APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2006; **20**: 538–544
Published online in Wiley InterScience
(www.interscience.wiley.com) DOI:10.1002/aoc.1075

Materials, Nanoscience and Catalysis AOC

Isolation of monomethylarsonic acid-mineralizing bacteria from arsenic contaminated soils of Ohkunoshima Island†

Teruya Maki*, Noriko Takeda, Hiroshi Hasegawa and Kazumasa Ueda

Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, 920-1192, Japan

Received 11 October 2005; Accepted 18 January 2006

Chemical warfare agents, composed of harmful organoarsenic compounds have contaminated the soils of Ohkunoshima Island with high levels of arsenic. As a basic research establishing useful bioremediation techniques, environmental factors such as arsenic concentrations and bacterial biomass in the soils were investigated. Among the five stations of Ohkunoshima Island, the soils of four stations were contaminated by high levels of arsenic compounds at concentrations of 125, 12.7, 3.29 and 0.504 g/kg soil, while the other station with low arsenic concentrations of 0.007 g/kg soil was considered an uncontaminated area. The distribution of arsenic compounds originating from the chemical weapon agent differs among the various areas of Ohkunoshima Island. The cell densities of arsenate-resistant bacteria also varied among the five stations, ranging from 10^6 to 10^8 cells/g soil. In an attempt to isolate bacteria that strongly mineralize the organoarsenic compounds, the mineralization activities for monomethylarsonic acid [MMAA(V)] of 48 isolates of arsenate-resistant bacteria were determined. Only nine isolates reduced 140 μg/l of MMAA(V), giving decreasing percentages ranging from 5 to 100% within 14 days. Among the nine isolates, two remarkably converted 140 μg/l of MMAA to more than 71 μg/l of inorganic arsenic. Presumably only specific members of the environmental bacterial population have strong mineralization activities for MMAA. Phylogenetic analysis using 16S rDNA sequences showed that the two isolates belonged to the *Pseudomonas putida* strains, which are known to have strong mineralization activity for various organic compounds. In the soil contaminated by arsenic at a high level, few bacteria in the arsenate-resistant bacterial group would significantly mineralize organoarsenic compounds. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: organoarsenic; monomethylarsonic acid; MMAA mineralization; bacteria; arsenic contaminated soil

INTRODUCTION

The release of organoarsenic compounds from soil contaminated by harmful organoarsenic compounds, such as chemical warfare agents and arsenical herbicides, endangers neighboring areas and aquifers.[1–3] Ground water contaminated by diphenylarsinic acid caused a poisoning incident in Kamisu-machi, Ibaraki Prefecture, Japan.[4] The patients who suffered the arsenic poisoning showed dysfunction of the central nervous system.[4] Diphenylarsinic acid and Lewisite (2-chloro-ethenyl dichloro arsine) were demonstrated to reduce vital activities of human cells and to change cell structures.[4,5] Bioremediation, the use of bacteria for environmental restoration, has been proposed as a cost-effective alternative technology to reduce the toxic activity of harmful metal compounds in the contaminated soils.[6,7]

The microorganisms used in the bioremediation could mineralize the harmful organoarsenic compounds to inorganic arsenic, which is less toxic than its precursors. Terrestrial

† This paper is based on work presented at the 12th Symposium of the Japanese Arsenic Scientists' Society (JASS) held 5–6 November 2005 in Takizawa, Iwate Prefecture, Japan.

*Correspondence to: Teruya Maki, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, 920-1192, Japan.
E-mail: makiteru@t.kanazawa-u.ac.jp
Contract/grant sponsor: Grant-in-aid for Encouragement of Young Scientists, Ministry of Education, Science, Sports and Culture; Contract/grant number: 14780441.
Contract/grant sponsor: Salt Science Research Foundation; Contract/grant number: 0424.
Contract/grant sponsor: Nissan Science Foundation.

