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Identification of Iron Transporters Expressed in the Magnetotactic Bacterium Magnetospirillum magnetotacticum

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Abstract   The magnetotactic bacterium Magnetospirillum magnetotacticum MS-1 mineralizes the magnetite (Fe_3O_4) crystal and organizes a highly ordered intracellular structure, called the magnetosome. However, the iron transport system, which supports the biogenesis of magnetite, is not fully understood. In this study, we first identified the expressions of both the ferric and the ferrous iron transporter proteins in M. magnetotacticum. The cellular protein compositions of ferric and ferrous iron-rich cultures were examined using two-dimensional electrophoresis. According to the gel patterns, two outer-membrane ferric-siderophore receptor homologues were identified as proteins strongly induced in the ferrous iron-rich condition. Also, we identified for the first time that the ferrous iron transport protein, FeoB, is expressed in the M. magnetotacticum cytoplasmic membrane using immunoblotting.

Introduction

Iron is an essential nutrient for most living microorganisms. Despite the fact that it is one of the most abundant elements in the Earth's crust, iron is biologically unavailable in an oxidizing atmosphere and at neutral pH due to the poor solubility of ferric hydroxides [8]. On the other

hand, intracellular ferrous iron can be extremely toxic, because reactive oxygen species are formed via the Fenton reaction from Fe^{2+} and hydrogen peroxide [22]. To avoid hazards of both iron starvation and toxicity, bacteria have various iron transport mechanisms and regulate the iron uptake systems [2, 8]. Often multiple iron transport systems are present in a given bacterial species for the uptake of various forms of iron. For scavenging insoluble ferric iron, many bacteria excrete siderophores, ferric chelators, and take up ferric-siderophore complexes using TonB-dependent outer-membrane receptors, whereas soluble ferrous iron can be directly transported, in many cases, via the Feo (ferrous iron transport) system [2, 7, 8].

Magnetotactic bacteria are widespread aquatic eubacteria that synthesize intracellular magnetic crystals, magnetite or greigite, in order to navigate along the geomagnetic field. These magnetic crystals are enveloped with lipid membrane vesicles, termed magnetosomes, which are aligned in a chain-like structure [3]. The biosynthesis of the intracellular magnetic crystals involves significant amounts of iron acquisition from the environment. Indeed, the iron content in the magnetotactic bacterium, Magnetospirillum magnetotacticum MS-1, is up to 2% of the dry weight of the cell, which is 10 times higher than the nonmagnetotactic heterotrophic bacteria [4]. However, knowledge about the iron transport mechanism of magnetotactic bacteria is still limited. It is reported that two of the three model Magnetospirillum spp. bacteria (M. magnetotacticum MS-1 and M. magneticum AMB-1) produce siderophores, indicating that they can take up ferric iron [5, 6, 15]. Although siderophore production was not detected in the other model magnetotactic bacterium, M. gryphiswaldense MSR-1, ferric iron was taken up by energy-dependent process [18]. On the other hand, ferrous iron uptake was also reported in M. gryphiswaldense MSR-1 [18]. Very recently, Rong et al. demonstrated that the Feo system plays a significant role to take up iron for magnetosome formation in M. gryphiswaldense MSR-1 using the feoB1 gene deletion mutant [16]. Yang et al. reported that supplementation of ferrous sulfate enhanced the magnetosome production of M. magneticum AMB-1 [23]. In addition, Suzuki et al. reported that the M. magneticum AMB-1 mutant NMA61, harboring a defective gene for cytoplasmic ATPase involved in ferrous iron uptake, contained no magnetosomes in the cell [19]. They also demonstrated that ferrous iron-uptake genes were induced under iron-rich culture condition [20]. From these results, they speculated that the ferrous iron uptake system was coupled to magnetosome synthesis within M. magneticum AMB-1.

Although many studies on iron uptake of magnetotactic bacteria have been reported, there have been no reports on the identification of iron transporter proteins that are major components of

iron uptake mechanisms. In the present study, we revealed that two TonB-dependent ferric-siderophore receptor homologues are highly expressed in the M. magnetotacticum MS-1 cell. Also, we identified the expression of the ferrous iron transport protein, FeoB, in the M. magnetotacticum MS-1 cytoplasmic membrane.

