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Differences in the ability of two marine annelid species, *Thalassodrilides* sp. and *Perinereis nuntia*, to detoxify 1-nitronaphthalene

Katsutoshi Ito [a, *], Mana Ito [a], Toshimitsu Onduka [a], Kohei Ohta [b], Takaaki Torii [c], Takeshi Hano [a], Kazuhiko Mochida [a], Nobuyuki Ohkubo [a], Takeshi Miura [b], Kazunori Fujii [a]

[a] National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, 2-17-5, Maruishi, Hatsukaichi, Hiroshima 739-0452, Japan
[b] South Ehime Fisheries Research Center, Ehime University, 1289-1, Funakoshi, Ainan, Ehime 798-4292, Japan
[c] IDEA Consultants Inc., 1334-5, Riemon, Yaizu-shi, Shizuoka 421-0212, Japan

HIGHLIGHTS

- The ability of annelids to biotransform 1-nitronaphthalene (1NN) was analyzed.
- An oligochaete *Thalassodrilides* sp. biotransformed 99% of 1NN to nontoxic products.
- A polychaete, *P. nuntia* biotransformed 40% of 1NN in 3 days.
- This is the first study to examine detoxification of 1NN by an oligochaete.

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ABSTRACT

Bioremediation is a promising method for remediating environmentally polluted water. We investigated the abilities of two benthic annelid species to biotransform 1-nitronaphthalene, a nitrated polycyclic aromatic hydrocarbon. We used an oligochaete, *Thalassodrilides* sp. (Naididae), collected from the sediment beneath a fish farm and a polychaete, *Perinereis nuntia*, which was obtained from a commercial source. Populations of both organisms were exposed to 1400 μg L^{-1} of 1-nitronaphthalene in seawater for 3 days in the dark at 20 °C. The concentration of the pollutant decreased to 12 μg L^{-1} in the seawater containing the *Thalassodrilides* sp. and to 560 μg L^{-1} in the seawater containing *P. nuntia*. The 1-nitronaphthalene concentration in the bodies of the animals increased from 12 to 94 μg kg^{-1} in *Thalassodrilides* sp. and from 0.90 μg kg^{-1} to 38,000 μg kg^{-1} in *P. nuntia*. After 3 days, 99% and 40% of the 1-nitronaphthalene had been biotransformed in the *Thalassodrilides* sp. and *P. nuntia* experimental groups, respectively. We then tested the acute toxicity of residual 1-nitronaphthalene from the same water using mummichog (fish) larvae. After the larvae had been exposed for 96 h, the percentage of apparently unaffected larvae remaining was 83.3% in *Thalassodrilides* sp. group but only 16.7% in the *P. nuntia* group. Clearly, of the two species we studied, *Thalassodrilides* sp. had a superior ability to convert 1-nitronaphthalene into substances that were nontoxic to mummichog larvae. Therefore, we recommend the use of this species for bioremediation of chemically polluted sediments.

1. Introduction

Many persistent chemical pollutants are deposited in estuarine and coastal sediments, where they cause harm to the benthic environment and have the potential to become biomagnified through the food chain, ultimately posing risks to human health as well. Because aquatic annelids tend to be more resistant to environmental pollution than are other benthic organisms, such as clams and crustaceans, they are able to inhabit areas that are contaminated by a variety of pollutants (Tsutsumi, 1990; Chang

* Corresponding author. 2-17-5, Maruishi, Hatsukaichi, Hiroshima 739-0452, Japan.
E-mail address: katsuit@affrc.go.jp (K. Ito).

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et al., 1992; Heilskov et al., 2006; Ito et al., 2011). The resistance of annelids to pollution may be explained by the fact that cytochrome P450, which transforms hydrophobic pollutants into more hydrophilic metabolites, has been identified in the polychaetes *Nereis virens*, *Capitella* sp., and *P. nuntia*. This hemeprotein may grant these polychaetes the ability to degrade chemical pollutants (Li et al., 2004; Jørgensen et al., 2008; Zheng et al., 2013). For example, Jørgensen et al. (2005) reported the biotransformation of pyrene, a common polycyclic aromatic hydrocarbon (PAH) pollutant, by the marine polychaete *Nereis virens*.

Thus, marine annelids appear to be suitable animals for bioremediation of sediment that is polluted by PAHs. However, much about bioremediation in annelids remains unknown, including the exact mechanism by which these organisms biotransform chemical pollutants.

One pollutant of interest is 1-nitronaphthalene (1NN), a nitrated polycyclic aromatic hydrocarbon (nitro-PAH) that is formed during the incomplete combustion of organic compounds (Nielsen, 1984). Such compounds are formed mainly by the reaction of PAHs with nitrogen oxides in polluted air (Atkinson and Arey, 1994). Nitro-PAH products are more toxic to many organisms than are the parent PAHs (Yaffe et al., 2001). The acute toxicity (96-h LC_{50}) of 1NN to a species of marine fish, the mummichog (*Fundulus heteroclitus*), has been reported to be 560 μg L^{-1} (Onduka et al., 2012). This value is in the "acute-I" category, which is the most hazardous category in the classification system of theOrganisation for Economic Co-operation and Development (2001). 1NN has been detected in river water in Japan at 3.7 ng L^{-1} (Murahashi et al., 2001) and in marine mussels (7.6 ng g^{-1} dry weight) and oysters (3.8 ng g^{-1} dry weight) collected in Osaka Bay, Japan (Uno et al., 2011). Yaffe et al. (2001), using an environmental model, reported that 1NN had the highest concentration potential among nitro-PAHs in the Los Angeles Basin in Southern California, USA. Thus, 1NN was used as a representative pollutant in this research.