microorganisms have been reported to mineralize the organic arsenical herbicides such as cacodylic acid and sodium methanearsenate to arsenate.[8,9] A bacterial isolate obtained from sludge water, strain ASV2, mineralizes arsenobetaine to inorganic arsenic, metabolizing the arsenobetaine as a carbon source.[10] Lehr *et al*. reported that *Mycobacterium neoaurum* demethylates 0.5 mg/l of monomethylarsonic acid [$CH_3AsO(OH)_2$; MMAA(V)] to inorganic arsenic, also using MMAA(V) as a carbon source, and the yields of inorganic arsenic were 27% from arsenate and 43% from arsenite.[11] However there are few reports on the biomass and distribution of organoarsenic-mineralizing bacteria. In a previous study, the biomass and composition of bacteria mineralizing dimethylarsinic acid [$(CH_3)_2AsO(OH)$; DMAA(V)] were investigated in lakes, and a bacterial population composed of various bacterial species was demonstrated to contribute to the mineralization cycle of organoarsenic in the aquatic environment.[12,13] To establish useful bioremediation techniques, bacteria strongly mineralizing the organoarsenic compounds have to be isolated, and environmental information about organoarsenic-mineralizing bacteria is required.

On Ohkunoshima Island (Hiroshima prefecture, Japan), chemical warfare agents were produced during World War II. However, no scientific investigation of the arsenic contamination in the soil has been performed. In this study, the total concentrations of arsenic compounds in the soil of Ohkunoshima Island were determined using an atomic absorption spectrometer with a cold trap method. After the bacterial biomass in the soils was determined and the arsenate-resistant bacteria were isolated from the contaminated soils, the MMAA-mineralization activity of each isolate was estimated by culture experiments. MMAA, which has a simple chemical structure, was used as a model of organoarsenic compounds. Moreover, the isolates with high MMAA-mineralization activities were identified using phylogenetic analysis using 16S rDNA sequences.

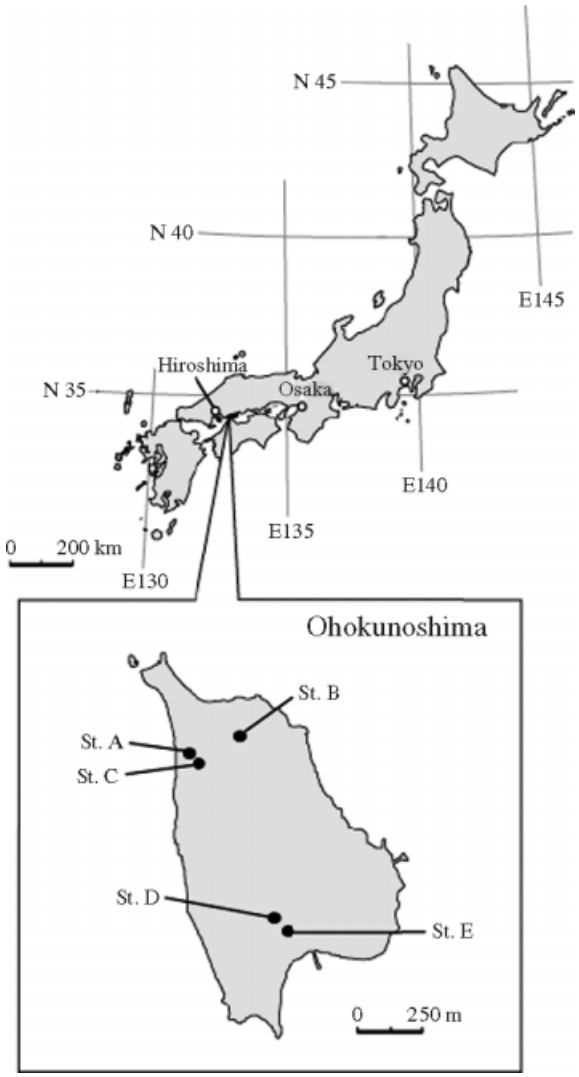

Figure 1. Sampling area and a station location (Ohkunoshima Island).

MATERIALS AND METHODS

Sampling

Soil samples were collected from the five stations located in Ohkunoshima Island (Hiroshima Prefecture, Japan; Fig. 1) in May 2003. The total arsenic concentrations in the soil samples were measured using an atomic absorption spectrometer with a cold trap method.

