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Short Communication

Effects of iron on the degradation of triphenyltin by pyoverdins isolated from *Pseudomonas chlororaphis*

Yukiho Yamaoka[1]*, Hiroyuki Inoue[1], Osamu Takimura[1] and Sinji Oota[2]

[1]National Institute of Advanced Industrial Science and Technology, 2-2-2 Hiro-Suehiro, Kure, Hiroshima 737-0197, Japan
[2]Instrument Center for Chemical Analysis, Hiroshima University, Higashi-Hiroshima, Hiroshima, 739-0046 Japan

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The yellow compound pyoverdin was isolated from the bacteria *Pseudomonas chlororaphis*, isolated from mud in Japan. A study of the effects of iron, phosphorus, manganese and zinc on degradation of triphenyltin (TPT) by pyoverdin (20 mg) was carried out in distilled water (30 ml) containing 6 μg l^{-1} concentration of TPT at 20°C for 48 or 96 h. The organotins in water were analyzed by gas chromatograph–mass spectrometry in the selected ion mode. The degradation of TPT by pyoverdin decreased with increase of phosphorus at 0–35 mg l^{-1} and Fe-EDTA at 0–2 mg l^{-1} concentrations. Also, degradation of diphenyltin by pyoverdin decreased with increase of Mn-EDTA at 0–1 mg l^{-1} and Zn-EDTA at 0–1 mg l^{-1}. On the other hand, degradation of TPT by pyoverdin was found to be unaffected by manganese and zinc in water. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: triphenyltin; degradation; pyoverdin; iron; phosphorus; manganese; zinc

INTRODUCTION

Organotin compounds have been used as biocides in antifouling paints applied to surfaces on ship bottoms and fishing nets.[1] The various environmental problems produced by organotin compounds include bioaccumulation of organotin,[2] organotin pollution in sediments,[3] and imposex in marine invertebrates.[4] Previous studies focused on the biodegradation of organotins have been made using microorganisms.[5–7] Our studies develop microbial remediation processes of organotin-polluted environments. We have previously demonstrated that triphenyltin (TPT) species were degraded with a culture solution of the bacteria *Pseudomonas chlororaphis, Pseudomonas aeruginosa* and *Pseudomonas fluorescens*,[8] and that TPT or/and diphenyltin (DPT) species break down to monophenyltin (MPT) with pyoverdin from *P. chlororaphis*.[10] Pyoverdin,[11] a bacterial siderophor and iron chelator, consists of three distinct structural parts, *viz*. a dihydroxyquinoline chromophore responsible for the fluorescence, a peptide chain comprising seven amino acids bound to the carboxyl group, and a small dicarboxylic acid (or its monoamide) connected amidically to the NH_2 group (Fig. 1). 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).[11,12] However, information on the effect of the element (iron, etc.) on the degradation of TPT compounds in water by pyoverdins is not well known.

In this paper we describe the effects of iron, manganese, zinc and phosphorus concentrations on the degradation of TPT compounds in water by pyoverdins obtained from *P. chlororaphis* (TPT species are known to be degraded by pyoverdin and could be studied for TPT bioremediation).

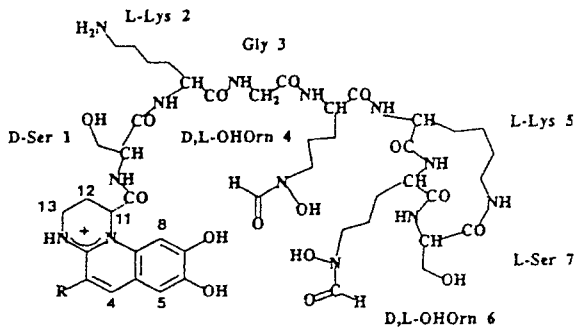

Figure 1. The chemical structure of pyoverdin (R=NH—CO—CH_2—CH_2—COOH; MW: 1161)[11] isolated from *P. chlororaphis*, isolated from mud in Japan.

*Correspondence to: Y. Yamaoka, National Institute of Advanced Industrial Science and Technology, 2-2-2 Hiro-Suehiro, Kure, Hiroshima 737-0197, Japan.
E-mail: yamaoka@cniri.go.jp.

