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Soil microbial communities: Influence of geographic location and hydrocarbon pollutants

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Abstract

The importance and relevance of the geographical origin of the soil sample and the hydrocarbons in determining the functional or species diversity within different bacterial communities was evaluated using the community level physiological profiles (CLPP) and Polymerase Chain Reaction–Denaturing Gradient Gel Electrophoresis (PCR-DGGE). Hydrocarbon contaminated and uncontaminated soils from different geographical locations were used in the study. In addition, the influence or relevance of the geographical location of the sample was further evaluated by artificially contaminating soils from different geographical locations with different petroleum products. The hydrocarbons rather than the geographical origin of the sample appear to be more important in determining functional or species diversity within the bacterial communities. Cluster analysis of the different community profiles using both functional and molecular responses revealed that the samples from different locations were as different as samples from the same location but from contaminated versus uncontaminated soils. The results of the soils from different locations artificially contaminated by different hydrocarbons also reached the same conclusion. The samples from different soils were as different as samples from the same soil contaminated by different petroleum products. In addition, the removal rate of the different hydrocarbons in the artificially contaminated soil was different. The results suggest that the pollutants rather than the geographical origin of the sample might be more important in determining the functional or species diversity within bacterial communities.

Keywords: Microbial communities; Geographical location; Hydrocarbons; Functional diversity; Species diversity; Biodegradation; DGGE

1. Introduction

The impact of petroleum hydrocarbons on soil microbial diversity has been the subject of investigation in recent years. The changes in hydrocarbon content in soils results in characteristics shifts in microbial populations and the abundance of hydrocarbon utilising bacteria (Atlas et al., 1991; Wünsche et al., 1995). Hydrocarbon contamination selects for a less diverse but catabolically versatile bacterial community (Atlas et al., 1991; Lindstrom et al., 1999). However, information about the importance of geographical origin of the soil and the hydrocarbons in determining the functional and species diversity within bacterial communities is not well documented. There is a need to understand the importance of geographical origin of the soil and the hydrocarbons when assessing the different soil environments contaminated by hydrocarbons. The improved knowledge of the influence of the geographical origin of the soil and the hydrocarbons on microbial diversity can help to improve microbial process used in the removal of hydrocarbons from the soil.

Bundy et al. (2002) used community level physiological profiles (CLPP) and phospholipids fatty acid (PLFA) to study the effect of diesel on microbial communities and reported that microbial communities in different soil types do not converge after diesel contamination. However, the soil used in the study was artificially contaminated and could therefore, not adequately reflect the contaminated field sites.

Juck et al. (2000) found that at two oil contaminated Arctic sites investigated by DGGE and Biolog analysis, absolute diversity was decreased at one site and remained the same or increased at the other. However, the study was conducted using the cold adapted microbial communities.

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In this study, we evaluated soil microbial diversity of different geographic locations contaminated by similar hydrocarbons. We also investigated the biodegradation efficiency and microbial diversity of different soils artificially contaminated by different hydrocarbons. The aim of the study was to characterise the microbial diversity of different hydrocarbon contaminated soil environments to establish the importance and relevance of the geographic locations in relation to the stressor. The community level physiological profile and Polymerase Chain Reaction–Denaturing Gradient Gel Electrophoresis (PCR-DGGE) were used to characterise the microbial communities.

2. Material and methods

2.1. Soil and soil sampling

The soil samples were taken in sterile bags from a hydrocarbon-contaminated site in Secunda (Mpumalanga Province), Coalbrook (Free State Province), and Rosslyn (Gauteng Province), South Africa. The Secunda and Rosslyn soils were predominantly sandy loam while Coalbrook soil was a predominantly loam soil. The soils had a Total Petroleum Hydrocarbon (TPH) concentration during the day of sampling of 1.2 g kg soil^{-1}, 1 g kg soil^{-1} and 2.5 g kg soil^{-1}, respectively. Both contaminated and uncontaminated soils were collected. The uncontaminated soil was collected from the CSIR (Council for Scientific and Industrial Research) site in Gauteng Province, SA. The sites where the soil samples were collected is shown in Fig. 1. The samples were stored at 4 °C until further analysis.

