

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

著者	Maki Teruya, Takeda Noriko, Hasegawa Hiroshi, Ueda Kazumasa
journal or publication title	Applied Organometallic Chemistry
volume	20
number	9
page range	538-544
year	2006-09-01
URL	http://hdl.handle.net/2297/2800

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 $\mu\text{g/l}$ of MMAA(V), giving decreasing percentages ranging from 5 to 100% within 14 days. Among the nine isolates, two remarkably converted 140 $\mu\text{g/l}$ of MMAA to more than 71 $\mu\text{g/l}$ 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

1 INTRODUCTION

2
3 The release of organoarsenic compounds from soil contaminated by harmful organoarsenic compounds, such as

4
5
6 *Correspondence to: Teruya Maki, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, 920-1192, Japan.

7 E-mail: makiteru@t.kanazawa-u.ac.jp

8
9
10 [†]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. E-mail: yamaoka-yu@aist.go.jp.

11 Contract/grant sponsor: Grant-in-aid for Encouragement of Young Scientists, Ministry of Education, Science, Sports and Culture; Contract/grant number: 14780441.

12 Contract/grant sponsor: Salt Science Research Foundation; Contract/grant number: 0424.

13 Contract/grant sponsor: Nissan Science Foundation.

16 chemical warfare agents and arsenical herbicides, endangers 16
17 neighboring areas and aquifers.^{1–3} Ground water contami- 17
18 nated by diphenylarsinic acid caused a poisoning incident in 18
19 Kamisu-machi, Ibaraki Prefecture, Japan.⁴ The patients who 19
20 suffered the arsenic poisoning showed dysfunction of the 20
21 central nervous system.⁴ Diphenylarsinic acid and Lewisite 21
22 (2-chloro-ethenyl dichloro arsine) were demonstrated to 22
23 reduce vital activities of human cells and to change cell 23
24 structures.^{4,5} Bioremediation, the use of bacteria for envi- 24
25 ronmental restoration, has been proposed as a cost-effective 25
26 alternative technology to reduce the toxic activity of harmful 26
27 metal compounds in the contaminated soils.^{6,7} 27

28 The microorganisms used in the bioremediation could min- 28
29 eralize the harmful organoarsenic compounds to inorganic 29
30 arsenic, which is less toxic than its precursors. Terrestrial 30

1 microorganisms have been reported to mineralize the organic
2 arsenical herbicides such as cacodylic acid and sodium
3 methanearsenate to arsenate.^{8,9} A bacterial isolate obtained
4 from sludge water, strain ASV2, mineralizes arsenobetaine
5 to inorganic arsenic, metabolizing the arsenobetaine as a
6 carbon source.¹⁰ Lehr *et al.* reported that *Mycobacterium*
7 *neoaurum* demethylates 0.5 mg/l of monomethylarsonic acid
8 [CH₃AsO(OH)₂; MMAA(V)] to inorganic arsenic, also using
9 MMAA(V) as a carbon source, and the yields of inorganic
10 arsenic were 27% from arsenate and 43% from arsenite.¹¹
11 However there are few reports on the biomass and distri-
12 bution of organoarsenic-mineralizing bacteria. In a previous
13 study, the biomass and composition of bacteria mineraliz-
14 ing dimethylarsinic acid [(CH₃)₂AsO(OH); DMAA(V)] were
15 investigated in lakes, and a bacterial population composed
16 of various bacterial species was demonstrated to contribute
17 to the mineralization cycle of organoarsenic in the aquatic
18 environment.^{12,13} To establish useful bioremediation tech-
19 niques, bacteria strongly mineralizing the organoarsenic
20 compounds have to be isolated, and environmental informa-
21 tion about organoarsenic-mineralizing bacteria is required.