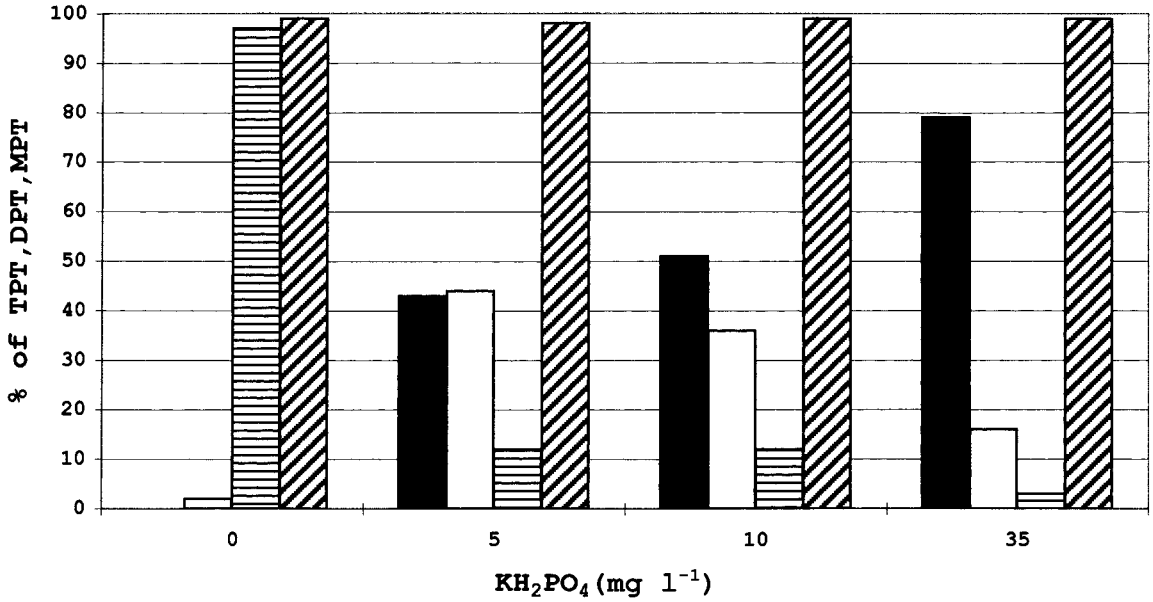

Figure 2. Effect of KH_2PO_4 on degradation of TPT chloride in water by pyoverdins. Degradation of TPT by pyoverdins (20 ± mg) was carried out at in distilled water (30 ml) containing a 6 μg l^{-1} concentration of TPT chloride and KH_2PO_4 (0, 5, 10, or 35 mg l^{-1}) at 20 °C for 96 h under aerobic conditions. Control TPT Chloride: no added pyoverdin. TPT: ■; DPT: □; MPT: ▤; control: ▨.

EXPERIMENTAL

Materials and methods

Many bacteria samples from muds were able to grow in a medium supplemented with 130 μmol TPT chloride. As a result of the TPT degradation, *P. chlororaphis* was isolated from muds in Chugoku National Industrial Research Institute.[8] The medium consisted of 0.4% succinis acid, 0.1% glycerol, 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.1% $(NH_4)_3SO_4$, 0.05% yeast extracts, 0.04% $MgCl_2$ and pH 7.0. *P. chlororaphis* was incubated in the medium at 27 °C for 3 days. The pH of the culture was periodically adjusted to 7.0 by careful addition of 6 mol l^{-1} hydrochloric acid. After 72 h, the culture medium was centrifuged at 5000 rpm for 20 min at 4 °C. The yield of pyoverdins was 280 mg per 1000 ml of culture medium.[10] The identity of the yellow compound (pyoverdin[11] Fig. 1) obtained from *P. chlororaphis* was confirmed by the UV spectrum, fast-atom bombardment mass spectrometry (FAB-MS), and amino acid analysis.[10]

Determination of organotin in water

Determination of organotin compounds was essentially performed by following the method of Iwamura[13] and Carlier-Pinasseau *et al.*,[14] with a slight modification of the extraction solvent (*n*-hexane) and equipment used. TPT, DPT and MPT chlorides in water were derivatized to diethyl-TPT, diethyl-DPT and diethyl-MPT using sodium tetraethhylborate ($NaB(C_2H_5)_4$), and analyzed using gas chromatography–MS 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.[13]

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

Authentic standards

TPT chloride was purchased from Tokyo Kasei Company Ltd (Tokyo). DPT and MPT chlorides were purchased from Aldrich Chemical Company (Milwaukee, WI).

