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Degradation of triphenyltin compounds in sea water by pyoverdins from *Pseudomonas chlororaphis*

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The yellow compound species pyoverdin was isolated from *Pseudomonas chlororaphis*. Degradation of triphenyltin (TPT) by pyoverdin (20 mg) was carried in distilled water (30 ml) containing a 6 μg l^{-1} concentration of TPT at 20 °C for 96 h in aerobic conditions. The organotins in water and sea water were analyzed by gas chromatograph–mass spectrometry in selected ion mode. TPT and diphenyltin (DPT) in sea water were degraded to monophenyltin (MPT) with pyoverdins isolated from *P. chlororaphis*. Degradation of TPT in sea water increased with increasing temperature between 4 and 37 °C. Optimum degradation of TPT in sea water was at pH 7–8.5. Degradation of TPT and DPT in distilled water can be faster than in sea water. Also, degradation of TPT in both water and sea water was faster than that of DPT. Tributyltin, dibutyltin, monobutyltin and MPT in water and sea water were not degraded by pyoverdins isolated from *P. chlororaphis*. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords: organotin; degradation; pyoverdin; *Pseudomonas chlororaphis*

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INTRODUCTION

Tin and organotins are toxic to a wide variety of marine organisms at levels present in polluted environments and thus may be an ecological and safety problem. Organotin compounds have been used as biocides in antifouling paints applied to surfaces on ship bottoms and fishing nets.[1] However, some organotin derivatives (TPT), such as triphenyltin are also comparatively highly toxic to mammals, and cases of poisoning have been described.[2,3] Organotin species are many more orders of magnitude more toxic than inorganic tin.

The various environmental problems produced by organotin compounds include bioaccmulation of organotin,[4] organotin pollution in sediments,[5] and imposex of the roll shell.[3] Since 1980, the majority of studies on the biodegradation of organotins have been made using microorganisms.[6–8] These studies have investigated tributyltin (TBT) breakdown with bacteria in soils and water.[7] Phenyltin species break down naturally to inorganic tin. We have previously demonstrated that TPT is degraded with a culture solution of *Pseudomonas chlororaphis*.[9] It is reported that yellow fluorescent compounds (pyoverdins) are produced by *Pseudomonas aeruginosa*,[10] *Pseudomonas fluorescens*,[10] and *Pseudomona tolaashir*.[11] It has also been confirmed that five kinds of pyoverdin are produced by *P. fluorescens*.[12] The properties of pyoverdins are consistent with their role as siderophores with a very high affinity for iron (III), together with a lack of affinity for iron (II).[13]

In this paper we describe the effects of salinity, temperature and pH on the degradation of TPT compounds in sea water by pyoverdins obtained from *P. chlororaphis*.

EXPERIMENTAL

Materials and methods

Many samples from mud were able to grow in a medium supplemented with 130 μmol TPT. *P. chlororaphis*, isolated from muds, was used for this work at the Chugoku National Industrial Research Institute.[9] The medium consisted of 0.4% succinate acid, 0.1% glycerol, 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.1% $(NH_4)_3SO_4$, 0.05% yeast extracts and 0.04% $MgCl_2$, at pH 7.0. *P. chlororaphis* was incubated in the medium at 27 °C for 3 days. The pH of the culture was adjusted periodically to 7.0 by careful addition of 0.6 mol dm^{-3} hydrochloric acid. After 72 h, the culture medium was centrifuged at 5000 rpm for 20 min at 4 °C. The aqueous phase was filtered with a glass fiber filter (Whitman GF/F (0.4 μm) in order to remove suspended materials. Next, the pyoverdins in the filtered aqueous phase were adsorbed with Sep-Pak tC18 (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 diameter × 25 cm) made up in 0.05 mol dm^{-3} pyridine/acetate pH 5.0.[14] The column was first eluted isocratically with the same buffer (60 ml), then with a linear 0.05–2 mol dm^{-3} gradient of pyridine/acetate pH 5.0. The fractions (5 ml) were monitored by UV–vis spectrophotometry at 380 nm.[14] Pyoverdins in the fractions were concentrated by rotary evaporation. The yield of pyoverdins was 280 mg per 1000 ml of culture medium.

