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Arsenic resistance and removal by marine and non-marine bacteria

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Abstract

Arsenic resistance and removal was evaluated in nine bacterial strains of marine and non-marine origins. Of the strains tested, *Marinomonas communis* exhibited the second-highest arsenic resistance with median effective concentration (EC_{50}) value of 510 mg As l^{-1}, and was capable of removing arsenic from culture medium amended with arsenate. Arsenic accumulation in cells amounted to 2290 μg As g^{-1} (dry weight) when incubated on medium containing 5 mg As l^{-1} of arsenate. More than half of the arsenic removed was related to metabolic activity: 45% of the arsenic was incorporated into the cytosol fraction and 10% was found in the lipid-bound fraction of the membrane, with the remaining arsenic considered to be adsorbed onto the cell surface. Potential arsenic resistance and removal were also examined in six marine and non-marine environmental water samples. Of the total bacterial colony counts, 28–100% of bacteria showed arsenic resistance. Some of the bacterial consortia, especially those from seawater enriched with arsenate, exhibited higher accumulated levels of arsenic than *M. communis* under the same condition. These results showed that arsenic resistant and/or accumulating bacteria are widespread in the aquatic environment, and that arsenic-accumulating bacteria such as *M. communis* are potential candidates for bioremediation of arsenic contaminated water.

Keywords: Arsenic; Removal; Accumulation; Resistance; Bioremediation; Bacteria

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1. Introduction

Arsenic, which is derived from both natural geothermal and anthropogenic sources, is widely distributed in the environment (Smedley and Kinniburgh, 2002). Due to its toxicity to humans, arsenic causes serious health problems in many parts of the world (Mushtaque and Chowdhury, 2004). In addition to arsenic, several other metals and metalloids are widely distributed as pollutants in environments such as groundwater and soils, and removal of these metals has become essential for keeping drinking water safe and preserving aquatic environments. In general, metals in contaminated-water are removed by methods such as chemical precipitation, ion-exchange or adsorption. However, these methods have disadvantages such as high cost and a generation of the secondary contaminants. Recent recognition of the need to develop low cost environmentally friendly technologies for water treatment has stimulated interest in studies on the bioremediation of metals (Clausen, 2000; Srinath et al., 2002; Tsuruta, 2004). Bacteria capable of removing arsenic from their surroundings could thus be ideal candidates for bioremediation, and could therefore be used as an alternative or to supplement existing physico-chemical methods of arsenic removal. However, except for some genetically engineered microorganisms (Sauge-Merle et al., 2003; Kostal et al., 2004), no bacteria that could be applied for this purpose have been found to date.

Metal-accumulating bacteria are often found among metal-resistant bacteria (Pümpel et al., 1995; Srinath et al., 2002; Hussein et al., 2005). Although arsenic is toxic to many bacteria, some bacteria are resistant to arsenic either due to the presence of a strictly phosphate-specific transport system, which prevents the uptake of arsenate which is analogous to phosphate (Willsky and Malamy, 1980), or due to an efflux system mediated by the plasmid- or chromosomally-encoded *ars* operon (Cervantes et al., 1994; Diorio et al., 1995; Cai et al., 1998). In the above-mentioned arsenic-resistant systems, arsenic is not accumulated in the bacterial cells. Recently, existence of a yet unknown arsenic resistance system has been suggested (Cai et al., 1998). It has been argued that arsenic resistance could be acquired as a result of exposure to high concentrations of arsenic in a specific environment. Zelibor et al. (1987) found that arsenic-resistant population correlated positively with the ambient arsenic concentration in arsenic-contaminated groundwater. Arsenic-resistant bacteria have been isolated from arsenic rich environments such as arsenic contaminated soils (Turpeinen et al., 2004) or hydrothermal vent (Jeanthon and Prieur, 1990). However, recent studies revealed large proportions of arsenic-resistant bacteria (7–50% of the total viable count) in arsenic free soils (Jackson et al., 2005). These suggest a variety of arsenic-resistant system and wide distribution of arsenic-resistant bacteria in the environment.

Although it is known that a variety of marine organisms such as fish, invertebrates and algae accumulate arsenic mainly as organo-arsenicals, the reason underlying this bioaccumulation is not yet clear (Francesconi and Edmonds, 1998). However, the accumulation of arsenic by a number of marine organisms suggests that they may have an affinity for this element for some biological purposes, indicating that the marine environment may harbor arsenic resistant and/or arsenic accumulating bacteria. However, to date, very few studies have focused on marine bacteria within this context.

