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Degradation of dibutyltin in sea water by pyoverdins isolated from *Pseudomonas chlororaphis*

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The yellow compounds pyoverdins were isolated from *Pseudomonas chlororaphis*, which was isolated from mud in Japan. Degradation of tributyltin (TBT), dibutyltin (DBT), and monobutyltin (MBT) by pyoverdin (20 mg) was carried in sea water (30 ml) containing a 6 μg l^{-1} concentration of TBT, DBT, and MBT at 24 °C for 24 h in aerobic conditions. TBT, DBT, and MBT in sea water were analyzed by gas chromatography–mass spectrometry in selected ion monitoring mode. DBT in sea water was degraded to MBT by pyoverdins isolated from *P. chlororaphis*. However, TBT and MBT in sea water was not degraded by pyoverdins. The optimum degradation of DBT in sea water was at pH 4.8–8.2, at a temperature 25–30 °C. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: dibutyltin; degradation; pyoverdin; *Pseudomonas chlororaphis*

INTRODUCTION

Organotin compounds have been used as biocides in antifouling paints applied to surfaces on ship bottoms and fishing nets.[1] However, some organotin derivatives such as tributyltins (TBTs), are also comparatively highly toxic to mammals, and cases of poisoning have been described.[2,3] Various environmental problems have been produced with the organotin compounds, viz. bioaccmulation of organotin, organotin pollution in sediments, and Impo-sex of the roll shell.[4] Previous studies focused on the biodegradation of organotins have investigated (TBT) break down with bacteria in mud and water.[4] Our studies develop microbial remediation processes for organotin-polluted environments. We have previously demonstrated that triphenyltin (TPT) and diphenyltin (DPT) were degraded by pyoverdins (*m/e* 1161) isolated from *Pseudomonas chlororaphis*.[5–7] Pyoverdins have a molecular mass of 1000 to 1500 Da and are constituted of a chromophore, structurally based on 2,3-diamino-6,7-dihydroxyquinoline, bound to a peptide of six to ten amino acids via the N-termin.[8] Pyoverdins are called siderophores and have a strong affinity for ferric iron.[9] However, no information has been reported on this degradation of butyltin (tributyltin (TBT), dibutyltin (DBT), and microbutyltin (MBT)) in sea water by pyoverdins. In this paper we describe the effect of temperature and pH on the degradation of DBT in sea water by pyoverdins obtained from *P. chlororaphis*.

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EXPERIMENTAL

Pyoverdins

The proverdins were isolated from *P. chlororaphis* that had been isolated from surface muds (aerobic conditions) in Japan. The isolations were carried out at the Chugoku National Industrial Research Institute, Japan. The medium consisted of 0.4% succinic acid, 0.1% glycerol, 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.1% $(NH_4)_2SO_4$, 0.05% yeast extracts, 0.04% $MgCl_2$, at pH 7.0. *P. chlororaphis* was incubated in the medium at 27 °C for 3 days. After 3 days, the culture medium was centrifuged at 5,000 rpm for 20 min at 4 °C. The aqueous phase was filtered using a Whatman GF/F (0.4 μm)) glass fiber filter in order to remove suspended materials. Next, pyoverdins in the filtered aqueous phase were adsorbed with a Sep-Pak tC18 column (30 ml) and eluted using a mixture of solutions (methanol: water, 1:1). After evaporation under reduced pressure of the eluted aqueous phase containing pyoverdins the pyoverdins were chromatographed on a CM-Sephadex C-25 column (3.0 cm × 25 cm) made up in 0.05 M pyridine/acetate at pH 5.0.[10] The column was first eluted isocratically with the same buffer (60 ml), then with a linear 0.05–2/M gradient of pyridine/acetate (pH

5.0). The fractions (5 ml) were monitored at 380 nm.[5] The identity of the pyoverdin[11] obtained from *P. chlororaphis* was identified by the UV spectrum, fast-atom bombardment mass spectrometry (FAB-MS), NMR, and amino acid analysis.[5,12] The pyoverdin had a UV spectra maximum at (λ_{max} = 400 nm) and m/z = 1161 (M^+).

