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Transcription of a novel P450 gene varies with some factors (pollutant exposure, temperature, time, and body region) in a marine oligochaete (*Thalassodrilides* sp.)

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ABSTRACT

Cytochrome P450 (CYP) enzymes play important roles in the metabolism of exogenous compounds such as polycyclic aromatic hydrocarbons (PAHs). A novel, full-length CYP gene (*CYP4V30*) was identified in the oligochaete *Thalassodrilides* sp. *CYP4V30* mRNA expression was studied in worms exposed to PAH-polluted (Σ16PAHs; 37441 ng/g dry weight) or unpolluted (Σ16PAHs; 19 ng/g dry weight) sediment. *CYP4V30* expression was much higher in worms exposed to contaminated sediments than in those exposed to unpolluted sediments at some temperatures (20 and 25 °C) and exposure durations (11-fold increase at 20 °C, 10-day exposure), but not at 15 °C or other exposure durations ($P < 0.05$). *CYP4V30* mRNA expression was higher in the middle of the body than in the posterior ($P < 0.05$). The variation in transcriptional response with exposure time, temperature, and body region indicates that these factors should be considered when monitoring marine sediment pollution.

1. Introduction

Aquatic environments near areas of human habitation have been contaminated by pollutants such as antifouling materials, metals, and compounds derived from oil or human waste. In particular, sediment contamination by polycyclic aromatic hydrocarbons (PAHs) with low polarity, and high hydrophobicity and persistency, has increased in coastal areas worldwide over the past 30 years (WHO, 1998). PAHs are major components of crude oil, and PAH contamination could have both petrogenic and pyrogenic sources such as oil leaks and fossil fuel combustion (Mehdinia et al., 2015; Van Metre et al., 2000). PAHs are included in the European Union and US EPA priority pollutant lists, which mandate their monitoring for regulatory administration because PAHs cause high toxicity effects on biota, including mutagenesis, carcinogenesis, and teratogenesis (WHO, 1998).

PAHs can be biotransformed and biodegraded by organisms in the environment through various pathways. Cytochrome P450 (CYP) enzymes are important in these processes, and they are widely distributed in organisms, including bacteria, plants, and animals. CYP enzymes transform both endogenous compounds (e.g., fatty acids and steroids) and xenobiotics (e.g., PAHs, pesticides, and other pollutants) (Buhler and Williams, 1988). In fact, they are known to play important roles in the initial step (phase I) of PAH hydroxylation in organisms (Buhler and Williams, 1988; Jørgensen et al., 2008; Karigar and Rao, 2011). Phase II involves the conjugation of molecules to the hydroxyl group to enhance water solubility of the phase I product, resulting in excretion from the body (Carrasco Navarro, 2013).

Sediment-dwelling marine invertebrates are exposed to PAHs contained in the sediment. CYP genes and proteins, which mediate the metabolism of xenobiotics, have been found in marine invertebrate phyla, including Annelida (e.g., polychaetes), Arthropoda (e.g., crustaceans), Mollusca (e.g., abalones, mussels, and octopuses), and Echinodermata (e.g., starfish and urchins) (Rewitz et al., 2006; Snyder, 2000), as well as terrestrial and freshwater organisms. Genes belonging to the CYP2, CYP4, and CYP331 families have been identified, and the induction of *CYP4* gene expression in marine invertebrates by some pollutants has been frequently reported (Li et al., 2004; Rewitz et al., 2004; Zheng et al., 2013). Moreover, Jørgensen et al. (2005) showed that the CYP4 enzyme is involved in the biotransformation of pyrene (a type of PAH) in polychaetes.

The biotransformation of PAHs by polychaetes such as *Capitella* sp. I, *Nereis virens*, and *Nereis diversicolor*, which are dominant species in polluted sediments, is well understood, and phase I (e.g., CYP) and phase II enzymes are involved (Forbes et al., 2001; Jørgensen et al., 2008; Malmquist et al., 2013; McElroy, 1990). *Capitella* sp. I removed 63% of the added PAH fluoranthene, and *N. diversicolor* removed 25% of the added PAH pyrene in sediment microcosms (Madsen et al., 1997;

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Christensen et al., 2002), indicating that they can contribute to the degradation of PAHs in sediment.

Although polychaetes are the dominant annelids in marine sediments, various oligochaetes also inhabit these habitats (Diaz and Erséus, 1994; Giere, 1975). However, only information about the biotransformation of xenobiotics is available, and information about the roles of CYP enzymes is limited to a single freshwater oligochaete, *Lumbriculus variegatus* (Lyytikäinen et al., 2007; Mäenpää et al., 2009; Carrasco Navarro et al., 2011). In Japan, *Thalassodrilides* sp. (Annelida, Oligochaeta) (Fig. 1) inhabits marine sediments at high densities. Our previous research revealed that this species could inhabit highly hypoxic and sulfidic sediments contaminated by various pollutants (Ito et al., 2015), and this is similar to another marine oligochaete *Tubificoides benedii* (Giere et al., 1999). Moreover, *Thalassodrilides* sp. could biotransform 1-nitronaphthalene (1NN), a nitrated PAH, into substances that were nontoxic to fish (Ito et al., 2016). Although, CYP enzymes might contribute to the biotransformation and detoxification of 1NN, the presence and increased expression of CYP genes upon exposure to pollutants has been not demonstrated in this species. Furthermore, the determination of the full-length DNA sequences of CYP genes and their inducibility by pollutants is important for understanding their role in the biotransformation and biodegradation of pollutants in contaminated sediments. However, no full-length CYP gene sequence has been determined for any oligochaete.

