Development of a Cell Surface Display System in a Magnetotactic Bacterium, “Magnetospirillum magneticum” AMB-1†‡

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Bacterial cell surface display is a widely used technology for bioadsorption and for the development of a variety of screening systems. Magnetotactic bacteria are unique species of bacteria due to the presence of magnetic nanoparticles within them. These intracellular, nanosized (50 to 100 nm) magnetic nanoparticles enable the cells to migrate and be manipulated by magnetic force. In this work, using this unique characteristic and based on whole-genomic and comprehensive proteomic analyses of these bacteria, a cell surface display system has been developed by expressing hexahistidine residues within the outer coiled loop of the membrane-specific protein (Msp1) of the “Magnetospirillum magneticum” (proposed name) AMB-1 bacterium. The optimal display site of the hexahistidine residues was successfully identified via secondary structure prediction, immunofluorescence microscopy, and heavy metal binding assay. The established AMB-1 transformant showed high immunofluorescence response, high Cd2+ binding, and high recovery efficiency in comparison to those of the negative control when manipulated by magnetic force.

The first surface display system was developed in a bacteriophage in which peptides and small proteins were fused with the pIII protein of the filamentous phage and displayed on the surface of the bacteriophage (30). The application of this technology provided extra functional characteristics to the host without causing metabolic abnormalities. By enabling the manipulation of diverse molecules, the technology has made possible a wide range of applications in the biotechnological and industrial fields, such as the recovery of harmful chemicals and heavy metals, live-vaccine development, and screening of peptide libraries (2, 10, 15, 32). In recent years, many functional proteins and peptides have been displayed on the surface of various hosts, including viruses, bacteriophages, bacteria, and yeasts.

Focusing on bacteria, several types of display systems have been introduced. A recombinant Escherichia coli strain displaying the MerR protein that has high binding affinity with mercury has been developed (2). Binding assays that were performed against various heavy metals suggested that the developed recombinant E. coli bound a 100-times-greater quantity of mercury than the other heavy metals. Subsequently, the hepatitis B surface antigen or a core protein of the hepatitis C virus was successfully displayed on the oral vaccine strain Salmonella enterica serovar Typhi Ty21a (15). Vaccination of mice with the live recombinant S. enterica serovar Typhi Ty21a vaccine elicited high levels of systemic serum antibodies against hepatitis B surface antigen and the hepatitis C virus core protein. Apart from the display of single functional molecules, combinatorial libraries anchored on the periplasmic face of the inner membrane of E. coli were also constructed and screened, with which the affinity of a neutralizing antibody to the anti-Bacillus anthracis protective antigen scFv was improved more than 200-fold (10). Disruption of the outer membrane by Tris-EDTA-lysozyme treatment was conducted, and the experimental results indicated that the spheroplasts showed high affinity with fluorescently labeled ligands and were screened via flow cytometry. Lastly, the internalization of E. coli into eukaryotic cells was demonstrated by virtue of the cell surface-displayed peptides (32). From this analysis, novel entry motifs, as well as integrin-binding Arg-Gly-Asp (RGD) motifs, were verified as promoting a high degree of bacterial entry into HeLa cells.

As described above, although cell surface display technology has been developed and applied within bioadsorbent and screening systems or other related systems, cell recovery has been a bottleneck, as large machines and a decrease in throughput are often necessary. Therefore, in order to overcome this problem, a highly applicable and versatile method or technology is demanded. Magnetotactic bacteria are known to synthesize intracellular magnetic nanoparticles that allow them to be directly manipulated by magnetic force (8). Magnetotactic bacteria can be easily collected and concentrated by magnetic separation. This unique characteristic of the bacteria suggests that they serve as an ideal candidate for cell surface display.