Determination of organotin in sea water

Determination of TBT, DBT, and MBT was essentially performed by following the method proposed by Iwamura[13] and Carlier-Pinasseau *et al.*,[14] with a modification of extraction solvent (*n*-hexane) and equipment used. To 100 ml of sea water in a 100 ml separation funnel, 0.3 g of sodium chloride, 0.6 g of TBT, DBT, and MBT was added. Next, 3 ml of acetic acid–sodium acetate buffer solution (pH 5) and 0.5 M of 2% sodium tetraethylborate ($NaBEt_4$) solutions were added to the sea water to adjust the pH 5, which was monitored by putting a drop of the sample solution on a pH sensor, and the mixture was shaken for 10 min. 10 ml of *n*-hexane was added, and then the mixture was shaken. After centrifugation, the organic layer was collected; this procedure was repeated twice. The combined extracts were dried over Na_2SO_4 and then concentrated, using a decompression KD concentrator, to 2 ml, and analysed using gas chromatography–MS in selected ion monitoring mode. All sample analyses were done in duplicate, and the data are reported as the mean. Standard solutions for calibration were prepared by ethylation of TBT, DBT, and MBT salts as described earlier.[10]

The capillary columns used were a cross-linked 5% phenyl methyl silicon (DB-5; J&W Scientific, Folsom, CA; 0.25 mm (i.d.) × 30 m × 0.25 μm (film thickness)) Operating conditions were as follows: column oven, programmed from 60 °C (hold 1 min) at a rate of 20 °C min^{-1} to 130 °C (hold 0 min), followed by a rate of 10 °C min^{-1} to 210 °C (hold 0 min), followed by a rate of 5 °C min^{-1} to 260 °C (hold 0 min), followed by a rate of 10 °C min^{-1} to 300 °C (hold 2 min); injection port (splitless), injection temperature: 290 °C, ion source temperature: 230 °C, interface temperature: 280 °C. Injection volume: 1 μ. SIM monitor ion: MBT, 233 m/e DBT, 261 m/e; TBT, 263 m/e; internal standard, tetraphenyltin (Tetra-PT).

Authentic Standards

Tributyltin chloride (TBT, 96%), dibutyltin dichloride (DBT, 96%) and monobutyltin trichloride (MBT, 95%) were purchased from Tokyo Kasei Company Ltd (Tokyo). $NaBEt_4$ was obtained from Hayashi Pure Chemical (Tokyo).

RESULTS AND DISCUSSION

Degradation of DBT by pyoverdins

Degradation of DBT by pyoverdin (20 mg) was carried out in sea water (30 ml) containing a 6 μg l^{-1} concentration of DBT at 24 °C for 24 h in aerobic conditions. The experimental results are shown in Fig. 1. The faction of DBT was decreased from 100 to 1% over 0–24 h of reaction. In contrast to DBT, MBT was increased from 0 to 99% over 0–24 h. DBT in the control sample (no pyoverdin added) was not changed over the 0–24 h. On the other hand, degradation of TBT and MBT in sea water by pyoverdins did not occur. This result shows that DBT in sea water with pyoverdins degraded to MBT with the release of butane (Scheme 1). The degradation rate of DBT was 98% day^{-1}. It is reported that the degradation rate of TPT and DPT by pyoverdin was 28% day^{-1} and 12% day^{-1} respectively.[5] This result shows that the degradation of DBT in sea water by pyoverdins was faster than that of TPT and DPT in sea water by pyoverdins. Also, it was shown that TPT, DPT, and DBT in sea water and pyoverdins was selectively degraded to MPT and MBT. The properties of pyoverdins are consistent with their role as siderophores with a strong affinity for ferric iron.[9] Yamaoka *et al.*[6] found that degradation of TPT by pyoverdins was inhibited by iron in water. In conclusion, the results suggest that DBT and TPT was reacted with the iron chelate site on pyoverdins. However, the detail of the degradation mechanism of DBT by pyoverdins is the subject of further study.

