

Magnetosome Formation and Expression of *mamA*, *mms13*, *mms6* and *magA* in *Magnetospirillum magneticum* AMB-1 Exposed to Pulsed Magnetic Field

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Abstract To investigate the effects of pulsed magnetic field on magnetosome formation in *Magnetospirillum magneticum* AMB-1, cultures inoculated with either magnetic or non-magnetic pre-cultures were incubated under 1 mT pulsed magnetic field. Magnetism of cells was measured by using spectrophotometer coupled with applied magnetic fields and the values were described as C_{mag} . Magnetosome in cells was counted by transmission electron microscopy observation. The results showed that pulsed magnetic field did not affect cellular growth, but enhanced magnetosome formation. The applied pulsed magnetic field might exceed the chain of magnetosomes and change the homogeneity of the magnetosome particles. The results implied that magnetite precipitation induced by the adjacent magnetosome was affected by pulsed magnetic field. Moreover, the applied pulsed magnetic field up-regulated the *magA* and *mamA* expression in cells, which might account for the increasing number and the exceeding chain of magnetosomes in cells.

Introduction

All magnetotactic microbes synthesize unique intracellular structures called magnetosomes [14]. The unique properties of magnetosomes have made them a potential biomarker for geobiologists and an ideal system for studying biomineralization and some medical applications such as drug delivery, magnetic resonance imaging, and array-based assaying [8].

Previous study has shown that magnetosomes membrane is derived from the cytoplasmic membrane and contains a unique set of proteins that are thought to direct the biomineralization of magnetite crystals [10]. So far, 48 proteins have been identified as magnetosome specific proteins in *Magnetospirillum magneticum* AMB-1, and at least 13 proteins are potentially involved in formation of magnetosome [4, 7]. Most of the genes that are essential for magnetosome formation could be assigned to “magnetosome island” [3]. Among the genes, *magA*, *mms6*, *mamA* and *mms13* are involved in iron uptake [9], synthesis of magnetite crystals in a uniform size [2], magnetosome assembly [6], and formation of magnetosome [15], respectively.

Magnetosomes in magnetotactic bacteria respond to magnetic fields by arranging the magnetosomes in chains. The magnetotactic bacteria use magnetosome for orientation provides both a simple biophysical mechanism for magnetoreception and an unambiguous example of an Earth-strength magnetic effect on biology [5]. Research over the past 20 years has progressed steadily from the initial discoveries to a rudimentary understanding of the function of this sensory system [1, 5]. It would be interesting to evaluate the effects of external magnetic field on magnetosome formation in *M. magneticum* AMB-1. In this article, 1 mT pulsed magnetic field was applied to cultures

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of AMB-1. Magnetosome in cells were inspected and counted, the expression of *mamA*, *mms13*, *mms6*, and *magA* were analyzed by quantitative RT-PCR.

Materials and Methods

Strains and Growth Conditions

Magnetospirillum magneticum, AMB-1 (ATCC 700264) was grown microaerobically at 28°C in Enriched Magnetic Spirillum Growth Medium (EMSGM) [16]. Magnetic and non-magnetic pre-cultures were prepared by incubating cells microaerobically in EMSGM and aerobically in EMSGM without ferric quinate at 28°C, respectively.

Pulsed Magnetic Field Exposure Treatment

Pulse magnetic field exposure was performed on a special instrument that designed and constructed at Institute of Electrical Engineering, Chinese Academy of Science. This apparatus was composed of a pair of Helmholtz coils which provided a highly homogenous pulsed magnetic field. For measurement of flux density, an F.W.BELL 7010 Gauss/Tesla meter was applied. The instrument was kept at 28°C in a constant temperature chamber. Single continuous exposures were carried out at a pulsed magnetic field intensity of 1 mT.

Measurement of Coefficient of Magnetism (C_{mag} Value)

Magnetic and non-magnetic pre-cultures were inoculated into EMSGM media (after inoculation $OD_{600} = 0.05$) and incubated under pulse magnetic field or geomagnetic field at 28°C microaerobically. Samples were taken every 2 h for up to 48 h. C_{mag} values of cells were assayed according to the methods described by Schuler [12]. $C_{\text{mag}} = 0$ was assumed for non-magnetic cells; $C_{\text{mag}} = 1$ corresponded to approximately 10 particles per cell.