The aim of the present study was to investigate arsenic resistance and removal by representative pure cultures and bacteria in environmental water samples from marine and non-marine environments.

2. Materials and methods

2.1. Arsenic resistance and arsenic removal by bacterial strains

Bacterial strains were obtained from the National Collection of Industrial and Marine Bacteria Ltd. (NCIMB, Aberdeen, Scotland), and the Institute of Molecular and Cellular Biosciences (IAM), the University of Tokyo (Tokyo, Japan). The bacterial strains used in this study included *Vibrio alginolyticus* NCIMB 1903, *Halomonas marina* IAM 14107, *Alteromonas macleodii* IAM 12920 and *Marinomonas communis* IAM 12914 as representatives of marine bacteria, and *Methylosinus trichosporium* OB3b NCIMB 11131, *Escherichia coli* IAM 12119, *Pseudomonas aeruginosa* IAM 1514, *Bacillus subtilis* IAM 12118 and *Rhodococcus equi* IAM 12426 as representatives of

non-marine bacteria. Among the marine strains, *V. alginolyticus* was grown in medium 209 (NCIMB Ltd., 2002), while the other marine strains were grown in Marine broth (Difco 2216). Among the non-marine strains, *M. trichosporium* OB3b was grown in nitrate mineral salts (NMS) medium (NCIMB Ltd., 2002) under a methane–air (20:80) atmosphere, while the other non-marine strains were grown in B-1 medium (IAM, 1998). All strains were incubated at 30 °C with shaking at 120 rpm.

As arsenate, sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) was purchased from Wako Pure Chemical Industries (Osaka, Japan), and a stock solution (50,000 mg As l^{-1}) was prepared by dissolving it in distilled water, sterilized by filtration through a membrane filter (0.22 μm, Nuclepore Corp., CA, USA) and then used to add to the medium. Each culture medium was amended with arsenate to give final concentrations of 0, 5, 50 and 250 mg As l^{-1}. Because no growth inhibition for *V. alginolyticus* was observed even at 250 mg As l^{-1}, higher concentrations of arsenate (500 and 750 mg As l^{-1}) were also tested for this strain. Bacterial growth was monitored by measuring the optical density (OD) of the cultures at 660 nm using a spectrophotometer (UV-1700, Shimadzu, Japan). All incubations were stopped when the culture without arsenate reached stationary phase. As an indicator of arsenic resistance, median effective concentration (EC_{50}, mg As l^{-1}) was calculated from the final OD values by probit analysis.

Arsenic removal was assessed by monitoring the decrease in the arsenic concentration of the culture medium after incubation. Before and after incubation, subsamples were taken and filtered with a membrane filter (0.22 μm, Nuclepore Corp., CA, USA). Total arsenic concentrations in the filtrates were determined with inductively coupled plasma mass spectrometry (ICP-MS, ICPM-8500, Shimadzu, Kyoto, Japan). Arsenic removal was observed for *M. communis*. Therefore, arsenic removal at lower concentration of arsenate (0.07 mg As l^{-1}) was also tested for this strain. The arsenic concentrations in *M. communis* cells incubated on a medium amended with 5 mg As l^{-1} arsenate were quantitatively measured. Bacterial cells were incubated in 300 ml medium in triplicate with shaking at 100 rpm and harvested at 5000 rpm for 20 min at 4 °C. Prior to ICP-MS analysis, the harvested cells were washed once with artificial seawater (Kester et al., 1967) and twice with deionized water, dried at 100 °C in an oven, weighed, and then digested with nitric acid.

