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Adsorption of tributyltin by tributyltin resistant marine *Pseudoalteromonas* sp. cells

Haruo Mimura [a,*], Ryusei Sato [a], Yuichi Furuyama [a], Akira Taniike [a], Masahiro Yagi [b], Kazutoshi Yoshida [c], Akira Kitamura [a]

[a] Graduate School of Maritime Sciences, Kobe University, 5-1-1, Fukae, Higashinada, Kobe 658-0022, Japan

[b] Department of Environmental Chemistry, Kobe Institute of Health, 4-6, Minatojima, Chuo, Kobe 650-0046, Japan

[c] Hyogo Prefectural Institute of Technology, 3-1-12, Yukihira, Suma, Kobe 654-0037, Japan

*Corresponding author.
Tel.: +81-78-431-6344; Fax: +81-78-431-6365.
E-mail address: hmimura@maritime.kobe-u.ac.jp (H. Mimura).

Abstract

The isolate, *Pseudoalteromonas* sp. TBT1, could grow to overcome the toxicity of tributyltin chloride (TBTCl) up to 30 μM in the absence of Cl^- in the medium until the cells reached an exponential phase of growth. The viability, however, was reduced after the cells reached a stationary phase. The degradation products, such as dibutyltin (DBT) and monobutyltin (MBT), were not detected in the growth medium, indicating that the isolate has no ability to degrade TBT into less toxic DBT and MBT. Up to about $10^{7.5}$ TBT molecules were adsorbed by a single cell. The observation of morphological changes with an electron microscope showed that the cell surface became wrinkled after exposure to the lethal concentration of 10 mM TBTCl. These results indicate that the resistance of the isolate toward the toxicity of TBTCl is not related to the degradation of TBT to less toxic compounds but, rather, is closely related to the unique cell surface, which seems to play an important role in preventing the diffusion of TBTCl into the cytoplasm.

Key words: Accelerator analysis; Adsorption; Tributyltin chloride; *Pseudoalteromonas* sp.; Seawater

Introduction

Organotin compounds have been extensively used worldwide due to their wide applications in the industrial and agricultural fields as industrial catalysts, polyvinyl chloride stabilizers, biocides for fungi, bacteria, and insects, and wood preservatives (Hoch, 2001). Tributyltin (TBT) oxide, TBT chloride (TBTCl), and triphenyltin chloride (TPhTCl) had been used particularly for the prevention of biofouling on ship hulls since the 1970s, which resulted in the global distribution of these organotin compounds into the marine environment (Guruge and Tanabe, 2001; Nemanic et al., 2002; Sudaryanto et al., 2004; Shim et al., 2005; Murai et al., 2005; Bhosle et al., 2006). Recently, these organotin compounds were detected even in deep-sea gastropods and crabs (Harino et al., 2005) as well as in marine mammals, such as whales (Harino et al., 2007), and in tuna (Ueno et al., 2004).

The induction of imposex, an abnormally progressive formation of a penis and a sperm duct in females, was observed in abalone (Horiguchi et al., 2002), ivory shells (Horiguchi et al., 2006), and gastropods (Horiguchi, 2006) by exposure to TBTCl and TPhTCl. Moreover, the testosterone metabolic processes in *Daphnia magna* were affected by exposure to sublethal concentrations of TBT (Oberdörster et al., 1998). Consequently, the toxicity of these compounds can be included with the action of endocrine disrupters. Organotin compounds also have mutagenicity (Hamasaki et al., 1993). Therefore, the International Maritime Organization (IMO) prohibited the use of such

organotin compounds as antifouling biocides after 1 January 2008 in order to prevent terrestrial pollution (IMO, 2001). While the efflux of TBTCl into the marine environment has to be avoided, Lewis et al. have pointed out the importance of preventing biofouling on ship hulls in order to prevent the biological invasion of non-indigenous marine organisms in Antarctica (2004).