Determination of organotin in sea water

Determination of organotin compounds was essentially performed following the method proposed by Iwamura[15] and Carlier-Pinasseau *et al.*,[16] with a slight modification of the extraction solvent (*n*-hexane) and equipment used. To 1000 ml of sea water in a 1000 ml separatory funnel, 3 g of sodium chloride and 0.6 μg of organotin compounds was added. Next, 3 ml of acetic acid–sodium acetate buffer solution (pH 5) and 0.5 mol of 2% sodium tetraethylborate ($NaB(C_2H_5)_4$) solutions were added to sea water to adjust the pH to 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. Then 10 ml of *n*-hexane was added, and the mixture 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–mass spectrometry in selected ion mode (GC–MS-SIM). All sample analyses were done in duplicate, and data are reported as the mean. Standard solutions for calibration were prepared by ethylation of organotin salts as described previously.[15]

Capillary columns were used: viz. 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 μl. SIM monitor ion: monophenyltin (MPT), 253 *m/e*; diphenyltin (DPT), 303 *m/e*; TPT, 351 *m/e*; internal standard, tetraphenyltin (Tetra-PT).

Authentic standards

TPT, TBT, dibutyltin (DBT) and monobutyltin (MBT) were purchased from Tokyo Kasei Company Ltd (Tokyo). DPT and MPT were purchased from Aldrich Chemical Company (Milwaukee, WI).

RESULTS AND DISCUSSION

Identification of pyoverdins

The identify of the yellow compounds obtained from *P. chlororaphis* was confirmed by the UV spectrum, fast atom bombardment mass spectrometry and (FAB-MS), amino acid analysis. The yellow compounds isolated from *P. chlororaphis* had similar UV spectra ($\lambda_{max} = 400$ nm) to pyoverdin 1e[12] from *P. fluorescens* ATCC 13525.[12] FAB-MS of the yellow compounds isolated from *P. chlororaphis* gave a molecular peak at $m/z = 1161$ (M^+) as for pyoverdin from *P. fluorescens* ATCC 13525.[12] Total acid hydrolysis (48 h, 6 mol dm^{-3} HCl, 110 °C) of the yellow compounds of *P. chlororaphis* indicated that they

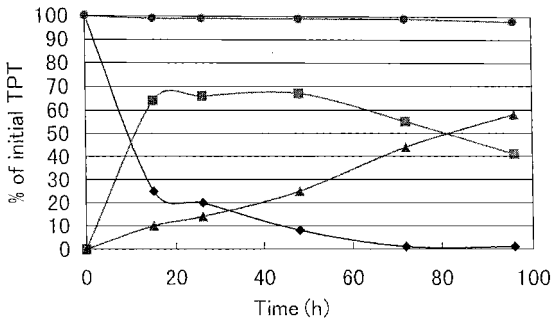

Figure 1 Degradation of TPT in pure water by pyoverdins and formation of DPT and MPT. ◆: TPT; ■: DPT; ▲: MPT; ●: s-TPT (no added pyoverdin). Degradation of TPT by pyoverdin (20 mg) was carried out in distilled water (30 ml) containing a 6 μg l^{-1} concentration of TPT at 20 °C for 96 h.

are constituted of three amino acids, possessing 2 mol of serine, 2 mol of lysine and 1 mol of glycine. Analysis of a 47% HI hydrolysate revealed 2 mol of hydroxyornithine (HOornicine). The present study clearly indicates that the yellow compounds isolated from *P. chlororaphis* were in accordance with pyoverdin 1e[12] of *P. fluorescens*.[13] The pyoverdin structure shows a fluorescent chromophore, which is a quinoline derivative, and a peptide arm of six to nine amino acid residues.[12] The results suggested that the yellow compound isolated from *P. chlororaphis* was pyoverdin 1e.[12]