2.2. Secondary ion mass spectrometry (SIMS) analysis of *M. communis* cultured with arsenic

To confirm arsenic removal by *M. communis*, cells incubated with arsenate (5 mg As l^{-1}) were harvested and washed as described above. For comparison, and to search for more arsenic-accumulating bacteria, six arsenic-resistant bacteria were isolated from a soil sample taken from Ibaraki Prefecture, Japan. Arsenic-resistant bacteria were isolated with a heterotrophic medium (0.5 g peptone, 0.1 g yeast extract, 0.1 g iron citrate, and 5 g agar in 950 ml deionized water and 50 ml aged seawater (coastal seawater stored in the dark for more than 1 month)) amended with arsenate (250 mg As l^{-1}). Each strain was processed similarly to *M. communis*. After washing with deionized water, approximately 10 μl of each cell suspension was dropped onto a silicone wafer, air-dried, and coated with gold (30 nm thickness) for semi-quantitative analyses of arsenic by secondary ion mass spectrometry (CAMECA IMS-1270 at the Geological Survey of Japan, AIST, Ibaraki, Japan). Positive secondary ions of $^{12}C^+$ and $^{75}As^+$ were obtained by sputtering samples with O^- primary ion (25 μm beam, 1 nA). Several other ions (^{23}Na, ^{24}Mg, ^{28}Si, ^{31}P, ^{32}S, ^{39}K, ^{40}Ca and ^{56}Fe) were simultaneously monitored together by a magnetic field scan and a growth of ^{28}Si signal from base Si-wafer was used as an indicator of sputtering through the bacteria samples. Mass resolving power of SIMS was first set to 4500 and the samples that exhibited arsenic-like signals were further analyzed with an increased mass resolving power of 8000 in order to accurately separate molecular interferences in $^{75}As^+$ peak. Other analytical conditions used were similar to Kita et al. (2004) who studied trace element analyses of silicate minerals. Since a suitable standard with known compositions of As and similar major elements in bacteria samples was not available, it was not possible to obtain an absolute concentration of As in samples. Therefore, we were only able to compare relative $^{75}As/^{12}C$ signal ratios among different bacteria to evaluate which bacteria had more potential in removing arsenic.

2.3. Fractionation of arsenic in M. communis

M. communis incubated with arsenate (5 mg As l^{-1}) was harvested and washed as described above, and then used for fractionation analysis, the details of which are given below.

2.3.1. Cytosol and membrane fractions

Bacterial cells incubated with arsenate were disrupted with a sonifier (S-250, Branson Ultrasonics, CT, USA) for 6 min at a duty cycle of 50%. Cells remained undisrupted were removed by centrifugation at 10,000 rpm for 10 min. The supernatant was centrifuged at 80,000 rpm for 20 min to separate cytosol and membrane fractions. The cytosol fraction was obtained as supernatant; a part of this fraction was used to determine total arsenic concentration by the ICP-MS, while another part of this fraction was used for arsenic speciation analysis. Membrane fraction was digested with nitric acid and total arsenic concentration was measured with the ICP-MS.

2.3.2. Arsenic speciation analysis in cytosol fraction and culture broth

A portion of the cytosol fraction and the filtrated culture broth after incubation with 5 mg As l^{-1} of arsenate were used for arsenic speciation analysis by high performance liquid chromatography-ICP-MS (HPLC-ICP-MS), the details of which have been described previously (Takeuchi et al., 2005).

2.3.3. Water-soluble and lipid-bound fraction

The modified Bligh and Dyer method (Bligh and Dyer, 1959) was used to separate water-soluble and lipid-bound arsenic in the cell. To the harvested cells incubated in presence of arsenate (5 mg As l^{-1}), 20 ml of methanol, 10 ml of chloroform and 8 ml of distilled water were added; the mixture was sonicated for 10 min, and then left undisturbed for 3 h. Next, chloroform and distilled water (10 ml each) was added, and the mixture was allowed to settle for 24 h. The extract was filtrated with No. 5B filter (Advantec, Tokyo, Japan), and a separating funnel was used to separate methanol–water phase from the chloroform phase. Each phase was centrifuged at 5000 rpm for 20 min, and the supernatant was dried and digested with nitric acid, and then total arsenic concentration was measured with the ICP-MS.

2.4. Physico-chemical adsorption of arsenate and arsenite

Physico-chemical adsorption of arsenate and arsenite to the cell surface of *M. communis* was determined. As arsenite, sodium arsenite ($NaAsO_2$) was purchased from Wako Pure Chemical Industries (Osaka, Japan). A stock solution (50,000 mg As l^{-1}) was prepared in distilled water, which was stored at −20 °C until use. Overall removal of arsenic which includes both adsorption and accumulation was measured with cells incubated with arsenate (5 mg As l^{-1}) until the OD of the culture reached 0.6. For the measurement of physico-chemical adsorption, *M. communis* was grown to the stationary phase without arsenic and harvested. Half of the harvested cells were treated at 100 °C for 10 min (heat-killed cells). Since the heat treatment may change the property of the cell surface, both the live and the heat-killed cells were examined. The live and heat-killed cells were resuspended in media amended with either arsenate (5 mg As l^{-1}) or arsenite (5 mg As l^{-1}), at the OD of the culture 0.6, and reacted with shaking at 100 rpm for 4 h. Cells were harvested, and the arsenic concentrations in the cells were measured with the ICP-MS.