Transmission Electron Microscopy (TEM) Images

The morphologies of cell and magnetosome were observed using a Philips CM120 TEM operated at 100 kV. The TEM samples taken at 16 h were adsorbed on carbon-coated copper grids. Grids were washed gently and negatively stained with 1% (w/v) phosphotungstic acid.

Quantitative RT-PCR

Total RNA was extracted from the samples taken at 16 h after exposure of pulsed magnetic field with the RNeasy Kit (Qiagen, Germany) and treated with RNase free

DNaseI during the isolation step according to the manufacturer's instructions. The isolated RNA was quantified by spectrophotometry at 260 nm. Equal amount of total RNA (1 µg) was used to synthesize the first cDNA strand of the genes, a Reverse Transcription System Kit (Superscript II, Invitrogen) was used with the protocol provided by the manufacturer. The expression of *mamA*, *mms13*, *mms6*, and *magA* was analyzed by qRT-PCR. The specific primers designed to target the genes (*mamA*, *mms13*, *mms6*, and *magA*) are presented in Table 1. PCR was performed on the LightCycler instrument (Roche Applied Science) with the following cycling program: 95°C for 10 min followed by 40 cycles of 10 s at 95°C, 5 s at 60°C, and 15 s at 72°C. The products were analyzed by melting-curve analysis by applying 95°C for 1 min, 60°C for 2 min, and 50°C for 2 s, followed by an increase in temperature from 75 to 95°C and continuous fluorescence recording. Gene expression levels obtained by qRT-PCR were normalized using the *16SrRNA* gene. Each DNA sample was analyzed at least three times in separate reactions. Average of three replicates was obtained for each gene and binned into four groups according to the values of expression ratio r between pulsed magnetic group and geomagnetic group: 'down-regulated' ($r < 0.7$), 'unchanged' ($0.7 < r < 1.4$), 'weakly up-regulated' ($1.4 < r < 2$) and 'strongly up-regulated' ($r > 2$) [13].

Statistical Analysis

All statistical analyses were performed by using Sigma Stat 3.0 (SPSS Inc., Chicago, IL, USA). The determined P values of the statistical significance were analyzed using two-way ANOVA for time course of growth and C_{mag} , and one way ANOVA for the other experiment data. Results were expressed as mean \pm SD. Significance was set to $P < 0.05$.

Table 1 List of primers and their region of transgene coverage

Genes	Sequence of the primers (5' → 3')	PCR products
16srRNA (D17514.1)	FP1 GCTCGTGTCTGAGATGTTG	237 bp
	RP1 TCCGAAGTCTGAGATGGCTTAT	
<i>magA</i> (AB001696.1)	FP2 ACGCGGGAGCCGTTCAAA	311 bp
	RP2 CGGGCCTCGTCGCCATACA	
<i>mms13</i> (AB096081.1)	FP3 AGAACGTCCGCTCCTGAA	272 bp
	RP3 GTCCCGCAAGATGTCCTCG	
<i>mms6</i> (AB096081.1)	FP4 GGTCGTCGGTGGAAACCATCTG	164 bp
	RP4 CGACTTCCTCGTCGCTCTGC	
<i>mamA</i> (AY508230.1)	FP5 CGATGTGGATGTGGCTCTG	214 bp
	RP5 GTTGAAATTGATGGGATTGG	

Results

C_{mag} Values and Growth of *M. magneticum* AMB-1 Exposed to Pulsed Magnetic Field

To investigate effects of pulsed magnetic field on growth of *M. magneticum* AMB-1, magnetic or non-magnetic pre-cultures were inoculated into EMSGM media. After inoculation ($OD_{600} = 0.05$), it was divided into two groups with one for pulsed magnetic field exposure and the other for geomagnetic field exposure and cultivated at 28°C microaerobically. Cell growth was determined by measuring the optical density at 600 nm. C_{mag} was assayed as described above. Figure 1a and b showed that there was no effect on growth of *M. magneticum* AMB-1 when cells were incubated in pulsed magnetic field.

