Adsorption of rare earth ions onto the cell walls of wild type and lipoteichoic acid-defective strains of *Bacillus subtilis*

Hiroshi Moriwaki*, Remi Koide, Ritsuko Yoshikawa, Yuya Warabino,

Hiroki Yamamoto

Shinshu University, Faculty of Textile Science and Technology, Division of Applied Biology, 3-15-1, Tokida, Ueda 386-8567, Japan

---

* To whom correspondence should be addressed.

E-mail: moriwaki@shinshu-u.ac.jp

Abstract

The aim of this study is to investigate the potential of cell walls of wild type and lipoteichoic acid-defective strains of *Bacillus subtilis* 168 to adsorb rare earth ions. Freeze-dried cell powders prepared from both strains were used for the evaluation of adsorption ability for the rare earth ions, namely, La(III), Eu(III), and Tm(III). The rare earth ions were efficiently adsorbed onto powders of both wild type strain (WT powder) and lipoteichoic acid -defective strain (ΔLTA powder) at pH 3. The maximum adsorption capacities for Tm(III) by WT and ΔLTA powders were 43 and 37 mg·g^{-1}, respectively. Removal (%) of Tm(III), La(III), and Eu(III) from aqueous solution by WT powder was greater than by ΔLTA powder. These results indicate that rare earth ions are adsorbed to functional groups, such as phosphate and carboxyl groups, of lipoteichoic acid.

We observed coagulated ΔLTA powder in the removal of rare earth ions (1-20 mg·L^{-1}) from aqueous solution. In contrast, sedimentation of WT powder did not occur under the same conditions. This unique feature of ΔLTA powder may be caused by the difference of the distribution between lipoteichoic acid and wall teichoic acid. It appears that ΔLTA powder is useful for removal of rare earth ions by adsorption, because aggregation allows for rapid separation of the adsorbent by filtration.

Keywords rare earth ion · *Bacillus subtilis* 168 · lipoteichoic acid · lipoteichoic acid-defective strain · adsorbent

Introduction

Rare earth metals are widely used in various technological devices, including superconductors, magnets, catalysts, and batteries (Andrianov et al. 2011; Molander and Romero 2002). The global demand for rare earth metals has been increasing (Du and Graedel 2011). There is concern that the increased demand for rare earth metals leads to environmental exposure or water pollution from rare earth metal mines and various commercial products. In one report, high levels of rare earth metals were detected in hair from the scalp of children living in a rare earths mining area in China (Tong et al. 2005). It is known that rare earth metals exhibit hepatotoxic and neurotoxic effects (Pałasz and Czekaj 2000; Basu et al. 1982). Therefore, useful techniques to extract or remove rare earth metal from the environment are needed.

There are many examples of functionalized polymers or phosphates used for the extraction of rare earth ions (Buchmeiser et al. 1998; Sun et al. 2012). However, most of these reagents are expensive and may be toxic themselves. Under these circumstances, it is very important to develop low-cost and environmentally-friendly removal methods for rare earth ions from water.

It is benefical to use bioresource materials as adsorbents for environmental pollutants because of their safety and cost effectiveness. Recently, various bioresource materials have been used as adsorbents for pollutants, such as oil, pesticides, and metal ions (Moriwaki et al. 2009; Senthilkumaar et al. 2010; Ghimire et al. 2003). Bacteria are regarded as effective adsorbents for dissolved metals because of their high surface area per unit weight. Several studies concerned with the adsorption of rare earth ions onto bacterial cells have been conducted (Merroun et al. 2003; Takahashi et al. 2005; Ozaki et al. 2006). It has been suggested that carboxylate and phosphate groups are mainly responsible for the adsorption of rare earth ions onto bacterial cell surfaces (Takahashi et al. 2010). However, more information is needed to clarify the details of the mechanism

of adsorption of rare earth ions onto bacteria in order to apply them effectively in environmental remediation.

In this study, wild type and lipoteichoic acid-defective strains of *Bacillus subtilis* 168, which do not produce extracellular polymers such as polyglutamic acid, were investigated for their potential use as adsorbents of rare earth ions. Freeze-dried cell powders prepared from both strains were used for evaluating their ability to adsorb rare earth ions, namely, La(III), Eu(III), and Tm(III).

Teichoic acids are bacterial polysaccharides that consist of glycerol phosphate or ribitol phosphate. These polyphosphate compounds constitute a major component of the cell wall in most gram-positive bacteria, including *Bacillus subtilis*. Teichoic acids come in 2 distinct forms depending on whether they are linked to the head groups of membrane lipids (lipoteichoic acids: LTAs) or to the wall peptidoglycan (wall teichoic acids: WTAs) (Neuhaus et al. 2003). Peptidoglycan is also major structural compound in the cell walls of bacteria. The contribution of LTAs to the adsorption of rare earth ions onto bacterial cells is not clear, although it has been reported that phosphates on the cell wall contribute to the process (Takahashi et al. 2010). By comparing the adsorption of rare earth ions onto the cell surfaces of wild type and lipoteichoic acid-defective strains, it was expected that the role of LTAs in the adsorption of rare earth ions onto the bacterial cell walls could be further elucidated.