The completion of the whole-genome sequence of “Magnetospirillum magneticum” (proposed name) AMB-1 opened doors to molecular and proteomic possibilities in further understanding the characteristics of the AMB-1 bacterium (18). Among the proteins discovered from the various and widely conducted proteomic analyses, several proteins located in the...
outer membrane were successfully identified (31). One of these outer membrane-localized proteins, the membrane-specific protein (Msp1) (Amb0025) (NCBI GeneID 3806647), shared homology with the outer membrane porin precursor of *Rhodospirillum rubrum* (E value, 5.3E-25) and was found to be highly expressed in *M. magnetotacticum* AMB-1. The high level of expression of the Msp1 protein allows it to be a possible carrier protein or candidate in constructing an effective surface display system on *M. magnetotacticum* AMB-1. Similarly, homologues of the Msp1 protein have also been identified in other magnetotactic bacteria, such as the hypothetical protein Magn03010474 from *Magnetospirillum gryphiswaldense* MS-1 (E value, 0.0) and the outer membrane protein (porin) (EMBL accession no. CU459003.1) from *Magnetospirillum gryphiswaldense* MSR-1 (E value, 5E-107). The identification of these homologues suggests that the development of a cell surface display system in *M. magnetotacticum* AMB-1 using the Msp1 protein as a carrier molecule could demonstrate possibilities for the development of similar systems within the respective hosts.

A heterologous-molecule expression system within *M. magnetotacticum* AMB-1 using an *E. coli*-AMB-1 shuttle vector that was constructed from pUC19 and pMGT has been reported and established (22). Based on this technology, many applications using functional-molecule-expressing bacterial magnetic particles have been developed and reported (13, 19, 35, 36). Though it is a powerful technology for many assay systems, the difficulty of preparing a great variety of bacterial magnetic particles still remains. In contrast, the cell surface display system has an advantage for establishing cell libraries expressing different types of molecules. Here, for the first step in the development of a cell surface display system using magnetotactic bacteria as a whole, this report focuses on the identification of an optimal display site within the Msp1 protein, successful expression and display of a metal-binding polypeptide, and magnetic separation of magnetotactic bacteria from water samples.

MATERIALS AND METHODS

**Bacterial strains and culture conditions.** *E. coli* strain DH5α was used as a host for gene cloning for which the cells were cultured in LB medium containing ampicillin (50 μg/ml) at 37°C. *M. magnetotacticum* AMB-1 was microaerobically cultured in magnetic spirillum growth medium at 28°C as previously described (20). Microaerobic conditions were established by purging the cultures with argon gas. *M. magnetotacticum* AMB-1 transformants were cultured under the same conditions as the wild-type strain, supplemented with 5 μg/ml of ampicillin.

**Construction of expression vectors for cell surface display system on *M. magnetotacticum* AMB-1.** The plasmid pUMP1 was designed based on the pUGM plasmid (Ap', 6.4 kbp) (22, 34). For the construction of the pUMP1Msp1 and pUMP1OmpA expression plasmids, the msp1 and ompA genes were obtained by PCR amplification using LA Taq polymerase (TAKARA). *M. magnetotacticum* AMB-1 and *E. coli* genomic DNA were used as templates, with the respective primer sets comprised of Msp1_f (5'-ATGCAATATGGAAGAAAGATCTTTTGTACG' -3') and Msp1_r (5'-ATGCAATATGGAAGAAAGATCTTTTGTACG' -3') for the amplification of msp1 and OmpA_f (5'-ATGCAATATGGAAGAAAGATCTTTTGTACG' -3') and OmpA_r (5'-ATGCAATATGGAAGAAAGATCTTTTGTACG' -3') for the amplification of ompA (the underlined sequence in the primer pairs indicates the NsiI site). The amplified genes were then cloned into the vector pGEM-T Easy (Promega, Madison, WI). Site-specific insertion of the NsiI site and hexahistidine residues into the msp1 and ompA genes was performed according to the standard PCR-based protocol as previously described, using the various primer sets shown in Table S1 in the supplemental material (7). Vector construction and mutations were confirmed by agarose gel electrophoresis after digestion with NsiI and KpnI. As the KpnI site exists within the pUMP vector, the Msp1 and OmpA genes were cloned into the vector pUC19 and pMGT. *E. coli* transformants were then cultured under the same conditions as the wild-type strain, supplemented with 5 μg/ml of ampicillin.