Organotin compounds released from ship hulls are stable and exist in the marine environment for prolonged periods (Hoch, 2001), which may cause an increase in the frequency of the occurrence of TBT-resistant marine microorganisms (Wuertz et al., 1991; Suzuki et al., 1992). A TBT-resistant marine *Alteromonas* sp., which grew in the presence of 125 μM of TBTCl, has been isolated from coastal seawater (Fukagawa et al., 1992), and a protein deduced from the cloned genes responsible for TBT resistance was related to Na^+/H^+ antiporters and various Ca^{2+} transporters (Fukagawa and Suzuki, 1993). A multidrug efflux pump was shown to be essential for the TBT resistance of *Pseudomonas stutzeri* (Jude et al., 2004). Recently, a TBT-degrading *Aeromonas veronii*, which used TBT as carbon source, was isolated from an estuarine environment (Cruz et al., 2007).

We isolated a TBT-resistant eutrophic marine bacterium from the sediment in a ship's ballast tank. The isolate grew in the presence of 150 μM of TBTCl in the liquid medium. The resistant mechanism toward the toxicity of TBT, however, is not clear. Kubota et al. (2004) demonstrated that the number of cell-associated Sn elements originating in TBTCl was counted with an

accelerator. Therefore, in this study, the adsorption ability of the isolate toward TBTCl was examined in response to the changes in the concentrations of externally added TBTCl. The ability to degrade TBT to less toxic dibutyltin (DBT) and monobutyltin (MBT) was also examined. The morphological changes of the cells after exposure to a lethal concentration of TBTCl were observed with a scanning electron microscope.

Materials and Methods

Identification of the isolate

The identification of the isolate, *Pseudoalteromonas* sp. TBT1, was carried out on the basis of the partial 16S rDNA gene sequences as well as the biochemical and the physiological properties (Krieg and Holt, 1984). Sequencing was performed by a company commercially. Briefly, a partial 16S rDNA gene fragment from the V1 to V3 region (about 500 bp) was amplified by PCR and sequenced with a MicroSeq® 500 16S rDNA kit (Applied Biosystems, CA, USA). The sequencing data obtained were used for the homology analysis, which was carried out with MicroSeq® Microbial Identification System Software and MicroSeq® Bacterial 500 Library (Applied Biosystems, CA, USA). A phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987).

Growth and medium composition

A growth medium containing 5 g Bacto peptone per liter (Difco, MD, USA), 1 g yeast extract per liter (Difco, MD, USA), 0.4 M NaCl, 10 mM KCl, 10 mM $CaCl_2 \cdot 2H_2O$, 53 mM $MgCl_2$, and 28 mM Na_2SO_4 was used in the experiments unless otherwise noted. Pre-incubation was carried out for one day at 30˚C in a liquid medium in the absence of TBTCl. The medium pH was adjusted to 7.8 using tetramethylammonium hydroxide (TMAH). Incubation was carried out after the addition of a cell suspension to the growth medium with or without TBTCl to give a one-thousandth dilution.

For the accelerator analysis, cells grown in the presence of TBTCl were harvested by centrifugation (10,000 × g, 5 min) at an early stationary phase of growth. Cells were washed three times with distilled water and resuspended in about 200 μl of distilled water. The samples thus obtained were kept at -20˚C until use.

Resting cells were prepared from cells grown until the early stationary phase in the absence of TBTCl. Cells in the growth medium (1 ml) were harvested by centrifugation and washed twice with a solution containing 0.4 M NaCl, 10 mM KCl, 10 mM $CaCl_2 \cdot 2H_2O$, 53 mM $MgCl_2$, and 28 mM Na_2SO_4, where the pH was adjusted to 7.8 using 10 mM HEPES - TMAH. The cells thus obtained were resuspended in 1 ml of the same solution. Tributyltin chloride was added to the cell suspension to obtain specific concentrations. The sample was then vigorously shaken for about 1 min. Washing was carried out three times with distilled water, and the cells were resuspended in

about 200 μl of distilled water. The samples thus obtained were stored at -20˚C until use.

Enumeration of the number of colony-forming cells

A one-tenth serial dilution with the solution described above was obtained. The cells were then spread onto agar plates containing the growth medium and agar (15 g $liter^{-1}$). Plates were incubated at 30˚C for 1 day, and the colonies on the plates were counted. The data are shown as colony-forming units (CFU) ml^{-1}.

