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Removal of nonylphenol by earthworms and bacterial community change

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ABSTRACT

Nonylphenol (NP), resulting from surfactants used in industrial and domestic products, is an endocrine disruptor, and its presence in the environment has garnered much concern. We assessed combinations of soil, compost, and sludge with living earthworms or earthworm casts for NP degradation and accompanying changes in bacterial community composition. NP could be removed in sludge and compost with living earthworms. The removal rate was higher with sludge than compost. The addition of earthworm casts to soil enhanced NP removal. We used a 454 pyrosequencing-based metagenomic approach to characterize NP-degrading associated bacterial communities and obtained 58,529 16S rRNA gene sequences from 25 experimental samples. Pearson correlation analysis revealed that the sequence frequency for 61 bacterial genera may be associated with NP degradation; 11 of these genera were reported to be involved with NP degradation. This is the first work to use living earthworms and earthworm casts for NP degradation and suggests a novel way to obtain bacteria with NP degradation ability. These results may have great potential for the effective removal of organic toxic chemicals in the environment.

Introduction

Nonylphenol polyethoxylates (NPEOs) are widely used as surfactants in industrial and domestic products. The degradation of NPEOs creates products such as nonylphenol (NP), nonylphenol monoethoxylate, and nonylphenol diethoxylate, which are more persistent, lipophilic, and toxic than the parent compounds (Ahel et al., 1994). NP can mimic natural hormones by interacting with estrogen receptors (Soto et al., 1991). In wastewater treatment plants, because of their low solubility, the substances tend to be precipitated from the wastewater and concentrated in sludge. Sludge is routinely applied to agricultural soils in many countries to improve soil structure and add nutrients, but possible unwanted environmental effects result from trace organic contaminants added to soil (Wang and Jones, 1994).

Because of their biological, chemical and physical actions, earthworms can be directly used for bioremediation to promote degradation of organic contaminants (Contreras-Ramos et al., 2006). Earthworms can retard the binding of organic contaminants to soils, release previously soil-bound contaminants for subsequent degradation, and promote and disperse organic contaminant-degrading microorganisms (Hickman and Reid, 2008). The co-application of both compost and earthworms could be advantageous. Compost provides additional microbial numbers and diversity, nutrients, pH buffering, and improved moisture retention (Semple et al., 2003). Earthworm digestive actions result in increased soil particle surface areas, which may improve the accessibility of bound or sequestered contaminants to degrading microorganisms (Gevao et al., 2001).

Earthworm casts are nutritionally rich, and their deposition on burrow walls, within burrows or on the soil surface can significantly affect the chemical and physical composition of the surrounding soil in terms of altered C:N ratios and higher pH (Brown and Doube, 2004). Earthworm casts could be used to improve chemical conditions to aid bioremediation and improve the overall soil condition.

Many studies have used PCR-denaturing gradient gel electrophoresis (PCR-DGGE) to examine the effect of toxic chemicals on bacterial communities in sludge (Castle et al., 2006; Li et al., 2013; Zhang et al., 2013). However, because of the limited resolution of DGGE, detailed information about the diversity, composition and structure of bacterial communities from NP-degrading microcosms and their links with environmental parameters remains elusive. The method 454 pyrosequencing of 16S rRNA genes has been developed as a high-throughput metagenomic technology for

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profiling microbial communities (Claesson et al., 2010; Xu et al., 2012) with resolution at the genus level.

Earthworms have been used to remediate contaminated soils (Hickman and Reid, 2008). Use of the earthworm *Eisenia fetida* accelerated the removal of polycyclic aromatic hydrocarbons (PAHs; Matscheko et al., 2002). However, little is known about the use of earthworms to remove NP in sludge and soil. We studied whether *E. fetida* could be used for NP degradation. We examined the degradation with soil, compost, and sludge along with living earthworms or earthworm casts. In addition, we used 454 pyrosequencing to characterize bacteria communities associated with NP-degradation.

Materials and methods

Chemicals

NP with 98.0% analytical purity was from Aldrich Chemical Co. (Milwaukee, WI). Solvents were from Mallinckrodt, Inc. (Paris, KY). All other chemicals were from Sigma Chemical Co. (St. Louis, MO).

Experimental design

Mature compost (C:N ratio 19:1; moisture content 70% of maximum water holding capacity [WHC]; pH 7.2) was produced from brewer's wheat residue (barley, wheat and rye) and spent mushroom compost that underwent rapid composting by use of an enclosed force-aerated system. The composted material continued to "mature" outdoors for approximately 3 months before use.

