Environmental Impact of Tributyltin-Resistant Marine Bacteria in the Indigenous Microbial Population of Tributyltin-Polluted Surface Sediments

HARUO MIMURA1*, MASAHIRO YAGI2, AND KAZUTOSHI YOSHIDA3

1Graduate School of Maritime Sciences, Kobe University, Kobe 658-0022, Japan
2Department of Environmental Chemistry, Kobe Institute of Health, Kobe 650-0046, Japan
3Hyogo Prefectural Institute of Technology, Kobe 654-0037, Japan

Received 4 August, 2015/Accepted 19 November, 2016

We compared the TBT-resistant ability of resting cells prepared from isolates that formed colonies on nutrient agar plates containing 100 µM tributyltin (TBT) chloride, such as Photobacterium sp. TKY1, Halomonas sp. TKY2, and Photobacterium sp. NGY1, with those from taxonomically similar type strains. Photobacterium sp. TKY1 showed the highest ability among those three isolates. The number of surviving Photobacterium sp. TKY1 cells was hardly decreased after 1 h of exposure to 100 µM TBTCl, regardless of the number of resting cells in the range from 10^{9.4} to 10^{4.2} CFU mL^{-1}. In such an experimental condition, the maximum number of TBT molecules available to associate with a single cell was estimated to be approximately 6.0 \times 10^{11.8}. Resting cells prepared from type strains Photobacterium ganghwense JCM 12487^T and P. halotolerans LMG 22194^T, which have 16S rDNA sequences highly homologous with those of Photobacterium sp. TKY1, showed sensitivity to TBT, indicating that TBT-resistant marine bacterial species are not closely related in spite of their taxonomic similarity. We also estimated the impact of TBT-resistant bacterial species to indigenous microbial populations of TBT-polluted surface sediments. The number of surviving Vibrio natriegens ATCC 14048^T cells, 10^{6.2^{\pm 0.3}} CFU mL^{-1}, was reduced to 10^{4.4^{\pm 0.4}} CFU mL^{-1} when TBT-resistant Photobacterium sp. TKY1 cells, 10^{9.1^{\pm 0.2}} CFU mL^{-1}, coexisted with 10^{9.4^{\pm 0.2}} CFU mL^{-1} of V. natriegens ATCC 14048^T cells in the presence of 100 µM TBTCl. These results indicate that the toxicity of TBT to TBT-sensitive marine bacterial populations might be enhanced when a TBT-resistant marine bacterial species inhabits TBT-polluted surface sediments.

Key words : TBT-resistant marine bacteria / Busy ports / TBT-polluted surface sediments / Survivability.

INTRODUCTION

The usage of organotin compounds had expanded worldwide because of their broad applications in industry and agriculture as catalysts, polyvinyl chloride stabilizers, biocides for fungi, bacteria, and insects, and wood preservatives (Hoch, 2001). In particular, tributyltin (TBT) oxide, TBT chloride (TBTCl), and triphenyltin (TPhT) chloride were used to prevent biofouling on ship hulls since the 1970s, resulting in extensive pollution by these organotin compounds in the marine environment (Sudaryanto et al., 2002; Murai et al., 2005; Furdek et al., 2012). Pollution by organotin compounds has already spread to the Antarctic Ocean. In sediments close to the channels of Antarctica, high concentrations of organotin compounds, up to 2,290 µg Sn kg^{-1} sediment, were detected (Negri et al., 2004). Therefore, marine pollution by TBT originating from antifouling paints is still a great concern (Antizar-Ladislao, 2008).

Bioaccumulation of organotin compounds causes chronic toxicity in living things (Ebdon et al., 1989; Ueno et al., 2004; Harino et al., 2007). The compounds also act as endocrine disrupters (Oberdörster et al., 1998; Horiguchi et al., 2002). In addition to their toxicity, mutagenic effects on the Bacillus subtilis Rec^+ or Rec^-
strain (Hamasaki et al., 1992), Salmonella Typhimurium (Hamasaki et al., 1993) and the marine worm (Hagger et al., 2002) have been confirmed. The International Maritime Organization (IMO) has since prohibited the use of such organotin compounds as antifouling biocides through the International Convention on the Control of Harmful Anti-fouling Systems on Ships (IMO, 2008). While the use of TBT must be avoided, Lewis et al. (2004) emphasized the importance of preventing biofouling on ship hulls to prevent the invasion of non-indigenous marine organisms.

