Cm^r	Present study
M. gryphiswaldense MSR-1 PWT	Wild-type carrying plasmid pfp, Nx^r, Cm^r	Present study
Escherichia coli DH5α	*endA1 hsdR17 [r−m+] supE44 thi-1 recA1 gyrA [NalR] relA1* Δ *[lacZYA-argF] U169 deoR [Ø80*Δ*{lacZ} M15]*	[10]
E. coli S17-1	*thi endA recA hsdR* with RP4-2-Tc::Mu-Km::Tn*7* integrated in chromosome, Sm^r	[25]
E. coli H1771	MC4100 *aroB feoB7 fhuF*::λp*lac* Mu, Km^r	[11,13]
Plasmids		
pGEM®-T Easy	Cloning vector, Amp^r	Promega
pTfeoB6	pGEM®-T Easy carrying MSR-1 *feoB1* 675 bp fragment, Amp^r	Present study
pUX19	Suicide vector for *M. gryphiswaldense* MSR-1, Km^r	[30]
pUX19f6e	675 bp fragment of MSR-1 was subcloned into pUX19, Km^r	Present study
pAfeo	A 10Kb plasmid contain intact *feoAB1* gene and pUX19, Km^r	Present study
pSUP202	Suicide vector for *M. gryphiswaldense* MSR-1, Cm^r, Tc^r, Amp^r	[20]
pSUPF	pSUP202 containing MSR-1 *feoB1* upstream region, downstream region, Cm^r, Amp^r	Present study
pSUPFG	pSUPF containing Gm cassette, Cm^r, Gm^r, Amp^r	Present study
pPR9TT	Broad-host-range plasmid, promoterless *lacZ*, Amp^r Cm^r	[21]
pPfeoK	pPR9TT with MSR-1 *feoB1* and kan promoter, Amp^r Cm^r	Present study
pFp	pPR9TT carrying MSR-1 *feoAB1* promoter, Amp^r, Cm^r	Present study
pWKS30	Low-copy-number cloning vector, Amp^r	[29]
p30M	pWKS30 carrying MSR-1 *feoAB1* and *feoAB1* promoter	Present study