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Comparative bioaugmentation with a consortium and a single strain in a phenanthrene-contaminated soil: Impact on the bacterial community and biodegradation

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ABSTRACT

The efficiency of two inoculation strategies, using a consortium (CON) or an isolated strain (AM), on phenanthrene-contaminated soil was determined with special concern on the study of the bacterial community composition by PCR-DGGE and pyrosequencing of 16S rRNA gene fragments.

Both strategies stimulated the phenanthrene degradation, increasing the cultivable heterotrophic bacteria number and biological activity. At the end of the treatments, the microcosms inoculated with AM reached the lowest values of phenanthrene but also the lowest dehydrogenase activity.

In DGGE patterns a reduction in number of bands in the contaminated and inoculated microcosms was observed, being the most significant differences attributed to inoculation with AM.

The pyrosequencing technique yielded results that correlated with the fingerprint, showing that the bacterial community composition based on relative abundance was significantly modified by treatments.

Sphingomonadales and Burkholderiales were highly stimulated by phenanthrene contamination and inoculation. In the Phe microcosm, the higher increase in Actinomycetales (mainly *Arthrobacter*) was observed.

Effectively, the use of the strain AM as inoculant became the best strategy to remediate the soil mainly based on the degradation efficiency, however it caused more drastic changes in microbial community than inoculation with CON, what can be compromising the ulterior functionality of the soil.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are amongst the most widespread organic contaminants in soils, water, and wastewater (Puglisi et al., 2007). The presence of PAH in contaminated soils and sediments poses a serious risk to human environments, since they have ecotoxic, mutagenic, and, in some cases, carcinogenic effects (ATSDR, 2005).

Although biological processes have received much scientific attention, the development of efficient in situ bioremediation processes for decontamination of hydrocarbon-contaminated soils still remains a challenging task (Szulc et al., 2014). The most commonly used type of eco-friendly strategies holding the promise of epitomizing in situ bioremediation are, bioaugmentation by inoculation with PAH degrading strains and biostimulation by supplementation with nutrients to stimulate indigenous microbial activity during bioremediation (Tyagi et al., 2011; Simarro et al., 2013).

Bioaugmentation efficiency is a function of the ability of the inoculated microbial degraders to remain active in the natural environment (Alexander, 1999). In most cases, failure was related to poor survivability and adaptability of the introduced microorganisms due to improper strain selection (Szulc et al., 2014). In previous works, we studied the bioaugmentation with a single degrading strain, *Sphingomonas paucimobilis* 20006FA. The study revealed a reduction in genetic and functional diversity of soil, which could have caused an accumulation of toxic phenanthrene metabolites reducing efficiency in phenanthrene degradation during the inoculation period (Coppotelli et al., 2008).

Although several PAH degrading bacterial species have been isolated (Samanta et al., 2002), it is not expected that a single

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isolate would exhibit the ability to degrade completely all PAH. As has been established, bioaugmentation requires different species of introduced PAH-degrading microorganisms, which can compete with the indigenous microbial community in PAH-contaminated soil, especially if they are to participate in the main carbon and energy flux processes and enhance PAH removal (Dejonghe et al., 2001). The benefits of using consortia for bioaugmentation have been extendedly discussed, since they can share biochemical steps in order to completely mineralize recalcitrant and/or toxic substrates (Mrozik and Piotrowska-Seget, 2010) and they can better overcome the barriers present in the new ecological and physicochemical environments. Many studies indicated that the use of consortia of aromatic-degrading bacteria has been more effective in removing pollutants as compared with selected single strains (Ghazali et al., 2004; Jacques et al., 2008).

The ultimate goal of any remediation process must be not only to remove the contaminant(s) from the polluted soil but also, most importantly, to restore the capacity of the soil to function according to its potential (Epelde et al., 2009). The measurement of microbiological parameters such as microbial biomass, enzyme activities and the diversity of soil microbial communities may serve as a good index of the impact of pollution on soil health (Labud et al., 2007).

The use of culture-independent methods to study microbial diversity has expanded our view of the microbial world and allowed access to extreme and difficult environments to study (Amann et al., 1995). Fingerprinting molecular biology techniques, like PCR-DGGE were employed to investigate the soil community structure; however, it only allows an assessment of the dominant members of communities (de Araujo and Schneider, 2008). Recently, pyrosequencing has emerged as the most powerful tool to analyze complex microbial communities in different ecosystems (Hervé et al., 2014), allowing inferring the biological function(s) of organisms and their contribution to the functional status of the sampled environment (Sun et al., 2013).