Measurements of arsenic species

To evaporate the whole carbon source, 1 g of the soil sample was dried at a temperature of 160 °C for 2 h, then heated at a temperature of 600 °C for 6 h. The residue compounds in the treated soils were dissolved in concentrated HNO_3 solution[14]. The solution was used to measure arsenic concentration. The treated soil samples or the untreated bacterial cultures were filtered with a 0.2 μm nuclepore filter (Advantec, Tokyo, Japan). After the volumes of filtrates were adjusted to 40 ml by the dilution using pure water, 5 ml of 0.2 mol/l Na_2 EDTA and 5 ml of 5 mol/l HCl were added to the filtrates. Next the filtrates were reacted with 10 ml of 0.1 g/ml sodium tetrahydroborate, and the arsines produced were swept using a flow of He gas into a cold trap. This trap was cooled by liquid nitrogen, before being gently warmed by electrical heating. Arsines, such as inorganic arsine and MMAA, were released into a quartz-T tube heated in a C_2H_2–air flame and monitored using an atomic absorption spectrometer Z-8100 (Hitachi Co., Chiba, Japan). An atomic absorption spectrometry technique combined with a cold trap method was employed.[15,16] A mixed solution of arsenate, MMAA and DMAA was used as a standard for the determination of arsenic concentrations in the samples, and additional amounts of 250, 100 and 50 nmol of each standard arsenic compound in the reaction solutions provided a linear line to calibrate

DOI: 10.1002/aoc

the measurements. Moreover, after arsenate, MMAA and DMAA were added to a soil sample including low levels of arsenic compounds, 85.0 ± 3.0% of the additional amounts of each arsenic compound added could be detected by this measurement. In addition, the weights of additional arsenic compounds in the samples were also linear to the values of measurements.

Viable bacterial count and bacterial isolation

The arsenate-resistant bacteria in the soil sample were counted using the spread-plate method. One gram of the soil sample was resuspended in sterile water and vortexed in order to detach the bacteria from the sediment particles. Serial 10-fold dilutions were prepared, and 0.1 ml aliquots were plated in duplicate onto an agar plate of ST 10^{-1} culture medium (tripticase peptone 0.1 g/l, yeast extract 0.01 g/l), including arsenate (Wako, Osaka, Japan) at final concentration of 140 μg/l. The bacteria that could grow on the culture medium plates were defined as arsenate-resistant bacteria. After the culture-medium plates were incubated at 20 °C under dark conditions for 7 days, colonies were counted, and the bacterial cell densities in the soils were calculated using the numbers of colonies. Distinct colonies were selected from each soil sample and isolated in pure culture on an agar plate. Purified strains were then stocked in nutrient broth with 15% glycerol at −20 °C.

MMAA-mineralization and arsenate-resistances of isolates

With regard to the bacterial culture, arsenate-resistant isolates were incubated in a liquid ST 10^{-1} culture medium with 140 μg/l of MMAA (Roth, Karlsruhe, Germany) for about 7 days. For the evaluation of the MMAA-mineralization activities of arsenate-resistant isolates, 1 ml of each isolate culture was inoculated into 19 ml of liquid ST 10^{-1} culture medium including MMAA at final concentrations of 140 μg/l. After 14 days of incubation, 2 ml of the bacterial culture were used for the measurement of inorganic arsenic and MMAA. After the bacterial cultures were filtered with a 0.2 μm nuclepore filter (Advantec, Tokyo, Japan), the concentration of MMAA and inorganic arsenic in bacterial cultures was determined by the atomic absorption spectrometer with a cold trap method. The percentage decreases of MMAA were calculated by dividing the concentrations of MMAA by the initial concentrations of MMAA. Isolates producing high concentrations of inorganic arsenic were inoculated into a liquid ST 10^{-1} culture medium with 140 μg/l of MMAA again, and the concentrations of arsenic compounds and the bacterial growths were determined at the 0 day, the 1st day, the 3rd day, the 7th day, and the 14th day. The bacterial growths were determined by absorbance at 550 nm in the bacterial culture. Moreover, for investigation of arsenate resistances of the isolates, the bacterial growths were monitored in the culture medium, including 0, 0.142, 1.42, 14.2 and 142 mg/l of arsenate, over 14 days. All bacterial culture were incubated at 20 °C on a rotary shaker under dark conditions. Moreover, all experiments were performed in duplicate and the data reported in this study are the average of these two bacterial cultures.

Sequencing of 16S rDNA and phylogenetic analysis

Isolates with high activities of MMAA-mineralization were identified by phylogenetic analysis using 16S rDNA sequences. Isolates cultivated in an ST 10^{-1} culture medium overnight were pelleted by centrifugation at 15 000*g* for 15 min. The bacterial cell pellets were lysed with SDS, proteinase K and lysozyme. Genomic DNAs were purified by phenol–chloroform extraction, chloroform extraction and ethanol precipitation.