**Subcellular fractionation of magnetotactic bacteria.** Stationary-phase cultures of wild-type AMB-1 or transformants harboring each expression plasmid were harvested by centrifugation (8,000 × g for 10 min at 4°C), resuspended in 40 ml of 10 mM HEPES buffer (pH 7.4), and disrupted by three passes through a French press at 1,500 kg/cm² (Ohtake Works Co., Ltd., Tokyo, Japan). The subcellular fractions, including the outer membrane fraction, were obtained as previously described (22, 31, 36).

**Structural prediction analysis of Msp1 protein.** The Msp1 protein, the protein with the highest expression level identified within the outer membrane fraction, as previously described, is comprised of 436 amino acids (Fig. 1A) (31, 34) and shows homology with the outer membrane porin precursor of *R. rubrum* (E value, 5.3E-25). The porin protein family, which is one of the major outer membrane protein families, has a structure comprised of β-strand and coiled-loop repeats (1, 24). In order to identify the applicability of pUC19 for cell surface display system on *M. magnetotacticum* AMB-1, the Msp1 protein was predicted using the PSIPRED program and targeted hexahistidine insertion sites. (A) Protein profile of outer membrane fraction from *M. magnetotacticum* AMB-1. The main band, indicated by the arrow, is the Msp1 protein. (B) Secondary structural prediction of Msp1. Arrows indicate the insertion sites of hexahistidine and the NheI restriction site. Shaded amino acid sequences represent the β-strand regions.
display, the secondary structure of the protein was predicted with the PSIPRED program (11, 12), available at http://bioinf.cs.ucl.ac.uk/psipred/psiform.html. Other prediction programs, including PROF (23), PHDsec (27, 28), and SSpro and SSpro8 (25), provided similar results. Based on the predicted secondary structure, functional molecule insertion sites were determined within several of the speculative outer membrane-exposed coiled-loop regions.

Western blot analysis of Msp1-hexahistidine and OmpA-hexahistidine hybrid proteins. Proteins from the cytoplasmic-periplasmic, undisrupted cell, and inclusion body and cell membrane fractions of the AMB-1 transformants were each mixed with sodium dodecyl sulfate (SDS) sample buffer (62.5 mM Tris-HCl [pH 6.8], 5% 2-mercaptoethanol, 2% SDS, 25% glycerol, and 0.004% bromophenol blue), denatured, and subjected to SDS-polyacrylamide gel electrophoresis on a 12.5% (wt/vol) polyacrylamide gel. For immunostaining of Western blots, mouse anti-hexahistidine antibody (1:2,000 dilution; Calbiochem) and goat alkaline phosphatase-labeled anti-mouse immunoglobulin G (IgG) antibody (1:2,500 dilution; Invitrogen) were used. The blotted and immunostained membrane was developed by using BCIP/NBT-Blue (substrate solution; Sigma, St. Louis, MO).

Immunofluorescence microscopy of hexahistidine expressed on cell surface. Following overnight culture, cells (1.0 × 10^10) collected by centrifugation (8,000 × g for 10 min at 4°C) were resuspended and harvested in phosphate-buffered saline (PBS) containing 2% bovine serum albumin. Intact cells were then incubated with mouse anti-hexahistidine antibody (1:1,000) for 4 h at 4°C. The cells were extensively washed twice with PBS by centrifugation (8,000 × g for 10 min at 4°C), resuspended in PBS containing secondary goat anti-mouse IgG antibody conjugated with phycoerythrin (PE; Beckman Coulter) at 1:1,000, and incubated for an additional 4 h at 4°C. The cells were washed again twice with PBS by centrifugation. The total fluorescence was measured from a population of cells which had been counted by using a fluorescence reader (Fluorostar Galaxy; BMG). Photographs of the AMB-1 transformant showing the highest fluorescence intensity and nonfluorescent cells (negative control) were captured by using a fluorescence microscope (BX51; Olympus).