In shallow marine environments, water temperature varies seasonally. Temperature affects metabolic rate in all organisms, including marine invertebrates (Brockington and Clarke, 2001; Gillooly et al., 2001). Temperature also alters the toxicity and the biological disposition (e.g., absorption, metabolism, and excretion) of chemical contaminants (Hooper et al., 2013). However, the effect of temperature on the expression levels of genes involved in biotransformation and detoxification of xenobiotics is not well known.

The objectives of this study were as follows: 1) identification of the full sequence of a CYP gene from *Thalassodrilides* sp.; 2) analysis of the variation in *CYP4* expression in *Thalassodrilides* sp. exposed to environmental sediments contaminated with PAHs; and 3) investigation of whether temperature affected *CYP4* mRNA expression.

2. Materials and methods

2.1. Animals

The oligochaete *Thalassodrilides* sp. (Naididae) (Fig. 1) was collected from the sediment around a fish farm in the Uwakai Sea on the southern side of Ehime, Japan. *Thalassodrilides* sp. is a dominant organism there, with >100.000 individuals per square meter. The worms were brought back to the laboratory and maintained in seawater under a natural photoperiod at 20 °C in a continuous flow-through system, and were fed commercial fish food (N400; Kyowa Hakko, Tokyo, Japan) once daily.

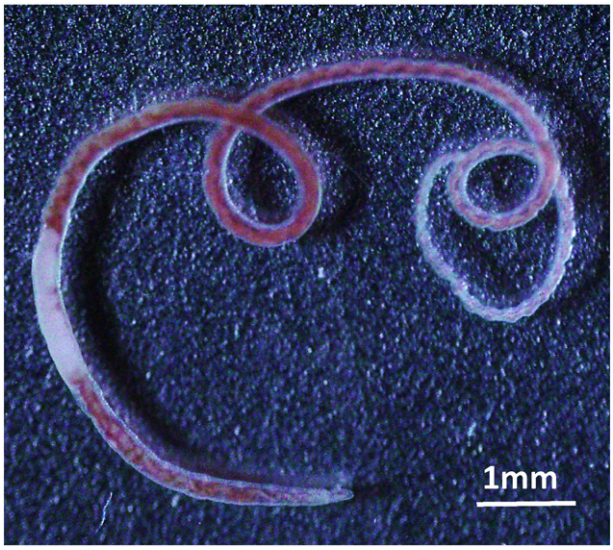

Fig. 1. A mature individual of the oligochaete *Thalassodrilides* sp.

2.2. Sediment

Polluted sediment was collected from Osaka Bay, which is near industrial factories and frequently anchored by many ships. Sediment from the fish farm in the Uwakai Sea that is inhabited by *Thalassodrilides* sp. was used as unpolluted sediment for control experiments. These sediments were sieved through a 1-mm mesh sieve, and were frozen at −20 °C until use to eliminate macro- and meiobenthos. The concentrations of 16 PAHs contained in the sediments were analyzed by gas chromatography mass spectrometry (GC/MS), and the results are shown in Table 1.

2.3. Exposure design

Microcosms were constructed as follows: a clear glass column (diameter 8 cm, height 10 cm) was filled with 70 g (wet weight) of polluted or unpolluted (control) sediment and 280 mL of seawater, which had been filtered through sand and activated carbon. Approximately 200 *Thalassodrilides* sp. individuals (0.72 ± 0.08 mg wet weight per individual, which was calculated by weighting 17–20 individuals 15 times) were added to each microcosm. Worms were acclimated for 3 days in aquaria at the experimental temperatures (15, 20, and 25 °C) before use. Three columns were prepared for each temperature, and the columns were kept in a dark incubator at different temperatures (15, 20, and 25 °C) without added food after the worms were added. The overlying water was slightly aerated, and the worms were sampled immediately before (initial) and at 1, 3, and 10 days of exposure to the sediment. At each time point, 60 individuals were distributed among three tubes (~20 per tube), frozen in liquid nitrogen, and then stored at −80 °C until extraction of total RNA.

To identify which parts of the worm express *CYP*, 30 worms were exposed to the polluted sediment at 20 °C. After 10 days of incubation, worms were cut into anterior, middle, and posterior thirds, frozen in tubes (~10 individuals) with liquid nitrogen, and then stored at −80 °C until extraction of total RNA.

2.4. cDNA cloning and sequencing

Total RNA was isolated from worms using ISOGEN (Nippon-Gene, Tokyo, Japan), and was subsequently treated with DNase I. It was then converted to cDNA using the Super Script First-Strand Synthesis System for RT-PCR kit (Roche, Indianapolis, IN, USA), following the manufacturer's instructions. A partial *CYP* gene sequence was obtained using PCR with a degenerate primer set (forward: 5′-GAYACITTYATGTTYGARGGNCAYGAYAC-3′ and reverse: 5′-GCDATYTTYTGNCCDATRCARTT-3′) designed against conserved regions (Rewitz et al., 2003). To obtain the 5′ and 3′ ends, primer sets were designed from the partial cDNA sequences, and the 5′ rapid amplification of cDNA ends (RACE) System and 3′ RACE System (Invitrogen, Carlsbad, CA, USA) were used with the primer set according to the manufacturer's instructions. The products amplified by 5′-RACE and 3′-RACE PCR reactions were cloned into the pCRII-TOPO vector (Invitrogen), and were then sequenced using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.5. Sequence analysis

The homology search for the amino acid sequence deduced from the full-length cDNA was carried out using NCBI BLAST Servers. For comparison with other CYP sequences, amino acid sequences of different animal species stored on GenBank and a CYP4V25 sequence from Dejong