Materials and methods

Reagents

Nitric acid, manganese(II) sulfate, iron(II) sulfate, lanthanum(III) chloride, europium(III) chloride, and thulium(III) chloride were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Distilled water was produced by an automatic water distillation apparatus (WG203; Yamato Scientific, Tokyo, Japan).

Construction of the mutant strains

The strains of *B. subtilis* and *Escherichia coli*, and plasmids used in this study are listed in **Table 1**. All chemical reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise noted. *B. subtilis* 168 was used as the parent strain throughout the study. *B. subtilis* strains were grown in Luria Bertani (LB) medium (Sambrook *et al*. 1989) at 37˚C. To culture a quadruple mutant of the *ltaS* paralogous genes (RY1604), erythromycin was added to final concentrations of 0.30 μg mL^{-1}. *E. coli* strains harboring plasmids were cultured in LB medium at 37˚C, and ampicillin was added to a final concentration of 100 μg mL^{-1}. DNA manipulations and *E. coli* transformation were performed by standard methods (Sambrook et al. 1989). The conventional transformation method (Anagnostopoulos and Spizizen 1961) was used for *B. subtilis* transformation.

Primers used in this study are listed in **Table 2**. After the polymerase chain reaction (PCR)-amplified fragments were digested with restriction enzymes purchased from Takara (Kyoto, Japan), all DNA fragments were separated by 1% agarose gel electrophoresis and purified with QIAquick gel extraction kit (QIAGEN) according to the manufacturer's instructions.

To construct a triple mutant for *yfnI*, *yqgS*, and *yvgJ* without any antibiotic resistance markers, we used an efficient allelic replacement method with pMAD (Arnaud *et al*. 2004). For the construction of pMADΔyfnI, upstream (530 bp) and downstream (509 bp) regions of the *yfnI* gene were amplified with 2 sets of primers, yfnIf-Bf and yfnIf-PEr, and yfnIb-ESf and yfnIb-NKr, respectively, and 168 chromosomal DNA (chrDNA) as a template. The amplified fragments were digested with *Eco*RI. The fragments were then ligated and the ligation mixture was used as a

template for a 2nd PCR with yfnIf-Bf and yfnIb-NKr. After digestion with *Bam*HI and *Nco*I, the resulting 1.0-kb DNA fragment was cloned into the corresponding sites in pMAD to obtain pMADΔyfnI. Similar procedures were used to construct pMADΔyqgS and pMADΔyvgJ. For pMADΔyqgS, primers used for the upstream (510 bp) and downstream (511 bp) fragments were yqgSf-Bf and yqgSf-PEr, and yqgSb-ESf and yqgSb-NKr, respectively. For pMADΔyvgJ, primers used for the upstream (508 bp) and downstream (519 bp) fragments were yvgJf-Bf and yvgJf-Pr, and yvgJb-PSf and yvgJb-NKr, respectively. The amplified fragments were digested with *Pst*I. Then the fragments were ligated and the ligation mixture was used as a template for a 2nd PCR with yvgJf-Bf and yvgJb-NKr. After the amplified 1.0-kb fragment was digested with *Bam*HI and *Nco*I, the fragment was cloned into the corresponding sites in pMAD to obtain pMADΔyvgJ. After sequencing the inserts of these pMAD-derivatives, the plasmids were used for transformation of *E. coli* C600 to generate concatemeric DNAs.

For the construction of an IPTG-inducible *ltaS*-conditional mutant, the 5'-terminal region, including the ribosome-binding site, was amplified by PCR using 168 chrDNA as the template, and primers yflE-SD-EF and yflE-p2-BR. The amplified 311-bp fragment was digested with *Eco*RI and *Bam*HI, and the resultant fragment was cloned into the corresponding sites of pMUTIN4 (Vagner *et al*. 1998) to obtain pM4ΔNyflE. After sequencing the insert DNA fragment, the plasmid was used for transformation of *E. coli* C600 to generate a concatemeric DNA.

The sources of donor DNAs and recipient cells used for *B. subtilis* mutant construction are listed in **Table 1**. To construct a triple null mutant of *yfnI*, *yqgS* and *yvgJ*, *B. subtilis* RY1603, without any antibiotic resistance genes, we used an efficient allelic replacement method, step by step, with pMAD-derived plasmids (Arnaud *et al*. 2004). First, to construct a *yvgJ* null mutant (ΔyvgJ), *B. subtilis* 168 was transformed with pMADΔyvgJ. A blue colony was selected on LB plates containing 0.3 μg·mL^{-1} of erythromycin and 200 μg·mL^{-1} of X-gal (5-bromo-4-chloro-3-