Cd^{2+} binding to cells with surface-expressed hexahistidine. To perform Cd^{2+} binding, 1.0 × 10^10 cells from overnight cultures of the AMB-1 transformant harboring the pUMP1Msp1-225H expression plasmid (pUMP1Msp1-225H transformant) and the AMB-1 transformant harboring the pUMP1 plasmid (pUMP1 transformant) were collected with centrifugation (8,000 × g for 10 min at 4°C), washed twice with 100 μM HEPES buffer (pH 7.4), resuspended in the same buffer containing Cd^{2+} (100 μM), and gently agitated for 3 h. In this case, the pUMP1 transformant was used as a negative control. After the agitation, cells were washed twice with HEPES buffer, whereby the supernatant was collected for the analysis of Cd^{2+} binding. The cells with Cd^{2+} attached were then treated twice with EDTA (25 mM, pH 7.0, 1 ml), which is a metal ion chelator, and Cd^{2+} was removed from the cells surfaces. The supernatant after each EDTA treatment was also collected for analysis. Finally, the supernatants obtained after HEPES wash, supernatants after EDTA treatment, and the final cell precipitate were dried and dissolved with nitric acid solution (0.1 N) on an oil bath. These samples were analyzed by atomic absorption spectrophotometry (AA-660G Shimadzu). All assays were performed three times.

Magnetic recovery assay of the pUMP1Msp1-225H transformant in the presence of Cd^{2+}. To verify the ability to recover cells of wild-type AMB-1 and the pUMP1Msp1-225H transformant in the presence of Cd^{2+} using magnetic force, a magnetic cell recovery assay was conducted. The AMB-1 wild-type strain and the transformant were harvested at the late logarithmic phase of growth, and cells were counted and adjusted to 1.0 × 10^8 cells/ml magnetic spurilum growth medium in the presence of Cd^{2+} at different concentrations (0 μM, 10 μM, and 100 μM). Three milliliters of each sample was then transferred to separate test tubes (7 mm in diameter and 7.5 cm in height), and each test tube was sealed with a rubber cork. Cylindrical neodymium-boron magnets (15 mm in diameter and 1 cm in height) were placed in the middle of each sample-containing test tube, and the test tubes were set upright to allow cell recovery to take place. At the designated times (0.5 h, 1 h, 2 h, 4 h, 6 h, 12 h, and 24 h), culture medium from each test tube was collected by inserting a syringe through the rubber cork and extracting culture medium (20 μl) near the surface. The cell count was performed against the extracted culture medium samples accordingly.

RESULTS

Secondary structural prediction of Msp1 and confirmation of vectors for hexahistidine insertion within various coiled-loop regions of Msp1 and OmpA. Secondary structure predic-