RESULTS AND DISCUSSION

Effect of concentration of phosphorus on degradation of TPT by 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 chloride and 0, 5, 10, 35 mg l^{-1} concentrations of phosphorus (as KH_2PO_4) at 20 °C for 48 h under aerobic conditions. The experimental results are shown in Fig. 2. The total phenyltin level in water after degradation without

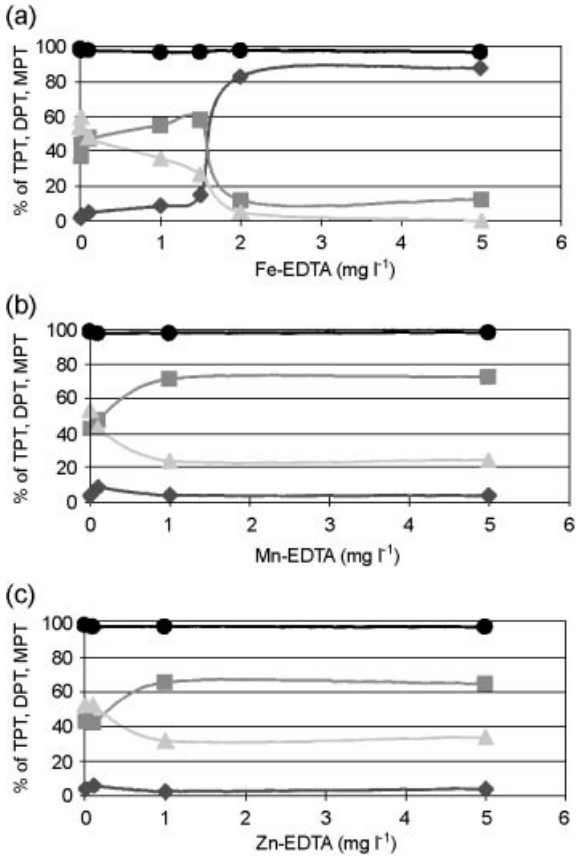

Figure 3. Effect of iron, manganese and zinc concentrations on degradation of TPT in water by pyoverdins. Degradation of TPT by pyoverdin (20 mg) was carried out in water (30 ml) containing a 6 μg l^{-1} concentration of TPT and 0, 1, 1.5, 2, or 5 mg l^{-1} concentrations of Fe-EDTA (a), Mn-EDTA (b) and Zn-EDTA (c) at 20°C for 48 h under aerobic conditions. Control TPT: no added pyoverdin, TPT: ◆; DPT: ■; MPT: ▲; control: ●.

phosphorus was composed of 0% TPT, 3% DPT and 97% MPT, whereas the total phenyltin level in high phosphorus (KH_2PO_4 35 mg l^{-1}) water was composed of 79% TPT, 17% DPT and 17% MPT. Inoue *et al.*[8] and Yamaoka *et al.*[10] reported that pyoverdin directly catalyzed the dephenylation of TPT to produce DPT and MPT. This result shows that degradation of TPT by pyoverdin is inhibited with increase of phosphorus concentration in water. Generally, the concentrations of phosphorus in sediments were higher than in water. These results show that degradation of TPT by pyoverdin is faster in water than in sediments.

Effect of concentration of manganese, zinc and iron on degradation of TPT by pyoverdin

In order to demonstrate the effect of iron, manganese and zinc on degradation of TPT by pyoverdin, degradation experiments of TPT by pyoverdin (20 mg) were carried out in distilled water (30 ml) containing a 6 μg l^{-1} concentration of TPT and 0, 1, 1.6, 2, or 5 mg l^{-1} concentration of Fe-EDTA, Mn-EDTA or Zn-EDTA at 20°C for 48 h under aerobic conditions. The experimental results are shown in Fig. 3. The amount of DPT in water increases with increase of Fe-EDTA in the range of 0–1.5 mg l^{-1} reaching a maximum at a 1.5 mg l^{-1} concentration of Fe-EDTA, and then decreasing with further increases of Fe-EDTA (>1.5 mg l^{-1}). The amount of TPT increased with increase of Fe-EDTA, and was a maximum at 2 mg l^{-1} of Fe-EDTA. On the other hand, MPT in water decreases with an increase of Fe-EDTA concentration from 0 to 2 mg l^{-1} and was zero at 5.0 mg l^{-1}. This result shows that iron in Fe-EDTA reacts with pyoverdins in proportion to their concentration in the water. These results, obtained from the effect of iron on degradation of TPT with pyoverdin, clearly indicate that only iron reacts with pyoverdin. In conclusion, the results suggest that the inhibition of iron on the degradation of TPT by pyoverdin was accomplished by the normal reaction pathway for iron on siderephores.[11]

On the other hand, TPT was not changed with increase of Mn- and Zn-EDTA. The amounts of DPT in water increased abruptly with increase of Mn- and Zn-EDTA at 0–1 mg l^{-1} and was not changed at 1 to 5 mg l^{-1} of Mn- and Zn-EDTA. These results show that the degradation of TPT by pyoverdin was not influenced by addition of manganese and zinc. However degradation of DPT was little influenced by addition of manganese and zinc. In conclusion, the order of degradation of DPT by pyoverdins was zinc > manganese > iron. Details of the degradation mechanisms of TPT and DPT by pyoverdins will be studied further in the future.

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