Degradation of phenyltin by pyoverdins

Degradation of TPT was investigated by using pyoverdins isolated from *P. chlororaphis*. Degradation of TPT by pyoverdins (20 mg) was carried out in distilled water (30 ml) containing a 6 μg l^{-1} concentration of TPT at 20 °C for 96 h under aerobic conditions. TPT in water degraded to DPT and MPT (Fig. 1). The degradation process of TPT in water by pyoverdin was similar to that in sea water. The amount of TPT decreased from 100 to 2% during the 0–96 h of reaction. In contrast to TPT, DPT increased from 0 to 64% during the 0–15 h period and decreased from 65 to 40% during the 48–96 h period. MPT increased from 0 to 58% during the 0–96 h of reaction.

TPT in the control sample (no added pyoverdin) did not changed during the 0–96 h. Also, MPT was not degraded by pyoverdins (see Table 1). That is to say, MPT increased with decrease of TPT. As to the formation of DPT from TPT by pyoverdins, it shows that TPT and DPT were degraded to phenyltin with release of benzene. Regarding the degradation mechanism of TPT, it is necessary to examine this aspect in more detail in the future.

Table 1 Degradation of organotins by pyoverdins isolated from P. chlororaphis[a]

Compound	Solution	Rate of compound degradation(%)/day
Tetra-PT	Water	0
TPT	Sea water	28
	Water	44
DPT	Sea water	12
	Water	20
MPT	Sea water	0
	Water	0
TBT	Water	0
DBT	Water	0
MBT	Water	0

[a] Degradation of organotins (Tetra-PT, TPT, DPT, MPT, TBT, DBT, and MBT) by pyoverdins (20 mg) was carried in sea water and/or distilled water (30 ml) containing a 6 μg/l^{-1} concentration of organotins at 20 °C for 96 h.

Effect of salinity on degradaton of phenyltin by pyoverdins

Degradation of TPT by pyoverdins (20 ng) was carried in distilled water (30 ml) containing a 6 μg l^{-1} concentration of TPT and NaCl (0, 3, 6 or 10 mg) at 20 °C for 48 h under aerobic conditions. The relationship between the degradation of TPT by pyoverdins and the concentration of sodium chloride in water is shown in Fig. 2. Degradation of TPT by pyoverdins decreased with increase of NaCl concentration in water. The total phenyltin level in water was composed of 89% TPT, 9% DPT and 2% MPT, whereas the total phenyltin level in high salinity water (3.8% NaCl) was composed of 54% TPT, 37% DPT and 9% MPT. The degradation of TPT by pyoverdins shows that it is inhibited with increase of salinity. These results show that the degradation of TPT in water by pyoverdins is faster than in sea water (Table 1).

Effect of temperature on degradation of phenyltin by pyoverdins

In general, the degradation of organotins is affected greatly by changes in temperature. Therefore, the relationship between temperature and the degrada-

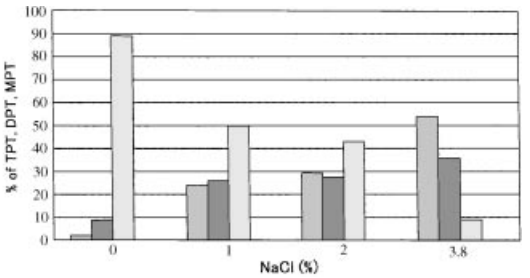

Figure 2 Effect of NaCl on degradation of TPT in pure water by pyoverdins. Degradation of TPT by pyoverdins (20 ng) was carried out in distilled water (30 ml) containing a 6 μg l^{-1} concentration of TPT and NaCl (0, 3, 6 or 10 mg) at 20 °C for 48 h.