Tributyltin-resistant, or TBT-degrading, bacteria have been isolated from marine environments. A TBT-resistant marine Alteromonas sp., growing in the presence of 125 µM TBTCl, was isolated from coastal seawater (Fukagawa et al., 1992). A protein from the cloned genes deduced to be responsible for TBT resistance was found to be related to Na⁺/H⁺ antiporters and various Ca²⁺ transporters (Fukagawa and Suzuki, 1993). A multidrug efflux pump was shown to be essential to TBT resistance in Pseudomonas stutzeri (Jude et al., 2004). Adsorption of TBT molecules is an important function of cell walls for Pseudoalteromonas sp. to prevent the inflow of molecules into the cytoplasm (Kubota et al., 2004; Mimura et al., 2008a; Mimura et al., 2008b). Recently, a TBT-degrading Aeromonas veroni, which uses TBT as a carbon source, was isolated from an estuarine environment (Cruz et al., 2007). It seems that the strain could be applied to repair to TBT-polluted sediments.

Organotin compounds are stable and have existed mainly in sediments, rather than in seawater, for prolonged periods (Dowson et al., 1993; Hashimoto, 1998; Hoch, 2001; Saeki et al., 2007; Antizar-Ladislao, 2008), indicating that the frequency of mutations related to TBT resistance might occur in indigenous marine bacteria in sediments (Wuertz et al., 1991; Suzuki et al., 1992; Chen and White, 2004) and that sublethal concentrations of TBT in such sediments give considerable damage to the bacterial ecosystems. Therefore, in this study, we compared survivability of an isolate, from sediments of busy ports such as Tokyo Bay and Ise Bay, with taxonomically similar type strains in the presence of TBT to know whether or not the tolerance is in the genus level. In addition, the impact of an indigenous TBT-resistant bacterium was estimated for microbial populations in TBT-polluted surface sediments.

MATERIALS AND METHODS

Isolation of TBT-resistant marine bacteria

Sampling of surface sediments was carried out in 2008 using a Van Dorn water sampler (5026, Rigosha Co., Ltd., Tokyo, Japan) on board a training ship when anchored at the quarantine anchorages in Tokyo Bay (35.58 N, 139.85 E) and Ise Bay (34.97 N, 136.78 E), Japan. The depth of the water at the sampling sites was 14 to 15 m, and the seawater temperature was 20 to 24°C. Each surface sediment sample was poured into an autoclaved bottle with a screw cap. The samples thus obtained were stored on board up to 7 days at 4°C. When the ship called at a port, samples kept in a cool box were sent to the laboratory.

One hundred µL of the sample was taken after vigorous shaking and spread onto a nutrient agar plate containing 5.0 g Bacto Peptone (Difco, Sparks, MD, USA), 1.0 g yeast extract (Difco, Sparks, MD, USA), 15 g agar in seawater (liter⁻¹), and 100 µM TBTCI as a final concentration. TBTCI was added to the medium after it was autoclaved. After the sample was spread onto an agar plate, it was incubated for 5 days at 25°C, and a single colony formed on the plate was picked up with an autoclaved toothpick and streaked onto a new agar plate. Three TBT-resistant species thus obtained were used for identification and experiments.

Identification of isolates

Identification of the isolates was carried out commercially on the basis of partial 16S rDNA sequences. Briefly, a partial 16S rDNA 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, Foster City, CA, USA). The sequencing data obtained were used for homology analysis using the database, Apron DB-BA Version 4.0 (TechnoSuruga Laboratory, Shizuoka, Japan). A phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987). Partial 16S rDNA sequences of Photobacterium sp. TKY1, Halomonas sp. TKY2, and Photobacterium sp. NGY1 were deposited in the DDBJ/GenBank/EMBL under the accession numbers of AB501122, AB501213, and AB511030, respectively.