![FIG. 2. Western blot analysis of Msp1-hexahistidine and OmpA-hexahistidine hybrid protein expression. Lane 1, total protein extracts from transformant; lane 2, cell debris and inclusion body; lane 3, cytoplasmic-periplasmic fraction; lane 4, cell membrane fraction. Each lane was loaded with 40 μg of protein. Mouse antihexahistidine antibody and horseradish peroxidase-conjugated goat anti-mouse IgG antibody were used as the primary and secondary antibodies, respectively.](image-url)
Immunofluorescence staining and microscopy of various Msp1-hexahistidine and OmpA-hexahistidine hybrid proteins. Although many outer membrane proteins have been analyzed for cell surface display in gram-negative bacteria (5, 26), it is still difficult to specify the best site within the carrier protein for the insertion of functional molecules from the amino acid sequence. Lately, using bioinformatic tools, such as PSIPRED (11, 12), PROF (23), PHDsec (27, 28), and SSpro and SSpro8 (25), information about the predicted secondary structures of proteins can be obtained easily. Unfortunately, the difficulty still lies in the prediction of the conformation of the coiled loop, since the site is thought to be a good site for displaying functional molecules. Whether the functional molecule should be inserted on the inside or outside of the outer membrane is very important for the display of the molecule on the cell surface. The next difficulty would be whether the length of the coiled loop is sufficient to result in molecule functionality. Therefore, in this study, in order to verify whether the functional molecule was successfully displayed and functional, immunofluorescence staining and microscopic observation of the various Msp1-hexahistidine and OmpA-hexahistidine hybrid protein-expressing AMB-1 transformants were conducted (Fig. 3). In the results of the comparative assay, based on binding affinity with an antihexahistidine antibody, the transformant harboring the pUMP1Msp1-225H expression plasmid (pUMP1Msp1-225H transformant) showed the highest fluorescence intensity. This transformant was also observed with fluorescence microscopy (Fig. 4). The pUMP1Msp1-225H transformant showed higher fluorescence intensity than the AMB-1 transformant harboring the pUMP1 plasmid (pUMP1 transformant).

Cd\textsuperscript{2+} binding experiments with the pUMP1Msp1-225H and pUMP1 transformants. In order to investigate whether the recombinant magnetotactic bacteria had gained an increased capability to adsorb divalent heavy metal ions, a Cd\textsuperscript{2+} bioadsorption assay was employed. The pUMP1 transformant used as a control and the pUMP1Msp1-225H transformant were grown to similar cell densities and were tested for the ability to adsorb Cd\textsuperscript{2+}. As shown by the results presented in Fig. 5, the pUMP1Msp1-225H transformant adsorbed a larger amount of Cd\textsuperscript{2+} than the control transformant. The majority of the Cd\textsuperscript{2+} remained tightly adsorbed onto the AMB-1 transformants even after they were washed with HEPES. When the cells were washed with EDTA, approximately $3.8 \times 10^6$ molecules per cell were recovered from the pUMP1Msp1-225H transformant and the adsorption ability was increased by approximately 40% in comparison to that of the control transformant.

Magnetic cell recovery assay of the pUMP1Msp1-225H transformant in the presence of Cd\textsuperscript{2+}. Showing that magnetotactic bacteria can be recovered by magnetic force magnifies the possibilities of applications in bacterial surface display systems. The results shown in Fig. 6 demonstrate that upon collection of bacteria with magnetic force, over 80% of the bacteria were successfully recovered within 10 h. In addition, the recovered cells were also counted at the end point to further verify the accuracy of this assay. The amount of cadmium ion depleted from the medium was also determined; a total of approximately 2.8 nmol of cadmium ion was absorbed onto the AMB-1 transformant from each 3 ml of cadmium solution. Similarly, the results shown in Fig. 5 indicate that magnetotactic bacteria can be applied in bioremediation, even in the presence of Cd\textsuperscript{2+}, and that cells can be recovered efficiently.
DISCUSSION

Bacterial surface display systems have been implemented by expressing a heterologous peptide or protein as a fusion protein with various carrier proteins or their fragments to add additional functions to microorganisms (16). Depending on the characteristics of the carrier protein, C-terminal fusion, N-terminal fusion, or sandwich fusion strategies can be considered. In the case of C-terminal or N-terminal fusion, the display site of the functional molecule is localized at the opposite end of the signal peptide. However, many cell surface proteins, such as outer membrane proteins (Omps), required the whole structure of the protein to be assembled within the outer membrane (33) in order to prevent misconformation or misfolding. Sandwich fusion or site-directed insertion is an effective means to overcome this difficulty. In fact, PhoE (14), FliC (17), and OmpC (33) have been shown to be good examples of sandwich carriers for functional molecules. It is also possible to speculate that the use of carrier proteins derived from other organisms often causes different physiological effects or even crucial cellular damage within the host cell. Therefore, for the development of a cell surface display system, utilizing carrier proteins native to the host cell is always the best alternative.