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

37

38

39

40

MATERIALS AND METHODS

41

Sampling

42

43

44

45

46

47

48

Measurements of arsenic species

49

50

51

52

53

54

55

56

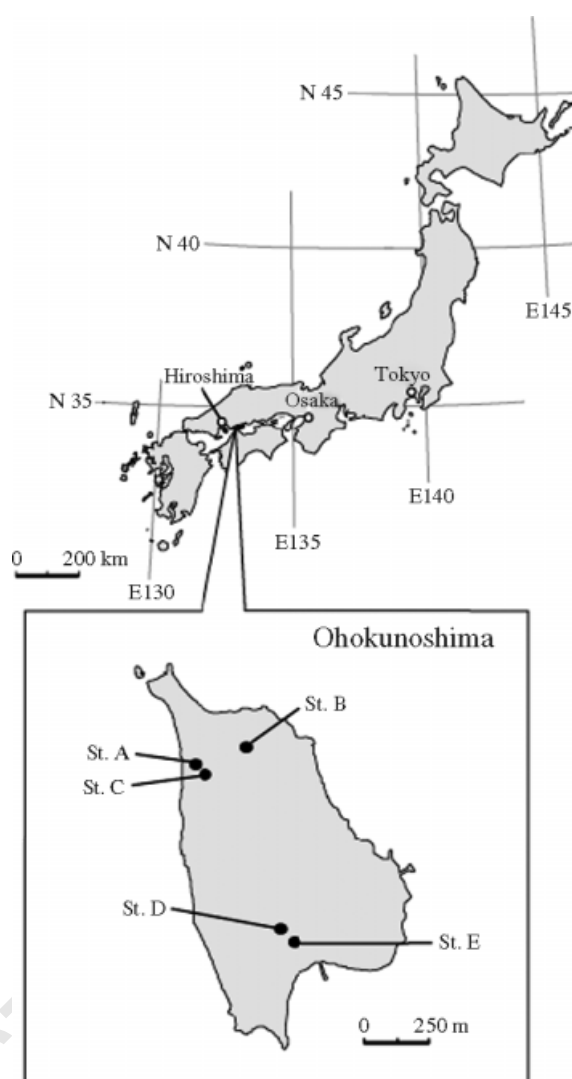


Figure 1. Sampling area and a station location (Ohkunoshima Island).

Japan). After the volumes of filtrates were adjusted to 40 ml
by the dilution using pure water, 5 ml of 0.2 mol/l Na₂
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 C₂H₂-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

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

1 the measurements. Moreover, after arsenate, MMAA and
2 DMAA were added to a soil sample including low levels of
3 arsenic compounds, $85.0 \pm 3.0\%$ of the additional amounts
4 of each arsenic compound added could be detected by this
5 measurement. In addition, the weights of additional arsenic
6 compounds in the samples were also linear to the values of
7 measurements.

9 Viable bacterial count and bacterial isolation

10 The arsenate-resistant bacteria in the soil sample were
11 counted using the spread-plate method. One gram of the
12 soil sample was resuspended in sterile water and vortexed
13 in order to detach the bacteria from the sediment particles.
14 Serial 10-fold dilutions were prepared, and 0.1 ml aliquots
15 were plated in duplicate onto an agar plate of ST 10^{-1}
16 culture medium (trypticase peptone 0.1 g/l, yeast extract
17 0.01 g/l), including arsenate (Wako, Osaka, Japan) at final
18 concentration of 140 $\mu\text{g/l}$. The bacteria that could grow on
19 the culture medium plates were defined as arsenate-resistant
20 bacteria. After the culture-medium plates were incubated
21 at 20 °C under dark conditions for 7 days, colonies were
22 counted, and the bacterial cell densities in the soils were
23 calculated using the numbers of colonies. Distinct colonies
24 were selected from each soil sample and isolated in pure
25 culture on an agar plate. Purified strains were then stocked in
26 nutrient broth with 15% glycerol at $-20\text{ }^\circ\text{C}$.