Materials and Methods

Microorganisms and Culture Conditions

Magnetospirillum magnetotacticum MS-1 (ATCC 31632) was cultivated in a chemically defined liquid medium (MS-1 medium) containing 0.1 mM sodium ascorbate as a reductant under an O_2 (1%)/N_2 (99%) atmosphere at 25°C in the dark [4]. For the ferric iron-rich culture condition, sodium ascorbate was omitted from the medium. The spontaneous nonmagnetic mutant occurred during subcultivation of M. magnetotacticum MS-1. Nonmagnetic cells were separated from magnetic cells using a bar magnet, and subjected to sequential subcultivations in MS-1 medium containing sodium ascorbate. Escherichia coli was grown at 37°C in Luria-Bertani (LB) medium [17]. When necessary, ampicillin (50 μg/ml) was supplemented.

Two-Dimensional Electrophoresis (2-DE)

Cells were collected in the late logarithmic growth phase and used to prepare protein extracts. Sample preparations and 2-DE were performed by the method of O'Farrell [14]. Isoelectric focusing on an Ampholine, pH 5-8 or pH 5–7 (GE Healthcare Biosciences), pH gradient was used for the first dimension. Protein spots were sequenced using an ABI model 476A N-terminal amino acid sequencer.

Physical and Chemical Measurements

Protein contents were determined by the bicinchoninic acid method (BCA Protein Assay Kit; Pierce). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli [10]. The iron concentration of the cell-free spent culture was measured photometrically as described previously [5], with slight modifications. For measurement of ferrous iron concentration of the cell-free spent culture, the reductant,

hydroxylamine hydrochloride, was omitted. The BigDye terminator v 3.1 cycle sequencing kit (Applied Biosystems) and capillary sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) were used for DNA sequencing.

Protein Expression and Purification

For expression of the His-tagged N-terminal region of M. magnetotacticum FeoB1 (N-FeoB1; amino acid residues 1–244), the 5′ fragment of the feoB1 gene was subcloned into pET-15b (Novagen). Primers containing the restriction sites for NdeI (underlined) and BamHI (double-underlined) were used for subcloning: feoB1_f,

5′-GGAATTC<u>CATATG</u>AAATCCCCCGTCACCG-3′; and feoB1_r,

5′-CG<u>GGATCC</u>CTAATGCTCGTGGGCCAGGGCG-3′. N-FeoB1 protein was overexpressed in E. coli BL21(DE3). At the midexponential growth phase, protein expression was induced by the addition of 1 mM isopropyl-β-d-thiogalactopyranoside. After 1 h of induction, cells were harvested and N-FeoB1 was purified from the inclusion bodies. Upon purification, E. coli cells were suspended in 10 mM Tris-HCl buffer (pH 8.0) and disrupted using sonication. Inclusion bodies were collected by centrifugation at 8000 g for 15 min and suspended in 15 ml of 20 mM Tris-HCl (pH 8.0). The His-tagged N-FeoB1 protein was purified by Ni^{2+}-affinity chromatography (Ni-NTA agarose; QIAGEN) under the denaturing condition according to the QIAGEN technical manual. After the purified protein was treated with the Thrombin Kit (Novagen) to remove His-tag, the sample was resolved on SDS-PAGE. Protein bands of N-FeoB1 were excised from the gel, and then N-FeoB1 was extracted with a minimal volume of 10 mM Tris-HCl buffer (pH 8.0) containing 0.1% SDS. The resulted sample was used as the antigen.

Generation of Antibody

Polyclonal antibodies against N-FeoB1 were generated by surgically implanting the antigen in a rabbit. The antigen (100 mg) was dissolved in phosphate-buffered saline with 0.1% SDS, then emulsified and injected into the rabbit, with booster immunizations delivered at approximately 2 weeks.

Immunoblotting

Immunoblot analysis was performed as previously described [21]. Immunoreactivity species of anti-N-FeoB antibodies were detected at a dilution of 1:10,000.