Preparation of samples for gas chromatography-mass spectrometry analysis

Cells were separated from the supernatant by centrifugation (13,000 × g, 7 min) after the cells were grown for one, two, and three days in a growth medium (100 ml) containing 150 μM of TBTCl. The ethylated organotin compounds, which had been extracted by the procedure described below, were measured using gas chromatography (GC) - mass spectrometry (MS). A cell pellet (0.1 ml) was suspended in a solution (3 ml) containing one volume of 1 M hydrobromic acid dissolved in methanol and one volume of ethyl acetate. After the insoluble fraction was removed by filtration, a saturated sodium bromide solution (5 ml) was added to the solution. Next, a solution (1.5 ml) containing three volumes of ethyl acetate and two volumes of hexane was added to the solution. After vigorous shaking, the hexane portion was recovered. This procedure was repeated twice. The samples obtained were mixed and dehydrated with anhydrous sodium sulfate. One ml of a 2 M acetic acid-sodium acetate buffer, pH 5.0, containing 2% sodium

tetraethylborate was added to the sample followed by the addition of 1 ml of ethanol. The ethylated organotin compounds were extracted into hexane. After dehydration with anhydrous sodium sulfate, the total volume of the extract was adjusted to 10 ml using hexane. As for the organotin compounds in the supernatant, one ml was pipetted out and mixed with 0.01 ml of a 2 M acetic acid-sodium acetate buffer, pH 5.0, containing 2% sodium tetraethylborate. The extraction process of the ethylated organotin compounds was the same as that used for the pellet described above. The samples thus obtained were quantified with the GC/MS (GC5890II-MS5971A, Hewlett-Packard, USA).

Accelerator analysis

Each sample was dropped onto a hollow (2 mm diameter × 0.5 mm depth) in a carbon plate (20 mm × 100 mm × 2 mm thickness) by pipetting and gently dried for about 40 min. Some repetitions of this process were required to finish loading the sample, during which process the volume of the sample was drastically reduced in the hollow (Kubota et al., 2004).

Quantitative analysis of the element of Sn originated in TBTCl adsorbed by the cells as well as of the elements with medium mass in the cells was performed by the analysis of particle-induced X-ray emission (PIXE). The experimental setup is shown in Fig. 1. The samples were analyzed in a vacuum chamber connected to the M30 beam line of a 5SDH-2 Pelletron accelerator under a pressure of 1.0×10^{-4} Pa. The sample target was exposed to a probe beam of 3.0 MeV protons at

an incident angle of 0˚ with a beam current of 2 nA and a beam diameter of 1 mm. Proton incidence of 3.0 μC was necessary to obtain reasonable statistical accuracy of the X-ray yields from Sn and other atomic species measured under the present condition. A Si-PIN photodiode detector was positioned at a 135˚ angle to the incident direction of the probe beam. A piece of aluminum foil with 90 μm thickness was mounted in front of the detector to reduce low-energy background X-rays and scattered protons. The solid angle subtended by the Si-PIN detector was 5.4×10^{-2} sr.

Preparation of samples for scanning electron microscopy

Cells at the early stationary phase of growth (10 ml) were filtrated with a membrane filter (pore size, 0.45 μm) and washed with 50 ml of a solution containing 0.4 M NaCl, 10 mM KCl, 10 mM $CaCl_2 \cdot 2H_2O$, 53 mM $MgCl_2$, and 28 mM Na_2SO_4, in which the pH was adjusted to 7.8 using 10 mM HEPES - TMAH. Then, cells trapped on the membrane filter were resuspended in 10 ml of the same solution.

Cells were fixed in 1% (vol/vol) glutaraldehyde for 1 h at 4˚C after exposure to 10 mM of TBTCl for 10 min, washed twice with the solution described above, dehydrated once for 1 h in 50, 70, 90, 95, and 100% of ethanol, and suspended in 100% of *t*-butyl alcohol. After being freeze-dried, they were pasted on a carbon tape and coated with Pt-Pd particles under vacuum. The samples thus obtained were observed under a scanning electron microscope (XL30 CP, FEI

Company, the Netherlands).