28 MMAA-mineralization and arsenate-resistances 29 of isolates

30 With regard to the bacterial culture, arsenate-resistant isolates
31 were incubated in a liquid ST 10^{-1} culture medium with
32 140 $\mu\text{g/l}$ of MMAA (Roth, Karlsruhe, Germany) for about
33 7 days. For the evaluation of the MMAA-mineralization
34 activities of arsenate-resistant isolates, 1 ml of each isolate
35 culture was inoculated into 19 ml of liquid ST 10^{-1} culture
36 medium including MMAA at final concentrations of 140 $\mu\text{g/l}$.
37 After 14 days of incubation, 2 ml of the bacterial culture were
38 used for the measurement of inorganic arsenic and MMAA.
39 After the bacterial cultures were filtered with a 0.2 μm
40 nucleopore filter (Advantec, Tokyo, Japan), the concentration
41 of MMAA and inorganic arsenic in bacterial cultures was
42 determined by the atomic absorption spectrometer with a
43 cold trap method. The percentage decreases of MMAA were
44 calculated by dividing the concentrations of MMAA by the
45 initial concentrations of MMAA. Isolates producing high
46 concentrations of inorganic arsenic were inoculated into a
47 liquid ST 10^{-1} culture medium with 140 $\mu\text{g/l}$ of MMAA again,
48 and the concentrations of arsenic compounds and the bacterial
49 growths were determined at the 0 day, the 1st day, the 3rd
50 day, the 7th day, and the 14th day. The bacterial growths
51 were determined by absorbance at 550 nm in the bacterial
52 culture. Moreover, for investigation of arsenate resistances
53 of the isolates, the bacterial growths were monitored in the
54 culture medium, including 0, 0.142, 1.42, 14.2 and 142 mg/l
55 of arsenate, over 14 days. All bacterial culture were incubated
56 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 000g 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. The 16S 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

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 normal bacteria were counted using ST 10^{-1} culture medium.

^b The arsenate-resistant bacteria were counted using ST 10^{-1} culture medium including 142 $\mu\text{g/l}$ of arsenate.

1 to 125 g/kg soil among the five stations of Ohkunoshima
 2 Island (Table 1). High levels of arsenic contamination were
 3 found in the four stations A–D, at total concentrations of
 4 125, 12.7, 3.29 and 0.504 g/kg soil, respectively. The soils of
 5 the four stations included at least two orders higher concentra-
 6 tions of arsenic compounds than the averages of natural
 7 soils, which generally contain arsenic compounds at con-
 8 centrations of the mg/kg order.^{21,22} In contrast, the other
 9 station E indicated a low concentration of 0.007 g/kg soil,
 10 the natural soil level, suggesting that this station is not con-
 11 taminated by arsenic compounds. The soils of station A and
 12 station B were composed of sand and clay, respectively,
 13 and the both soils included ash. The residues of chemical
 14 weapon agents in the ash would cause a concentrated con-
 15 tamination of arsenic compounds. Moreover, the distribution
 16 of arsenic compounds was different among the areas of in
 17 Ohkunoshima Island. Accordingly, the high level of arsenic
 18 compounds contamination occurred in specific areas, where
 19 the chemical weapon agent was synthesized from arsenic
 20 compounds or disposed of at the end of World War II. All
 21 soils from stations C–E contained no ash, and indicated the
 22 same characteristics containing a mixture of silt and humus.
 23 The arsenic compounds originating from stations A or B
 24 would have spread to stations C and D. The cell densities
 25 of arsenate-resistant bacteria were also different among the
 26 five stations, ranging from 6×10^6 to 4.8×10^8 cells/g soil
 27 (Table 1). In particular, arsenate-resistant bacterial cell den-
 28 sities and the normal bacterial cell densities of the highly
 29 arsenic contaminated areas such as stations A and B were
 30 lower than at the other stations. In stations A and B, the
 31 sand and clay including low amounts of carbon sources do
 32 not allow bacterial growth, and the high arsenic concentra-
 33 tions limit the bacterial growth. In contrast, in stations C–E,

the humus with rich carbon sources induce bacterial growth, 35
 supporting the occurrence of arsenate-resistant bacteria. 36

After the bacterial counts using the spread plate method, 37
 we obtained a total of 48 isolates of arsenate-resistant bacteria 38
 from the five stations. For the investigation of the MMAA- 39
 mineralization activities of 48 isolates, each isolate was 40
 inoculated into the culture medium, including 140 $\mu\text{g/l}$ of 41
 MMAA, and the concentration of MMAA was measured 42
 after 14 days of incubation. As a result, only nine isolates 43
 among 48 significantly reduced 140 $\mu\text{g/l}$ of MMAA by 44
 percentages ranging from 5 to 100% within 14 days (Table 2). 45
 Consequently, the nine isolates of arsenate-resistant bacteria 46
 may be able to mineralize MMAA, while the other 39 isolates 47
 have no or very weak mineralization activities. A previous 48
 study reported that nine of 10 isolates from lake water slightly 49
 mineralized 138 $\mu\text{g/l}$ of DMAA at mineralization percentages 50
 of less than 40% within 14 days.¹³ Sanders suggested that 51
 microorganisms in natural water would mineralize DMAA 52
 at a slow rate of approximately 1.1 ng/l/day.²³ Presumably, 53
 large parts of the environmental bacterial population have 54
 low or no mineralization activities for methylarsenic. 55