16S rDNA fragments (ca.1450 bp) of bacteria were amplified by a polymerase chain reaction (PCR). Reaction mixtures (final volume, 100 μl) contained 200 μM of dNTPs, 0.5 units of Ex *Taq* polymerase (Takara BIO Inc., Ohtsu, Japan), and 0.2 μM of each oligonucleotide primer, 27F and 1492R. These primers specifically bind to eubacterial 16S rDNA.[17] Genomic DNA of bacteria was added at a final concentration of 10 ng/μl. Thermal cycling was performed using a Program Temp Control System PC-700 (Astec, Fukuoka, Japan) under the following conditions: denaturation at 95 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 2 min, for a total of 30 cycles. The16S rDNA fragments (approximately 1450 bp) in PCR amplicons were separated using the agarose gel electrophoresis, and were purified by phenol–chloroform extraction and chloroform extraction followed by ethanol precipitation. Partial sequences (ca. 500 bp) of 16S rDNA fragments were determined using a Dye DeoxyTM Terminator Cycle Sequencing Kit (ABI, CA, USA) with a 27F sequencing primer and a DNA auto-sequencing system (model 373A) according to the recommended protocol. The sequences determined were compared with a DDBJ (DNA Data Bank of Japan) database using the BLASTA and FASTA SEARCH programs.[18]

For phylogenetic analyses, the DNA sequences were aligned using the CLUSTAL W version 1.7 (European Bioinformatics Institute).[19] A phylogenetic tree including the isolates was constructed according to the neighbor-joining algorithmic method (PHYLIP computer program package, version 3.6a2),[20] using the partial sequences of 16S rDNA. The root position was estimated by using the 16S rDNA sequence of *Bacillus subtilis* as an outgroup.

Nucleotide sequence accession numbers

The DDBJ accession numbers for the new 16S rDNA sequences of C-1 and D-7 are AB236664 and AB236665, respectively.

RESULTS AND DISCUSSION

The total concentrations of arsenic compounds in the soil samples indicated wide ranges of values from 0.007 g/kg soil

DOI: 10.1002/aoc

Table 1. Total concentrations of arsenic compounds and bacterial cell densities in soils, and numbers of obtained isolates of arsenate-resistant bacteria, at five stations in Ohkunoshima Island

Stations	A	B	C	D	E
Total concentrations of arsenic compounds (g/kg soil)	125	12.7	3.29	0.504	0.007
Normal bacterial cell densities (10^7 cells/g soil)[a]	5.2	24	48	150	53
Arsenate-resistant bacterial cell densities (10^7 cells/g soil)[b]	1.3	0.6	7.1	1.9	48
Numbers of isolates	12	8	9	10	9

[a] The nomal bacteria were counted using ST 10^{-1} culture medium.
[b] The arsenate-resistant bacteria were counted using ST 10^{-1} culture medium including 142 μg/l of arsenate.

to 125 g/kg soil among the five stations of Ohkunoshima Island (Table 1). High levels of arsenic contamination were found in the four stations A–D, at total concentrations of 125, 12.7, 3.29 and 0.504 g/kg soil, respectively. The soils of the four stations included at least two orders higher concentrations of arsenic compounds than the averages of natural soils, which generally contain arsenic compounds at concentrations of the mg/kg order.[21,22] In contrast, the other station E indicated a low concentration of 0.007 g/kg soil, the natural soil level, suggesting that this station is not contaminated by arsenic compounds. The soils of station A and station B were composed of sand and clay, respectively, and the both soils included ash. The residues of chemical weapon agents in the ash would cause a concentrated contamination of arsenic compounds. Moreover, the distribution of arsenic compounds was different among the areas of in Ohkunoshima Island. Accordingly, the high level of arsenic compounds contamination occurred in specific areas, where the chemical weapon agent was synthesized from arsenic compounds or disposed of at the end of World War II. All soils from stations C–E contained no ash, and indicated the same characteristics containing a mixture of silt and humus. The arsenic compounds originating from stations A or B would have spread to stations C and D. The cell densities of arsenate-resistant bacteria were also different among the five stations, ranging from 6×10^6 to 4.8×10^8 cells/g soil (Table 1). In particular, arsenate-resistant bacterial cell densities and the normal bacterial cell densities of the highly arsenic contaminated areas such as stations A and B were lower than at the other stations. In stations A and B, the sand and clay including low amounts of carbon sources do not allow bacterial growth, and the high arsenic concentrations limit the bacterial growth. In contrast, in stations C–E, the humus with rich carbon sources induce bacterial growth, supporting the occurrence of arsenate-resistant bacteria.