tion of TPT by pyoverdins was studied. Degradation of TPT by pyoverdins (20 mg) was carried out in sea water (30 ml) containing a 6 μg l^{-1} concentration of TPT at 4, 15, 20, 24, 27, and 37 °C for 96 h under aerobic conditions. The relationships between the temperature and the degradation of TPT by pyoverdins are shown in Fig. 3. The total phenyltin level in sea water was composed of 62% TPT, 38% DPT and 0% MPT at 4 °C. These results suggest that TPT was degraded to DPT at 4 °C, and not degraded to MPT. The total phenyltin level in sea water was composed of 29% TPT, 63% DPT and 8% MPT at 15 °C. The TPT and DPT levels in sea water did not change in the range from 15 to 24 °C. The DPT level decreased above this temperature, but TPT did not decrease rapidly. The total phenyltin level in sea water was composed of 21% TPT, 37% DPT and 42% MPT at 37 °C. There is a tendency shown for the rapid

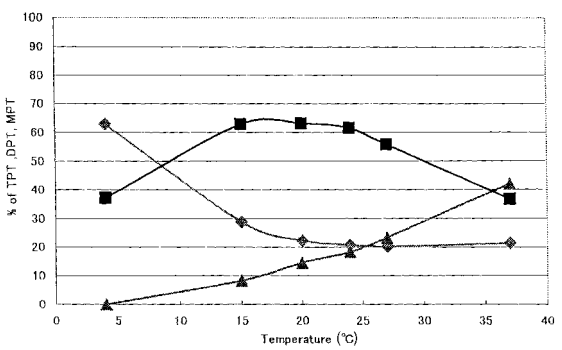

Figure 3 Effect of temperature on degradation of TPT in sea water by pyoverdins. ◆: TPT; ■: DPT; ▲: MPT. Degradation of TPT by pyoverdins (20 mg) was carried out in sea water (30 ml) containing a 6 μg l^{-1} concentration of TPT at 4, 15, 20, 24, 27, and 37 °C for 96 h.

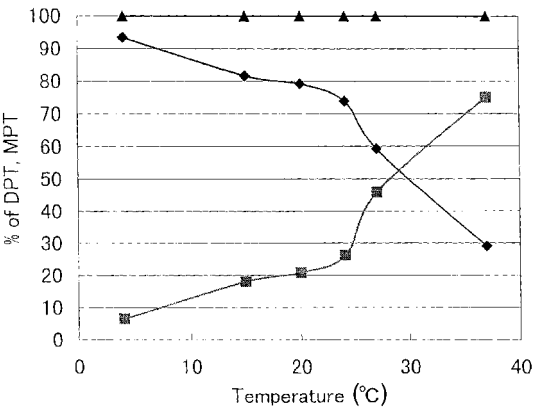

Figure 4 Effect of temperature on degradation of DBT in sea water by pyoverdins. ◆: DPT; ■: MPT, ▲: s-MPT (no added pyoverdin). Degradation of DPT and MPT by pyoverdins (20 mg) was carried out in sea water (30 ml) containing a 6 μg l^{-1} concentration of TPT at 4, 15, 20, 24, 27, and 37 °C for 96 h.

degradation of TPT to MPT in the range 24–37 °C. On the other hand, the relationship between the temperature and the degradation of DPT and MPT by pyoverdins is shown in Fig. 4. DPT decreased from 93 to 28% in the range 4–37 °C. In contrast to DPT, MPT increased from 7 to 82% with increase in temperature from 4 to 37 °C. However, the MPT concentration in sea water remained constant within the range at 4–37 °C. These results show that MPT cannot be degraded by pyoverdin. Also, these results suggest that degradation of TPT and DPT by pyoverdin depends upon temperature.