indolyl-β-D-galactopyranoside). We performed the subsequent integration and excision procedures as described by Arnaud *et al*. (2004). After the excision procedure, we selected some white and erythromycin-sensitive colonies to obtain *B. subtilis* ΔyvgJ. We then confirmed the proper null mutation at the *yvgJ* locus by PCR with primers YvgJ-up and YvgJ-down. Next, *B. subtilis* ΔyvgJ was transformed with pMADΔyqgS to obtain a *yvgJ yqgS* double null mutant, *B. subtilis* RY1602. After integration and excision procedures, we checked the proper null mutation at the *yqgS* locus by PCR with primers YqgS-up and YqgS-down. Finally, *B. subtilis* RY1602 was transformed with pMADΔyfnI to obtain a triple null mutant of *yfnI*, *yqgS* and *yvgJ*, *B. subtilis* RY1603. After integration and excision procedures, we checked the proper null mutation at the *yfnI* locus by PCR with primers YfnI-up and YfnI-down.

For construction of an *ltaS*-conditional mutant, *B. subtilis* 168 was transformed with pM4ΔNyflE to obtain *B. subtilis* LTASp. After selection on LB agar plates containing erythromycin (0.30 μg·mL^{-1}) and IPTG (1 mM), appropriate integration of the plasmid DNA at the *ltaS* locus was confirmed by PCR. To obtain a quadruple mutant of the *ltaS* paralogous genes (RY1604), *B. subtilis* RY1603 was transformed with LTASp chrDNA as a donor DNA. The resulting transformants were selected on LB agar plates containing erythromycin (0.30 μg·mL^{-1}) and IPTG (1.0 mM). We subsequently confirmed the proper mutations of four loci (*ltaS*, *yfnI*, *yqgS* and *yvgJ*) by PCR. In addition, after the inducer (IPTG) was removed, we also confirmed that the quadruple mutant cells exhibited slow growth and aberrant filamentous clumps twisted around their long axis as described previously (Schrner *et al*. 2009).

Preparation of WT and ΔLTA powder

B. subtilis strains were cultured in 6 mL of LB medium at 30˚C over night. When a quadruple

mutant of the *ltaS* paralogous genes (RY1604) was first cultured, erythromycin and IPTG were added to final concentrations of 0.30 μg·mL^{-1} and 1.0 mM, respectively. Cells corresponding to 1 mL of the cultures were harvested by centrifugation (Tabletop centrifuge-CT15RE, Hitachi Koki, Tokyo, Japan) and washed once with 1 mL of fresh LB medium to remove the inducer. The cells were then inoculated at an OD_{600} of 0.001 into 1 L of fresh LB medium. After incubation for 3 h at 37˚C, LTA-deficient cells were harvested by centrifugation (himac CR21, Hitachi Koki, Tokyo, Japan). The cell pellet was suspended with 30 mL of ultrapure water and boiled for 20 min. After cooling, cells were precipitated by centrifugation (himac CR21, Hitachi Koki, Tokyo, Japan), washed once with 30 mL of ultrapure water, and freeze-dried. Then, the solid materials were crushed with a medicine spoon and sieved. The diameter of the powders ranged from 53 to 100 μm. Powders from the wild type strain (WT powder) and the lipoteichoic acid-defective strain (ΔLTA powder) were obtained.

The powders were visualized using a scanning electron microscope (SEM; JSM-6010LA; JEOL, Tokyo, Japan). The samples were sputtered with platinum using a JFC-1600 instrument (JEOL, Tokyo, Japan).

Adsorption test

The general procedure for evaluating the removal of Tm(III), La(III), Eu(III), Fe(II), or Mn(II) using WT powder or ΔLTA powder as a sorbent was as follows: 20 mg of WT powder or ΔLTA powder was added to 20 mL of a metal ion solution (20 μg·mL^{-1}, pH 3) in a conical flask. It is known that rare earth ions form complexes with hydroxide or carbonate at pH greater than approximately 5.5 (Liu and Byrne, 1998). In order to keep rare earth metals as free cations, the pH level of the tested solutions was set at 3 for the adsorption test. The suspensions were mixed for 30 min at 10 rpm and

then filtered with filter paper (suspended particle size: 7μm; No. 5A, Advantec, Tokyo, Japan). The adsorption (%) of a metal ion to the membrane was below 10% under the experimental condition. The filtrate was centrifuged at 4000 rpm (Tabletop centrifuge-2410, Kubota Corporation, Tokyo, Japan) for 20 min. A 5.0 mL aliquot of the supernatant was added to 5 mL of nitric acid, and heated. The resulting solution was diluted with distilled water to a final volume of 20 mL, and then quantitatively analyzed for the metal ion concentration using inductively coupled plasma atomic emission spectrometry.

The adsorption capacities of the WT and ΔLTA powder for Tm(III) were determined in the original Tm(III) solution under various conditions. All experiments were conducted at 25 ^{o}C in thermostatic chamber.

