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Bacterial community structure in soils contaminated by polycyclic aromatic hydrocarbons

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Abstract

Bacterial community structure was examined in polycyclic aromatic hydrocarbon (PAH) contaminated soil taken from a timber treatment facility in southern Ireland. Profiles of soil bacterial communities were generated using a molecular fingerprinting technique, terminal restriction fragment length polymorphism (TRFLP), and results were interpreted using sophisticated multivariate statistical analysis. Findings suggested that there was a correlation between PAH structure and bacterial community composition. Initial characterisation of soil from the timber treatment facility indicated that PAH contamination was unevenly distributed across the site. Bacterial community composition was correlated with the type of PAH present, with microbial community structure associated with soil contaminated with two-ringed PAHs only being distinctly different to communities in soils contaminated with multi-component PAH mixtures. Typically the number of bacterial ribotypes detected in samples did not appear to be adversely affected by the level of contamination.

Keywords: Polycyclic aromatic hydrocarbon (PAH); Terminal restriction fragment length polymorphism (TRFLP); Creosote; Bacterial community structure; Contaminated soil

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic xenobiotic fused-ring aromatic compounds consisting of hydrocarbon molecules of two or more fused benzene and/or pentacyclic rings in linear, angular or cluster formation (Cerniglia, 1992). They are widespread environmental pollutants (Keith and Telliard, 1979), with particularly the higher molecular weight compounds representing a significant threat to human health due to their mutagenic and carcinogenic properties (Phillips, 1983). PAHs are formed, in large part, from the incomplete combustion of fossil fuels and are present in petroleum products and wood preservatives such as creosote (Cerniglia, 1992; Maliszewska-Kordybach, 1999). Creosote is a persistent chemical mixture composed of approximately 85% PAHs, 10% phenolic compounds and 5% N-, S- and O-heterocyclics, and has been extensively used worldwide as a commercial timber preservative (Carriere and Mesania, 1995). This has led to widespread contamination of soils and groundwaters proximal to wood treatment plants. Microbial degradation of PAHs has been widely reported and offers an attractive approach to the removal of these compounds from contaminated sites (Bossert and Compeau, 1995; Alexander, 1999). However, very little is known about the long term effects these compounds have on soil microbiota.

Recent molecular advances now allow the successful profiling of soil microbial communities, based upon culture-independent techniques such as cloning, or fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE), automated ribosomal intergenic spacer analysis (ARISA) or terminal restriction fragment length polymorphism (TRFLP) (Tiedje et al., 1999; Torsvik and Ovreas, 2002). Application of these techniques yields

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information that can be used to assess how environmental factors contribute to changes in microbial community structure (Kennedy et al., 2004). Although a considerable amount is known about how culturable bacteria respond to anthropogenic agents (Heitkamp et al., 1988; Juck et al., 2000; Margesin et al., 2000; Blakely et al., 2002; Sarkar et al., 2005) little is known about how xenobiotics influence the structure of soil microbial communities *in situ*. At present, bioremediation strategies rely on the stimulation of *in situ* microbial populations, but in most cases there is no knowledge of how existing populations respond either to the presence of the xenobiotic or the treatment applied. It has been suggested that microbial community structure in polluted environments is influenced by the complexity of chemical mixtures present and time of exposure (Langworthy et al., 1998; MacNaughton et al., 1999), and is thought generally to lead to a reduction in microbial diversity. The objective of this study was to determine whether the amount of PAH contamination together with the PAH compound present significantly affected microbial community structure in PAH contaminated soils.

2. Materials and methods

2.1. Site and soil sampling

Soil was sampled at a timber preservation plant. The soil type was a grey-brown podzol overlying limestone and glacial till. Fourteen samples were randomly taken from across the site from the 0–15 cm soil horizon, with triplicate samples taken from each sampling site (over a 0.5 m^2 area). These were aseptically harvested into plastic bags, transported to the laboratory in a cool box and stored at 4 °C prior to processing and analysis. Material used for molecular analyses was stored at −20 °C prior to analysis.