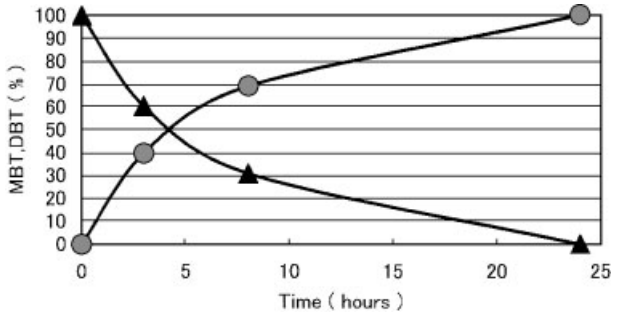

Figure 1. Degradation of DBT in sea water by pyoverdins and formation of MBT. ▲: DBT; ●: MBT. Degradation of DBT by pyoverdin (20 mg) was carried out in sea water (30 ml) containing a 6 μg l^{-1} concentration of TPT at 24 °C for 24 h.

$$(CH_2CH_2CH_2CH_3)_2Sn^{2+} \rightarrow CH_3CH_2CH_2CH_2Sn^{3+} + CH_3CH_2CH_2CH_3$$

Scheme 1. Proposed degradation pathway of DBT in sea water by pyoverdin.

Effect of temperature on degradation of DBT by pyoverdin

Degradation of TPT and DPT in sea water by pyoverdins is affected greatly by changes in temperature.[5] However, no information has been reported on the effect of temperature on the degradation of DBT by pyoverdin. Therefore, the relationship between the temperature and the degradation of

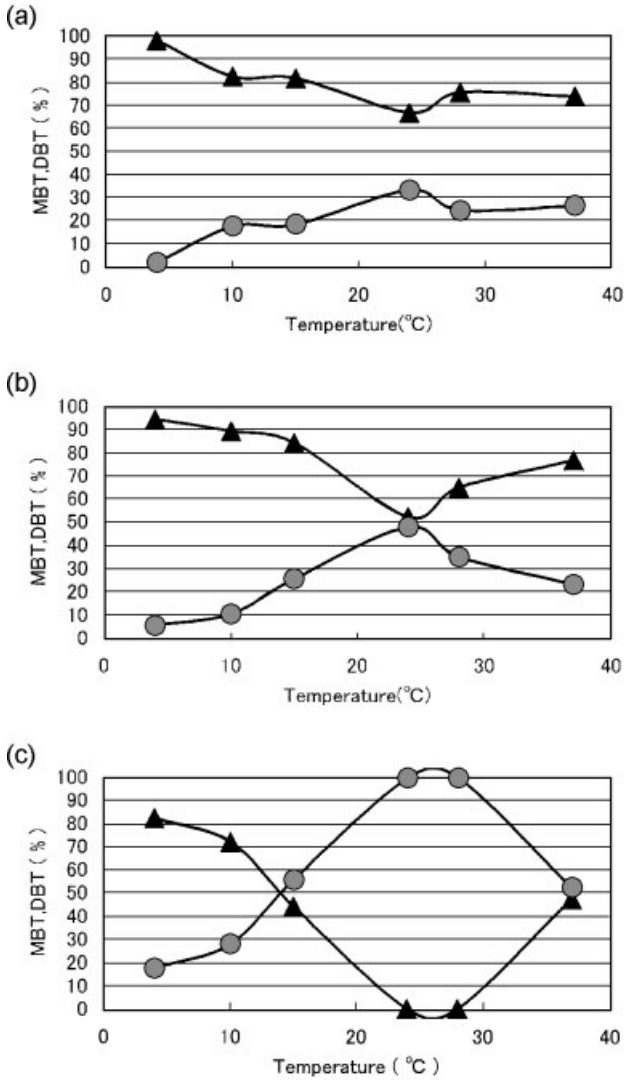

Figure 2. Effect of temperature on degradation of DBT in sea water by pyoverdins ▲: DBT; ●: MBT. Degradation of DBT by pyoverdin (2 mg) was carried out in sea water (10 ml) containing a 6 μg l^{-1} concentration of DBT at 4, 10, 15, 24, and 27 °C for (a) 3, (b) 5, and (c) 24 h.

DBT by pyoverdins was studied. Degradation of DBT by pyoverdin (2 mg) was carried out in sea water (10 ml) containing a 6 μg l^{-1} concentration of DBT at 4, 10, 15, 24, and 27 °C for 24 h in aerobic conditions. The relationship between the temperature and the degradation of DBT by pyoverdins is shown in Fig. 2. After 24 h, DBT decreased from 80 to 0% with increase in temperature from 4 to 24 °C. In contrast to DBT, MBT increased from 20 to 100% with increase in temperature from 4 to 24 °C. Figure 2 shows that the optimum degradation of DBT was at a temperature of 25–30 °C. These results suggest that degradation of DBT by pyoverdin depends upon temperature.

