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Growth of *Pseudomonas* sp. TX1 on a wide range of octylphenol polyethoxylate concentrations and the formation of dicarboxylated metabolites

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ABSTRACT

Pseudomonas sp. TX1, is able to use octylphenol polyethoxylates ($OPEO_n$, or Triton® X-100; average $n = 9.5$) as a sole carbon source. It can grow on 0.05–20% of $OPEO_n$ with a specific growth rate of 0.34–0.44 h^{-1}. High-performance liquid chromatography–mass spectrometer analysis of $OPEO_n$ degraded metabolites revealed that strain TX1 was able to shorten the ethoxylate chain and produce octylphenol (OP). Furthermore, formation of the short carboxylate metabolites, such as carboxyoctylphenol polyethoxylates ($COPEO_n$, $n = 2, 3$) and carboxyoctylphenol polyethoxycarboxylates ($COPEC_n$, $n = 2, 3$) began at the log stage, while octylphenol polyethoxycarboxylates ($OPEC_n$, $n = 1–3$) was formed at the stationary phase. All the short-ethoxylated metabolites, $OPEO_n$, $OPEC_n$, $COPEO_n$, and $COPEC_n$, accumulated when the cells were in the stationary phase. This study is the first to demonstrate the formation of $COPEO_n$ and $COPEC_n$ from $OPEO_n$ by an aerobic bacterium.

1. Introduction

Alkylphenol polyethoxylates ($APEO_n$), an important group of nonionic surfactants, have been extensively used as detergents, emulsifiers, solubilizers, and dispersants of pesticides for the last forty years (Ahel et al., 1994a,b; Petrovic and Barcelo, 2001). Included in this surfactant family are octylphenol polyethoxylates ($OPEO_n$, Triton® X-100; structure in Fig. 1) and nonylphenol polyethoxylates ($NPEO_n$, Triton® N-101), which are commercially used in domestic, industrial, and agricultural activities. A significant portion of $APEO_n$ is discharged into the sewage treatment plants. In addition, $APEO_n$ are often used to enhance the activity of various pesticides. Previous studies have indicated that approximately 60% of $APEO_n$ entering wastewater treatment plants are released into the aqueous environment as APEO metabolites (Ahel et al., 1994a). Some of the $APEO_n$ metabolites, such as nonylphenol and octylphenol (OP, structure in Fig. 1), have chronic ecotoxicity and they are potential endocrine disrupters to aquatic organisms, wildlife, and humans (Klecka et al., 2008; Liney et al., 2006; Nimrod and Benson, 1996; Pachura-Bouchet et al., 2006; Petrovic and Barcelo, 2000; Staples et al., 2004; White et al., 1994). Common $APEO_n$ degradation products have been detected in such sources as sewage sludges and effluents, aquatic sediments, groundwater, surface water and drinking water (Benotti et al., 2009; Hawrelak et al., 1999; Klecka et al., 2007; Ying et al., 2002).

The fate and biodegradability of $APEO_n$ and alkylphenol (AP) in the environment have received much attention over the last decade (Klecka et al., 2008; Nimrod and Benson, 1996; Staples et al., 1999, 2008; Zhao et al., 2006). The biodegradation of $APEO_n$ has been studied using isolated bacterial strains that grow solely on $OPEO_n$ or $NPEO_n$. Most isolates belong to the genus *Pseudomonas* (John and White, 1998; Maki et al., 1994; Nguyen and Sigoillot, 1997; Turkovskava et al., 1996). *Pseudomonas* sp. strain TR01 and a *Pseudomonas putida* strain isolated from activated sludge were shown to transform $NPEO_n$ by shortening the ethoxylate chain to form the dominant intermediate nonylphenol diethoxylate ($NPEO_2$) (John and White, 1998; Maki et al., 1994; Turkovskava et al., 1996). Nguyen and Sigoillot (1997) isolated four *Pseudomonas* strains from coastal seawater that grew on $OPEO_n$ and generated OPEO with 4–5 units of the ethoxylate chain as the end products. Another study examined one strain of *Burkholderia cepacia* and ten isolates of *P. putida* from the topsoil of paddy fields. These strains transformed $OPEO_n$ to form $OPEO_2$ and $OPEO_3$, the dominant metabolites that accumulate under aerobic conditions (Nishio et al., 2002). *Sphingomonas macrogoltabidus* was demonstrated to degrade 4-*tert*-octylphenol polyethoxylates to form $OPEO_1$ (Nishio et al., 2005). Recently, *Bacillus* sp. LY was found to degrade $NPEO_n$ without forming carboxylated metabolites (Lu et al., 2008).

Most of the bacteria capable of degrading long-chain AP belong to the sphingomonads and pseudomonads. The formation of the