2.2. Microbial community level physiological profiles

Microbial suspensions were prepared from soil as described by Wünsche et al. (1995). After appropriate dilutions in sterile saline solution, the cell suspensions were used to determine the number of culturable heterotrophs and to inoculate BIOLOG GN micro plates. The number of culturable heterotrophs, expressed as CFU, was determined by spreading 0.1 ml cell suspension on to a nutrient agar (Biolab Diagnostics) medium, amended with cycloheximide (200 μg ml^{-1}) to suppress fungal growth. Three replicates were spread on agar plates and incubated for 24 h at 28 °C. The results of the culturable heterotrophs for the different soil samples are shown in Fig. 2.

To obtain a substrate utilisation fingerprint of the microbial communities, three replicates of all the soil extracts were inoculated in BIOLOG GN microtiter plates (Biolog Inc., Hayward Calif) containing 95 different sole-carbon sources and a control without a carbon source. The dilutions yielding similar TRHs numbers were used to inoculate the plates. The BIOLOG GN plates were incubated at 28 °C and readings done using a Bio-Tek Elx800 (Bio-Tek Instruments Inc) micro plate reader at 600 nm after 24, 48 and 72 h. Once the raw data from the replicate Biolog plates was collected, an average of the three plates was calculated using MS Excel. Further statistical analyses were done using STATISTICA for Windows release 5.1.

2.3. Soil DNA extraction and purification

Total DNA was isolated from the soil using the Bio101 extraction kit (Bio Inc.). One gram of soil was used for DNA extraction. The extracted DNA was examined by horizontal electrophoresis in 1% agarose.

2.4. Community fingerprinting by PCR-DGGE

2.4.1. PCR conditions

A 1-μl volume of extracted DNA was amplified by PCR with a 9600 thermal cycler (Perkin-Elmer/Cetus). The PCR mixture used contained 100 μmol of each primer, 100 mM each deoxy-nucleoside triphosphate, 5 μl 10× PCR buffer, 0.25 μl hot start polymerase (5 U μl^{-1}), (Perkin-Elmer, Roche Molecular Systems, Branchburg, NJ), 2.5 μl 2% bovine serum albumin and 40 μl sterile water, to a final volume of 50 μl. The 16S rRNA genes from soil microbial communities were amplified by PCR using the primers, pA8f-GC (5′-CGC-CCG-CCG-CGC-GCG-GCG-GGC-GGG-GCG-GGG-GCA-CGG-GGG-GAG-AGT-TTG-ATC-CTG-GCT-CAG-3′) and KPRUN518r (5′ATTACC GCGGCTGCTGG-3′), which have been found to be useful for 16S rRNA gene amplification in ecological and systematic studies (Øvreås and Torsvik, 1998). Samples were amplified as follows: 95 °C for 10 min, 30 s cycles of denaturation (1 min at 94 °C), annealing (30 s at 51 °C), and extension (1 min at 72 °C), and a final extension at 72 °C for 10 min. Amplified DNA was examined by horizontal electrophoresis in 1% agarose with 5-μl aliquots of PCR product.

2.4.2. Denaturing gradient gel electrophoresis

DGGE was performed using a Hoefer SE600 vertical dual cooler system (Hoefer Scientific, San Francisco, CA). PCR samples were loaded onto 8% (w/v) polyacrylamide gels in 0.5× TAE (20 mM Tris, 10 mM acetate, 0.5 mM Na-EDTA, pH 7.4). The polyacrylamide gels (bisacrylamide gel stock solution, 37.55:1; BioRad Laboratories) were prepared with a 20–55% gradient of denaturant (urea and formamide) and allowed to polymerise. Electrophoresis was run at 60 °C, first for 10 min at 20 V and then overnight at 70 V. After electrophoresis, the gels were stained for 15 min in SYBR Green I nucleic acid gel stain, rinsed in distilled water for 1 min and photographed with a Polaroid MP4 Land camera. The gels were analysed using a software program Gel2K (www.im.uib.no) developed by Svein Norland (Department of Microbiology, University of Bergen, Norway), where presence/absence of bands was recorded. Clustering was based on the simple matching