After the bacterial counts using the spread plate method, we obtained a total of 48 isolates of arsenate-resistant bacteria from the five stations. For the investigation of the MMAA-mineralization activities of 48 isolates, each isolate was inoculated into the culture medium, including 140 μg/l of MMAA, and the concentration of MMAA was measured after 14 days of incubation. As a result, only nine isolates among 48 significantly reduced 140 μg/l of MMAA by percentages ranging from 5 to 100% within 14 days (Table 2). Consequently, the nine isolates of arsenate-resistant bacteria may be able to mineralize MMAA, while the other 39 isolates have no or very weak mineralization activities. A previous study reported that nine of 10 isolates from lake water slightly mineralized 138 μg/l of DMAA at mineralization percentages of less than 40% within 14 days.[13] Sanders suggested that microorganisms in natural water would mineralize DMAA at a slow rate of approximately 1.1 ng/l/day.[23] Presumably, large parts of the environmental bacterial population have low or no mineralization activities for methylarsenic.

Among the nine isolates, the two isolates, C-1 and D-7, completely eliminated 140 μg/l of MMAA in the culture medium after 14 days of incubation, and produced inorganic arsenic at concentrations of more than 70 μg/l (Table 2). After both of the two isolates were inoculated into the culture medium including 140 μg/l of MMAA again, the concentrations of inorganic arsenic and MMAA were monitored at the day 0, and the 1st, 3rd, 7th and 14th days. As a result, in the culture of C-1, the concentration of MMAA gradually decreased to below the limit of detection after 14 days, while that of inorganic arsenic increased to 90.9 μg/l from the 7th to the 14th day (Fig. 2). The culture of D-7 indicated that the MMAA disappeared within 7 days,

Table 2. Concentrations of MMAA and inorganic arsenic in the bacteria culture medium after 14 days of incubation. Each of 48 isolates of arsenate-resistant bacteria was inoculated into culture medium including 140 μg/l of MMAA, and data for the nine isolates remarkably reducing MMAA are shown in this table

Isolates	A-11	C-1	C-2	C-4	D-7	E-2	E-3	E-4	E-5
Concentrations of MMAA (μg/l)	113	0	109	129	0	71.4	123	112	70.0
Concentrations of inorganic arsenic (μg/l)	<14	87	<14	<14	71	<14	<14	<14	<14

DOI: 10.1002/aoc

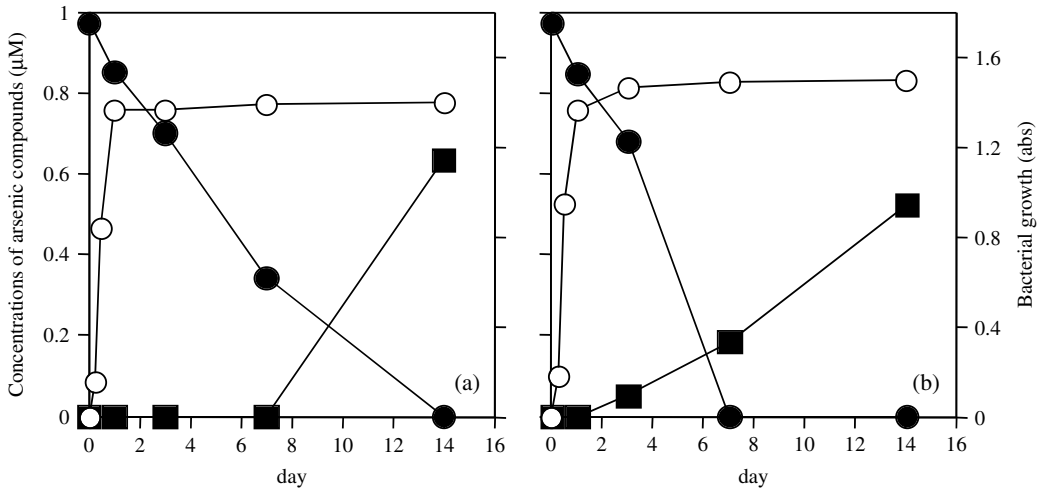

Figure 2. Changes in concentrations of MMAA (solid circles) and inorganic arsenic (solid squares), and bacterial growths (open circles), in bacterial cultures during the 14 days of incubation. The isolates of arsenate-resistant bacteria, C-1 (a) and D-7 (b), were inoculated to the culture medium including 1 μM MMAA.