Results and Discussion

The isolate was Gram-negative, rod-shaped, and motile and not spore-forming. The isolate had catalase and oxidase activities but not glucose fermentation activity. Further identification was carried out with molecular techniques. Although we did not obtain 100% homologous sequences in the database (Applied Biosystems, CA, USA), the isolate was related to several species of the genus *Pseudoalteromonas*, such as *P. nigrifaciens* [92.15%], *P. atlantica* [91.95%], *P. undina* [91.76%], and *P. carrageenovora* [91.57%]. The isolate constructed a unique branch in the phylogenetic tree. These results indicate that the isolate is a novel strain and belongs to the genus *Pseudoalteromonas*. The partial rDNA sequence of the isolate, *Pseudoalteromonas* sp. TBT1, was deposited in the DDBJ/GenBank/EMBL under the accession number **AB298440**.

The colony-forming ability of the isolate on nutrient agar plates containing various concentrations of TBTCl was examined after the cells were streaked on the plates (Table 1). It took about 3 days for the cells to succeed in making colonies in the presence of 150 μM of TBTCl. Even in the presence of 700 μM of TBTCl, the isolate could make colonies on the plate. These results indicate that the isolate has highly resistant ability toward the toxicity of TBTCl.

We quantified the concentrations of TBT and the degradation products, such as DBT and MBT,

in the supernatant and the cell pellet with GC/MS after the cells reached the stationary phase (Table 2). The values in the cell pellet were within 90 to 107 μmol (g dry weight)$^{-1}$ throughout the incubation period. In the samples obtained from the supernatant, TBT was not detected, except for the one taken after 3 days of incubation. The detected value of 1 nmol ml^{-1} was less than 0.7% at the initial concentration of 150 nmol ml^{-1}. Dibutyltin and monobutyltin were not detected at all from any of the samples quantified.

The concentration of TBT remaining in the supernatant was negligible, indicating that almost all the TBT molecules in the growth medium were adsorbed by the cells. The degradation products, such as DBT and MBT, were not detected from samples prepared from either the cell pellet or the supernatant throughout the incubation period. These results indicate that the isolate cannot degrade TBT into less toxic DBT and MBT and the resistant ability toward the toxicity of TBT is not related to the detoxification of TBT.

The growth curve in the presence of 30 μM of TBTCl is shown in Fig. 2 when the isolate was grown in a medium without the addition of chloride anions (Cl^-). The growth of the cells reached the stationary phase after around 37 hours of incubation at 30˚C regardless of the presence of TBTCl, at which time the number of colony-forming cells was $10^{8.0\pm0.2}$ ml^{-1} (n = 3). After the cells reached the stationary phase, the values were reduced to $10^{7.4\pm0.3}$ and $10^{4.9\pm0.5}$ ml^{-1} at 61 and 92 hours of incubation, respectively, in the presence of TBTCl. The values at the stationary phase

of growth were maintained until 92 hours of incubation in the absence of TBTCl.

In order to count the number of the Cl element which is originated in TBTCl in the cell pellet with the accelerator, cells were grown in a medium without the external addition of Cl^-. Since the isolate required Na^+ and Mg^{2+} for growth, NaCl and $MgCl_2$ in the medium were replaced with Na_2SO_4 and $MgSO_4 \cdot 7H_2O$. In the medium, the sensitivity of the isolate toward the toxicity of TBTCl was enhanced by the absence of Cl^-, and the maximum concentration of TBTCl at which the cells could grow was reduced to 30 μM. Tokunaga et al. reported that the multidrug efflux pump in the moderately halophilic *Chromohalobacter* sp. was induced by exposure to 2. 0 M NaCl (2004). As for the isolate, Cl^- as well as Na^+ might be important for the induction and/or the activation of an efflux pump in relation to resistance toward the toxicity of TBTCl.

The values of the areal densities for the elements interacting with the cells are shown in Table 3. The areal density of Sn in TBT, which was obtained from the spectrum of PIXE, was $10^{16.3}$ cm^{-2}. The spectrum of Cl originated in TBTCl, however, was not detected at all from any of the samples. The areal densities of the elements with medium mass, such as Cu, Fe, Ni, and Zn, were in the order of 10^{14}, 10^{15}, 10^{14}, and 10^{15}, respectively, regardless of the presence of TBTCl.

The number of the element of Br detected in the presence of TBTCl was $10^{4.5}$ $cell^{-1}$, i.e., ten times higher than that in the absence of TBTCl. The amounts of Cu and Fe interacting with a single cell were reduced to $10^{-0.4}$ times from the control values, $10^{5.3}$ for the Cu $cell^{-1}$ and $10^{5.9}$ for

the Fe cell^{-1}. For Ni and Zn, the interaction of those elements with a single cell was increased $10^{0.2}$ and $10^{0.5}$ times, respectively, from the control values of $10^{4.8}$ for the Ni cell^{-1} and $10^{5.7}$ for the Zn cell^{-1}.