The objective of this research was to elucidate the abilities of an oligochaete *Thalassodrilides* sp. and of a polychaete, *P. nuntia*, to biotransform 1NN. *Thalassodrilides* sp. is the dominant species throughout the year in the sampling field, with populations reaching 100,000 individuals and 50 g biomass m^{-2}. *P. nuntia* is a common species that inhabits the sand and gravel within the tidal zones in the coastal areas of Japan. These species have been suggested to be potentially useful for bioremediation in the field.

To assess the detoxification ability of these species, juveniles of a marine teleost fish were exposed to seawater containing 1NN previously used in aquaria containing the two annelid species. Although both annelid species demonstrated some bioremediation ability, the experiment showed that *Thalassodrilides* sp. was clearly superior and was able to render the water nontoxic to this fish within 3 days.

2. Materials and methods

2.1. Animals

The oligochaete *Thalassodrilides* sp. (Naididae) (Fig. 1A), identified only to the genus level, was collected from the sediment beneath a fish farm in Uwakai Sea of the southern part of Ehime, Japan (Fig. 2). Estimates of the population density within the field from which the samples were taken were over 100,000 individuals per square meter. The organisms were brought back to the laboratory and maintained under laboratory conditions at 20 °C before use.

Specimens of the polychaete *P. nuntia* (Fig. 1B) were purchased from Kochi Prefecture Gokai-Seisan Union (Kochi, Japan). They were acclimatized for a few weeks in aquaria at 20–25 °C under a natural photoperiod and were fed a commercial diet (C-700,

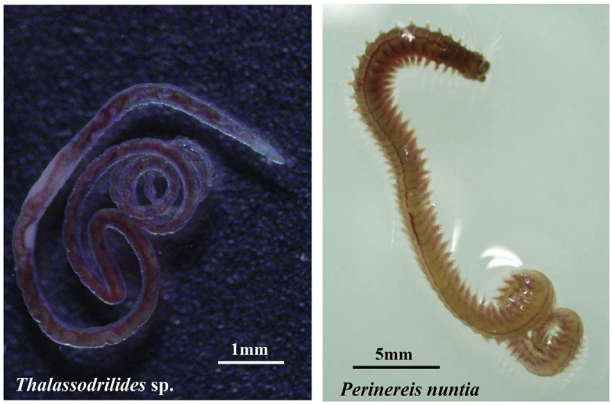

Fig. 1. Oligochaete *Thalassodrilides* sp. collected from the sediment beneath a fish farm. And a polychaete, *Perinereis nuntia*, that was obtained from a commercial source.

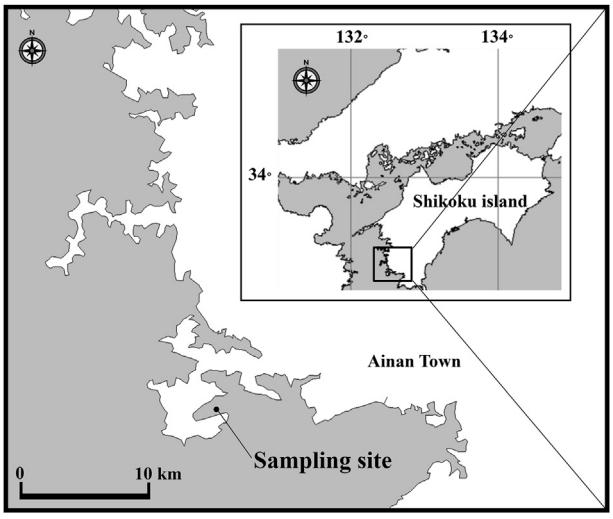

Fig. 2. Sampling sites offshore of Ainan Town, Ehime, Japan.

particle size of 425 μm-710 μm; Kyowa Hakko, Tokyo, Japan) once per day until use.

Individuals of the Arasaki strain of the mummichog (*F. heteroclitus*) (Shimizu, 1997) that had been bred in our laboratory for several years were used as a test organism for toxicity experiments. Juveniles (2–3 weeks after hatching; 15 mg body weight) were used in the toxin metabolism experiments. Hatched larvae were used in the bioremediation test with seawater containing 1NN following detoxification by *Thalassodrilides* sp.

2.2. Chemicals

1NN (99% purity) was obtained from Sigma–Aldrich (St. Louis, MO, USA). A stock solution was created consisting of filtered seawater with 1NN at its solubility limit (aqueous solubility = 9.18 mg/L). 1NN test solutions were prepared by adding the needed amount of stock solution to pre-filtered (GFC filter, Whatman, Maidstone, UK) seawater. Deuterium-labeled 1NN (1NNd7) and deuterium-labeled 3-nitrofluoranthene (3NFd9) were purchased from CDN Isotopes Inc. (Pointe-Claire, Canada). These stock solutions (1 mg/L) of 1NNd7 and 3NFd9 were prepared in acetone (Wako Pure Chemical Industries, Osaka, Japan) and were used as a surrogate and an internal standard, respectively, for mass spectrometry analysis.