2.5. Arsenic resistance and removal by bacteria in the environmental samples

The distribution of arsenic (arsenate)-resistant bacteria was determined in two freshwater, one brackish water and three seawater samples. Coastal seawater samples were collected from the Ibaraki and Okinawa Prefectures and Tokyo, Japan. Freshwater samples were collected from the Lake Kasumigaura in Ibaraki Prefecture, Japan and the Lake Inawashiro in Fukushima Prefecture, Japan. Brackish water (with small effect of seawater, showing electronic conductivity of 837 μS cm^{-1}) was taken from the Lake Jyusan in Aomori Prefecture, Japan. Samples filtered with No. 5B filter papers were used for viable counts on heterotrophic agar plates (0.5 g peptone, 0.1 g yeast extract, 0.1 g iron citrate, and 5 g agar in 950 ml deionized water and 50 ml aged seawater) amended with arsenate to yield concentrations of 0, 1, 5, 50 and 250 mg As l^{-1} each prepared in triplicate. In the case of seawater samples, 25 g NaCl and 2 g $MgSO_4 \cdot 7H_2O$ were also added to the medium. Plates

were aerobically incubated at 25 °C. The total number of viable bacteria and the number of arsenic-resistant bacteria were determined by counting the number of colonies formed during 2 weeks of incubation.

Water samples were also used to determine the potential of bacterial arsenic removal. Samples were filtered with No. 5B filter papers to remove most of the protists, and inoculated in 300 ml of heterotrophic liquid medium described above but without agar, amended with arsenate (1 mg As l^{-1}) in triplicate, and incubated at 25 °C for 2–3 days. Bacterial cells were harvested by centrifugation at 5000 rpm for 20 min at 4 °C. Bacterial cells from seawater samples were washed once with artificial seawater and twice with deionized water, while cells from fresh and brackish water were washed three times with deionized water. The washed cells were processed to measure arsenic concentrations. Arsenic removal by *M. communis* cultured with the same concentration of arsenate (1 mg As l^{-1}) was similarly evaluated for a comparison.

3. Results

3.1. Arsenic resistance of bacterial strains

Arsenic resistance of each strain was exhibited as EC_{50} calculated by probit analysis. The EC_{50} of nine strains was calculated to be in a 20–730 mg As l^{-1} range (Table 1). Chromosomal *ars* operon has been reported for *E. coli*, *P. aeruginosa* and *B. subtilis* (Diorio et al., 1995; Cai et al., 1998; Mukhopadhyay et al., 2002). Therefore, these three strains are designated as arsenic-resistant strains. *R. equi* and *M. trichosporium* showed higher EC_{50} than those of *E. coli* and *P. aeruginosa*, but lower than that for *B. subtilis*. All the marine strains (*Vibrio alginolyticus*, *Halomonas marina*, *Alteromonas macleodii* and *Marinomonas communis*) showed similar or higher arsenic resistance (EC_{50} of 210–730 mg l^{-1}) than arsenic-resistant strains.

Table 1
Median effective concentration (EC_{50}) calculated for bacterial strains by probit analysis

Strains	EC_{50} (mg As l^{-1})
Vibrio alginolyticus	730
Halomonas marina	310
Alteromonas macleodii	210
Marinomonas communis	510
Escherichia coli	20
Pseudomonas aeruginosa	100
Rhodococcus equi	140
Methylosinus trichosporium	150
Bacillus subtilis	220

Each strain was cultured with medium amended with arsenate at 0, 5, 50 and 250 (and 500 and 750 mg As l^{-1} for *V. alginolyticus*) mg As l^{-1}.

