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Profiling 16S bacterial DNA and RNA: Difference between community structure and transcriptional activity in phenanthrene polluted sand in the vicinity of plant roots

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Abstract

Biodegradation of polycyclic aromatic hydrocarbons (PAH) in soil is mainly performed by endogenous bacteria. The density and activity of soil bacteria are usually increased in the rhizosphere. A compartmentalized device was used to follow biodegradation of phenanthrene and bacterial community structure as a function of distance from roots. Isolation of total DNA and RNA, followed by PCR-TTGE and RT-PCR-TTGE on a 16S rDNA partial sequence, was performed to describe the structure of bacterial community and of active species, respectively. After 4 weeks, active species profiles in the immediate vicinity of roots were the same as in non-planted treatment, with similar biodegradation efficiency (approx. 60%) whereas community structure clearly indicated that bacterial populations were different. On the opposite, at a furthest distance (6–9 mm) from the roots where biodegradation was lower, bacterial community structure was similar to the non-planted treatment whereas active species differed. A specie activity factor (S_{af}) was calculated for five relevant species to follow their transcriptional state in the rhizosphere and showed spatial variations as a function of species and distance from roots. We conclude that depending on distance from roots, the rhizosphere selected different bacterial communities, and different active species within these communities, resulting in different degradation values. Potential biases of molecular protocols used in this study are discussed as well as their relevance to describe the bacterial component of the rhizosphere involved in PAH biodegradation.

Keywords: Bacterial community; DNA/RNA; Polycyclic aromatic hydrocarbon; Rhizodegradation; RT-PCR; TTGE

1. Introduction

In soil, polycyclic aromatic hydrocarbons (PAH) biodegradation is mainly mediated by microbial activities. These microorganisms, fungi and bacteria, are indeed able to degrade PAH and use these compounds as source of carbon. Additional C sources increase PAH biodegradation (Carmichael and Pfaender, 1997; Chen and Aitken, 1999) in combination with mineral nutrients. The positive effect of plant introduction was observed in situ and under controlled conditions (Alkorta and Garbisu, 2001; Dzantor et al., 2000; Günther et al., 1996; Tesar et al., 2002). In pot experiments, PAH dissipation increased in the rhizosphere (Binet et al., 2000; Günther et al., 1996; Joner et al., 2001). Plant roots create oriented fluxes of nutrients and water and translocate high amounts of organic substances such as sugars, amino-acids, phenolic acids in the surrounding soil (Lynch, 1990; Rovira, 1969). This massive input of organic substrate in soil is known to induce a selection of rhizospheric communities (Siciliano et al., 2001; Fons et al., 2003) and increase the diversity and the number of total bacteria (Smalla et al., 2001) resulting in higher bacterial activity (Lynch and Whipps, 1990). Root exudation is hypothesized to be the main parameter increasing PAH biodegradation in the rhizosphere by acting on PAH degrading bacteria (Anderson and Coats, 1994; Joner et al., 2002). However, bacterial

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community structure and activity in a PAH polluted rhizosphere remain poorly described. A device, which mimic a 2D rhizosphere, was previously designed to study biodegradation of PAH and bacterial density as a function of distance from roots (Corgié et al., 2003). In these devices, a spatial distribution of bacterial density was observed as well as gradual changes in bacterial communities with distance from roots (Corgié et al., 2004) emphasizing the ecological complexity of the rhizospheric ecosystem and the biodegradation process in presence of plant exudates.

To conduct functional ecology studies, populations should be fully described, their reaction to biotic and physical environmental pressure and their actions on environment quantified (Rasmussen and Sorensen, 2001). However, studying microbial communities, which present complex structure in soil (Wikström et al., 1999) still remains a challenge due to the high heterogeneity and the changeableness of that environment. Classical microbiology techniques, based on the ability of microorganisms to grow on more or less specific substrates, have partly reached their limits to describe bacterial populations in soil. Only 5–30% of total bacterial species are indeed cultivable from soil samples (Widmer et al., 2001). These non-exhaustive techniques are then limited to analyze population structures and dynamics. Molecular-based techniques, which provide a "snapshot" of microbial communities in their environment, have been successfully applied to soil samples (Macrae, 2000; Torsvik et al., 1990; Volossiouk et al., 1995) and have increased analytic capabilities in terms of population description by casting off bacteria cultivation steps (Felske et al., 1999). DNA analysis inform on community structure corresponding to species presence and abundance (McCaig et al., 2001) whereas analyzing RNA gives an access to the transcriptional state of previously detected species. Combining these two approaches is likely to provide more information on functional community structure.