Among the nine isolates, the two isolates, C-1 and 56
 D-7, completely eliminated 140 $\mu\text{g/l}$ of MMAA in the 57
 culture medium after 14 days of incubation, and produced 58
 inorganic arsenic at concentrations of more than 70 $\mu\text{g/l}$ 59
 (Table 2). After both of the two isolates were inoculated 60
 into the culture medium including 140 $\mu\text{g/l}$ of MMAA again, 61
 the concentrations of inorganic arsenic and MMAA were 62
 monitored at the day 0, and the 1st, 3rd, 7th and 14th 63
 days. As a result, in the culture of C-1, the concentration 64
 of MMAA gradually decreased to below the limit of detection 65
 after 14 days, while that of inorganic arsenic increased to 66
 90.9 $\mu\text{g/l}$ from the 7th to the 14th day (Fig. 2). The culture 67
 of D-7 indicated that the MMAA disappeared within 7 days, 68

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 $\mu\text{g/l}$ 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 ($\mu\text{g/l}$)	113	0	109	129	0	71.4	123	112	70.0
Concentrations of inorganic arsenic ($\mu\text{g/l}$)	<14	87	<14	<14	71	<14	<14	<14	<14

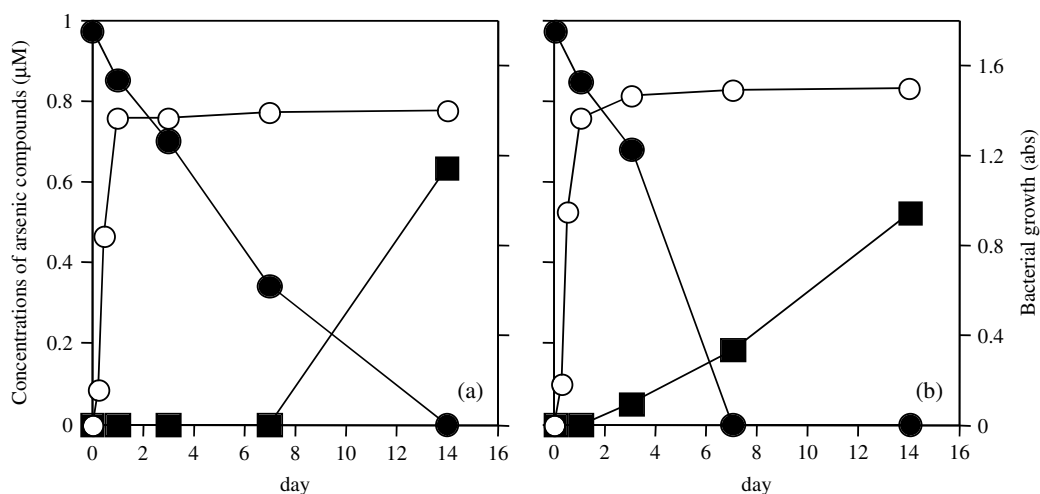


Figure 2. Changes in concentrations of MMAA (solid circles) and inorganic arsenic (solid squares), and bacterial growths (open circles), in bacterial cultures during the 14 days of incubation. The isolates of arsenate-resistant bacteria, C-1 (a) and D-7 (b), were inoculated to the culture medium including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