Enumeration of colony-forming cells after exposure to TBT

Resting cells prepared from the isolate and two type strains most like it based on partial rDNA sequences were examined. Cells grown until the early stationary phase of growth were harvested by centrifugation (10,000×g, 5 min), washed twice with 50 mM potassium-phosphate (K-Pi) buffer, pH 7.8, containing 0.5 M NaCl, and resuspended in the same volume with the buffer (1 mL). We varied the initial number of resting cells (mL⁻¹) by serial dilution prior to the addition of TBTCI at a final concentration of 100 µM. After exposure to TBT for 1 h, the cell suspension was diluted...
Measurement of cell-associated TBT

Photobacterium sp. TKY1 cells were pre-incubated for 1.5 days at 25°C in the medium containing 5.0 g Bacto Peptone, 1.0 g yeast extract, 20 g sucrose, 0.04 g bromothymol blue, and 15 g agar in seawater (liter⁻¹) to enumerate each number of surviving cells such as V. natriegens ATCC 14048ᵀ as well as Photobacterium sp. TKY1 in their mixture in the presence and absence of 100 µM TBTCl. We could distinguish colonies of TKY1 from those of Photobacterium sp. TKY1 because the former strain grew faster than the latter on the agar plate, and sucrose metabolites from V. natriegens ATCC 14048ᵀ acidified the medium pH, with changes in the color of a colony to yellow-orange.

Observation of morphological changes in isolates by scanning electron microscopy

Resting cells suspended in 50 mM K-Ph buffer, pH 7.5, that contained 0.5 M NaCl were prepared from Photobacterium sp. TKY1 cells grown in the medium described previously for 4 days at 25°C in the absence or presence of 100 µM TBTCl. Resting cells were also prepared from cells grown in the absence of TBTCl for 1.5 days at 25°C, and exposed to TBTCl at a final concentration of 100 µM for 1 h.

Resting cells prepared from V. natriegens ATCC 14048ᵀ reaching the early stationary phase of growth were mixed with those obtained from Photobacterium sp. TKY1 cells, and then the cell mixture was exposed to TBT at a final concentration of 100 µM for 1 h.

Cells examined were fixed with 1% (vol/vol) glutaraldehyde, as a final concentration, for 1 h at 4°C, dehydrated once for 1 h in 50, 70, 90, 95, and 100% ethanol, and suspended in 100% 1-butyl alcohol. After being freeze-dried, they were pasted on a carbon tape and coated with Pd-Pb particles under vacuum. Samples thus obtained were observed with a scanning electron microscope (XL30 CP, FEI Company, Eindhoven, the Netherlands).

Purchase of chemicals

Tributyltin chloride, of greater than 95% purity, was purchased from Wako Pure Chemical Industries, Ltd. (Wako, Japan). Other chemicals used were of high grade.
RESULTS AND DISCUSSION

Phylogenetic similarity of each isolate with type strains

The homologous percentage of the type strain to each isolate was more than 98.5%, based on partial 16S rDNA sequences, except for P. halotolerans LMG 22194T (94.2%) and P. damsela ATCC 33539T (95.2%) (Table 1). So far, Photobacterium sp. TKY1, Halomonas sp. TKY2, and Photobacterium sp. NGY1 were very close to P. ganghwense JCM 12487T, H. venusta DSM 4743T, and P. rosenbergii LMG 22228T, respectively.

| Strain isolated          | Type strains                  | Origin of the type strain | References                   |
|--------------------------|-------------------------------|---------------------------|------------------------------|
| Photobacterium sp. TKY1  | Photobacterium ganghwense JCM 12487T (99.4%) | Seawater                  | Park et al. (2006)           |
|                          | Photobacterium halotolerans LMG 22194T (94.2%) | Saline lake               | Rivas et al. (2006)          |
| Halomonas sp. TKY2       | Halomonas venusta DSM 4743T (99.6%) | Seawater                  | Baumann et al. (1972)        |
|                          | Halomonas alkaliphila DSM 16354T (99.6%) | Salt pool                 | Romano et al. (2006)         |
| Photobacterium sp. NGY1  | Photobacterium rosenbergii LMG 22228T (98.5%) | Bleached coral            | Thompson et al. (2005)       |
|                          | Photobacterium damsela ATCC 33539T (95.2%) | Damselfish skin ulcers    | Smith et al. (1991)          |

We selected two kinds of type strains, which showed high first and secondary similarities to the 16S rDNA sequences of each isolate, based on the database of Apron DB-BA Version 4.0 (TechnoSuruga Laboratory Co., Ltd.). The homologous percentage between the isolate and each type strain is shown in parentheses. The type strain listed in the upper line made a cluster with the isolate on the phylogenetic tree.