C_{mag} gradually declined during early growth after inoculation with magnetic cells. This was due to the dilution of magnetosome-bearing bacteria in the culture by cell division while the formation of new magnetite crystals was followed behind (Fig. 2a). The differences of C_{mag} values detected in the cultures from the two groups were shown to

be significant after an incubation time of about 12 h ($P < 0.05$). When cultures were inoculated with non-magnetic cells, C_{mag} kept zero during 0–10 h, then it increased rapidly and reach a peak at 20 h (Fig. 2b). The differences of C_{mag} value detected in the two groups were shown to be significant after an incubation time of about 20 h ($P < 0.05$). C_{mag} values detected in pulsed magnetic field group were higher than those in geomagnetic field group. The results demonstrated that pulsed magnetic field raised C_{mag} values of the AMB-1 cells, which means that the percentage of magnetosome-containing bacteria was increased.

Magnetosome Chain in *M. magneticum* AMB-1 Exposed to Pulsed Magnetic Field

To know influences of pulsed magnetic field on magnetosome morphology in *M. magneticum* AMB-1, samples were taken at 16 h after exposure of pulsed magnetic field or geomagnetic field and investigated by TEM observation. Typical images were presented in Fig. 3a and c and showed that magnetic crystals closely aligned in a chain when

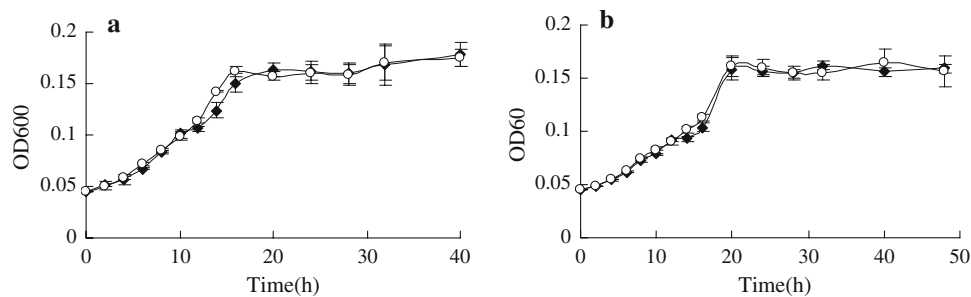


Fig. 1 Growth curves of *M. magneticum* AMB-1 exposed to pulsed magnetic field or geomagnetic field. **a**, Cultures inoculated with magnetic cells; **b** Cultures inoculated with non-magnetic cells. After inoculation ($OD_{600} = 0.05$), cultures were exposed to pulsed

magnetic field (filled diamond) or geomagnetic field (open circle) and incubated at 28°C microaerobically. Cell growth was determined by measuring the optical density at 600 nm