3.2. Arsenic removal by bacterial strains

Of nine bacterial strains examined, only *M. communis* showed significant decrease of arsenic concentration in the culture broth (Fig. 1). Percentage of arsenic removal at the end of the experiment (after all the cultures reached a stationary phase) was 3.5, 14.8, 16.1 and 15.5% when concentrations of added arsenate were 0.07, 5, 50 and 250 mg As l^{-1}, respectively. For cells incubated with 5 mg l^{-1} of arsenate, arsenic concentration in the cells was determined. The average value from two independent experiments each conducted in triplicate was 2290 ± 760 ($n = 6$) μg As g^{-1} dry weight of cells (dw). Arsenic removal by cells of *M. communis* was further confirmed by data from SIMS analysis. Eight other pure cultures tested for arsenic resistance and removal did not significantly remove arsenic (data not shown). Then, we attempted to isolate arsenic-resistant bacteria and test their ability to remove arsenic. Six arsenic-resistant strains were isolated from the soil with arsenate-amended medium

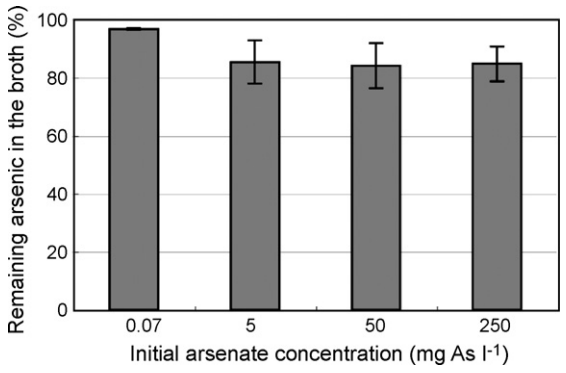

Fig. 1. Arsenic removal by *M. communis* grown with 0.07, 5, 50 and 250 mg As l^{-1} of arsenate. Remaining arsenic in the culture broth was calculated by considering the initial concentration to be 100%. Error bars show standard deviation of triplicates.

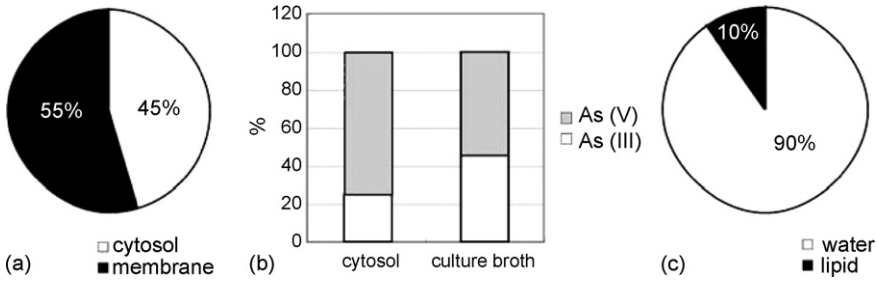

Fig. 2. Distribution of (a) total arsenic in the cytosol and membrane fractions, (b) arsenic species in the cytosol and the culture broth, (c) total arsenic in the water-soluble and lipid-bound fraction of *M. communis* cultured with 5 mg l^{-1} of arsenate.

and their arsenic removal was determined by SIMS. Although some strains showed arsenic-like signals with a mass resolving power of SIMS set at 4500, further accurate identification of ^{75}As with an increased mass resolving power of 8000 revealed that arsenic was detected only from *M. communis* with a relative $^{75}As/^{12}C$ signal ratio of 0.34. This value was at least 1–2 orders of magnitude higher than those of other strains, thus indicating the significant potential of *M. communis* for arsenic remediation. Since six arsenic-resistant strains isolated from soil did not show arsenic accumulation, microbiological characterization was not conducted for these isolates.

In *M. communis*, 45% of arsenic existed in the cytosol fraction, and 55% in the membrane-associated fraction (Fig. 2a). The amended arsenate was reduced to arsenite in the cytosol fraction and culture broth after incubation. Arsenite accounted for 25 and 45% of the total arsenic in the cytosol fraction and culture broth, respectively (Fig. 2b). No methylated forms of arsenic such as monomethyl arsonic acid, dimethyl arsinic acid and trimethylarsine oxide known to be produced by bacteria (Bentley and Chasteen, 2002) and often found in other marine organisms (Francesconi and Edmonds, 1998) were detected. Arsenic is known to exist as lipid-bound form in marine organisms probably because it acts as nitrogen and phosphorus analog (Morita and Shibata, 1990). Therefore, the ratio of water-soluble and lipid-bound arsenics was also determined. Of the total arsenic in the cell, water-soluble and lipid-bound arsenics accounted for 90 and 10%, respectively (Fig. 2c).