Tributyltin-resistant ability of resting cells

We examined changes in the TBT-resistant ability of each isolate as well as those of its type strains to a constant number of TBT molecules, 6.0 × 10^10 mL^-1 when the number of resting cells varied up to 10^14 CFU mL^-1 (Fig.1). The number of cell-associated TBT molecules with a single cell increases in response to the reduction of the number of resting cells in the experiment. As a result, the number of resting cells of Photobacterium sp. TKY1 was hardly reduced even when the initial number of resting cells varied from 10^11 to 10^12 CFU mL^-1, at which the number of surviving cells was 10^3.8 CFU mL^-1 (Fig.1A). In such experimental conditions, the number of cell-associated TBT molecules increased by

---

**TABLE 1.** List of two type strains with high similarities to the 16S rDNA sequences of each isolate

**FIG. 1.** Changes in the survivability of the isolate and the taxonomically similar type strains in relation to the given numbers of resting cells exposed to TBT. Surviving cells were counted by the colony-counting method after exposure to 100 µM of TBT for 1 h. The number of cell-associated TBT molecules varied with the number of resting cells exposed to TBT. Changes in the number of surviving cells of Photobacterium sp. TKY1 (○), P. ganghwense JCM 12487T (△), and P. halotolerans LMG 22194T (□) are shown in relation to given numbers of resting cells (Fig.1A). The results obtained from Halomonas sp. TKY2 (●), H. venusta DSM 4743T (△), and H. alkaliphila DSM 16354T (□) cells are shown in Fig.1B. For Photobacterium sp. NGY1 (●), P. rosenbergii LMG 22228T (△), and P. damsela ATCC 33539T (□), the number of surviving cells is shown in Fig.1C. The dotted line in each figure means perfect survivability, regardless of the initial number of resting cells.
5-orders of magnitude, indicating that the isolate was highly resistant to TBT. When resting cells of $10^{9.2}$ CFU mL$^{-1}$ were exposed to TBT for 1 h, surviving cells of *P. ganghwasense* JCM 12487$^\text{T}$ were reduced to $10^{6.5}$ CFU mL$^{-1}$. Survivability, however, drastically reduced from $10^{9.3}$ CFU mL$^{-1}$ to zero when the number of resting cells varied from $10^{7.9}$ to $10^{5.2}$ CFU mL$^{-1}$. *P. halotolerans* LMG 22194$^\text{T}$ also showed a result similar to that of *P. ganghwasense* JCM 12487$^\text{T}$. The number of surviving cells was $10^{6.3}$ CFU mL$^{-1}$ when resting cells of $10^{9.2}$ CFU mL$^{-1}$ were exposed to such a number of TBT molecules (mL$^{-1}$). The number of surviving cells was reduced to $10^{8.6}$ CFU mL$^{-1}$ when the resting cells of $10^{10}$ CFU mL$^{-1}$ were exposed to the same concentration of TBT.

The sensitivity of *Halomonas* sp. TKY2 and *H. venusta* DSM 4743$^\text{T}$ to TBT were enhanced in relation to the reduction of the initial number of resting cells (Fig.1B). When the resting cells of $10^{7.0}$ CFU mL$^{-1}$ were examined, the surviving cells of *Halomonas* sp. TKY2 and *H. venusta* DSM 4743$^\text{T}$ were reduced by 3- and 2-orders of magnitude, respectively, whereas the reduction of surviving cells prepared from each strain was repressed by 0.6-order of magnitude to the initial number of resting cells, $10^{5.9}$ CFU mL$^{-1}$. For *Halomonas* sp. TKY2 and *H. venusta* DSM 4743$^\text{T}$ cells, no surviving cells were observed when the number of resting cells was less than $10^{6.0}$ CFU mL$^{-1}$. Of the three *Halomonas* species examined thus far, *H. alkaliphila* DSM 16354$^\text{T}$ cells showed the highest survivability. The number of surviving cells hardly decreased when the number of resting cells varied from $10^{7.8}$ to $10^{7.3}$ CFU mL$^{-1}$. The reduction of surviving cells was on 0.3-order of magnitude, even when the resting cells, such as $10^{5.1}$ CFU mL$^{-1}$, were exposed to TBT. *H. alkaliphila* DSM 16354$^\text{T}$ was isolated from the water of a salt pool in Italy (Romano et al., 2006). It might be plausible to think that the highly resistant ability is not caused by prolonged exposure to sublethal TBT concentrations, but fundamentally possessed by the strain. Further study of the resistance of *H. alkaliphila* DSM 16354$^\text{T}$ to TBT is necessary.