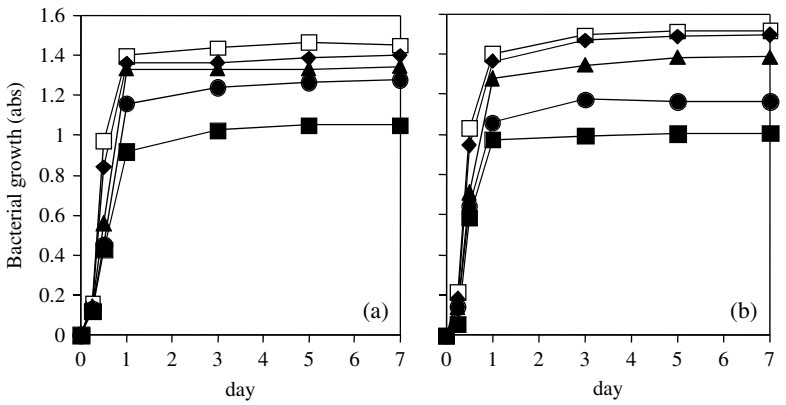

Figure 3. Changes in bacterial yields in bacterial cultures including arsenate at concentrations of 0 mg/l (open squares), 0.142 mg/l (solid diamonds), 1.42 mg/l (solid triangles), 14.2 mg/l (solid circles) and 142 mg/l (solid squares), during the 7 days of incubation. The isolates of arsenate-resistant bacteria, C-1 (a) and D-7 (b), were inoculated to the culture medium.

and the production of inorganic arsenic slightly increased to 72.8 μg/l for 14 days. The two isolates, C-1 and D-7, completely mineralized 140 μg/l of MMAA within 14 days, and converted it to inorganic arsenic at concentrations of 72.8 and 90.9 μg/l, respectively. Lehr *et al*. reported that *Mycobacterium meoaurum* converted about 500 μg/l MMAA to inorganic arsenic at a conversion percentage of 50% within 14 days.[11] The two isolates, C-1 and D-7, would have similar levels of MMAA-mineralization activities as *Mycobacterium meoaurum*. During the stationary phase in the cultures of two isolates, the MMAA level immediately decreased, while inorganic arsenic gradually increased. Furthermore, the concentrations of inorganic arsenic did not coincide with the initial concentration of MMAA. The arsenic within the bacterial cells could not be monitored in this study, because the bacterial cells were eliminated during filtration in arsenic measurement. Probably, the inorganic arsenic in bacterial cells was gradually released from the declining cells during the stationary phase, and the released inorganic arsenic could be slightly detected after the decrease of MMAA in the culture.

When the arsenate-resistances of C-1 and D-7 were estimated by monitoring the yields of bacteria in culture media including 0, 0.142, 1.42, 14.2 and 142 mg/l of arsenate, the bacterial yields of the both isolates during the stationary phase decreased in proportion to the concentration of arsenate in the culture medium (Fig. 3). Although the bacterial yields were reduced by arsenate, the two isolates, C-1 and D-7, grew during the first day and could survive in the culture medium until 142 mg/l of arsenate. The two isolates are strongly resistant to inorganic arsenic. In general, arsenate-resistant bacteria reduced the arsenate to arsenite within bacterial cells, and exported the arsenite out of cells.[24,25] Probably,