Analytical procedure

Metal ion concentrations in solutions were measured by inductively coupled plasma (ICP) atomic emission spectrometer (SPS 3100; SSI nanotechnology, Tokyo, Japan) under the following experimental operating conditions: RF power, 1.2 kW; plasma gas flow rate (Ar), 16 L·min^{-1}; carrier gas flow rate, 1.0 L·min^{-1}; auxiliary gas flow rate, 1.0 L·min^{-1}. Working wavelengths for iron, manganese, lanthanum, europium, and thulium were 260.569, 259.940, 379.478, 412.970, and 313.126 nm. The limits of quantification for Mn(II), Fe(II), La(III), Eu(III), and Tm(III) were 5, 2, 5, 7 and 3 ng·mL^{-1}, respectively.

Results

WT and ΔLTA powders

The surfaces of WT and ΔLTA powders were examined by SEM. Typical rod-shaped cells were observed in WT powder and slightly curved ones were found in ΔLTA powder, and it was confirmed that the structure of the cell walls was not disrupted by the preparation of the powders. Furthermore, it was determined that there were no significant differences in the surface areas between the WT and ΔLTA powders because the conformations of the surfaces were very similar. The powders were shaken in water for 30 min, filtered, dried, and reevaluated by SEM (**Fig.1** for ΔLTA powder). No changes in shape of the powders were observed after shaking in aqueous solution for 30 min.

Removal of rare earth metal ions by WT or ΔLTA powder

The adsorptive removals of La(III), Eu(III), Tm(III), Mn(II), and Fe(II) by the cell walls were studied in order to assess the extraction ability of the *B. subtilis* powders. The results are shown in **Table 3**.

The removal (%) of rare earth ions from aqueous solution (20 $\mu g \cdot mL^{-1}$) with WT or ΔLTA powder as the adsorbent were > 60% (entries 1, 2, and 4 in Table 3). On the other hand, the removal (%) of Fe(II) and Mn(II) were < 24% (entries 7 and 8). The percentage of Tm(III) removed was higher than the percentage of Fe(II) removed from the Fe(II) and Tm(III) binary solution. This suggests that Tm(III) was selectively removed by the *B. subtilis* powders (entries 9 and 10). These results indicate that the *B. subtilis* powders are effective adsorbents for rare earth ions.

The removal (%) of rare earth ions increased in the order of atomic number (La(III) < Eu(III) < Tm(III), entries 1, 2, and 4). As atomic number increases, the radius of each lanthanide ion (III) steadily decreases (lanthanide contraction), and electron affinity increases. Because of this feature of rare earth ions, the adsorptive association of heavy rare earth ions to the adsorbents would be greater

than that of light rare earth ions. Takahashi *et al.*(2010) have reported similar patterns of adsorption of rare earth ions by cell walls of gram-positive bacteria.

The removal (%) of metal ions by WT powder was greater than by ΔLTA powder. This result indicates that lipoteichoic acid contributes to the adsorption of rare earth ions onto the cell wall of *B. subtilis*. Furthermore, without LTA, the removal (%) of the rare earth ions was greater than that of Fe(II) and Mn(II) at pH 3. This indicates that WTA contributes to the adsorption of rare earth ions onto cell walls. It is well known that the cell wall of *B. subtilis* consists of peptidoglycan (65%) and teichoic acid (35%). However, the abundance ratios of LTA and WTA in *B. subtilis* 168 have not been clarified. Therefore, the contribution ratio to the adsorption of rare earth ions by LTA and WTA could not be quantified at this stage.

The effect of changing the duration of shaking on the adsorption of Tm(III) by the *B. subtilis* powders was studied in order to determine the equilibration time for adsorption (**Fig. 2**). The percentages of Tm(III) removed were 91 and 74% for WT and ΔLTA powders, respectively, and barely changed by varying the duration of shaking from 1 to 300 min. These results suggest that this method of adsorption does not require long equilibration times, and can therefore be used for the rapid removal of Tm(III) from water. The rapid removal of Tm(III) by the *B. subtilis* powders may be related to their high surface area per unit weight.

Next, the recovery of adsorbed Tm(III) by the powders was observed. WT or ΔLTA powder (20 mg) was added to 20 mL of La(III), Eu(III) or Tm(III) solution (20 $\mu g \cdot mL^{-1}$, pH 3), mixed for 30 min at 10 rpm and filtered with 5A filter paper. The used filter was eluted by nitric acid (2.7 M, 20 mL, 2 times), and the concentrations of rare earth ions in the eluates were analyzed. The total recoveries of adsorbed rare earth ions were > 94% for both WT and ΔLTA powders. This result indicates that rare earth ions in water can be extracted by adsorption to the powders and eluted with nitric acid.