Preparation of Cellular Components

Magnetospirillum magnetotacticum cells (ca. 25 g, wet weight) were suspended in 100 ml of 10 mM Tris-HCl (pH 8.0) and disrupted with a Branson model 450 ultrasonic oscillator (20 kHz, 80 W) for 10 min. Unbroken cells and magnetosomes were removed by centrifugation at 8000 g for 15 min. The supernatant was centrifuged at 100,000 g for 1 h. The pellet and supernatant were used as the membrane fraction and soluble fraction, respectively. Cytoplasmic membrane and outer-membrane fractions were prepared by the method of Mizushima and Yamada [12]. Magnetosomes were purified as described elsewhere [21].

Analysis of DNA and Protein Sequence Data

Peptide sequences determined were compared to the unfinished genome sequence of M. magnetotacticum MS-1 (NZ_AAAP00000000) using the BLAST algorithm [1]. Multiple sequences were aligned using the program EMBOSS Pairwise Alignment Algorithms [9]. Protein localization and signal peptide were predicted using the PSORT program [13].

Results and Discussion

Identification of Ferric-Siderophore Receptors

We compared the protein compositions of the cell-free extracts from M. magnetotacticum cells grown in ferrous and ferric iron-rich medium using 2-DE (Fig. 1a and b). For the ferrous iron-rich medium, sodium ascorbate was added to the MS-1 medium as a redundant, while sodium ascorbate was omitted from the ferric iron-rich medium. When the cells were harvested

during the late logarithmic growth phase, the ferrous iron content of the cell-free supernatant of the medium with sodium ascorbate was 95% of the total iron, while that of the medium without sodium ascorbate was 7% of the total iron. According to the 2-DE analyses, two protein spots, with apparent molecular masses of 76 and 70 kDa, were highly expressed under the ferrous iron-rich culture condition (Fig. 1c). The experiment was repeated three times using individual cultures to confirm the reproducibility of the 2-DE. The 76- and 70-kDa proteins were identified as the gene products of Magn03010691 (ZP_00055999) and Magn03011022 (ZP_00056323), respectively, based on homology searches using the determined N-terminal amino acid sequences: 76-kDa protein, *ADXATDMPEVVIREK* (X was not determined); and 70-kDa protein, *EDIPEVXITAPRFXV*. The predicted molecular masses, cleavage sites of the N-terminal signal peptides, and N-terminal amino acid sequences from the gene sequences Magn03010691 and Magn03011022 corresponded to those of the 76- and 70-kDa proteins, respectively. The amino acid sequence of the 76-kDa protein showed a similarity to the TonB-dependent receptor of Beijerinckia indica (46% identity), while that of the 70-kDa protein showed a similarity to the TonB-dependent receptor of Rhodopseudomonas palustris BisB18 (42% identity). Interestingly, both of the gene products were TonB-dependent outer-membrane ferric-siderophore receptor homologues. In the genomes of M. magnetotacticum MS-1 and M. magneticum AMB-1, three genes encoding TonB-dependent outer-membrane receptor homologues were conserved. In the present study, we confirmed that at least two of the three ferric-siderophore outer-membrane receptor homologues are highly expressed in cells cultured in ferrous iron-rich medium. These ferric iron transporters may be expressed to promote iron uptake in the ferrous iron-rich medium.

Furthermore, the expressions of these ferric-siderophore receptor protein homologues were lacking in spontaneously obtained nonmagnetic cells cultivated in the ferrous iron-rich medium (Fig. 2). The spontaneous nonmagnetic cells were isolated by magnetic selection and serial subcultivations (see Materials and Methods). Nonmagnetic cells showed no magnetic response under the microscope. They were brown in color, while the wild-type M. magnetotacticum MS-1 magnetic cells were black (Fig. 2a). This indicated that the nonmagnetic cells synthesized no magnetite crystals. These results suggest that the 70- and 78-kDa outer-membrane transporters are necessary for the iron acquisition system of M. magnetotacticum. Noteworthily, siderophore production of M. magnetotacticum MS-1 is induced under a high iron concentration (20 μM) rather than under a deficient iron concentration (5 μM) [15]. This is the opposite of the

pattern normally observed in nonmagnetic bacteria [2, 8]. This unusual result implies that the ferrisiderophore uptake system of the magnetotactic bacterium might be optimized to accumulate robust iron for biomineralization of magnetite.