This functional activity approach was used to detect the active species involved in the rhizodegradation of PAH. Using compartmentalized devices to spatially study rhizosphere gradients (Corgié et al., 2003), the present work aims to describe bacterial community structure and structure of active species as a function of the distance from the roots. Total genomic DNA and RNA were isolated from rhizosphere sections of lateral compartments spiked, or not, with phenanthrene. A partial sequence of the 16S rDNA gene was amplified and analyzed to profile the communities by temporal temperature gel electrophoresis (TTGE). In parallel, phenanthrene concentration was analyzed in the same rhizosphere sections.

2. Material and methods

2.1. Experimental design

Compartmentalized devices, as previously described by Corgié et al. (2003), were forming a vertical root compartment (RC: 3.5 cm diam, 250 cm^3) and 2 rhizosphere horizontal compartments (RHC: 3.5 cm diam, 30 cm^3) inserted at the bottom of the vertical one. In order to restrict root entry the root-free compartments were separated from the root compartment by a 37 μm nylon mesh. Ryegrass seeds (*Lolium perenne* L. cv. Barclay) were individually pre-grown in vermiculite for 2 weeks. One plant was transferred in each vertical root compartment and allowed to grow for another 2 weeks to ensure that the roots fully occupied the central compartment. The sterile nutrient solution (1 mM NH_4NO_3, 1 mM $Ca(NO_3)_2$, 1 mM Na_2HPO_4 2 H_2O, 1 mM K_2SO_4, 0.75 mM $MgSO_4$ 7 H_2O, 12.5 μM H_3BO_3, 2.5 μM $MnSO_4$ H_2O, 0.3 μM $CuSO_4$ 5 H_2O, 1 μM $ZnSO_4$ 7H_2O, 0.05 μM Na_2MoO_4 2 H_2O, 0.2 μM $CoSO_4$ 7 H_2O, 20 μM Fe-EDTA; pH 6) was refilled on a weekly basis and was provided by a cotton wick connected to a 250 ml reservoir.

Three treatments were carried out with four replicates: planted pots with non-polluted sand in the RHC (P-PHE), planted pots with polluted sand in RHC (P + PHE), and non-planted pots with polluted sand in RHC (NP + PHE). Washed quartz sand (0.125–2 mm) was spiked with PHE dissolved in chloroform (Fischer Scientific, 99% purity) to a final concentration of 500 μg g^{-1} sand. A bacterial inoculum was isolated as described by Corgié et al. (2003) from an industrial PAH-contaminated soil collected at an abandoned coke plant in Northern France (Joner et al., 2002). Briefly, 80 g of soil was agitated with glass beads (1 h, 500 ml of 0.8% NaCl) and the solution was filtered at 10, 5 and 2 μm to remove soil particles. The solution was finally filtered at 0.2 μm and bacteria were collected from the surface of the 0.2 μm filter and suspended in 100 ml of 0.8% NaCl. Bacteria cell concentration was immediately estimated on a Thoma cell. The microbial suspension was diluted and added to the polluted and unpolluted sands for a final concentration of approx. 10^5 cells g^{-1} sand. RHC were immediately added to the planted or unplanted RC and complete pots were incubated for another 4 weeks in a growth chamber (24/20 °C day/night, 16 h light period, 60–70% RH, 350 μmol m^{-2} s^{-1} PAR). At harvest, lateral compartments were removed and the sand cut in 3 mm layers, at three defined distances from the separating mesh: S1 (0–3 mm), S2 (3–6 mm) and S3 (6–9 mm). For each sample (approx. 6 g wet weight), 1 g was frozen at −80 °C prior to nucleic acid isolation. The remaining sand was air dried overnight and stored at 4 °C prior to PHE quantification using GC-MS (SARM, CRPG-CNRS, Vandoeuvre-lès-Nancy, France) after Soxhlet extraction with chloroform on 2 g dried samples.

2.2. Profiling of bacterial 16S rDNA and rRNA

2.2.1. Nucleic acid isolations

Total nucleic acid isolations were performed with a modified protocol of Corgié et al. (2004) based on a bead beating lysis and a phenol/chloroform purification. To inhibit the activity of nucleases and the loss of RNA, the entire protocol was performed at 4 °C. Briefly, sand sample (approx. 1 g) and 800 μl of extraction buffer (100 mM Tris,