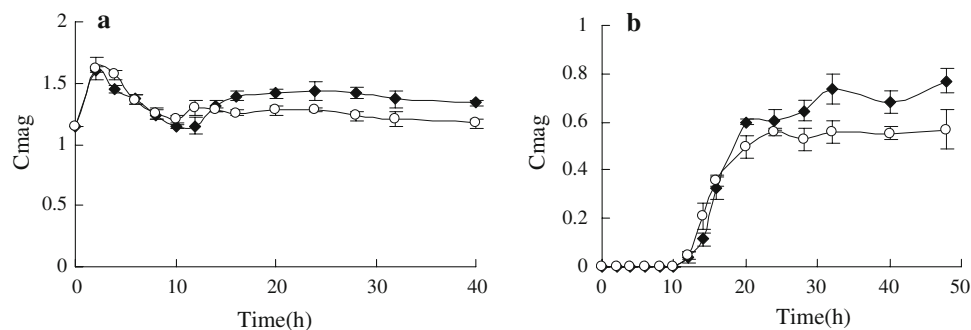
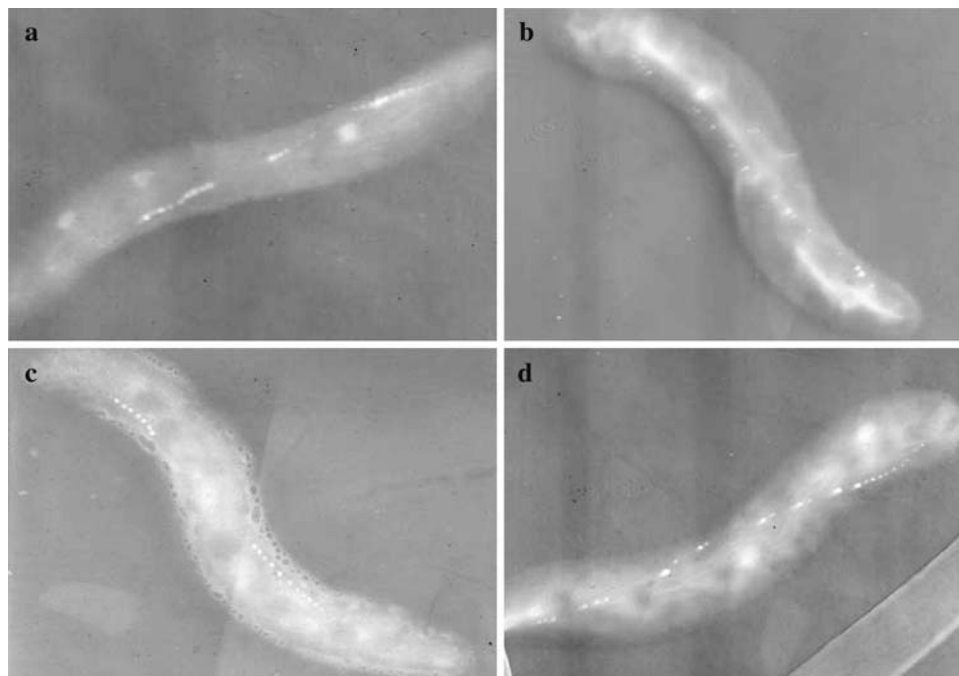


Fig. 2 C_{mag} time curves of *M. magneticum* AMB-1 under pulsed magnetic field or geomagnetic field. **a** Cultures inoculated with magnetic cells; **b** Cultures inoculated with non-magnetic cells. After inoculation ($OD_{600} = 0.05$), cells were exposed to pulsed magnetic

field (filled diamond) or geomagnetic field (open circle) and cultivated at 28°C microaerobically. C_{mag} was assayed as described above

Fig. 3 Transmission electron microscope images of magnetosome in *M. magneticum* AMB-1. Cultures inoculated with magnetic cells and exposed to geomagnetic field (a) or pulsed magnetic field (b). Cultures inoculated with non-magnetic cells and exposed to geomagnetic field (c) or pulsed magnetic field (d). A total of 1 ml of sample taken at 16 h after exposure to pulsed magnetic field or geomagnetic field were viewed and recorded with a transmission electron microscope



cultures were exposed to geomagnetic field. Most of the magnetic crystals have uniform particles morphologies with a unidirectional orientation. There are 1–3 gaps between magnetosomes in the chain. Whereas, when cells were exposed to pulsed magnetic field, the magnetic crystals showed un-consistent morphologies and arranged loosely in a long chain with no gap (Fig. 3b and d).

A total of 200 magnetosome-containing cells were selected randomly from each group and the number of the crystals was counted. The average number of magnetic particles was increased by 25% when pulsed magnetic field was applied to the cultures (Table 2). The results indicated that pulsed magnetic field stimulated magnetosome formation in AMB-1.

Analysis of Expression of *mamA*, *mms13*, *mms6*, and *magA* in *M. magneticum* AMB-1 Exposed to Pulsed Magnetic Field

To investigate the influence of pulse magnetic field on expression of magnetosome formation-associated genes in

M. magneticum AMB-1, samples were taken at 16 h after exposure to pulsed magnetic field or geomagnetic field. The expression of *mamA*, *mms13*, *mms6*, and *magA* were analyzed by qRT-PCR. Figure 4 showed that pulsed magnetic field up-regulated *mamA* and *magA* expression ($P < 0.05$) in AMB-1 cells.