Identification of Ferrous Iron Transporter FeoB

The ferrous iron transport system, Feo, is encoded in the feoAB gene cluster (feoABC in γ-proteobacteria). feoA encoded a small protein of unknown function, and feoB encoded a transmembrane protein essential for ferrous iron uptake, which is likely to act as the permease of the ferrous iron [7]. Two sets of feoAB gene homologues, feoAB1 (ZP_00208166 and ZP_00054713) and feoAB2 (ZP_00052875 and ZP_00052876 [this is the truncated feoB gene because of the draft genome sequence]), were recorded in the unfinished genome sequence of M. magnetotacticum MS-1. M. magnetotacticum FeoB1 shows 28% identity and 44% positivity to E. coli FeoB and has conserved motifs in FeoB proteins, N-terminal GTP binding motifs [11], and C-terminal two Gate motifs [7]. To examine the expression of the Feo system in M. magnetotacticum, we purified the recombinant N-terminal region of FeoB1 (N-FeoB1; amino acid residues 1-244) and generated polyclonal antibodies against the N-FeoB1. According to immunoblot analysis of the membrane fraction from M. magnetotacticum cells grown in ferrous iron-rich medium, we recognized expression of the FeoB protein as a single positive band with an apparent molecular mass of 73 kDa, which corresponded to the deduced molecular mass of the feoB1 gene product (Fig. 3a). Also, immunoblotting of the cellular components showed that FeoB is localized in the cytoplasmic membrane (Fig. 3b). This is the first report of FeoB protein expression in magnetotactic bacteria.

We are the first to identify the expressions of transporters for both ferric and ferrous iron in the magnetotactic bacterium M. magnetotacticum. The iron uptake system involving biomineralization is of substantial interest regarding iron cycling in nature and technical applications. To gain new insight into the iron-transport-coupled biomineralization mechanism, characterizations and mutational studies targeted at these transporter proteins are warranted.

References

1. Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410

2. Andrews SC, Robinson AK, Rodríguez-Quiñones F (2003) Bacterial iron homeostasis. FEMS Microbiol Rev 27:215–237

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

4. Blakemore RP, Maratea D, Wolfe RS (1979) Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium. J Bacteriol 140:720–729

5. Calugay RJ, Miyashita H, Okamura Y et al (2003) Siderophore production by the magnetic bacterium Magnetospirillum magneticum AMB-1. FEMS Microbiol Lett 218:371–375

6. Calugay RJ, Takeyama H, Mukoyama D et al (2006) Catechol siderophore excretion by magnetotactic bacterium Magnetospirillum magneticum AMB-1. J Biosci Bioeng 101:445–447

7. Cartron ML, Maddocks S, Gillingham P et al (2006) Feo-transport of ferrous iron into bacteria. Biometals 19:143–157

8. Crosa JH, Mey AR, Payne SM (2004) Iron transport in bacteria. ASM Press, Washington, DC

9. Labarga A, Valentin F, Anderson M et al (2007) Web services at the European Bioinformatics Institute. Nucleic Acids Research Web Services Issue 2007

10. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

11. Marlovits TC, Haase W, Herrmann C et al (2002) The membrane protein FeoB contains an intramolecular G protein essential for Fe(II) uptake in bacteria. Proc Natl Acad Sci USA 99:16243–16248

12. Mizushima S, Yamada H (1975) Isolation and characterization of two outer membrane preparations from Escherichia coli. Biochim Biophys Acta 375:44–53

13. Nakai K, Kanehisa M (1991) Expert system for predicting protein localization sites in gram-negative bacteria. Proteins 11:95–110

14. O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

15. Paoletti LC, Blakemore RP (1989) Hydroxamate production by Aquaspirillum magnetotacticum. J Bacteriol 167:73–76

16. Rong C, Huang Y, Zhang W et al (2008) Ferrous iron transport protein B gene (feoB1) plays an accessory role in magnetosome formation in Magnetospirillum gryphiswaldense strain MSR–1. Res Microbiol 159(7/8):530–536

17. Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

18. Schüler D, Baeuerlein E (1996) Iron-limited growth and kinetics of iron uptake in Magnetospirillum gryphiswaldense. Arch Microbiol 166:301–307

19. Suzuki T, Okamura Y, Arakaki A et al (2007) Cytoplasmic ATPase involved in ferrous ion uptake from magnetotactic bacterium Magnetospirillum magneticum AMB-1. FEBS Lett 581:3443–3448