The total number of TBT adsorbed by a single cell was quantified as $10^{6.8}$ (Table 3). Theoretically, the maximum number of TBT which can be delivered to a single cell is $10^{8.3}$ when 30 μM of TBTCl is in a cell suspension containing $10^{8.0}$ CFU ml^{-1}. As shown in Table 2, $10^{8.0}$ CFU ml^{-1} seems to be a large enough number for the complete adsorption of 30 nmol ml^{-1} of TBTCl. However, the number of TBT actually adsorbed was $10^{1.5}$ times smaller than the maximum number of TBT delivered to a single cell. The adsorption capacity of the cells toward TBT might be changed by growth in the absence of Cl^-. As for the other possibility, the washing process with distilled water, which was necessary to prepare the samples for the accelerator analysis, might affect the desorption of TBT molecules from associated cells.

The Cl in TBTCl, which was associated with the cells, was not detected with the accelerator. The dissociation of Cl from TBTCl might occur in the adsorption process of TBTCl to the cells. The low detection efficiency of the Si-PIN photodiode detector toward Cl should also be taken into consideration (Kubota et al., 2004).

The number of Br adsorbed by a single cell in the presence of TBTCl was obviously higher than that in the absence of TBTCl. Since the purity of TBTCl purchased from a chemical industry

was about 95%, tributyltin bromide (TBTBr) seemed to be contaminated in the reagent in the range of less than 5%. The Br detected from the samples seems to be originated in TBTBr.

No obvious differences in the numbers of elements with medium mass were observed between the values obtained in the presence and the absence of TBTCl, indicating that the interaction of those metal elements with the cells is independent of the action of TBTCl.

Various concentrations of TBTCl, from 3 μM to 10 mM, as final concentrations were externally added to the cell suspension, and the adsorption of Sn to the resting cells was measured with the accelerator (Fig. 3). The total number of Sn adsorbed by a single cell linearly increased with an increase in the concentrations of TBTCl externally added up to 3 mM, and the maximum number of Sn adsorbed was $10^{7.6}$ molecules $cell^{-1}$. The number of Sn originated in TBTCl became saturated in the presence of more than 3 mM of TBTCl.

Since the isolate could not degrade TBT (see Table 2), all of the Sn adsorbed is originated in TBT. For example, the number of TBT adsorbed by a single cell was $10^{6.5}$ molecules $cell^{-1}$ in the presence of 150 μM of TBTCl. However, the maximum number of TBT molecules which can be distributed to a single cell is estimated to be $10^{7.5}$ molecules $cell^{-1}$, i.e., $(150 \times 10^{-9}$ mol $ml^{-1} \times 6.0 \times 10^{23}$ molecules $mol^{-1}) / (10^{9.5}$ CFU $ml^{-1}) = 10^{7.5}$ molecules $cell^{-1}$. This value was ten times larger than that obtained experimentally. A difference of approximately ten times between the measured and the calculated values was observed for any concentrations of TBTCl externally added. The

same phenomenon has been observed for samples obtained from growing cells in the presence of 30 μM of TBTCl (see Table 3). It is difficult to explain why the measured values are about ten times smaller than those calculated. A possible explanation is that a certain number of TBT in the total TBT adsorbed by a single cell might be desorbed from the cells to reach a new equilibrium condition through the washing process, which contributes to the reduction of electrolytes in the cell suspension.

Morphological changes of the isolate are shown in Fig. 4. The cell surface became wrinkled and rough after exposure to a lethal concentration of 10 mM of TBTCl, while the untreated cells showed a smooth surface. I think that wrinkled cells as a result of exposure to a lethal concentration of TBTC1 are observed with disruption of osmotic regulation because, in general, high osmotic pressure in a cell suspension causes cells to wrinkle. Changes in the surface, such as wrinkles and roughness, appear to contribute to an increase in the surface area of the cells, which results in an increase in the adsorption capacity of the cell surface toward TBT.