DOI: 10.1002/aoc

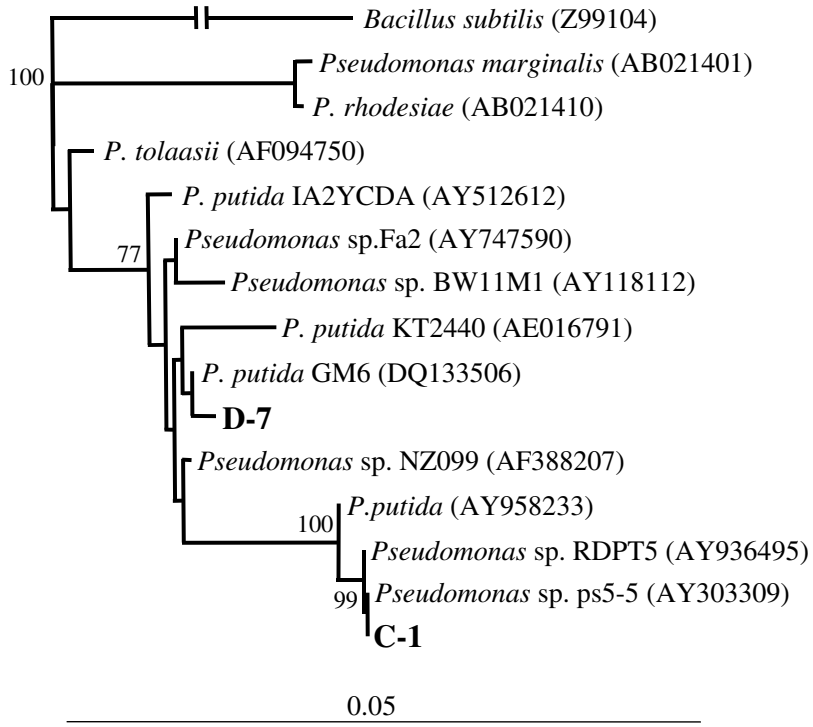

Figure 4. Phylogenetic tree for 16S rDNA sequence of the bacterial isolates, C-1 (a) and D-7. The tree was calculated from a dissimilarity matrix of ca. 500 bp alignment using a neighbor-joining algorithm. Bootstrap values larger than 50% (after 1000 resampling) are indicated on the branch.

the MMAA-mineralizing bacteria mineralize MMAA, and export the inorganic arsenic to protect their own cells from the arsenic compounds.

On the phylogenetic tree using the partial 16S rDNA sequences of the two isolates, C-1 and D-7, and known bacteria, C-1 was closely related to the strains RDPY5 and ps5-5 of the genus *Pseudomonas* at high similarities of 100%, and D-7 closely clustered with *Pseudomonas putida* strain GM6 at high similarity of 99.7% (Fig. 4). Moreover, the group of the genus *Pseudomonas* including the two isolates was composed of the strains of *P. putida*, indicating that the two isolates are identical to *P. putida*. Some strains of *P. putida* are known to have powerful oxygenase to mineralize stable chemical compounds such as chlorophenol at high activities.[26] According to the genome analysis, the metabolic enzymes, such as oxygenases and oxidoreductases, of *P. putida* were found to provide useful metabolic pathways for the transformation of aromatic compounds.[27] *P. putida* is currently regarded as an excellent organism for engineering of bioremediation capabilities.[28] This study is the first report indicating that *P. putida* mineralizes organoarsenic compounds. Possibly, the two isolates, C-1 and D-7, oxidize or demethylate various organoarsenic compounds.

In this study, although many parts of the bacterial biomass in the arsenic-contaminated soils would have low levels of organoarsenic-mineralization activities, bacteria of the genus *Pseudomonas* which mineralize MMAA remarkably well were isolated from the arsenic-contaminated soils. Previously, *Mycobacterium meoaurum* was also reported to be MMAA-mineralizing bacteria.[11] In aquatic environments, various species of bacteria are thought to contribute to the mineralization for DMAA.[12,13] Accordingly, the several bacterial species in arsenic-contaminated environments can mineralize harmful organoarsenic compounds. More work is needed to investigate the ecological characteristics of the organoarsenic-mineralizing bacteria to establishing effective and useful bioremediation.

Acknowledgments

This research was partly supported by a Grant-in-Aid for Encouragement of Young Scientists (17710061) from the Ministry of Education, Science, Sports and Culture. The Salt Science Research Foundation, no. 0424 and the Nissan Science Foundation also support this work.