20. Suzuki T, Okamura Y, Calugay RJ et al (2006) Global gene expression analysis of iron-inducible genes in Magnetospirillum magneticum AMB-1. J Bacteriol 188:2275–2279

21. Taoka A, Asada R, Sasaki H et al (2006) Spatial localizations of Mam22 and Mam12 in the magnetosomes of Magnetospirillum magnetotacticum. J Bacteriol 188:3805–3812

22. Touati D (2000) Iron and oxidative stress in bacteria. Arch Biochem Biophys 373:1–6

23. Yang C-D, Takeyama H, Tanaka T et al (2001) Effects of growth medium composition, iron sources and atmospheric oxygen concentrations on production of luciferase-bacterial magnetic particle complex by a recombinant Magnetospirillum magneticum AMB-1. Enzyme Microb Technol 29:13–19

Fig. 1 2-DE gel profiles of the proteins extracted from ferrous (a) and ferric (b) iron-rich cultures. They were subjected to isoelectric focusing (first dimension) using the pH range 5–8 and then to SDS-PAGE (second dimension). Proteins were stained with Coomassie Brilliant Blue R-250. (c) Enlargements of the boxes from in A (left) and B (right). The arrow and arrowhead show the protein spots of 76- and 70-kDa ferric-siderophore outer-membrane receptor homologues, respectively. They were strongly expressed in the ferrous iron-rich culture

Fig. 2 (a) Pellets of M. magnetotacticum nonmagnetic mutant (left) and wild-type (right) cells. 2-DE gel profiles of proteins extracted from nonmagnetic cells (b) and wild-type magnetic cells (c). Values at the right of the gel in C indicate molecular masses of the maker proteins. (d) Enlargements of the boxes in B (left) and C (right). The arrow and arrowhead show the protein spots of 76- and 70-kDa ferric-siderophore outer-membrane receptor homologues, respectively. (e) The same areas as in D, in 2-DE gels using pH range 5–7 isoelectric focusing

Fig. 3 (a) Immunoblotting analysis of the M. magnetotacticum membrane fraction with anti-N-FeoB1 antibody. Anti-N-FeoB1 recognized a single positive band with the apparent molecular mass of 73 kDa. This corresponded to the deduced molecular mass of the feoB1 gene product. Proteins (20 μg) extracted from the membrane fraction of M. magnetotacticum cells were loaded in the lane. Molecular masses of the standards are indicated on the left. (b) Localization of FeoB in M. magnetotacticum. Immunoblotting of proteins (10 μg/lane) extracted from the cytoplasmic membrane, outer membrane, magnetosomes, and soluble fractions with anti-N-FeoB1. The FeoB signal was detected in the cytoplasmic membrane fraction

(A)

(B)

(C)

(A)

(B)

(C)

97.4 kDa
66.3 kDa

42.4 kDa

30.0 kDa

20.1 kDa

(D)