Throughout the experiments, it became obvious that the cell surface of the isolate plays an important role in resistance toward the toxicity of TBTCl. Except for the high adsorption ability toward TBT, the isolate, *Pseudoalteromonas* sp. TBT1, might have membrane-binding proteins which pump TBT out from the cytoplasm (Jude et al., 2004). Further study is necessary to identify the resistant mechanism of the isolate toward the toxicity of TBTCl.

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Figure captions

Fig. 1. Plan view of the target setup for accelerator analysis.

Fig. 2. Changes in the number of viable cells during the incubation of *Pseudoalteromonas* sp. TBT1 in the presence of TBTCl.

Pre-incubation of the isolate was carried out for 2 days at 30˚C in a growth medium containing 5 g Bacto peptone (Difco, MD, USA), 1 g yeast extract (Difco, MD, USA) ($liter^{-1}$), 50 mM Na_2SO_4, 50 mM $MgSO_4 \cdot 7H_2O$, and 5 mM K_2SO_4. The medium pH was adjusted to 7.8 with 0.1 M Na_2CO_3. Incubation was started by the addition of the cell suspension into the growth medium with (closed circles) and without (open circles) 30 μM of TBTCl to give an initial number of $10^{4.1\pm0.2}$ CFU ml^{-1}.

Growth experiments were carried out three times independently, and each piece of data is shown as the averaged value ± SD (n = 3).

Fig. 3. Changes in the total numbers of the element of Sn originated in TBTCl adsorbed by a resting cell.

All the volume in a sample was loaded completely onto a hollow in a carbon plate. Total number of Sn adsorbed by a single cell in the presence of TBTCl was obtained by the following

equation: $A_{Sn} \times S / N_c$, where A_{Sn} is the areal density (cm^{-2}) of Sn in a hollow; S (= $10^{-1.5}$ cm^2) is the area of every hollow in the carbon plate; and N_c (= $10^{9.5}$ CFU) is the averaged number of colony-forming resting cells in the sample.

Experiments were carried out twice independently, and the averaged values are shown. The values of A_{Sn} scattered by about a factor of 2 at given concentrations of TBTCl externally added.

Fig. 4. Morphological changes of the cells after exposure to a lethal concentration of TBTCl.

The morphology was observed before (A) and after (B) exposure to 10 mM of TBTCl.

Fig. 1

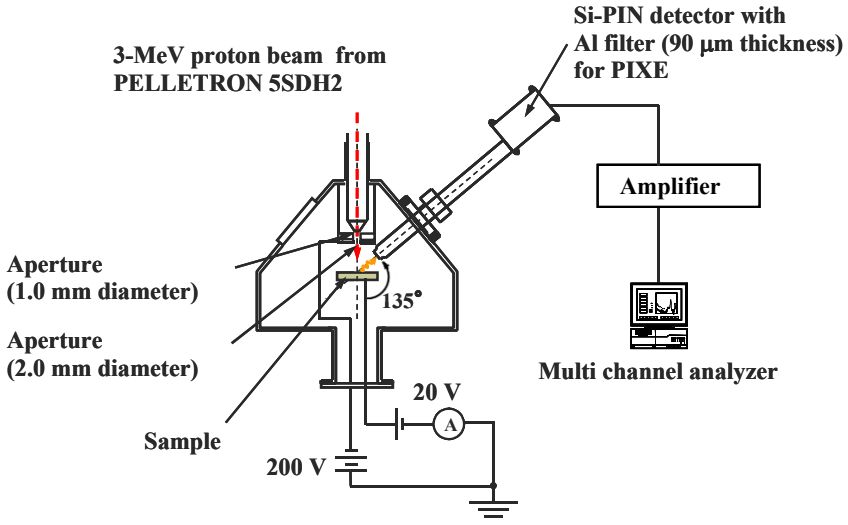
Si-PIN detector with
Al filter (90 μm thickness)
for PIXE
3-MeV proton beam from
PELLETRON 5SDH2
Amplifier
Aperture
(1.0 mm diameter)
Aperture
(2.0 mm diameter)
135°
Multi channel analyzer
20 V
A
Sample
200 V