Changes in the survivability of a given number of resting cells of *Photobacterium* sp. NGY1, *P. rosenbergii* LMG 22228$^\text{T}$, and *P. damsela* ATCC 33539$^\text{T}$ were examined (Fig.1C). The number of surviving cells of *Photobacterium* sp. NGY1 drastically decreased to $10^{4.2}$ CFU mL$^{-1}$ when the initial number of $10^{6.3}$ CFU mL$^{-1}$ was exposed to TBT for 1 h. *P. rosenbergii* LMG 22228$^\text{T}$ showed relatively higher survivability as compared to *Photobacterium* sp. NGY1. The numbers of surviving cells were $10^{4.8}$ and $10^{3.5}$ CFU mL$^{-1}$, respectively, when resting cells of $10^{9.2}$ and $10^{8.8}$ CFU mL$^{-1}$ were exposed to TBT for 1 h. Resting cells of *P. damsela* ATCC 33539$^\text{T}$ showed similar sensitivity to *P. rosenbergii* LMG 22228$^\text{T}$.

The isolates, *Photobacterium* sp. TKY1, *Halomonas* sp. TKY2, and *Photobacterium* sp. NGY1, formed colonies on the nutrient agar plate containing 100 µM TBT within 5 days of incubation at 25°C (see MATERIALS AND METHODS). The sensitivity to TBT of *Halomonas* sp. TKY2 and *Photobacterium* sp. NGY1, however, was enhanced with the reduction of the initial number of resting cells in the presence of 100 µM TBT, indicating that some kinds of amino acids might essential for the survival of the strains, i.e., syntheses of stress proteins for coping with the toxicity of TBT (Nováková et al., 2009). Resting cells prepared from the isolate, *Photobacterium* sp. TKY1, and the type strain, *H. alkaliphila* DSM 16354$^\text{T}$, showed high survivability to TBT, indicating that they might have constitutively expressed transporters such as the multidrug efflux pump, TbtABM, in the membrane found in *P. stutzeri* (Jude et al., 2004).

**Capacity of adsorption of TBT molecules as an indicator of tolerance to TBT**

Cell-associated TBT molecules were measured with a HPLC/MS/MS. As shown in Table 2, growing *Photobacterium* sp. TKY1 cells as well as resting cells, $10^{9.4}$ CFU mL$^{-1}$, adsorbed TBT. These results indicate that the isolate could grow in the nutrient liquid medium to cope with the adsorption of TBT molecules by the growing cells. The cell wall of the isolate might play an important role to prevent the interaction of TBT molecules with the cell membrane. The concentration of TBT recovered from the medium alone was found to be 65.0 nmol mL$^{-1}$. The concentration was lower than the adjusted concentration of 100 nmol mL$^{-1}$. It seemed that some of the TBT molecules were not recovered due to their adhesion onto the inside of the glass test tube and chips used for pipetting.

We have pointed out previously that the cell wall of a marine *Pseudoalteromonas* sp. plays an important role in preventing the inflow of TBT molecules into the cytoplasm across the membrane (Kubota et al., 2004; Mimura et al., 2008a; Mimura et al., 2008b). The isolate, *Photobacterium* sp. TKY1, could adsorb TBT molecules, approximately $3.5 \times 10^{9.6}$ (a single cell$^{-1}$), when resting cells were examined. The order of the value was consistent with that obtained from TBT-resistant marine *Pseudoalteromonas* sp. cells examined in the presence of 100 µM TBTCl (Mimura et al., 2008b). Those results indicate the importance of the cell wall’s function and capacity to adsorb TBT molecules on the surface in relation to the resistance of the species to TBT toxicity.