(E)
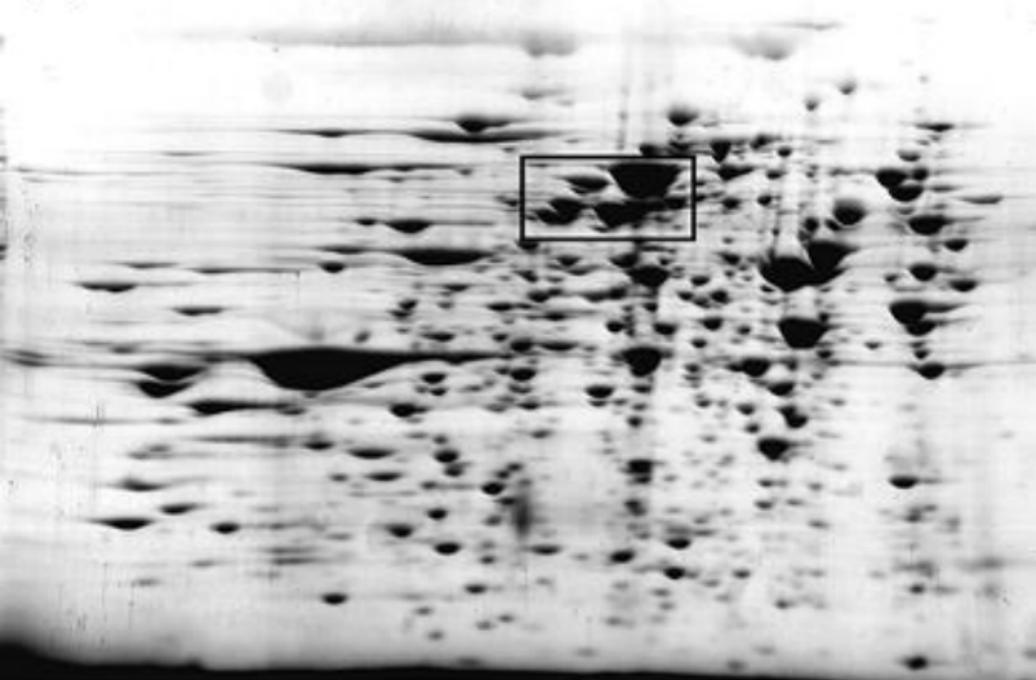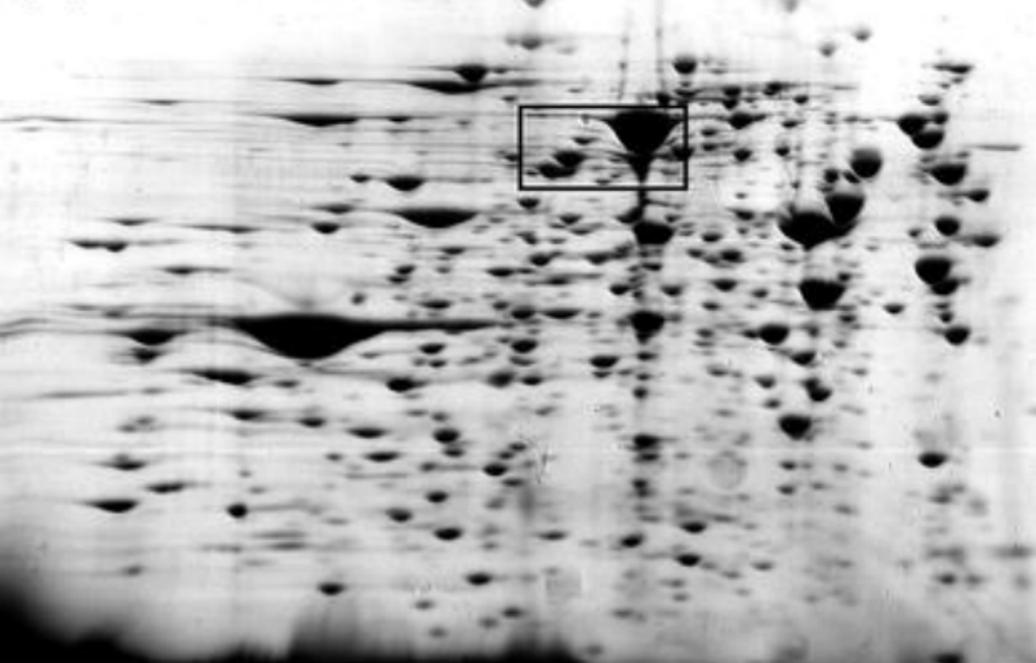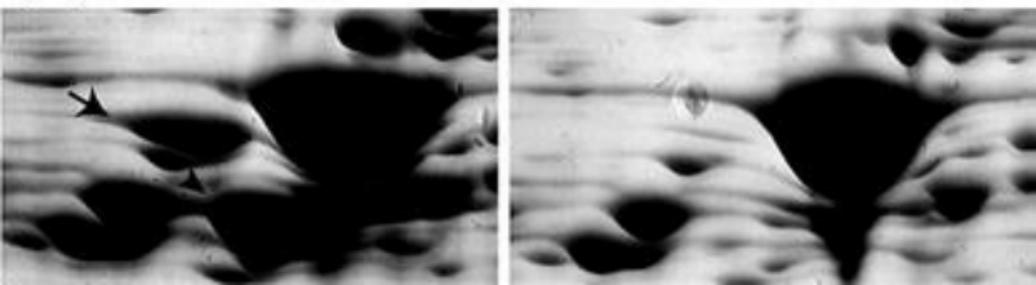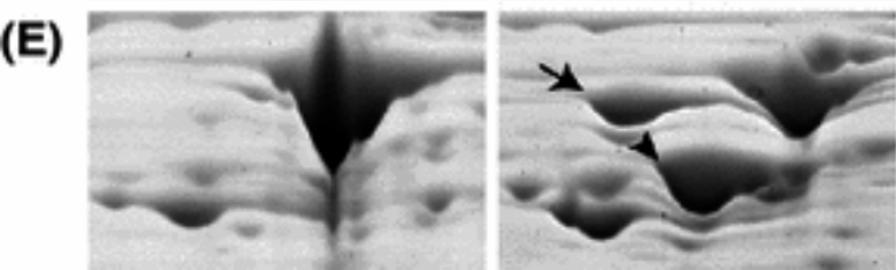