Physico-chemical adsorption of arsenate and arsenite onto the cell surface of *M. communis* was measured using live and heat-killed cells, and compared with the amount of arsenic removed by the cells incubated with arsenate. Adsorption was greater for arsenate than arsenite (Table 2). While the amount of arsenate adsorbed was 653 μg As g^{-1} dw for live-cells and 843 μg As g^{-1} dw for heat-killed cells, the amount of arsenite adsorbed was 119 μg As g^{-1} dw for live-cells and 81 μg As g^{-1} dw for heat-killed cells (Table 2). For both arsenate and arsenite, the differences in adsorption between live and heat-killed cells were not significant considering the standard deviation (Table 2), thus indicating that neither significant active uptake of arsenic took place during the adsorption experiment nor there was any effect of heat on the binding sites.

3.3. Potential of arsenic resistance and removal by bacteria in aquatic environments

Distribution of arsenic (arsenate)-resistant bacteria was determined in six marine and non-marine environmental samples using arsenate-amended agar plates. In general, the number of bacterial colony count decreased as arsenic concentration increased (Table 3). The number of colony forming units (CFUs) counted on agar

Table 2
Arsenic accumulated in the cell during growth, and arsenate (As(V)) and arsenite (As(III)) adsorbed onto live and heat-killed cells

	As (μg g dw^{-1})
Accumulated in the cell during growth	2290 (756)
Adsorbed onto live-cells, As(V)	653 (144)
Adsorbed onto heat-killed cells, As(V)	843 (58)
Adsorbed onto live-cells, As(III)	119 (33)
Adsorbed onto heat-killed cells, As(III)	81 (16)

Arsenic added was 5 mg As l^{-1} in all cases. Values in the parentheses show standard deviation between the triplicates.

Table 3
Number of arsenic (arsenate)-resistant bacteria (% of CFUs counted with arsenic free medium), and arsenic removal by bacterial consortium enriched from fresh, brackish and seawater samples with arsenate-amended medium (1 mg As l^{-1})

As (mg l^{-1})	Brackish	Fresh water		Seawater			*M. communis*
	Jyusan	Kasumigaura	Inawashiro	Tokyo	Okinawa	Ibaraki	
Number of arsenic-resistant bacteria (%)							
1	81	107	249	97	100	82	–
5	85	102	373	123	100	70	–
50	62	48	348	87	60	45	–
250	50	59	100	36	28	43	–
Arsenic removal (μg g^{-1} dw)	21 (3)	136 (65)	46 (10)	578 (414)	18 (14)	220 (81)	125 (118)

Arsenic concentration in *M. communis* cultured under the same conditions is also presented.

plates (% of CFUs counted with arsenic free medium) with 250 mg As l^{-1} of arsenate was 50–100% for fresh and brackish water samples, and 28–43% for seawater samples (Table 3).

Potential for arsenic removal was also examined for the bacterial consortia enriched from the above six environmental samples with arsenate (1 mg As l^{-1}). Arsenic concentrations in the bacterial consortia were 21–136 μg As g^{-1} dw of cells for fresh and brackish water and 18–578 μg As g^{-1} dw of cells for seawater samples (Table 3). Under the same growth condition (1 mg As l^{-1} of arsenate), *M. communis* accumulated arsenic at 125 μg As g^{-1} dw.

4. Discussion

The results demonstrated that *M. communis* is the first non-genetically engineered potent arsenic accumulating bacterium. When incubated with 5 mg As l^{-1} of arsenate, *M. communis* accumulated 2290 μg As g dw^{-1} of arsenic. This is the highest value ever reported in bacteria (110–765 μg As g dw^{-1}, Silver et al., 1981; Sauge-Merle et al., 2003; Kostal et al., 2004). The highest amount of arsenic accumulation reported in bacteria to date is 765 μg As g^{-1} dw for *E. coli* without *ars* operon. This was a result of the inefficiency of this bacteria to extrude arsenic. Other values were reported for engineered bacteria with the aim of enhancing arsenic accumulation. Kostal et al. (2004) engineered *E. coli* to overexpress ArsR, the regulatory protein of the *ars* operon that has a specific binding site available for arsenite (Kostal et al., 2004). Sauge-Merle et al. (2003) engineered *E. coli* to produce phytochelatin (PC) of *Arabidopsis thaliana* known to bind arsenite. Arsenic accumulation in the engineered cells from both of these studies was 110–173 μg As g^{-1} dw, which was markedly lower than that had accumulated in *M. communis*. Given the efficiency with which *M. communis* can accumulate arsenic, it is likely that the bacterium would be a strong candidate for bioremediation of arsenic-contaminated water.