**Estimation of the impact of a TBT-resistant bacterium on the indigenous microbial population**

We estimated the impact of a TBT-resistant marine bacterium on an indigenous microbial population in
Adsorption of TBT molecules by Photobacterium sp. TKY1 cells

| Strains and each number of resting cells (CFU mL⁻¹) | Surviving cells in the presence of TBTCl (100 µM)² |
|----------------------------------------------------|--------------------------------------------------|
| - Photobacterium sp. TKY1 (10⁶±2.0)⁵ | 10⁵±1.0² | 10⁶±4.0² |
| - Vibrio natriegens ATCC 14048ᵀ (10⁶±2.0)⁶ | 10⁰±3 | 10⁶±2.0³ |
| - Vibrio natriegens ATCC 14048ᵀ alone (10⁶±2.0)⁶ | - | 10⁰±3 |

¹Cells were grown in the presence of 100 µM TBTCl for 4 days at 25°C. After their harvest, TBT in the supernatant as well as the cell pellet were measured.
²Resting cells were prepared from the cells grown in the absence of TBTCl for 1.5 days at 25°C. After being harvested and washed twice, cells were suspended in 50 mM K-Pi buffer, pH 7.5, containing 0.5 M NaCl. TBT concentrations in supernatant and cell pellets were measured after the addition of 100 µM TBTCl, as the final concentration, for 1 h.
³TBTCl, as the final concentration of 100 µM, was added into the medium. After vigorous shaking, TBT in the sample was measured.
⁴Cell proteins of resting cells grown in the absence or presence of TBTCl were 0.29 mg mL⁻¹ and 0.42 mg mL⁻¹, respectively.

Changes in survivability of TBT-sensitive Vibrio natriegens ATCC 14048ᵀ cells when TBT-resistant Photobacterium sp. TKY1 cells coexist in the presence of TBTCl

| Strains and each number of resting cells (CFU mL⁻¹) | Surviving cells in the presence of TBTCl (100 µM)² |
|----------------------------------------------------|--------------------------------------------------|
| - Photobacterium sp. TKY1 | 10⁵±1.0² | 10⁶±4.0² |
| - Vibrio natriegens ATCC 14048ᵀ | 10⁰±3 | 10⁶±2.0³ |

Experiments were carried out three times independently, and the data are shown as the averaged value ± standard deviation.

No reduction of colony-forming Vibrio natriegens 14048ᵀ cells was observed after being mixed with Photobacterium sp. TKY1 cells for 1 h in the absence of TBTCl.

Morphological observation of Photobacterium sp. TKY1 and V. natriegens ATCC 14048ᵀ cells in the presence of TBT

Obvious morphological changes were not observed when Photobacterium sp. TKY1 cells were grown in the presence of 100 µM TBTCl (Fig. 2B) as well as after the addition of TBTCl to the resting cells (Fig. 2D). Morphological changes of TBT-sensitive V. natriegens ATCC 14048ᵀ cells mixed with those of Photobacterium sp. TKY1 were observed in the presence of TBTCl (Fig. 2F). After exposure to TBT, ruptured or wrinkled V. natriegens ATCC 14048ᵀ cells were observed, while Photobacterium sp. TKY1 cells in the mixture retained their normal cell morphology. Since the cells were suspended in the presence of 0.5 M NaCl, such changes in morphology seem to be closely related to the disruption of osmoregulation by the cation transport across the membrane (Fukagawa and Suzuki, 1993).

ACKNOWLEDGMENTS

We thank Dr. Takashi Miwa (of the National Institute for Sea Training at that time) for taking samples on board. This work was supported by a Grant-in-Aid for Scientific Research (C), JSPS.KAKENHI (21560830).
Dowson, P. H., Bubb, J. M., and Lester, J. N. (1993) A study of the partitioning and sorptive behavior of butyltins in the aquatic environment. Appl. Organomet. Chem., 7, 623-633.

Ebdon, L., Evans, K., and Hill, S. (1989) The accumulation of organotins in adult and seed oysters from selected estuaries prior to the introduction of U. K. regulations governing the use of tributyltin-based antifouling paints. Sci. Total Environ., 83, 63-84.