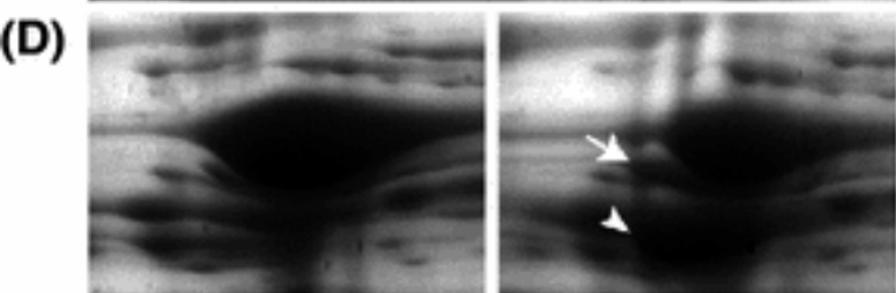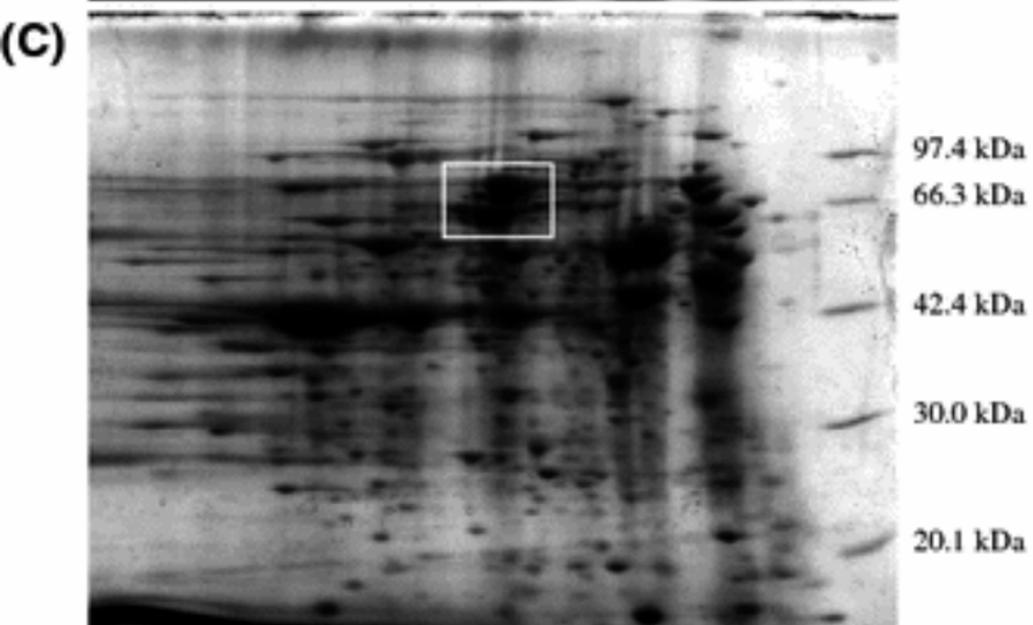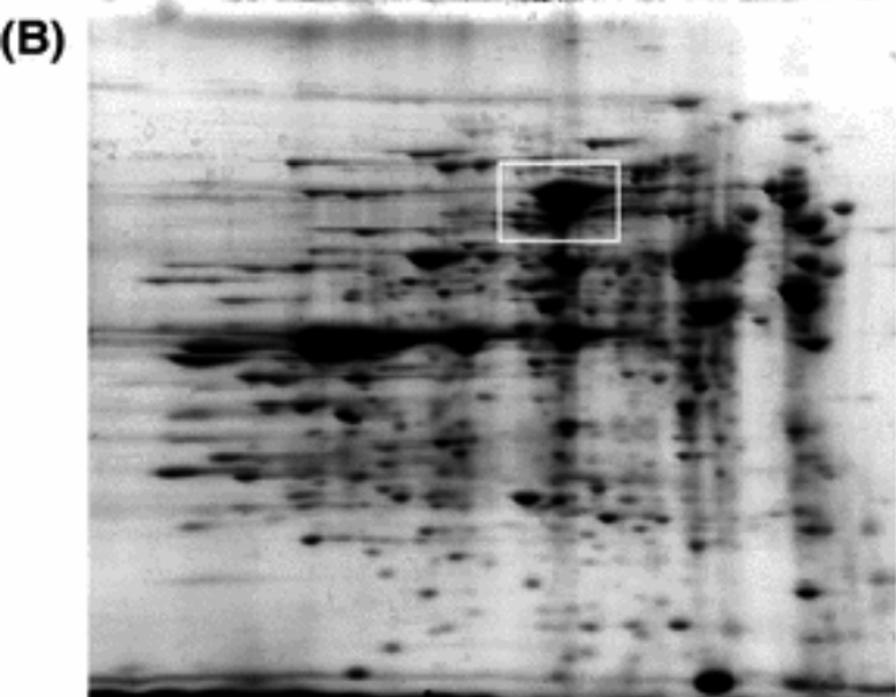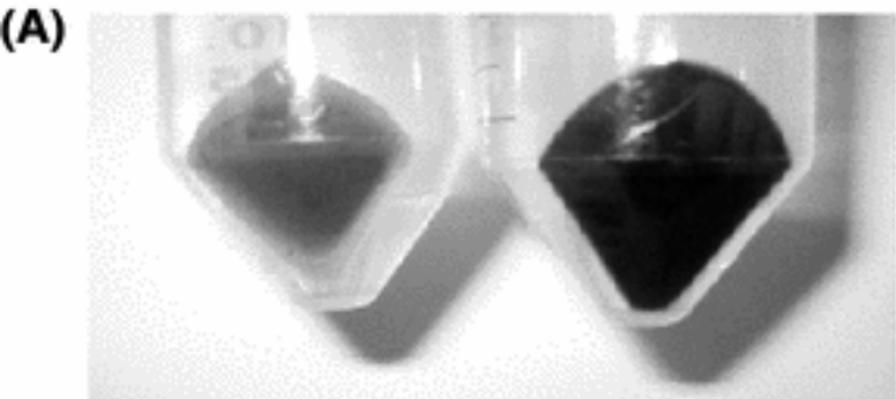
(A)