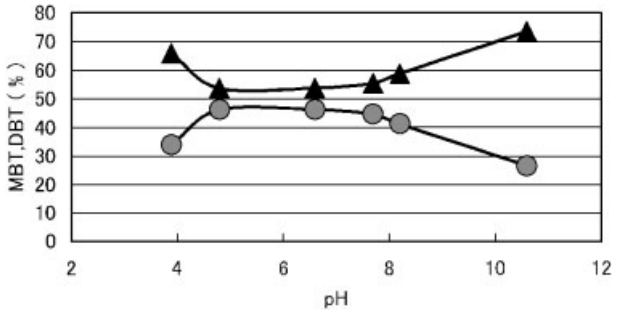

Figure 3. Effect of pH on degradation of DBT in sea water by pyoverdins ▲: DBT; ●: MBT. Degradation of DBT by pyoverdin (2 mg) was carried out in sea water (10 ml) containing a 6 μg l^{-1} concentration of DBT at 24 °C for 5 h, and pH was adjusted in the range 3.7–10.7 with 0.01 M NaOH and 0.01 M HCl.

Effect of pH on degradation of DBT by pyoverdin

Degradation of TPT and DPT in sea water by pyoverdins depends on the pH value of the sea water. However, no information has been reported on the effect of pH on degradation of DBT by pyoverdin. Degradation of DBT by pyoverdin (2 mg) was carried out in sea water (10 ml) containing a 6 μg l^{-1} concentration of DBT at 24 °C for 5 h in aerobic conditions, and pH was adjusted in the range 3.8–10.7 with 0.01 M NaOH and 0.01 M HCl solutions. The relationship between pH and the degradation of DBT by pyoverdins is shown in Fig. 3. The degradation of DBT in sea water decreased within the pH range 4.8 to 3.8 and at pH 8.2 it decreased. The total butyltin level in sea water was composed of 21% DBT and 62% MBT at pH 4.8–8.2. These results suggest that the optimum pH for the degradation of DBT by pyoverdins is 4.8 to 8.2 in sea water.

CONCLUSIONS

DBT in sea water was degradated to MBT by pyoverdins isolated from *P. chlororaphis*. The optimum degradation of DBT in sea water was at pH 4.8–8.2, and temperatures at 25–30 °C. TBT and MBT in sea water were not degraded by pyoverdins.

REFERENCES

1. Fent K. *Sci. Total Environ.* 1996; **185**: 151.
2. Takahashi S, Tanabe S and Kawaguchi K. *Environ. Sci. Technol.* 2000; **34**: 5129.
3. Lee RF. *Mar. Environ. Res.* 1991; **32**: 29.
4. Hirano H, Fukushima M, Kurokawa Y and Kawai S. *Environ. Pollut.* 1998; **98**: 163.
5. Yamaoka Y, Takimura O, Inoue H and Oota S. *Appl. Organomet. Chem.* 2001; **15**: 1.
6. Yamaoka Y, Takimura O, Inoue H and Oota S. *Appl. Organomet. Chem.* 2002; **16**: 1.

7. Inoue H, Takimura O, Fuse H, Murakami K, Kamimura K and Yamaoka Y. *Appl. Environ. Microbiol.* 2000; **66**: 3492.
8. Budzikiewicz H. *Z. Naturforsch. Teil C* 1997; **52**: 713.
9. Teintze M, Hossain MB, Barnes CL, Leong J and van der Helm D. *Biochemistry* 1981; **20**: 6446.
10. Demange P, Wendenbaum S, Linget C, Mertz C, Cung MT, Dell A and Abdallah MA. *Biol. Met.* 1990; **3**: 155.
11. Linget C, Azadi P, MacLeod JK, Dell A and Abdallah MA. *Tetrahedron Lett.* 1992; **33**: 1737.
12. Ueshima H, Yamaoka Y, Takimura O, Fuse H, Murakami K, Inoue H and Kamimura K. *Environmental Research in Japan*, vol. **2**. Ministry of the Environment. Japan, 2001; 50.
13. Iwamura Y, Kadokami K, Jinya D, Hanada Y and Suzuki M. *Bunseki Kagaku* 1999; **48**: 555.
14. Carlier-Pinasseau C, Lespets G and Astruc M. *Appl. Organomet. Chem.* 1996; **10**: 505.