Of the total arsenic removed, 45% was incorporated into the cytosol (Fig. 2a) and 10% incorporated as lipid-bound arsenic in the membrane (Fig. 2c). The rest of the arsenic (45%) was considered to be adsorbed onto the cell surface. Then, to confirm this, physico-chemical adsorption onto the cell surface was examined. Because nearly half of the arsenic was found as arsenite in the culture broth after incubation (Fig. 2b), adsorption was determined for both arsenate and arsenite. Adsorption of arsenite was smaller than adsorption of arsenate, and the maximum adsorption value of 843 μg As g^{-1} dw was obtained for arsenate (Table 2). This was within the range of estimated adsorption (45%, 1030 $\pm$ 340 μg As g^{-1} dw) calculated from the amount of arsenic accumulated by *M. communis* (2290 $\pm$ 760 μg As g^{-1} dw). Therefore, arsenic removal by *M. communis* was proved to be a result of both adsorption (45%) and active incorporation into the cell (55%). Although details of these mechanisms are not yet clear, accumulation of arsenic into the cell would be a result of higher uptake and lower efflux.

In this study, we employed SIMS analysis for screening of metalloid-accumulating bacteria for the first time. SIMS analysis has several advantages. We were able to circumvent labor intensive and time consuming work necessary in usual methods to quantify

metal concentrations in the cells which includes incubation in a large volume, harvesting of the cells, and digestion with acid and measurement. We were able to quantify carbon and arsenic simultaneously in a short time (approximately 10 min for one sample) with only a small amount of sample. Moreover, SIMS has a higher sensitivity (ppb level) compared to the other analytical equipment including the transmission electron microscope and energy dispersive X-ray spectroscopy (TEM-EDX). We could successfully detect arsenic accumulation by *M. communis* indicating that SIMS is an effective tool for screening metal(loid)-accumulating bacteria.

EC_{50} for the arsenic-resistant strains were 20–75 mg As l^{-1} (Table 1). Sensitivity for arsenic is reported to increase 10- to 100-fold in arsenic sensitive strains, mutants in the chromosomal *ars* operon (Diorio et al., 1995). Therefore, EC_{50} of 510 mg As l^{-1} observed for *M. communis* is significantly high suggesting an existence of a certain arsenic-resistant system such as *ars* operon. However, presence of *ars* operon in bacteria is known to extrude arsenate from the cell by an efflux system. Consequently, arsenic is not accumulated in bacteria (Cervantes et al., 1994; Mukhopadhyay et al., 2002). Moreover, the other arsenic-resistant system known to date, the phosphate-specific transport (Pst) system, would also lead to lower uptake of arsenate by the cells. Therefore, the present results obtained in *M. communis* with its higher resistance and higher accumulation of arsenic contradict the known arsenic-resistant systems, suggesting existence of an as yet unknown arsenic resistance system for this strain. Cai et al. (1998) also suggested the presence of a yet unknown arsenic resistance system from their observation of arsenic resistance in *Pseudomonas* strains without the *ars* operon. Further molecular and physiological studies on arsenic resistance in bacteria are necessary to increase our understanding.

Four common marine strains exhibited at least similar or higher EC_{50} than the arsenic-resistant strains (Table 1). Arsenic (arsenate)-resistant heterotrophs also comprised 28–43% of the total viable counts in marine environmental samples. This range is similar to that reported for one soil although arsenic concentration used in our study (250 mg As l^{-1}) was slightly lower than that in the previous study (323 mg As l^{-1}, Jackson et al., 2005) and our range is much higher than the values reported for other soils in another study (5.6–8.4% at 100 mg As l^{-1}, Wakao et al., 1995). These results suggest the wide distribution of the arsenic resistance in marine bacteria.

Although there were relatively high standard deviations in the values, bacterial consortium obtained from the two seawater samples exhibited higher arsenic accumulation than *M. communis* (Table 3). These results indicate the possibility that we could find more arsenic accumulating bacteria from the marine environment.

In conclusion, we reported *M. communis* as the first non-engineered and most powerful arsenic accumulating bacterium known so far. Further elucidation of the mechanism may lead to a new low cost technology for treatment of arsenic contaminated wastes.

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