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Comparative metagenomic analysis of PAH degradation in soil by a mixed microbial consortium

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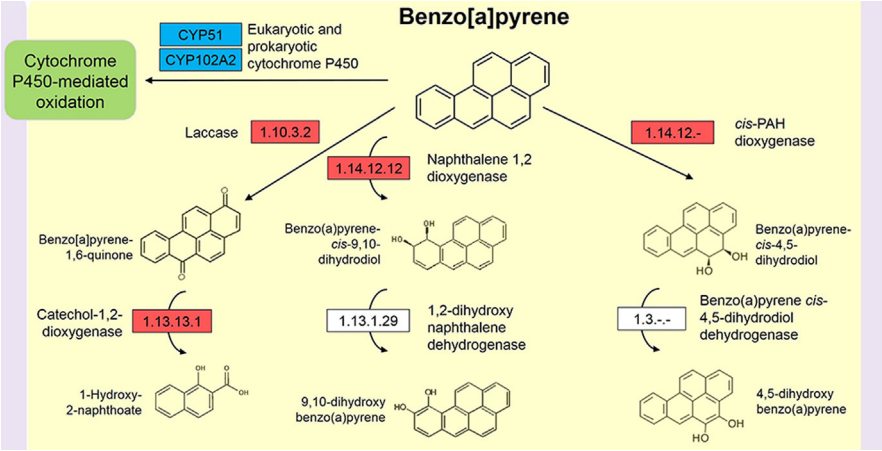
HIGHLIGHTS

- PAH degradation in soil by a microbial consortium studied by metagenomics.
- Consortium induced notable changes in the microbial diversity of polluted soils.
- Consortium shifted soil-native communities in favor of PAH-degrading populations.
- Concomitant degradation pathways taking place for Phe, Pyr and BaP degradation.
- High amounts of PAHs degraded in soils due to increased co-metabolic degradation.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we used a taxonomic and functional metagenomic approach to analyze some of the effects (e.g. displacement, permanence, disappearance) produced between native microbiota and a previously constructed Polycyclic Aromatic Hydrocarbon (PAH)-degrading microbial consortium during the bioremediation process of a soil polluted with PAHs. Bioaugmentation with a fungal-bacterial consortium and biostimulation of native microbiota using corn stover as texturizer produced appreciable changes in the microbial diversity of polluted soils, shifting native microbial communities in favor of degrading specific populations. Functional metagenomics showed changes in gene abundance suggesting a bias towards aromatic hydrocarbon and intermediary degradation pathways, which greatly favored PAH mineralization. In contrast, pathways favoring the formation of toxic intermediates such as cytochrome P450-mediated reactions were found to be significantly reduced in bioaugmented soils.

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PAH biodegradation in soil using the microbial consortium was faster and reached higher degradation values (84% after 30 d) as a result of an increased co-metabolic degradation when compared with other mixed microbial consortia. The main differences between inoculated and non-inoculated soils were observed in aromatic ring-hydroxylating dioxygenases, laccase, protocatechuate, salicylate and benzoate-degrading enzyme genes. Based on our results, we propose that several concurrent metabolic pathways are taking place in soils during PAH degradation.

1. Introduction

Contamination of soils with hydrocarbons has become a worldwide environmental issue because of the potential toxic effects on animals, humans, plants and microorganisms. Continuous contamination with crude oil and its derivatives favor the deposition and accumulation of xenobiotics and toxic compounds in soils. Polycyclic Aromatic Hydrocarbons (PAHs) are considered priority environmental pollutants because of their high toxicity and persistence. PAHs are molecules with physical and chemical characteristics that greatly contribute to their persistence in soil, possessing toxic, mutagenic and teratogenic properties [1]. During the last century there has been an increase in the amount of PAHs released into the environment from anthropogenic sources and atmospheric deposition from natural sources [2]. Microbial degradation is considered the main natural degradation form of hydrocarbons in soils [1,2]. Bioremediation, based on the use of microorganisms to degrade the contaminants, is a promising technology because of its high efficiency and cost-effectiveness. For over three decades it has been shown that microorganisms such as bacteria, fungi and algae possess specific catabolic activities that can be exploited for the remediation of soil and water impacted with low and high molecular weight PAHs [3,4].

Current knowledge about the microbial, functional and metabolic diversity and the impact produced by introduced microbial populations on native communities during the degradation of PAHs in soil is still limited. Metagenomics can be used to monitor microbial communities, providing access to the taxonomic and functional gene composition of soil microbial communities and thus giving information on potentially novel biocatalysts or enzymes and the phylogenetic and functional relationships between them [5]. Recent studies using Next-Generation Sequencing technologies during hydrocarbon bioremediation processes have shown the usefulness of these technologies to identify, monitor and estimate proportions of crude oil [6,7] and diesel degrading populations [8,9] present in soils and bioreactors. Thus, metagenomic sequencing of soil microbial communities involved in PAH degradation can provide insights regarding the microbial populations, functional and metabolic profiles and specific enzymes involved during the bioremediation of soils.