REFERENCES

1. Tychinin DN, Kosterin PV. *Environ. Sci. Pollut. Res.* 2000; **7**: 245.
2. Vilensky JA, Redman K. *Ann. Emerg. Med.* 2003; **41**: 378.
3. Ishii K, Tamaoka A, Otsuka F, Iwasaki N, Shin K, Matsui A, Endo G, Kumagai Y, Ishii T, Shoji S, Ogata T, Ishizaki M, Doi M, Shimojo N. *Japan. Ann. Neurol.* 2004; **56**: 741. DOI: 10. 1002/ana.20290.
4. Ochi T, Suzuki T, Isono H, Kaise T. *Toxicol. Appl. Pharmac.* 2004; **200**: 64. DOI: 10. 1016/j.taap.2004.03.014.

5. Henriksson J, Johannisson A, Bergqvist PA, Norrgren L. *Arch. Environ. Contam. Toxicol.* 1996; **30**: 213.
6. Gadd GM. *FEMS Microbiol. Rev.* 1993; **11**: 297.
7. Mulligan CN, Yong RN, Gibbs BF. *Engng Geology.* 2001; **60**: 193.
8. Von Endt DW, Kearney PC, Kaufman DD. *J. Agric. Food Chem.* 1968; **16**: 17.
9. Woolson EA, Kearney PC. *Environ. Sci. Technol.* 1973; **7**: 47.
10. Quinn JP, McMullan G. *Microbiology* 1995; **141**: 721.
11. Lehr CR, Polishchuk E, Radoja U, Cullen WR. *Appl. Organometal. Chem.* 2003; **17**: 831. DOI: 10. 1002/aoc.544.
12. Maki T, Hasegawa H, Watarai H, Ueda K. *Anal. Sci.* 2004; **20**: 61.
13. Maki T, Hasegawa H, Ueda K. *Appl. Organometal. Chem.* 2005; **19**: 231. DOI: 10. 1002/aoc.696.
14. Tessier A, Campbell PGC, Bisson M. *Anal. Chem.* 1979; **51**: 844.
15. Braman RS, Foreback CC. *Science* 1973; **182**: 1247.
16. Hasegawa H, Sohrin Y, Matsui M, Honjo M, Kawashima M. *Anal. Chem.* 1994; **66**: 3247.
17. Maidak BL, Olsen GJ, Larsen N, Overbeek R, McCaughey MJ, Woese C. *Nucl. Acids Res.* 1997; **25**: 109.
18. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. *J. Mol. Biol.* 1990; **215**: 403.
19. Thompson JD, Higgins DG, Gibson TJ. *Nucl. Acids Res.* 1994; **22**: 4673.
20. Saitou N, Nei M. *Mol. Biol. Evol.* 1987; **4**: 406.
21. Cullen WR, Reimer KJ. *Chem. Rev.* 1989; **89**: 713.
22. Francesconi KA, Kuehnelt D. Arsenic compounds in the environment. In *Environmental Chemistry of Arsenic*, Frankenberger WT Jr (ed.). Marcel Dekker: New York, 2002; 51–94.
23. Sanders JG. *Chemosphere.* 1979; **8**: 135.
24. Tamas MJ, Wysocki R. *Curr. Genet.* 2001; **40**: 2. DOI: 10. 1007/s002940100234.
25. Rosen BP. *FEBS Lett.* 2002; **529**: 86.
26. Wang SJ, Loh KC, Chua SS. *Enzyme Microbial. Technol.* 2003; **32**: 422. DOI: 10. 1016/S0141-0229(02)00315-0.
27. Nelson KE, Weinel C, Paulsen IT, Dodson RJ, Hilbert H, Martins dos Santos VA, Fouts DE, Gill SR, Pop M, Holmes M, Brinkac L, Beanan M, DeBoy RT, Daugherty S, Kolonay J, Madupu R, Nelson W, White O, Peterson J, Khouri H, Hance I, Chris Lee P, Holtzapple E, Scanlan D, Tran K, Moazzez A, Utterback T, Rizzo M, Lee K, Kosack D, Moestl D, Wedler H, Lauber J, Stjepandic D, Hoheisel J, Straetz M, Heim S, Kiewitz C, Eisen JA, Timmis KN, Dusterhoft A, Tummler B, Fraser CM. *Environ. Microbiol.* 2002; **4**: 799.
28. Lovley DR. *Nat. Rev. Microbiol.* 2003; **1**: 35. DOI: 10. 1038/nrmicro731.