Effect of pH on degradation of phenyltin by pyoverdins

Generally, degradation of TPT by pyoverdins depends on the pH value of sea water. The pH of sea water is 8.2. Degradation of TPT by pyoverdin (20 mg) was carried out in sea water (30 ml) containing a 6 μg l^{-1} concentration of TPT at 20 °C for 96 h under aerobic conditions and the pH was adjusted to 4.2–9 with 0.01 mol dm^{-3} NaOH and 0.01 mol dm^{-3} HCl solutions. The relationship between pH and degradation of TPT by pyoverdins is shown in Fig. 5. Degradation of TPT in sea water increased abruptly within the pH range 6.1 to 7, and at pH $\geq$ 8.2 it decreased. The total phenyltin level in sea water was composed of 21% TPT, 62% DPT and 17% MPT at pH 8.2, and 24% TPT, 60% DPT and 16% MPT at pH 7. These results suggest that the optimum pH for degradation of TPT by pyoverdins is 7 to 8.5 in sea water. In the following

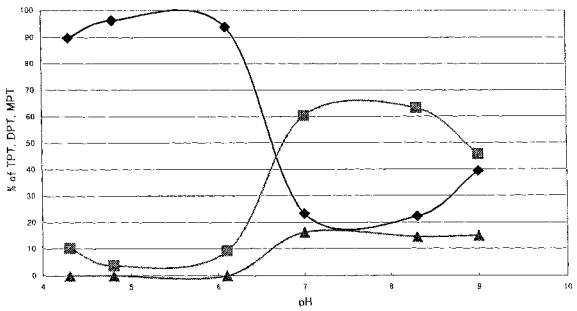

Figure 5 Effect of pH on degradation of TPT in sea water by pyoverdins. ◆: TPT; ■: DPT; ▲: MPT. Degradation of TPT by pyoverdins (20 mg) was carried out in sea water (30 ml) containing a 6 $\mu g\, l^{-1}$ concentration of TPT at 20 °C for 96 h; the pH was adjusted in the range 4.2–9 with 0.01 mol dm^{-3} NaOH and 0.01 mol dm^{-3} HCl.

experiments, to approximate the usual pH of sea water, the pH of degradation sea water was not adjusted.

Degradation of organotins by pyoverdins

Degradation of organotins (Tetra-PT, TPT, DPT, MPT, TBT, DBT, and MBT) by pyoverdins (20 mg) was carried out in sea water and/or distilled water (30 ml) containing a 6 $\mu g\, l^{-1}$ concentration of organotins and other triphenyl compounds at 20 °C for 96 h under aerobic conditions. The organotins and other triphenyl compound peaks on the GC–MS chromatogam obtained from the water sample were confirmed by comparing retention times and mass spectra with those of authentic standards (Tetra-PT, TPT, TBT, DPT, DBT, MPT, and MBT). The degradation rate of organotin compounds per day by pyoverdins was obtained as a percentage of the concentration in which TPT or DPT in the solution decreases. The degradation of organotins (Tetra-PT, TPT, DPT, MPT, TBT, DBT, and MBT), triphenylarsine and triphenylbismuth in sea water and distilled water by pyoverdins is shown in Table 1. The degradation rate of TPT was 44%/day in water and 28%/day in sea water. The degradation rate of DPT was 20%/day in water and 12%/day in sea water. The degradation of TPT and DPT in distilled water can be faster than in sea water. Also, the degradation of TPT in both water and sea water was faster than that of DPT. However, degradation of MPT, TBT, DBT, and MBT by pyoverdins did not occur. It is reported that pyoverdins were produced with *P. aeruginosa*,[10] and *P. fluorescens*,[10] and *P. tolaashii*.[13,14] It is shown that TPT and DPT in water and sea water were selectively degraded to MPT by pyoverdins from *P. chlororaphis*.

CONCLUSIONS

TPT and DPT in water were degraded to MPT with pyoverdins isolated from *P. chlororaphis*. The degradation of TPT in sea water was increased with increase of temperature in the range 4–37 °C. The optimum degradation of TPT in sea water was at pH 7–8.5. Degradation of TPT and DPT in distilled water can be faster than in sea water. Also, the degradation of TPT in both water and sea water was faster than that of DPT. TBT, DBT, MBT and MPT in water and sea water were not degraded by pyoverdins isolated from *P. chlororaphis*.

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