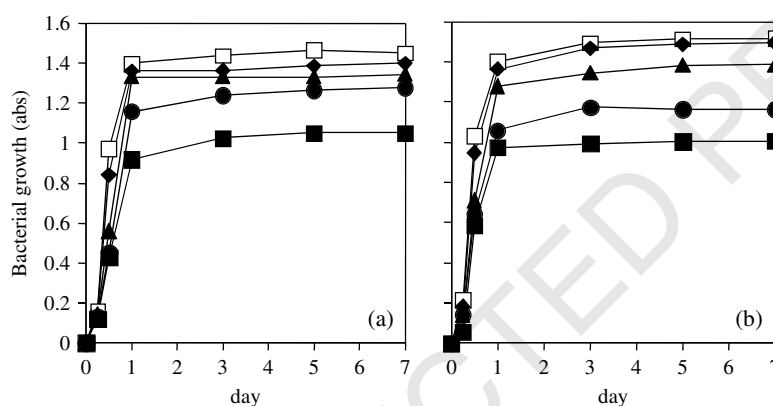


Figure 3. Changes in bacterial yields in bacterial cultures including arsenate at concentrations of 0 mg/l (open squares), 0.142 mg/l (solid diamonds), 1.42 mg/l (solid triangles), 14.2 mg/l (solid circles) and 142 mg/l (solid squares), during the 7 days of incubation. The isolates of arsenate-resistant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

1 and the production of inorganic arsenic slightly increased
 2 to 72.8 $\mu\text{g/l}$ for 14 days. The two isolates, C-1 and D-7,
 3 completely mineralized 140 $\mu\text{g/l}$ of MMAA within 14 days,
 4 and converted it to inorganic arsenic at concentrations of
 5 72.8 and 90.9 $\mu\text{g/l}$, respectively. Lehr *et al.* reported that
 6 *Mycobacterium meoaurum* converted about 500 $\mu\text{g/l}$ MMAA
 7 to inorganic arsenic at a conversion percentage of 50% within
 8 14 days.¹¹ The two isolates, C-1 and D-7, would have similar
 9 levels of MMAA-mineralization activities as *Mycobacterium*
 10 *meoaurum*. During the stationary phase in the cultures
 11 of two isolates, the MMAA level immediately decreased,
 12 while inorganic arsenic gradually increased. Furthermore,
 13 the concentrations of inorganic arsenic did not coincide with
 14 the initial concentration of MMAA. The arsenic within the
 15 bacterial cells could not be monitored in this study, because
 16 the bacterial cells were eliminated during filtration in arsenic

measurement. Probably, the inorganic arsenic in bacterial cells 17
 was gradually released from the declining cells during the 18
 stationary phase, and the released inorganic arsenic could be 19
 slightly detected after the decrease of MMAA in the culture. 20

When the arsenate-resistances of C-1 and D-7 were 21
 estimated by monitoring the yields of bacteria in culture 22
 media including 0, 0.142, 1.42, 14.2 and 142 mg/l of arsenate, 23
 the bacterial yields of the both isolates during the stationary 24
 phase decreased in proportion to the concentration of arsenate 25
 in the culture medium (Fig. 3). Although the bacterial yields 26
 were reduced by arsenate, the two isolates, C-1 and D-7, grew 27
 during the first day and could survive in the culture medium 28
 until 142 mg/l of arsenate. The two isolates are strongly 29
 resistant to inorganic arsenic. In general, arsenate-resistant 30
 bacteria reduced the arsenate to arsenite within bacterial 31
 cells, and exported the arsenite out of cells.^{24,25} Probably, 32