The experiment involved plastic vessels (5 L capacity) measuring 20, 20 and 30 cm in length, width, and height, respectively, with 3 experimental sets. Sets A and B involved 3 combinations of treatments: 1) 1000 g compost, 200 mg kg^{-1} NP and 30 earthworms; 2) 1000 g sludge, 200 mg kg^{-1} NP and 30 earthworms; and 3) 1000 g compost and 200 mg kg^{-1} NP. Set C involved 3 combinations of treatments as well: 0 g earthworm casts + 200 mg kg^{-1} NP + 20 g soil; 20 g earthworm casts + 200 mg kg^{-1} NP + 20 g soil; and 20 g earthworm casts + 200 mg kg^{-1} NP + 0 g soil. NP was dissolved in methanol and added to compost, casts or soil. Adult earthworms (*E. ferida*) were depurated (laid on damp filter paper in an enclosed plastic Petri dish) for 24 h before use. We added 30 earthworms per kilogram material (each depurated earthworm weighing 0.2–1.0 g) to each of the treatments and replicates. The earthworms were combined with the material, and perforated lids on vessels were sealed. Water was added periodically to maintain levels at 70% maximum WHC. All the experiments were conducted in the dark. We collected 2 g compost and sludge samples (experiment set A), 2 earthworm gut samples (experiment set B), or 2 g earthworm casts and soil samples (experiment set C) every 7 d to measure residual NP concentration and characterize bacterial communities.

Analytical methods

NP extraction and analysis were performed as we previously described (Chang et al., 2007). NP was extracted from samples twice with use of chloromethane, then again over 30 min at 30 °C with use of a Branson 5200 ultrasonic cleaner (Branson, USA), then extracted with a C18 solid-phase extraction cartridge. Extracts were analyzed by use of a Varian 3900 gas chromatograph connected to a Saturn 2100 ion-trap mass spectrometer (Varian, USA) equipped with a DB-5 MS capillary column (30 m × 0.25 mm I.D., 0.25 μm film; J&W, USA). The initial column temperature was set at 80 °C for 1 min, increased at 30 °C min^{-1} to 200 °C, maintained at 200 °C for 2 min, then increased at 10 °C min^{-1} to 300 °C, where it was held for 2 min. Injector temperatures were maintained at 300 °C. Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The splitter was opened for 30 s after injection at a split ratio of 20:1. Full-scan electron impact (EI) ionization data were collected under the following conditions: mass range 40–310 *m*/*z*, scan time 0.75 s, manifold temperature 60 °C, and emission current 4.0 μA. The average recovery was 92.2 ± 1.8%, and the detection limit was 50 μg L^{-1}.

DNA extraction, PCR, and 454 pyrosequencing

Total DNA for each enriched microcosm was extracted by the standard CTAB method. Partial 16S rRNA genes containing variable V5–V8 regions were amplified from the extracted DNA. The sequence for the 5′ primer comprised a 454 pyrosequencing adaptor, a unique 4-mer tag for each sample and 787F (5′-

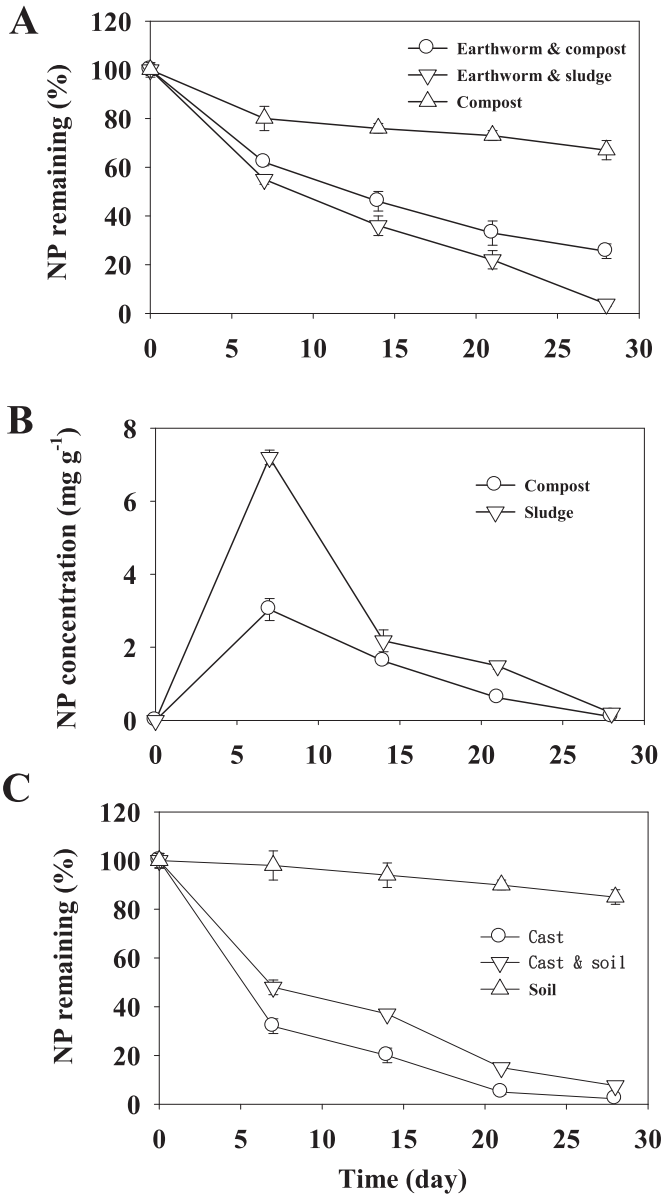

Fig. 1. Nonylphenol (NP) degradation and accumulation in earthworms. (A) NP degradation with earthworms in sludge or compost, without earthworms in compost; (B) NP accumulation in earthworms in sludge and compost; (C) NP degradation in earthworm casts and soil, earthworm casts, and soil. Data are mean ± SE from 3 independent experiments.