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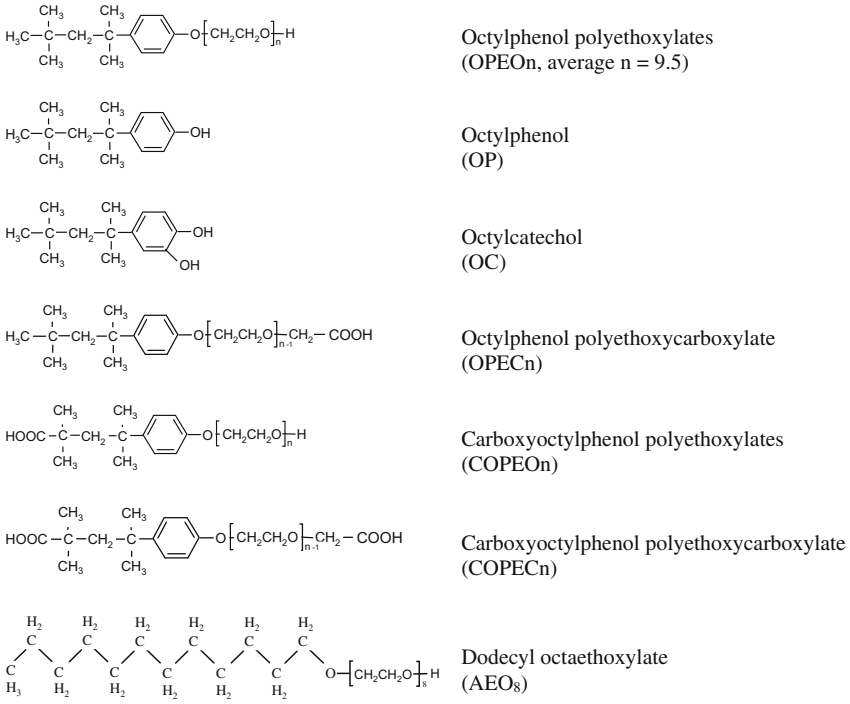

Fig. 1. Chemical structures of octylphenol polyethoxylates and related compounds. The exact structures of $COPEO_n$ and $COPEC_n$ compounds are not specified.

metabolic intermediate, 2, 4, 4-trimethyl-2-pentanol, has been demonstrated during the transformation of octylphenol (OP) and octylphenol monoethoxyl acetic acid ($OPEC_1$), suggesting that alkylphenol (AP) could be degraded by a central fission mechanism (Franska et al., 2003; Tanghe et al., 1999). The bacterial degradation of long-chain branched AP involves the hydroxylation of the aromatic ring and oxidation of alkyl chain (Corvini et al., 2006b; Montgomery-Brown et al., 2008). The cleavage of the benzylic carbon of AP and the formation of tertiary alcohols occurs via *ipso*-hydroxylation by *Shingomonas* sp. (Corvini et al., 2006a; Gabriel et al., 2005).

Previous studies showed that the primary degradation of $APEO_n$ by *Pseudomonas* species generally proceeds through gradual shortening of the ethoxylate chain. Furthermore, although previous reports have indicated that the ω/β-oxidative degradation of the hydrophobic alkyl chain does not seem to occur in the degradation of $APEO_n$ (White, 1993), Ding et al. detected dicarboxylated $APEO_n$ metabolites in tertiary treated wastewater effluents and in river samples (Cheng et al., 2006; Ding et al., 1996). Dicarboxylated $APEO_n$ has also been found in batch aerobic biodegradation studies of $NPEO_n$ (Di Corcia et al., 1998) and also in a soil aquifer treatment site (Montgomery-Brown et al., 2003). However, the formation of dicarboxylated metabolites has not been observed in a pure culture.

The objective of this study was to study *Pseudomonas* sp. TX1, to our knowledge the first bacterium studied to date that can grow on $OPEO_n$ (average $n = 9.5$, with a highly branched alkyl chain) at a wide range concentrations. We report the growth properties and kinetics of the formation of OP, carboxylated and dicarboxylated metabolites by a pure culture.

2. Methods

2.1. Bacterium and medium

Pseudomonas sp. TX1 was isolated from the sediment of a drainage system of rice field in northern Taiwan by the enrichment culture technique (Chen et al., 2005, 2006). It was grown in mineral salts basal (MSB) medium containing various concentrations of $OPEO_n$ (Triton® X-100, CASN 9002-93-1), which was purchased from Merck KGaA (Darmstadt, Germany) as the sole carbon source. The composition of MSB has been described previously (Chen et al., 2005). Luria–Bertani (LB) medium is composed of 10 g of tryptone, 5 g of yeast extract, and 10 g of sodium chloride per liter. LB medium containing 15% glycerol was used to store the bacteria at −70 °C. Bacterial growth was measured by removing 1 ml samples from the culture flask and adding methanol (50% v/v, final concentration) to solubilize the accumulated shorter ethoxylates created by degradation. This was done immediately prior to optical density measurement at 600 nm (John and White, 1998).

2.2. Identification of metabolites from the biodegradation of $OPEO_n$

The inoculum was prepared by harvesting strain TX1 at late-log phase from 50 ml MSB-0.5% $OPEO_n$ broth. The cell pellet was washed three times with 10 ml of 20 mM phosphate buffer (pH 7.0) and inoculated into 10 ml MSB-0.05% $OPEO_n$ broth (in 15 × 200 mm tube). The initial OD of each 10 ml culture was adjusted to 0.4. The tubes were incubated at 30 °C and shaken at 200 rpm and samples were taken within the period of transformation for a time-course analysis. To carry out the analysis of metabolites, each culture at its particular time point was mixed vigorously with 10 ml of 72.4% of $MgSO_4$ and 0.2 ml of 5 N H_2SO_4 followed by extraction with 25 ml of chloroform ($CHCl_3$) three times. A portion of the organic phase was collected and dried immediately using a rotary evaporator. Acetonitrile (2 ml) was then added to dissolve the dry residue for liquid chromatography–mass spectrometer (LC–MS) analysis (Nguyen and Sigoillot, 1997). $OPEO_n$ and its metabolites were analyzed by a high-performance liquid chromatography (HPLC) system (Waters Alliance 2690, Milford, MA, USA) equipped with an electrospray ionization–mass spectrometer (Platform LC; Micromass, Manchester, UK). The injection volume was 20 μl and the flow rate was set at 0.5 ml/min. A 5 μm C_{18} column (Waters μBondapak,