Fig. 2

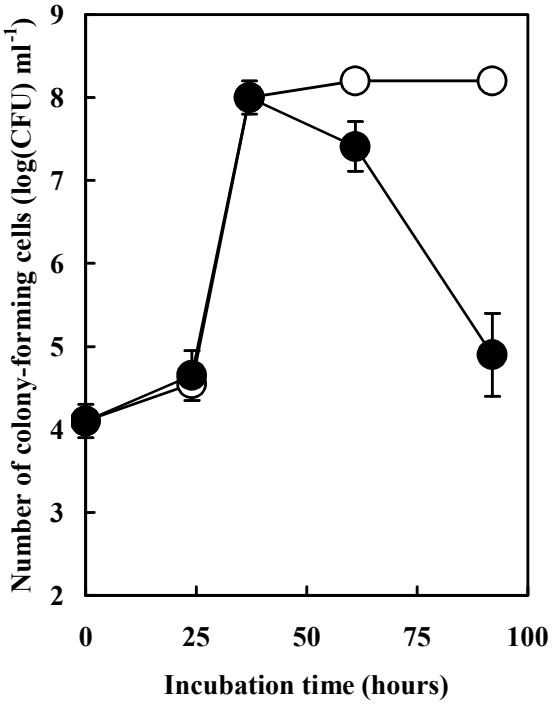
Number of colony-forming cells (log(CFU) ml-1)
9
8
7
6
5
4
3
2
0
25
50
75
100
Incubation time (hours)

Fig. 3

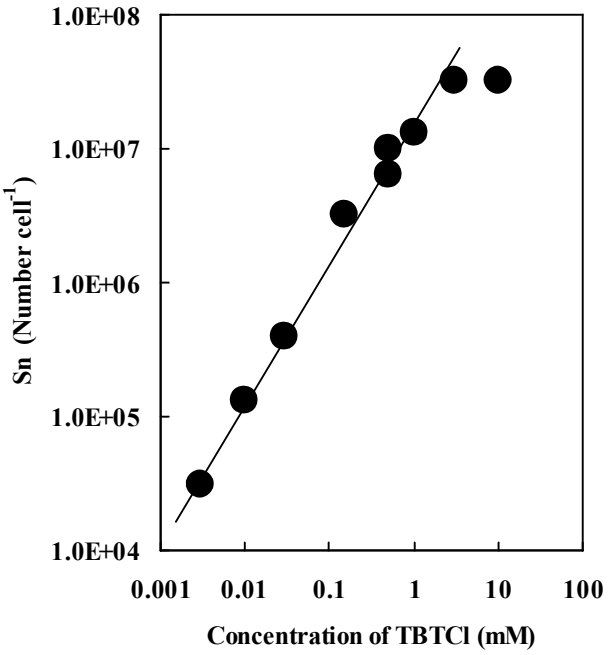
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
Sn (Number cell-1)
0.001
0.01
0.1
1
10
100
Concentration of TBTCl (mM)

Fig. 4

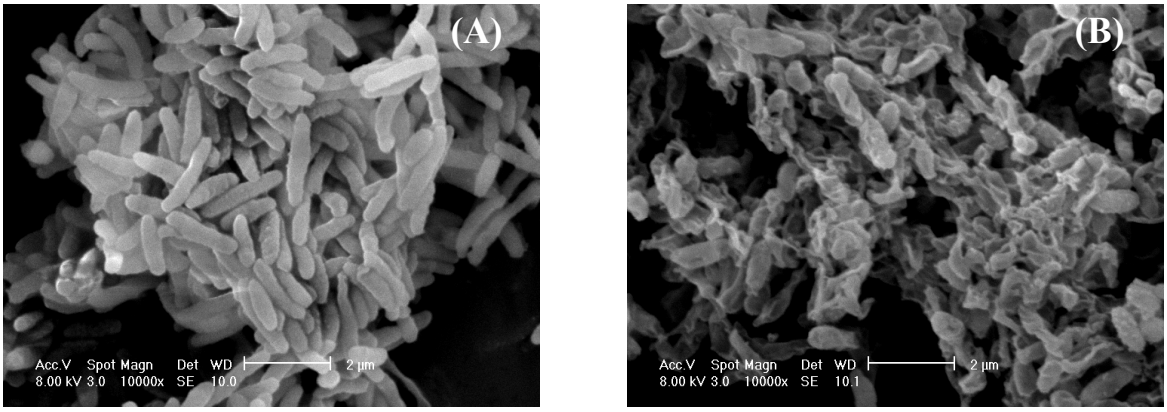
(A)
Acc.V Spot Magn Det WD 2 µm
8.00 kV 3.0 10000x SE 10.0
(B)
Acc.V Spot Magn Det WD 2 µm
8.00 kV 3.0 10000x SE 10.1