Fukagawa, T., Suzuki, S., Fukunaga, K., Suzuki, T., and Takama, K. (1992) Isolation and characterization of tributyltin chloride-resistant marine Vibrio. FEMS Microbiol. Lett., 93, 83-86.

Fukagawa, T., and Suzuki, S. (1993) Cloning of gene respon-
Horiguchi, T., Kojima, M., Kaya, M., Matsuo, T., Shirasaka, T., Sato, T., Nagase, H., and Kito, H. (2012) Organotin compounds in seawater and Mytilus galloprovincialis mussels along the Croatian Adriatic Coast. *Mar. Pollut. Bull.*, 64, 189-199.

Hagger, J. A., Fisher, A. S., Hill, S. J., Depledge M. H., and Jha, A. N. (2002) Genotoxic, cytotoxic and ontogenetic effects of tri-n-butyltin on the marine worm, Platynereis dumerilii (Polychaeta: Nereidae). *Aquat. Toxicol.*, 57, 243-255.

Hamasaki, T., Sato, T., Nagase, H., and Kito, H. (1992) The genotoxicity of organotin compounds in SOS chromotest and rec-assay. *Mutat. Res.*, 280, 195-203.

Hamasaki, T., Sato, T., Nagase, H., and Kito, H. (1993) The mutagenicity of organotin compounds as environmental pollutants. *Mutat. Res.*, 300, 265-271.

Harino, H., Ozhi, M., Wattayakorn, G., Adulyanukosol, K., Arai, T., and Miyazaki, N., 2007. Accumulation of organotin compounds in tissues and organs of stranded whales along the coasts of Thailand. *Arch. Environ. Contam. Toxicol.*, 53, 119-125.

Hashimoto, S., Watanabe, M., Noda, Y., Hayashi, T., Kurita, Y., Takasu, Y., and Otsuki, A., 1998. Concentration and distribution of butyltin compounds in a heavy tanker route in the Strait of Malacca and in Tokyo Bay. *Mar. Environ. Res.*, 45, 169-177.

Hoch, M. (2001) Organotin compounds in the environment: an overview. *Appl. Geochem.*, 16, 719-743.

Horiguchi, T., Kojima, M., Kaya, M., Matsuo, T., Shiraiishi, H., Morita, M., and Adachi, Y. (2002) Triphenyltin induce spermato genesis in ovary of female abalone, *Haliotis gigantea*. *Mar. Environ. Res.*, 54, 679-684.

International Maritime Organization (2008) International convention on the control of harmful anti-fouling systems on ships. Available in http://www.imo.org/OurWork/Environment/Anti-foulingSystems/Pages/Default.aspx. Accessed 27 July 2015.

Jude, F., Arpin, G., Brachet-Castang, C., Capdevey, M., Caumette, P., and Quentin, C. (2004) TbtABM, a multi-drug efflux pump associated with tributyltin resistance in *Pseudomonas stutzeri*. *FEMS Microbiol. Lett.*, 232, 7-14.

Kubota, N., Mimura, H., Yamauchi, T., and Kitamura, A. (2004) Accelerator analyses of tributyltin chloride associated with a tributyltin resistant marine microorganism. *Mar. Pollut. Bull.*, 48, 800-805.

Lewis, P. N., Riddle, M. J., and Hewitt, C. L. (2004) Management of exogenous threats to Antarctica and the sub-Antarctic Islands: Balancing Risks from TBT and Non-Indigenous Marine Organisms. *Mar. Pollut. Bull.*, 49, 999-1005.

Mimura, H., Sato, R., Furuyama, Y., Taniike, A., Yagi, M., Yoshida, K., and Kitamura, A. (2008a) Adsorption of tributyltin by tributyltin resistant marine *Pseudoalteromonas* sp. cells. *Mar. Pollut. Bull.*, 57, 877-882.

Mimura, H., Sato, R., Sasaki, Y., Furuyama, Y., Taniike, A., Yoshida, K., and Kitamura, A. (2008b) Accelerator analysis of tributyltin adsorbed onto the surface of a tributyltin resistant marine *Pseudoalteromonas* sp. cell. *Int. J. Mol. Sci.*, 9, 1989-2002.