Coagulation sedimentation of ΔLTA powder

The ΔLTA powder readily coagulated and sedimented in the presence of rare earth ions at 20 μg·mL^{-1} (**Fig. 3**). On the other hand, such coagulation and sedimentation did not occur with WT powder and rare earth ion aqueous solution under the same conditions. The times required for the filtration of the test solution (20 mL) by 5A filtration paper were 105 and 43 seconds for the WT powder and ΔLTA powder, respectively. This result suggests that ΔLTA powder is useful for the extraction of rare earth ions from water. The ΔLTA powder coagulates and sediments in the presence of Tm(III) at 1.0-200 μg·mL^{-1}.

Discussion

La(III), Eu(III), and Tm(III), but not Fe(II) or Mn(II), were removed effectively from water by WT and ΔLTA powder. It has been reported that cadmium ion adsorb preferentially onto phosphate groups of the *B. subtilis* cell walls at pH values lower than 4.5, and that the contribution of carboxylate groups increases as pH increases (Boyanov et al. 2003). In the present study, the contribution of phosphate groups to the adsorption of rare earth ions would be high because the adsorption activity was studied at pH 3. The unique properties of rare earth ions are their strong Lewis acidity and their affinity toward hetero atoms, such as oxygen. It is well known that rare earth ions interact with phosphate groups. It is thought that the adsorption of rare earth ions onto the oxygen atom of the phosphate group is more powerful than that of Fe(II) or Mn(II). That would cause the selective adsorption of rare earth ions onto the cell walls. When the adsorption of Mn(II) to the WT powder was studied at pH 5, the carboxyl group was easily ionized, and the removal (%) of Mn(II) was 75%. This result supports the ideas described above.

In order to evaluate the adsorption capacities the *B. subtilis* powders for Tm(III), the obtained adsorption data were fitted to the Langmuir and Freundlich adsorption isotherm equations. The adsorption of Tm(III) onto the WT or ΔLTA powder powders could be fitted well to Langmuir as well as Freundlich models (all r^2 values were > 0.97), and the adsorption mechanism of Tm(III) to the powders could not be discussed by using these adsorption isotherm equations. Here, we focused on the Langmuir constants of the *B. subtilis* powders. The parameters obtained from the Langmuir isotherm of the *B. subtilis* powders are summarized in **Table 4** along with the parameters obtained from the Langmuir isotherm of adsorbents, which have been previously reported. The *B. subtilis* powders had a maximum uptake capacity (q_e) of 43.1 and 36.9 $mg \cdot g^{-1}$ for WT powder and ΔLTA powder, respectively. The maximum uptake capacities of the *B. subtilis* powders for Tm(III) were comparable to those of other adsorbents for rare earth ions listed in **Table 4**. The K_L value of the *B. subtilis* powders for Tm(III) was also similar to those of the other adsorbents.

The difference of the coagulation and sedimentation properties between the WT powder and ΔLTA powder could be caused by the different distribution of teichoic acids. It is reported that LTA is a major component of the *B. subtilis* periplasm (Matias and Beveridge 2008), and it is believed that LTA localizes at the inner site of cell walls compared to WTA. In the case of ΔLTA powder, rare earth ions are adsorbed to WTA, which is located on the outer surface of the cell wall. That causes the interaction between ΔLTA powders to increase, and the aggregation to accelerate. On the other hand, in the WT powders, the rare earth ions adsorb to WTA and LTA. Therefore, compared to ΔLTA powder, coagulation sedimentation of WT powder is not likely to occur. The aggregation of WT powder occurred in solutions with Tm(III) concentrations greater than 50 $\mu g \cdot mL^{-1}$. This result supports the proposed mechnaism of the aggregation of the ΔLTA powder at lower levels of rare earth ions compared to the WT powder.

In conclusion, a novel application for the cell walls of wild type and lipoteichoic acid-defective

strains of *B. subtilis* 168 has been presented in this study. The procedure of culturing these *B. subtilis* strains has been well established, and it is not difficult to produce the powders described herein as adsorbent materials.

The comparison between the powders obtained from these *B. subtilis* strains revealed that lipoteichoic acid contributes to the adsorption of rare earth ions onto the cell walls of *B. subtilis*. In addition, we showed the novel finding, that in the presence of rare earth ions, the ΔLTA powder coagulates and sediment more easily than the WT powder.

It is anticipated that the *B. subtilis* powders could be useful not only for the removal of rare earth ions from environmental or waste water, but also for their extraction as valuable resources. The powders make several potential contributions to the development of water cleanup technology and environmental sciences.

The next step of this work is to investigate the potential of the cell walls of wall-teichoic acid -defective strains as adsorbents for rare earth ions and to understand the contribution of wall-teichoic acid to the adsorption of rare earth ions onto the cell wall.

Acknowledgement This work was supported by Shinshu University Satellite Venture Business Laboratory (SVBL). We wish to thank Mrs. Keiko Kakegawa (SVBL) for her generous SEM measurement. A part of this work was supported by Grants-in-Aid for Scientific Research (C) (19580085 and 23580107) and grants from the Kurata Memorial Hitachi Science and Technology Foundation, the Nagase Science and Technology Foundation, and the Research Foundation for the Electrotechnology of Chubu to H.Y.