Magnetotactic bacteria are known as a unique class of prokaryotes due to their ability to synthesize intracellular magnetic nanoparticles composed of core magnetites (Fe₃O₄), greigites (Fe₃S₄), or iron pyrite (FeS₂) (4, 6, 29), each individually enveloped by a phospholipid membrane. In the magnetotactic bacterium *M. magneticum* AMB-1, approximately 20 magnetic nanoparticles are synthesized per cell. These nanosized (50 to 100 nm) magnetite particles are aligned in chains perpendicular to the cell axis, enabling the cell to migrate along the Earth’s geomagnetic field lines and to maintain its position within the boundary of the oxic-anoxic transition zone (3). Using this unique characteristic, it is easy to collect and concentrate the cells with an external magnetic field. From the comparative proteomic analysis of cellular fractions of *M. magneticum* AMB-1, Msp1 was identified and was found to be expressed at high levels in the outer membrane fraction (31). The secondary structure of this outer membrane protein homologue, comprised of repeats of coiled loop and β-strand, was predicted with PSIPRED (Fig. 1B). Since the outside coiled loop is required for the cell surface display system, 11 sites of predicted coiled loops within Msp1 were selected as candidates. In addition, two coiled-loop sites of the OmpA protein from *E. coli*, for which the three-dimensional structure...
and success in several functional molecule displays have been reported (1, 9, 21, 24), were also constructed for this system. As it was of importance to our future work that the displayed peptide/protein should bind target molecules in free solution, an experiment was designed to evaluate the interaction between the antihexahistidine antibody and the AMB-1 transformants. In this simple quantitative evaluation of binding between the displayed peptide and the target protein, the pUMP1Msp1-225H transformant had the highest PE-conjugated anti-mouse IgG antibody fluorescence intensity (Fig. 3). Since the predicted sixth coiled loop is one of the long loops within this protein, it may have resulted in high antibody binding. On the other hand, although the expression levels of the OmpA proteins were similar to those of Msp1, the AMB-1 transformants harboring the pUMP1OmpA-66H and pUMP1OmpA-153H expression plasmids showed lower fluorescence intensities than those of the AMB-1 transformants harboring the Msp1 expression plasmids. Regarding this observation, as OmpA is a foreign protein to magnetotactic bacteria, it may have been difficult for the protein to be properly assembled in the outer membrane of the bacterium.