In this study the efficiency of a bioaugmentation strategy in phenanthrene-contaminated soil microcosms was determined using a phenanthrene-degrading consortium, enriched from a chronically contaminated petrochemical soil and previously characterized by Festa (Festa et al., 2013); and it was compared with the effect of bioaugmentation with a phenanthrene-degrading bacterium isolated from the consortium. We emphasize the study of the changes in soil bacterial community composition caused by phenanthrene contamination and bioaugmentation, using PCR-DGGE and pyrosequencing of PCR-amplified bacterial 16S rRNA gene fragments.

2. Materials and methods

2.1. Bacterial strains and growth conditions

A phenanthrene-degrading consortium (CON) was previously isolated from a chronically hydrocarbon-contaminated soil near La Plata city, Argentina. It was characterized in terms of structure, diversity and functionality, showing the capacity of degrading the 58% of the supplied phenanthrene in liquid mineral medium (LMM), in the first 7 days of incubation (Festa et al., 2013). One strain identified as *Sphingobium* sp. (AM), which was isolated from the CON, showed a degradation of the 75% of the supplied phenanthrene (Festa et al., 2013).

2.2. Soil characteristics and preparation

The soil selected for the study was an uncontaminated soil from an area near La Plata city, Argentina. It was analyzed in the Laboratory of Soil Science at the University of La Plata and showed the following physicochemical properties: a texture containing clay loam with a pH of 5.8–5.9, 3.60% organic carbon, 6.21% soil organic matter, 0.296% total nitrogen, 0.00042% available phosphorus, and 0.05 g kg^{-1} hydrocarbons. The soil was sieved in a 2-mm mesh and stored for 24 h at room temperature until use.

2.3. Preparation of cultures used as inocula

The CON inoculum was cultivated in LMM (Vecchioli et al., 1990) supplemented with 2000 mg l^{-1} of phenanthrene for 7 days at 28 °C and 150 rpm, then filtered (to eliminate phenanthrene crystals), centrifuged, washed and resuspended in 0.85% NaCl. The AM inoculum was cultured in R2 (Reasoner and Geldreich, 1985) for 24 h at 28 °C and 150 rpm, then centrifuged and washed to eliminate carbon sources and resuspended in 0.85% NaCl.

2.4. Preparation of soil microcosms

Soil microcosms consisted of 0.5 kg of sieved soil in a glass container with 1 kg capacity. They were artificially contaminated with 2000 mg of phenanthrene (Carlo Erba, Milan, Italy, >99.5% purity) per kilogram of dry soil. The phenanthrene was delivered in an acetone solution (150 mg/mL) and mixed into the soil manually with a spatula.

Three treatments were carried out in triplicates trays: (1) Phe: a contaminated and non-inoculated microcosm was used as a control of natural attenuation; (2) Phe + CON: microcosm was inoculated with 1×10^8 cells of CON per gram of dry soil one day after phenanthrene was added to the soil; (3) Phe + AM: microcosm was inoculated with 1×10^8 cells of AM strain per gram of dry soil one day after phenanthrene was added to the soil. In the two inoculated treatments a specific volume of the obtained cells suspension was added to the respective microcosms to achieve the desired inoculum density. A non-inoculated and non-contaminated microcosm (made in triplicate) was used as a control (Control). The same volume of bidistilled water was added to Phe and Control microcosms to standardize the moisture content. All microcosms were incubated at 24 ± 2 °C in the dark for 63 days and were mixed weekly for aeration. The moisture content of the soil was corrected when necessary to $20 \pm 2\%$ by adding distilled water. The 20% of water content correspond to a 65% of soil Water Holding Capacity.

2.5. Chemical analysis

A soil sample (25 g) was mixed with anhydrous sodium sulfate (25 g) and hydrocarbons were extracted for 6 h with ethyl acetate in a Soxhlet apparatus. The phenanthrene concentration in the ethyl acetate extracts was determined by HPLC (Coppotelli et al., 2010). The residual phenanthrene concentration was determined 2 days after the artificial contamination and every 7 days for the first 28 days and later on after 63 days. The statistical analysis of the phenanthrene degradation data was performed by parametric one-way ANOVA test followed by Tukey's honestly significant difference (HSD) post-hoc test, using XLStat-Pro statistical package version 7.5.2 (Addinsoft SARL, France).

2.6. Microbial enumeration and biological activity

Bacterial enumeration by CFU: In order to determine total heterotrophic cultivable bacteria and PAH-degraders, 10 g (wet weight) of soil sample were suspended in 100 ml of 0.85% NaCl, homogenized for 30 min on a rotary shaker (250 rpm) and decanted for 5–10 min. The total heterotrophic cultivable bacteria were quantified in duplicates by a successive 1/10 dilutions. These suspensions were spread on R2A medium plates (Reasoner and Geldreich, 1985) and after 7 days of incubation at 20 ± 2 °C colonies