Discussion

In this article, the effect of pulsed magnetic field on growth and magnetosome formation in *M. magneticum* AMB-1 was investigated. It was showed that pulsed magnetic field had no effect on growth of AMB-1, but raised C_{mag} value of the cultures, which means that the percentage of magnetosome-containing bacteria was increased. The results of TEM microscopy indicated that the magnetic crystals with un-uniform sizes arranged loosely in a long chain when cells were exposed to pulsed magnetic field. It was indicated that magnetite precipitation induced by the adjacent magnetosome was affected by pulsed magnetic field.

Table 2 The average numbers of magnetic particles in magnetosome-containing *M. magneticum* AMB-1 cultivated under pulsed magnetic field or geomagnetic field

Inoculated cells	Numbers of magnetic particles (particles/cell)		Increase of magnetic particles (%)
	Under geomagnetic field	Under pulsed magnetic field	
Magnetic cells	13.18 ± 3.86	16.55 ± 5.67	25.57
Non-magnetic cells	17.35 ± 4.89	21.73 ± 7.85	25.25

1 ml of samples taken at 16 h after exposure to pulsed magnetic field or geomagnetic field was counted with a transmission electron microscope. Experiments were performed three times

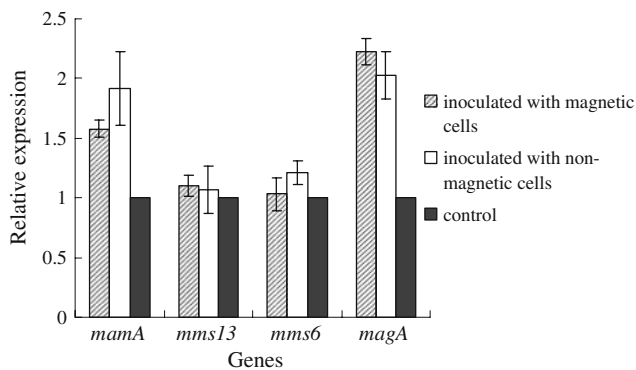


Fig. 4 Relative expression of *mamA*, *mms13*, *mms6* and *magA* in *M. magneticum* AMB-1 cultures inoculated with magnetic cells or non-magnetic cells. The ratio of genes expression under pulsed magnetic field and geomagnetic field was defined as relative expression. Relative expression of the control was 1; experiments were performed three times

Moreover, the average number of magnetic particles in magnetosome-containing cells increased by 25% when pulsed magnetic field was applied to the cultures. All these results indicated that the applied pulsed magnetic field enhanced magnetosome formation in *M. magneticum* AMB-1. Pulsed magnetic field might exceed the chain of magnetosomes and change the homogeneity of the magnetosome particles.

Beside the effects on crystallization, pulsed magnetic field influenced gene expressions. The results of qRT-PCR indicated that pulsed magnetic field up-regulated *mamA* and *magA* expressions in cells, which might account for the increasing number and the exceeding chain of magnetosomes in cells. The applied magnetic field affected the biosynthesis crystals differently depending on the initial stage of the magnetosomes. It is reported that MagA is involved in iron uptake and magnetite biomineralization [9]; Komeili [6] proposed that MamA may play a role in magnetosome assembly and maintenance processes or regulation of the lengths of magnetosomes. Taoka [15] demonstrated that MamA may function as a receptor for the protein–protein interaction in magnetosomes. It is known that the existing magnetic crystal might influence the formation of adjacent crystals [11]. Our results showed that pulsed magnetic field might counteract these effects, which might be the reason for the variation observed for the crystal size and the loosely spaced magnetosome chains in AMB-1 cells.

Together, the results showed that magnetic bacteria had the ability to adapt the changes of the external magnetic field and regulate the formation of the magnetosome in cells. On the other hand, the expression of *mamA* showed to be more active when cultures were inoculated with non-magnetic cells, which implied that *mamA* expression was more activated in the De Novo Synthesis pathway of

magnetosome. These results shed light on understanding the effects of the imposed magnetic field on biomineralization and suggest a new approach that may enhance formation of magnetosome.

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