References

Anagnostopoulos C, Spizizen J (1961) Requirements for transformation in *Bacillus subtilis*. J.

Bacteriol. 81: 741-746

Andrianov AV, Savel'eva OA, Bauer E, Staunton JB (2011) Squeezing the crystalline lattice of the heavy rare earth metals to change their magnetic order: Experiment and *ab initio* theory. Phys. Rev. B. 84: 132401

Arnaud M, Chastanet A, Débarbouillé M (2004) New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, Gram-positive bacteria. Appl. Environ. Microbiol. 70: 6887-6891

Basu A, Chakrabarty K, Chatterjee GC (1982) Neurotoxicity of lanthanum chloride in newborn chicks. Toxicol. Lett. 14: 21-25

Boyanov MI, Kelly SD, Kemner KM, Bunker BA, Fein JB, Fowle DA (2003) Adsorption of cadimium to Bacillus subtilis bacterial cell walls: a pH-dependent X-ray absorption fine structure spectroscopy study. Geochim. Cosmochim. Acta 67: 3299-3312

Buchmeiser MR, Tessadri R, Seebar G, Bonn GK (1998) Selective extraction of rare earth elements from rocks using a high-capacity cis-1,4-butanedioic acid functionalized resin. Anal. Chem. 70: 2130-2136

Chen Y, Zhu B, Wu D, Wang Q, Yang Y, Ye W, Guo J (2012) Eu(III) adsorption using di(2-thylhexly)phosphoric acid-immobilized magnetic GMZ bentonite. Chem. Eng. J. 181-182: 387-396

Du X, Graedel TE (2011) Global in-use-stocks of the rare earth elements: A first estimate. Environ. Sci. Technol. 45: 4096-4101

Ghimire KN, Inoue K, Yamaguchi H, Makino K, Miyajima T (2003) Adsorptive separation of arsenate and arsenite anions from aqueous medium by using orange waste. Water Res. 37: 4945-4953

Hang Y, Qin Y, Jiang Z, Hu B (2002) Direct analysis of trace rare earth elements by fluorination

assisted ETV-ICP-AES with slurry sampling through nano-sized TiO_2 separtion/preconcentration. Anal. Sci. 18: 843-846

Liu Z, Byrne RH (1998) Comprehensive investigation of yttrium and rare earth element complexation by carbonate ions using ICP-mass spectrometry. J. Solution Chem. 27: 803-815

Matias VRF, Beveridge TJ (2008) Lipoteichoic acid is a major component of the *Bacillus subtilis* periplasm. J. Bacteriol. 190: 7414-7418

Merroun ML, Chekroun KL, Arias JM, González-Munoz MT (2003) Lanthanum fixation by *Myxococcus xanthus*: cellular location and extracellular polysaccharide observation. Chemosphere 52: 113-120

Molander GA, Romero JAC (2002) Lanthanocene catalysts in selective organic synthesis. Chem. Rev. 102: 2161-2185

Moriwaki H, Kitajima S, Kurashima M, Hagiwara A, Haraguchi K, Shirai K, Kanekatsu R, Kiguchi K (2009) Utilization of silkworm cocoon waste as a sorbent for the removal of oil from water. J. Hazard. Mater. 165: 266-270

Neuhaus FC, Baddiley J (2003) A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. Microbiol. Mol. Biol. Rev. 67: 686

Ozaki T, Suzuki Y, Nankawa T, Yoshida T, Ohnuki T, Kimura T, Francis AJ (2006) Interactions of rare earth elements with bacteria and organic ligands. J. Alloys Compd. 408-412: 1334-1338

Pałasz A, Czekaj P (2000) Toxicological and cytophysiological aspects of lanthanides action. Acta Biochim. Polonica 47: 1107-1114

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press

Schirner K, Marles-Wright J, Lewis RJ, Errington J (2009) Distinct and essential morphogenic functions for wall- and lipoteichoic acids in *Bacillus subtilis*. EMBO J. 8: 830-842

Senthilkumaar S, Krishna SK, Kalaamani P, Subburamaan CV, G.-Subramaniam N (2010) Adsorption of organophosphorous pesticide from aqueous solution using “waste” Jute fiber carbon. Modern Appl. Sci. 4: 67-83

Sert Ş, Kütahyali C, İnan S, Talip Z, Çetinkaya B, Eral M (2008) Biosorption of lanthanum and cerium from aqueous solutions by *Platanus orientalis* leaf powder. Hydrometallurgy 90: 13-18

Sun X, Luo H, Dai S (2012) Solvent extraction of rare earth ions based on functionalized ionic liquids. Talanta 90: 132-137

Takahashi Y, Châtellier X, Hattori KH, Kato K, Fortin D (2005) Adsorption of rare earth elements onto bacteria cell walls and its implication for REE sorption onto natural microbial mats. Chem. Geol. 219: 53-67

Takahashi Y, Yamamoto M, Yamamoto Y, Tanaka K (2010) EXAFS study on the cause of enrichment of heavy REE on bacterial cell surfaces. Geochim. Cosmochim. Acta 74: 5443-5462

Tong SL, Zhu WZ, Gao ZH, Meng YX, Peng RL, Lu GC (2005) Distribution characteristics of rare earth elements in children’s scalp hair from a rare earths mining area in southern china. J. Envoron. Sci. Health A 39: 2517-2532

Vagner V, Dervyn E, Ehrlich D (1998) A vector for systematic gene inactivation in *Bacillus subtilis*. Microbiology 144: 3097-3104

Figure captions

Fig.1 Scanning electron micrographs of ΔLTA powders after shaking in water for 30 minutes followed by drying.