In this study, we used a taxonomic and functional metagenomic approach to analyze the metabolic profiles and main enzymes involved during the bioremediation of a soil polluted with low and high molecular weight PAHs, as well as some of the effects (e.g. displacement, permanence, disappearance) produced between soil native microbiota and a PAH-degrading microbial consortium.

2. Materials and methods

2.1. Soil samples

Uncontaminated soil samples obtained from the Xalostoc region in Tlaxcala, Mexico (19°24′08″N 98°02′54″W, 18 °C annual average temperature) were used in this study. Homogeneous samples were obtained at 30 cm depth in a simple random sampling, according to procedures described by US-EPA [10]. The soil sample composition was sandy loam with 2.4% organic matter, 1.4% total organic carbon, 0.063% nitrogen, 0.0023% phosphorous and a pH of 8.41. Soil samples were dried, homogenized and separated with a 2 mm test sieve. These soil samples were then spiked with 2500 mg kg^{-1} of a mixture of Phenanthrene (Phe), Pyrene (Pyr) and Benzo[a]pyrene (BaP) (1:1:1 ratio) using acetone as organic solvent and evaporated, as described by Ulla et al. [11].

2.2. PAH-degrading consortium and inoculum preparation

A microbial PAH-degrading consortium (C1), composed by four fungal (*Aspergillus flavus* H6, *Aspergillus nomius* H7, *Rhizomucor variabilis* H9, *Trichoderma asperellum* H15) and five bacterial native strains (*Klebsiella pneumoniae* B1, *Bacillus cereus* B4, *Pseudomonas aeruginosa* B6, *Klebsiella* sp. B10, *Stenotrophomonas maltophilia* B14), was used for the bioaugmentation of PAH-contaminated soils [12]. Individual strains composing the consortium were maintained aerobically at 30 °C in Potato Dextrose Agar (PDA) plates containing 0.1% Maya crude oil (fungi) or liquid Basal Saline Medium (g l^{-1}: NaCl, 0.3; $(NH_4)_2SO_4$, 0.6; K_2HPO_4, 0.75; KH_2PO_4; 0.25; $MgSO_4 \cdot 7H_2O$, 0.15; KNO_3, 0.6; yeast extract, 0.125) using 0.1% Maya crude oil as main carbon source (bacteria), as previously described [12]. Production of fungal spores was carried out in 250-mL flasks containing 30 ml of PDA medium, individually inoculated with each of the fungal strains and incubated at 30 °C. Spores were collected on day 4 with the addition of 20 ml of 0.1% Tween 80 solution, sterile glass beads and the flasks gently shaken for 2 min. The spore suspension concentration was quantified in a Neubauer haematocytometer chamber using an optical microscope. On the other hand, each of the bacterial strains was grown individually in 5 ml of liquid BSM at 30 °C with agitation at 200 rpm until cultures reached an optical density of 0.14 at 600 nm (comparable to a MacFarland standard No. 0.5; approx. cell density 1.5×10^7 CFU/ml). From these cultures, 10 μl of each strain (approximately 1.5×10^6 CFU) were sampled, mixed with the others (to give a final volume of 50 μl) and further used as inoculum in contaminated soil.

2.3. Microcosm treatability tests

Treatability assays were performed in microcosm solid culture systems using sterile corn stover (35.7% carbon, 0.465% nitrogen, 0.000031% phosphorous) for fungal growth support, texturizing and as a biostimulation agent. Corn stover (0.35 g dry weight) was placed in 100 ml glass flasks, moistened with 3 ml Czapeck medium (g l^{-1}: sucrose, 30; sodium nitrate, 3; dipotassium phosphate, 1; magnesium sulfate, 0.5; potassium chloride, 0.5; ferrous sulfate, 0.01; pH 7.3) to reach 30% moisture content and to promote germination of fungal spores and obtain the inoculum. Therefore, the flasks were inoculated with 2×10^6 spores g^{-1} of each fungal strain, hermetically sealed with sterile rubber caps and aluminum seal, then incubated for 5 d at 30 °C. Pre-inoculated corn stover was then mixed with 6.65 g of PAH-contaminated soil, inoculated with 2×10^6 CFU g^{-1} of each bacterial strain and sealed hermetically again. Controls consisted of non-inoculated systems (only

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