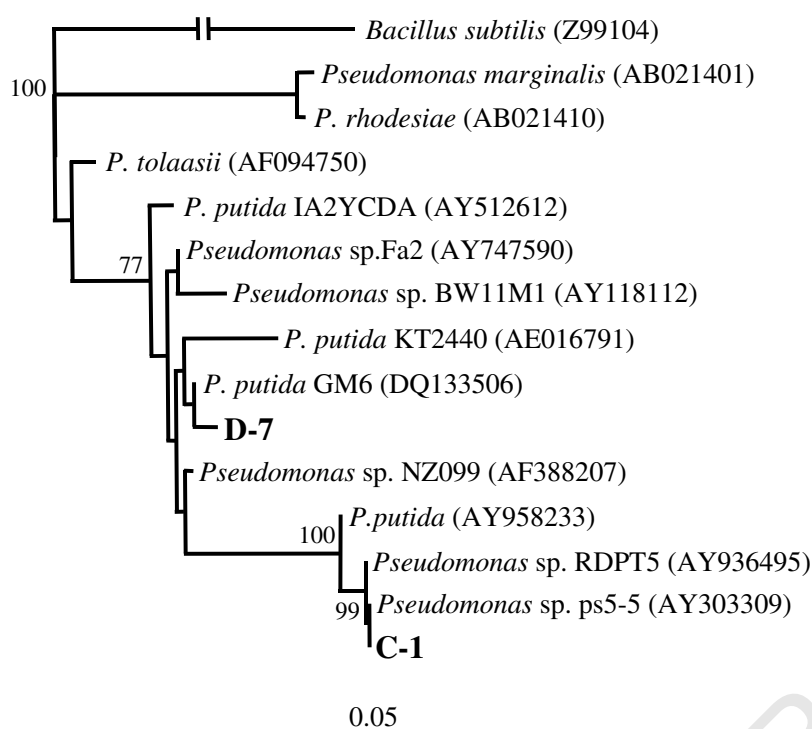


Figure 4. Phylogenetic tree for 16S rDNA sequence of the bacterial isolates, C-1 (a) and D-7. The tree was calculated from a dissimilarity matrix of ca. 500 bp alignment using a neighbor-joining algorithm. Bootstrap values larger than 50% (after 1000 resampling) are indicated on the branch.

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

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

24 In this study, although many parts of the bacterial biomass
25 in the arsenic-contaminated soils would have low levels
26 of organoarsenic-mineralization activities, bacteria of the
27 genus *Pseudomonas* which mineralize MMAA remarkably

well were isolated from the arsenic-contaminated soils. 28
Previously, *Mycobacterium meoaurum* was also reported to 29
be MMAA-mineralizing bacteria.¹¹ In aquatic environments, 30
various species of bacteria are thought to contribute to 31
the mineralization for DMAA.^{12,13} Accordingly, the several 32
bacterial species in arsenic-contaminated environments can 33
mineralize harmful organoarsenic compounds. More work 34
is needed to investigate the ecological characteristics of the 35
organoarsenic-mineralizing bacteria to establishing effective 36
and useful bioremediation. 37

Acknowledgment 38

This research was partly supported by a Grant-in-Aid for 39
Encouragement of Young Scientists (14780441) from the Ministry 40
of Education, Science, Sports and Culture. The Salt Science Research 41
Foundation, no. 0424 and the Nissan Science Foundation also support 42
this work. 43

REFERENCES 44

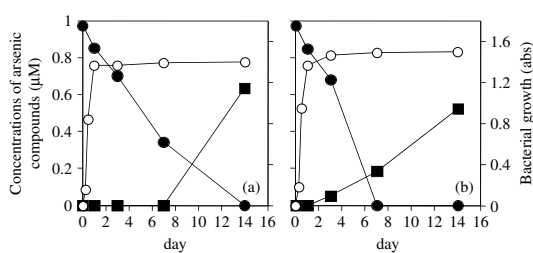
1. Tychinin DN, Kosterin PV. *Environ. Sci. Pollut. Res.* 2000; **7**: 245. 45
2. Vilensky JA, Redman K. *Ann. Emerg. Med.* 2003; **41**: 378. 46
3. Ishii K, Tamaoka A, Otsuka F, Iwasaki N, Shin K, Matsui A, 47
Endo G, Kumagai Y, Ishii T, Shoji S, Ogata T, Ishizaki M, Doi M, 48
Shimojo N. *Japan. Ann. Neurol.* 2004; **56**: 741. DOI: 10. 49
1002/ana.20290. 50
4. Ochi T, Suzuki T, Isono H, Kaise T. *Toxicol. Appl. Pharmac.* 2004; 51
200: 64. DOI: 10. 1016/j.taap.2004.03.014. 52