Fig. 2 Effect of duration of shaking on Tm(III) removal (%) by WT and ΔLTA powders.

(WT powder: □; ΔLTA powder: ○; Initial Tm(III) concentration: 20 μg·mL^{-1}; pH=3; adsorbent load: 20 mg/20 mL)

Fig.3 Photograph of the solution containing Tm(III) and the *Bacillus subtilis* cell powders (right: WT; left: ΔLTA) after shaking for 30 minutes.

(Initial Tm(III) concentration: 20 $\mu g \cdot mL^{-1}$; pH= 3; adsorbent loading: 20 mg/20 mL)

Table 1. Bacterial strains and plasmids used in this study.

Strain and plasmid	Relevant genotype	Source* or Reference
Bacillus subtilis		
168	*trpC2*	S. D. Ehrlich
ΔyvgJ	*trpC2* Δ*yvyJ*	pMADΔyvgJ→168
RY1602	*trpC2* Δ*yvyJ* Δ*yqgS*	pMADΔyqgS→ΔyvgJ
RY1603	*trpC2* Δ*yvyJ* Δ*yqgS* Δ*yfnI*	pMADΔyfnI→RY1602
LTASp	*trpC2 ltaS'*::*lacZ lacI bla ermC* P_{spac}-*ltaS*	pM4ΔNyflE→168
RY1604	*trpC2* Δ*yvyJ* Δ*yqgS* Δ*yfnI ltaS'*::*lacZ lacI bla ermC* P_{spac}-*ltaS*	LTASp→RY1603
Escherichia coli		
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi-1* Δ(*lac-proAB*) /F' [*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	TaKaRa
C600	*supE44 hsdR17 thi-1 thr-1 leuB6 lacY1 tonA21*	Laboratory stock
Plasmids		
pMAD	*bla ermC bgaB*	Arnaud et al., 2004
pMADΔyfnI	*bla ermC bgaB* Δ*yfnI*	This study
pMADΔyqgS	*bla ermC bgaB* Δ*yqgS*	This study
pMADΔyvgJ	*bla ermC bgaB* Δ*yvgJ*	This study
pMUTIN4	*lacZ lacI bla ermC*	Vagner et al., 1998
pM4ΔNyflE	pMUTIN4::Δ*ltaS* (containing *ltaS* Shine-Dalgarno sequence)	This study

* : Sources shown before and after the arrows indicate donor DNA and recipient cells on transformation, respectively.

Table 2. Primers used in this study.

Primer	Sequence(5'→3') *	Restriction site
yfnIf-Bf	gccggatccTACGCTAAGGATGTCTGG	*Bam*HI
yfnIf-PEr	gcgaattcctgcagCAGTATAACAGCCAGCAC	*Eco*RI
yfnIb-ESf	gcgaattcccgggCCGTTGATCCGTCAGAC	*Eco*RI
yfnIb-NKr	gccggtaccatggTCAGGCTGTTGTGCTTCC	*Nco*I
YfnI-up	GGCTGAGACATCTGCAG	
YfnI-down	CCCAGCATCTGTACCTC	
yqgSf-Bf	gccggatccTGTCAGCGCGAGATGTG	*Bam*HI
yqgSf-PEr	gcgaattcctgcagATACGTTTTCAGCCACATC	*Eco*RI
yqgSb-ESf	gcgaattcccgggACCAGAAAAGGCTGGAC	*Eco*RI
yqgSb-NKr	gccggtaccatggCTTCAGCTCCCAGATAGG	*Nco*I
YqgS-up	TGCTAAAAAGACGACACG	
YqgS-down	TGACGCGTTCATATGGAC	
yvgJf-Bf	gccggatccATACCCGATATCTGCCAC	*Bam*HI
yvgJf-Pr	gcgctgcagCATAAAAACCGCTGATTGTG	*Pst*I
yvgJb-PSf	cggctgcaggcctAATGGTGACCTGCTCAGG	*Pst*I
yvgJb-KNr	gccggtaccatggATCAATGTCGTTCTCCGC	*Nco*I
YvgJ-up	GCTTATGGCTGCGTCAG	
YvgJ-down	AAACGGATTTTGACAGGTG	
yflE-SD-EF	gcgcgaattcCGTTTCGCTCGAACTGGAT	*Eco*RI
yflE-p2-BR	gcgcggatccTGTAGTACACAATGTTGG	*Bam*HI

* : The additional sequence (lowercase) and restriction site (under line) are indicated.