Murai, R., Takahashi, S., Tanabe, S., and Takeuchi, I. (2005) Status of butyltin pollution along the coasts of western Japan in 2001, 11 years after partial restrictions on the usage of tributyltin. *Mar. Pollut. Bull.*, 51, 940-949.

Negri, A. P., Hales, L. T., Battershill, C., Wolff, C., and Webster, N. S. (2004) TBT contamination identified in Antarctic marine sediments. *Mar. Pollut. Bull.*, 48, 1142-1144.

Nováková, Z., Bobálová, J., Vidová, M., Hapala, I., and Smigáová, P. (2009) Tributyltin-resistant Methanotroph bacterium *ther- mothrophus* mutant with mutual retardation substitutions in the AIAO-ATP synthase. *FEMS Microbiol. Lett.*, 296, 255-259.

Oberdörster, E., Rittschof, D., and LeBlanc, G. A. (1998) Alteration of [11C]-testosterone metabolism after chronic exposure of *Daphnia magna* to tributyltin. *Arch. Environ. Contam. Toxicol.*, 34, 21-25.

Park, Y.-D., Baik, K. S., Seong, C. N., Bae, K. S., Kim, S., and Chun, J. (2006) *Photobacterium ganghwaense* sp. nov., a halophilic bacterium isolated from sea water. *Int. J. Syst. Evol. Microbiol.*, 56, 745-749.

Rivas, R., García-Fraile, P., Mateos, P. F., Martínez-Molina, E., and Velázquez, E. (2006) *Photobacterium halotolerans* sp. nov., isolated from Lake Martel in Spain. *Int. J. Syst. Evol. Microbiol.*, 56, 1067-1071.

Romano, I., Lama, L., Nicolaus, B., Poli, A., Gambacorta, A., and Giordano, A. (2006) *Halomonas alkaliphila* sp. nov., a novel halotolerant alkaliphilic bacterium isolated from a salt pool in Campania (Italy). *J. Gen. Appl.Microbiol.*, 52, 339-348.

Saeki, K., Nabeshima, A., Kunito, T., and Oshima, Y. (2007) The stability of butyltin compounds in a dredged heavily contaminated sediment. *Chemosphere*, 68, 1114-1119.

Saito, N., and Nii, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4, 406-425.

Smith, S. K., Sutton, D. C., Fuerst, J. A., and Reichelt, J. L. (1991) Evaluation of the genus *Listonella* and reassignment of *Listonella damsela* (Love et al.) MacDonell and Colwell to the genus *Photobacterium* as *Photobacterium damsela* comb. nov. with an emended description. *Int. J. Syst. Bacteriol.*, 41, 529-534.

Sudaryanto, A., Takahashi, S., Monirith, I., Ismail, A., MUCHTAR, M., Zheng, J., Richardson, B. J., Subramanian, A., Prudente, M., Huie, N. D., and Tanabe, S. (2002) Asia-Pacific mussel watch: Monitoring of butyltin contamination in coastal waters of Asian developing countries. *Environ. Toxicol. Chem.*, 21, 2119-2130.

Suzuki, S., Fukagawa, T., and Takama, K. (1992) Occurrence of tributyltin-tolerant bacteria in tributyltin- or cadmium-containing seawater. *Appl. Environ. Microbiol.*, 58, 3410-3412.

Thompson, F. L., Thompson, C. C., Naser, S., Hoste, B., Vandemeulebroecke, K., Munn, C., Bourne, D., and Swings, J. (2005) *Photobacterium rosenbergii* sp. nov. and *Enterobirrio coralli* sp. nov., vibrios associated with coral bleaching. *Int. J. Syst. Evol. Microbiol.*, 55, 913-917.

Ueno, D., Inoue, S., Takahashi, S., Ikeda, K., Tanaka, H., Subramanian, A. N., Fillmann, G., Lam, P. K., Zheng, J., Muchtar, M., Prudente, M., Chung, K., and Tanabe, S. (2004) Global pollution monitoring of butyltin compounds using skipjack tuna as a bioindicator. *Environ. Pollut.*, 127, 1-12.

Wuertz, S., Miller, C. E., Pfister, R. M., and Cooney, J. J. (1991) Tributyltin-resistant bacteria from estuarine and freshwater sediments. *Appl. Environ. Microbiol.*, 57, 2783-2789.