- 1 5. Henriksson J, Johannisson A, Bergqvist PA, Norrgren L. *Arch.*
2 *Environ. Contam. Toxicol.* 1996; **30**: 213.
- 3 6. Gadd GM. *FEMS Microbiol. Rev.* 1993; **11**: 297.
- 4 7. Mulligan CN, Yong RN, Gibbs BF. *Engng Geology.* 2001; **60**: 193.
- 5 8. Von Endt DW, Kearney PC, Kaufman DD. *J. Agric. Food Chem.*
6 1968; **16**: 17.
- 7 9. Woolson EA, Kearney PC. *Environ. Sci. Technol.* 1973; **7**: 47.
- 8 10. Quinn JP, McMullan G. *Microbiology* 1995; **141**: 721.
- 9 11. Lehr CR, Polishchuk E, Radoja U, Cullen WR. *Appl. Organometal.*
10 *Chem.* 2003; **17**: 831. DOI: 10.1002/aoc.544.
- 11 12. Maki T, Hasegawa H, Watarai H, Ueda K. *Anal. Sci.* 2004; **20**: 61.
- 12 13. Maki T, Hasegawa H, Ueda K. *Appl. Organometal. Chem.* 2005; **19**:
13 231. DOI: 10.1002/aoc.696.
- 14 14. Tessier A, Campbell PGC, Bisson M. *Anal. Chem.* 1979; **51**: 844.
- 15 15. Braman RS, Foreback CC. *Science* 1973; **182**: 1247.
- 16 16. Hasegawa H, Sohrin Y, Matsui M, Honjo M, Kawashima M. *Anal.*
17 *Chem.* 1994; **66**: 3247.
- 18 17. Maidak BL, Olsen GJ, Larsen N, Overbeek R, McCaughey MJ,
19 Woese C. *Nucl. Acids Res.* 1997; **25**: 109.
- 20 18. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. *J. Mol.*
21 *Biol.* 1990; **215**: 403.
- 22 19. Thompson JD, Higgins DG, Gibson TJ. *Nucl. Acids Res.* 1994; **22**:
23 4673.
- 24
- 25
- 26
- 27
- 28
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
20. Saitou N, Nei M. *Mol. Biol. Evol.* 1987; **4**: 406. 39
21. Cullen WR, Reimer KJ. *Chem. Rev.* 1989; **89**: 713. 40
22. Francesconi KA, Kuehnelt D. Arsenic compounds in the 41
environment. In *Environmental Chemistry of Arsenic*,
42 Frankenberg WT Jr (ed.). Marcel Dekker: New York, 2002;
43 51–94.
23. Sanders JG. *Chemosphere.* 1979; **8**: 135. 44
24. Tamas MJ, Wysocki R. *Curr. Genet.* 2001; **40**: 2. DOI: 10.
45 1007/s002940100234.
25. Rosen BP. *FEBS Lett.* 2002; **529**: 86. 46
26. Wang SJ, Loh KC, Chua SS. *Enzyme Microbial. Technol.* 2003; **32**:
47 422. DOI: 10.1016/S0141-0229(02)00315-0. 48
27. Nelson KE, Weinel C, Paulsen IT, Dodson RJ, Hilbert H, Martins
49 dos Santos VA, Fouts DE, Gill SR, Pop M, Holmes M, Brinkac L,
50 Beanan M, DeBoy RT, Daugherty S, Kolonay J, Madupu R,
51 Nelson W, White O, Peterson J, Khouri H, Hance I, Chris Lee P,
52 Holtzapple E, Scanlan D, Tran K, Moazzez A, Utterback T,
53 Rizzo M, Lee K, Kosack D, Moestl D, Wedler H, Lauber J,
54 Stjepandic D, Hoheisel J, Straetz M, Heim S, Kiewitz C, Eisen JA,
55 Timmis KN, Dusterhoft A, Tumbler B, Fraser CM. *Environ.*
56 *Microbiol.* 2002; **4**: 799.
28. Lovley DR. *Nat. Rev. Microbiol.* 2003; **1**: 35. DOI: 10.
57 1038/nrmicro731. 58
- 59
- 60
- 61
- 62
- 63
- 64
- 65
- 66
- 67
- 68
- 69
- 70
- 71
- 72
- 73
- 74
- 75
- 76

Applied Organometallic Chemistry

(Appl. Organometal. Chem.)

To establish useful bioremediation techniques for mineralizing harmful organoarsenic compounds, environmental factors such as arsenic concentrations and bacterial biomass in the soils of Ohkunoshima Island were investigated. The distribution of contamination levels of arsenic compounds was different in the various areas of the Island. Among the isolates of arsenate-resistant bacteria from the soils, a few isolates have remarkable reduction activities for monomethylarsonic acid, and the two isolates with strong activities belonged to the group of *Pseudomonas putida* strains.



T. Maki*, N. Takeda, H. Hasegawa and
K. Ueda000-000

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53

UNCORRECTED PROOFS