2.2. PAH extraction and analysis

PAH extraction was based on US EPA method no. 3541. Ten gram of soil and 10 g of sodium sulphate (Sigma) were mixed and placed in a cellulose extraction thimble, which was then placed in a Soxtec extraction system (Foss Soxtec Avanti 2055). This was extracted with a mixture of HPLC grade acetone and hexane (1:1) under the following conditions: 60 min boiling at 135 °C; 120 min solvent rinse at 135 °C. The extract was then evaporated to give a final volume of 25 ml. A 1 ml sample was then taken, sealed into a GC vial and stored at −20 °C. Dibutylphthalate (DBT) was used as an internal standard (80 mg l^{-1}) for subsequent GC analysis. A mean extraction efficiency of 86% was recorded.

Samples were measured using a gas chromatograph attached to a flame ionisation detector (Fisons GC8000), injected using an AS800 autosampler onto a HP-5MS silicone coated fused silica capillary column (Chrompack). The mobile gas phase was helium (90 kPa) and the make-up gas was nitrogen (100–150 kPa). The temperature gradient used was as follows: the initial oven temperature was held at 40 °C, which was raised to 270 °C at 10 °C min^{-1} increments and held at 270 °C. An injection volume of 1 μl of sample was applied to the column at an injection temperature of 250–300 °C, and a detector temperature of 300 °C. PAH concentration was calculated based on peak area and comparison with a standard solution (EPA 525 PAH Mix-B; Supelco).

2.3. Total soil DNA extraction and purification

Total soil DNA was extracted based upon a modification of a method of Griffiths et al. (2000). 0.5 g of sieved (<4 mm) soil was added to a 2 ml screw capped polyethylene micro-centrifuge tube, containing 0.5 g of 0.1 mm glass beads and 0.5 g of 0.5 mm zirconia beads, which had been autoclaved. Following the addition of 0.5 ml modified hexadecyltrimethylammonium bromide (CTAB; Sigma) extraction buffer (equal volumes of 10% CTAB in 0.7 M NaCl with 240 mM potassium phosphate buffer (pH 8.0)) and centrifugation at 3000*g* for 10 s, tubes were incubated in a 70 °C water bath for 10 min. After incubation, 0.5 ml of phenol:chloroform:isoamylalcohol (25:24:1) (Sigma) was added to tubes and these were shaken in a Hybaid Ribolyser at 5.5 m/s for 30 s. Tubes were then centrifuged at 16000*g* for 5 min at 4 °C. The top aqueous layer was removed and placed in a clean micro-centrifuge tube to which 0.5 ml of chloroform:isoamylalcohol (24:1) was added to remove residual phenol. Tubes were then centrifuged at 14000*g* for 1 min at 4 °C. This was repeated. The aqueous layer was again taken off and 50 μl of lysozyme (Sigma, 100 mg ml^{-1}) added. Tubes were then incubated at 37 °C for 1 h. Following incubation, tubes were centrifuged at 14000*g* for 5 min at 4 °C. The aqueous layer was taken off and purified using a Roche High Pure PCR Product Cleanup Kit as per manufacturer's instructions and eluted (10 mM Tris–HCl, pH 8.5) into a final volume of 50 μl.

2.4. Bacterial community fingerprinting by T-RFLP analysis

Terminal restriction fragment lengths were determined using a modification of the method of Liu et al. (1997). After extraction and purification of total DNA from soil, the bacterial 16S rRNA gene was amplified using primer set F27 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R1492 (5′-TACGGYTACCTTGTTACGACT-3′) (Lane, 1991). The forward primer F27 was labelled with Beckman Coulter fluorescent dye D4. PCR reactions were carried out in 50 μl volumes: consisting of 5 μl of each primer (30 pmoles each), 5 μl 10× Mg-free PCR buffer (Sigma), 5 μl of DMSO (dimethyl sulfoxide; Sigma), 2.5 μl $MgCl_2$ (25 mM; Sigma), 1 μl dNTPs (10 μmoles of each; Sigma), 25.25 μl distilled sterile H_2O, 2.5 U *Taq* DNA polymerase (Sigma) and ~10 ng extracted DNA. Thermocycling conditions were as follows: a hot-start at 94 °C for 3 min