Table 1

Growth ability of *Pseudoalteromonas* sp. TBT1 on nutrient agar plates containing various concentrations of TBTCl

TBTCl (μM)	Incubation time (day) at 30°C				
	0.5	1	2	3	5
None	+	++	++	+++	+++
150	w	+	+	++	++
300	w	+	+	++	++
500	-	w	+	+	+
700	-	-	w	+	+

The isolate was pre-incubated for 1 day on an agar plate in the absence of TBTCl at 30°C. A line with a length of about 6 mm was drawn on the new plates using an autoclaved toothpick to inoculate cells. Growth in the presence of TBTCl up to 700 μM was evaluated in comparison with that in the absence of TBTCl. The inoculated line on the plate becomes visible when the cells grow. Growth on a given day was classified into five different stages as follows: - (no growth), weak (weak growth), + (growth), ++ (satisfactory growth), and +++ (full growth).

Table 2

Adsorption of TBT by *Pseudoalteromonas* sp. TBT1 cells grown in the presence of TBTCl

Incubation (day)	TBT in cell pellet (μmol (g dry weight)$^{-1}$)	TBT in supernatant (nmol ml^{-1})
Control	0	150
1 day	107	ND[a]
2 days	90	ND
3 days	96	1

The growth medium contained 150 μM of TBTCl, and the numbers of colony-forming cells after 1 to 3 days of incubation changed from $10^{8.1}$ to $10^{8.2}$ CFU ml^{-1}.

Experiments were carried out twice independently, and the averaged value is shown. The difference between the averaged and the individual values was less than 6% for all samples.

[a] Not detected.

Table 3

The numbers of Sn and Cl originated in TBTCl and metal elements with medium mass adsorbed by *Pseudoalteromonas* sp. TBT1 cells grown in the presence and absence of TBTCl

Element	Cells were grown			
	in the presence of TBTCl (30 μM)		in the absence of TBTCl	
	Areal density (cm^{-2})	Number of adsorption[a] (number of element $cell^{-1}$)	Areal density (cm^{-2})	Number of adsorption[a] (number of element $cell^{-1}$)
Sn	$10^{16.3}$	$10^{6.8}$	ND	ND
Cl	ND[b]	ND	ND	ND
Br	$10^{14.0}$	$10^{4.5}$	$10^{13.0}$	$10^{3.5}$
Cu	$10^{14.4}$	$10^{4.9}$	$10^{14.8}$	$10^{5.3}$
Fe	$10^{15.0}$	$10^{5.5}$	$10^{15.4}$	$10^{5.9}$
Ni	$10^{14.5}$	$10^{5.0}$	$10^{14.3}$	$10^{4.8}$
Zn	$10^{15.7}$	$10^{6.2}$	$10^{15.2}$	$10^{5.7}$

The growth medium contained 5 g Bacto peptone, 1 g yeast extract ($liter^{-1}$), 50 mM Na_2SO_4, 50 mM $MgSO_4 \cdot 7H_2O$, and 5 mM K_2SO_4. The medium pH was adjusted to 7.8 with 0.1 M Na_2CO_3. Cells were grown for 37 hours at 30˚C in the presence and absence of TBTCl at a final concentration of 30 μM.

[a] Regardless of the presence of TBTCl, the value of $10^{8.0}$ CFU ml^{-1} was used as the number of colony-forming cells (see Fig. 2). For example, the number of Sn adsorbed by a single cell was calculated by the following equation: ($10^{16.3}$ areal density of Sn cm^{-2}) × ($10^{-1.5}$ cm^2) / ($10^{8.0}$ CFU) = $10^{6.8}$ Sn $cell^{-1}$, where the value of $10^{-1.5}$ means the area of a hollow in a carbon plate.

Experiments were carried out twice independently, and the areal density for each element is shown as the averaged value. Those of each element scattered by about a factor of 2.

[b] Not detected.