Table 3 Removal (%) of metal ions from water[a] using the *Bacillus subtilis* powders

Entry	Adsorbate	Initial conc. ($\mu g\ mL^{-1}$)	Removal (%)	
			WT powder	ΔLTA powder
1	La(III)	20	78	62
2	Eu(III)	20	89	74
3	Tm(III)	10	96	95
4	Tm(III)	20	90	74
5	Tm(III)	50	59	46
6	Tm(III)	100	35	29
7	Fe(II)	20	24	17
8	Mn(II)	20	24	17
9	Tm(III) + Fe(II)	20 ea.	89(Tm), 5.3 (Fe)	76(Tm), 8.1 (Fe)
10	Tm(III) + Fe(II)	50 ea.	54(Tm), 3.3 (Fe)	43(Tm), 4.0 (Fe)

[a] The pH value of the test solution was set at pH 3. The adsorbent (20 mg) was added to the aqueous solutions (20 mL).

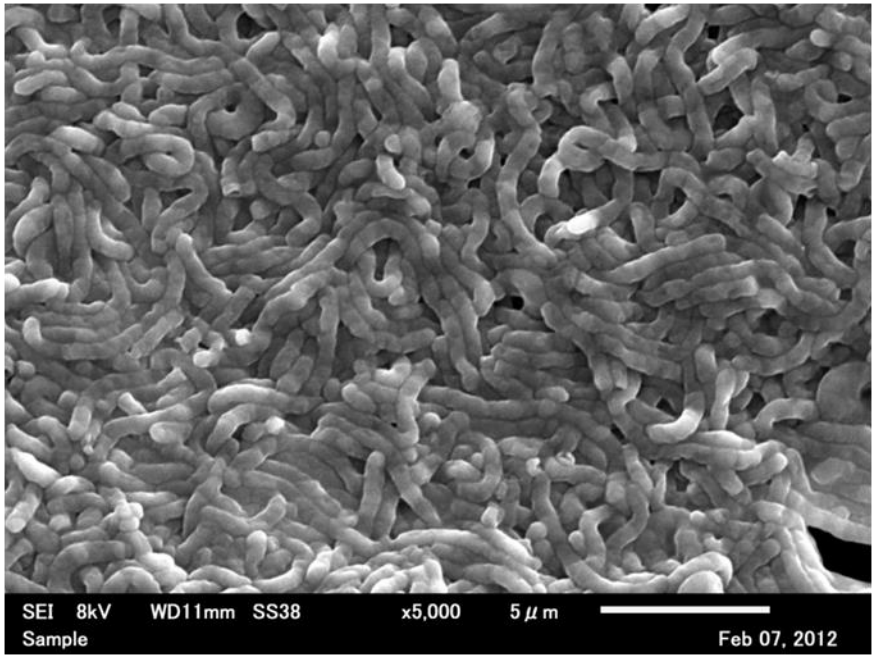

Fig.1

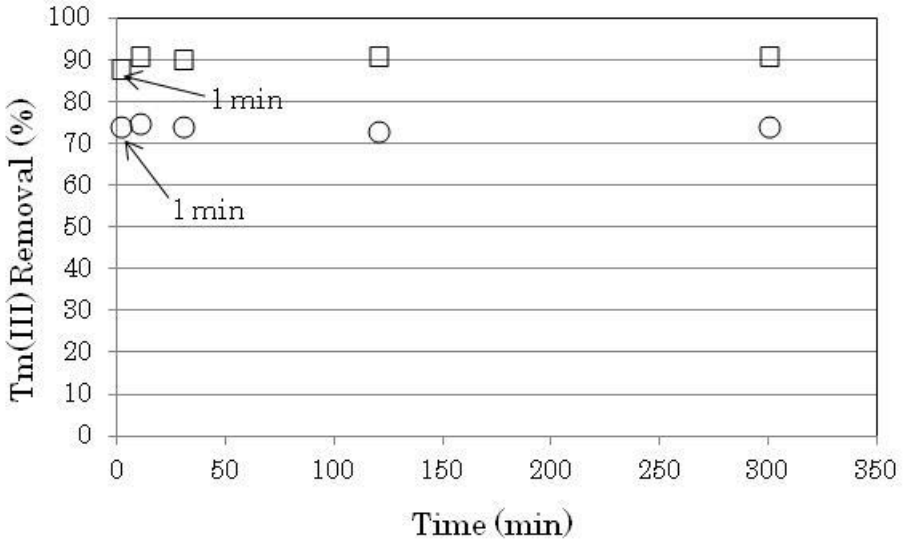
100
90
80
70
60
50
40
30
20
10
0
Tm(III) Removal (%)
1 min
1 min
0
50
100
150
200
250
300
350
Time (min)

Fig. 2

Fig. 3