Immunofluorescence microscopy was then performed to verify the binding of the displayed peptide with its specific antibodies. Cells were probed with mouse anti-hexahistidine as a primary antibody and fluorescently stained with PE-labeled goat anti-mouse IgG antibody. As shown by the results presented in Fig. 4, the pUMP1Msp1-225H transformant showed high fluorescence, indicating that the hexahistidine amino acids were successfully displayed on the cell surface. The pUMP1 transformants, on the other hand, were not stained at all with the PE-labeled secondary antibody. In order to further evaluate the AMB-1 transformant, the Cd\(^{2+}\) bioadsorption capability of whole cells expressing hexahistidine was tested by using atomic absorption spectroscopy to monitor their Cd\(^{2+}\) binding. Prior to performing this experiment with the *M. magneticum* AMB-1 cells, the effects of the Cd\(^{2+}\) binding over time and in different initial concentrations of Cd\(^{2+}\) were determined. The quantity of Cd\(^{2+}\) absorbed to wild-type *M. magneticum* AMB-1 cells reached maximal adsorption at about 15 min. In the case of the different initial concentrations of Cd\(^{2+}\), the binding of Cd\(^{2+}\) to 1.0 \times 10^{10} wild-type *M. magneticum* AMB-1 cells was highest at amounts of 60 nmol or more. Based on these data, a Cd\(^{2+}\) absorption assay was conducted to compare the maximum quantity of bound Cd\(^{2+}\) for the wild-type AMB-1 and the pUMP1Msp1-225H transformant. As shown by the results presented in Fig. 5, in comparison to a single magnetotactic bacterial cell composed of negatively charged molecules comprised of phospholipids and proteins, which adsorbs approximately 2.8 \times 10^{6} Cd\(^{2+}\) per cell, an additional 1.1 \times 10^{6} Cd\(^{2+}\) per cell (40% increase) were adsorbed onto the surface-displayed hexahistidine molecules of the AMB-1 pUMP1Msp1-225H transformants. Assuming that a maximum of six Cd\(^{2+}\) can bind to a hexahistidine molecule, approximately 1.8 \times 10^{5} hexahistidine molecules per cell were displayed on the cells. In a Western blot analysis, a six-His-tagged marker, in which an observed 50-kDa band within the marker represents 30 ng of protein or 3.6 \times 10^{11} molecules, was used as a control. Proteins extracted from 3.0 \times 10^{6} pUMP1Msp1-225H transformants were analyzed, and the Msp1-hexahistidine hybrid protein band of 43 kDa showed a band intensity similar to that of the referenced 50-kDa band of the control marker. Based on this observation, the number of hexahistidine molecules is then calculated to be 4.2 \times 10^{11} molecules in total or 1.4 \times 10^{9} molecules per single transformant. From the results of Western blot analysis, it was shown that 1.1 \times 10^{6} hexahistidine molecules were displayed on the cell surface of the pUMP1Msp1-225H transformant, suggesting that the 40% increase of Cd\(^{2+}\) was reasonable (data not shown).

*E. coli* cells are known to be good hosts for the display of functional proteins in which Bae et al. displayed a specific mercury-binding protein, showing a sixfold increase in mercury adsorption over control cells (2). The difference observed between the *E. coli* system and the use of *M. magneticum* AMB-1 with hexahistidine in this work would probably be directly affected by the different functional molecules and by the amounts of functional molecules expressed on the surface of the respective cells. Therefore, by optimizing the functional molecule and the expression and display of functional molecules on the surface of AMB-1 cells by genetic engineering techniques, such as analysis of promoter types, the AMB-1 cells can be further improved to obtain greater increases in the adsorption of Cd\(^{2+}\).

In the analysis of the possibility of recovering magnetotactic bacteria by magnetic force, over 80% of bacteria were successfully recovered within 10 h. It was shown that hexahistidine-displaying bacteria in the presence of Cd\(^{2+}\) can be recovered by magnetic force without an obvious drop in collection efficiency. In addition to the application of *M. magneticum* AMB-1 cells for Cd\(^{2+}\) adsorption, the high accumulation of arsenic and tellurium ions on the cell surface of AMB-1 cells has also been verified in basic preliminary work performed previously (data not shown).

In conclusion, a novel cell surface display system in which the Msp1 protein of *M. magneticum* AMB-1 was used as an anchoring motif has been developed. A hexahistidine amino acid was successfully inserted subsequent to the 225-amino-acid residue of the Msp1 protein and displayed on the cell surface, and the construct showed significant Cd\(^{2+}\) adsorption. Upon the successful identification of a functional molecule display site and the establishment of an AMB-1 transformant with high binding capability, it was shown in the results of this work that magnetotactic bacteria can be potential candidates for the display of other functional molecules and can possibly be applied in ligand screening and heavy metal adsorption. Furthermore, with their response to magnetic force, magnetotactic bacteria can be easily manipulated, showing that large machines, such as centrifuges, are unnecessary. It is believed that magnetotactic bacteria provide new advantages for the development of various bioaccumulation technologies and can play an important role in the future development of high-throughput screening or biosorption systems.

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