100 kDa —	
75 kDa —	
50 kDa —	
37 kDa —	
25 kDa —	
20 kDa —	
15 kDa —	
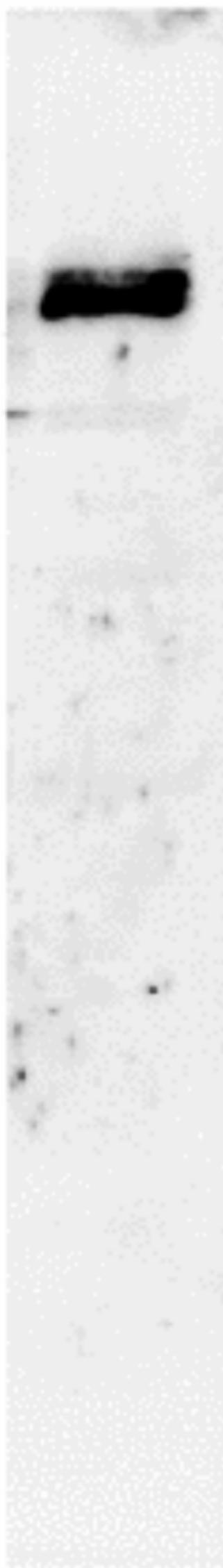
(B)

Cytoplasmic membrane Outer membrane Magnetosomes Soluble fraction