Characterization of the Diversity of Aromatic Ring-Dioxygenases among Soil Actinobacteria

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Abstract

Soil contamination with polycyclic aromatic hydrocarbons (PAHs) can result from direct exposure to fossil fuels or the incomplete combustion of organic matter and natural vegetation. Naphthalene is the simplest PAH (two fused benzene rings) and has been shown to be a component of both fossil fuels and wood char[1]. The Actinobacteria are ubiquitous Gram-positive, high G+C DNA, bacteria that have been shown to degrade a variety of PAHs and potentially play a significant role in the long-term degradation of PAHs. We are currently investigating the presence and diversity of Actinobacterial- and Pseudomonad-like aromatic ring dioxygenases (ARDs) in a variety of soils. These soils represent a range of exposure to naphthalene, including forested and urban, burned forest and unburned, and anthropogenically contaminated and those with no direct exposure to naphthalene. We designed degenerate PCR primers that target Actinobacterial aromatic ring dioxygenases (ARDs) and related enzymes (such as naphthalene and phthalate dioxygenases) based on the large subunit of *Rhodococcus* sp. NCIMB12038 NDO gene, *narAa* (**gb|AF082663)**. Ten Actinobacterial clone libraries have been completed. We have not detected *narAa*-like sequences in non-contaminated soils. The remaining distribution of Actinobacterial clone sequences shows significant associations with published phthalate-related dioxygenases, likely because phthalate can be an alternative to salicylate as an intermediate in the degradation of PAHs by some soil Actinobacteria[2]. This work will provide insight into the distribution of Actinobacterial dioxygenases and environmental factors controlling their abundance and distribution across a variety of soils.

Introduction

- Much information is available on the distribution of Proteobacterial aromatic dioxygenases; however, little information is available on the distribution of Actinobacterial aromatic dioxygenases, or factors that control their distribution in soil.
- The distribution of Actinobacterial aromatic ring dioxygenases may differ significantly from the more well studied Proteobacterial dioxygenases.
- We developed a set of degenerate PCR primers based on published Actinobacterial NDO and other gene sequence data on Genbank.
- These primers target a range of related aromatic dioxygenase genes of Actinobacterial origin.
- The primers were used to investigate distribution of the target genes in a range of soils, including soils with and without direct exposure to fuel oil.
- Forest fires are known to be a source of polyaromatic hydrocarbons to soils. Distribution of target genes in paired burned and unburned plots were also compared[1].

Hypotheses

- The distribution of detectable Actinobacterial aromatic dioxygenase genes in soils directly contaminated with fossil fuels differ from those in soils not directly contaminated by fossil fuels.
- The distribution of Actinobacterial aromatic dioxygenase genes will differ between soils subjected to controlled burns and those that are left unburned.

Figure 1. Binding sites for degenerative PCR primers. Primers were designed to include much of the Rieske center and enzyme active site[4,5].

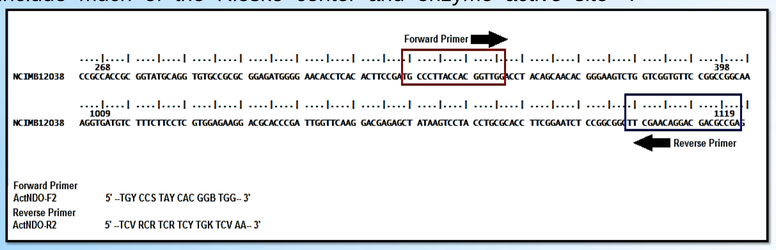
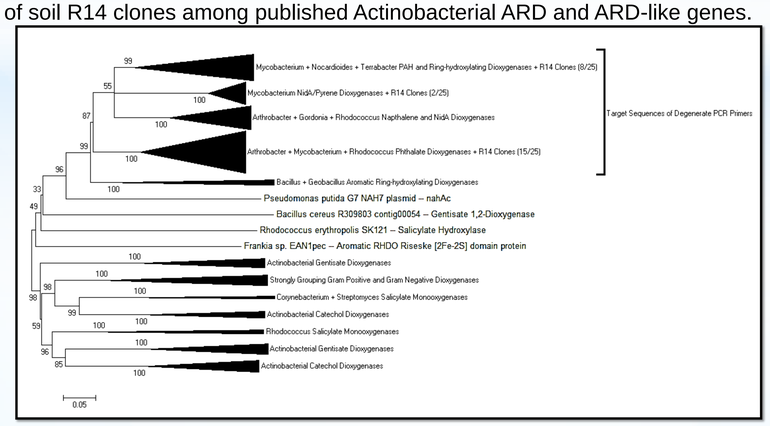


Figure 2. Geographical map of the continental United States showing sampling site locations.

Table 1. Descriptions of soils used in the experiment. ND, Not Determined; N/A , Not Applicable.

SOIL ID	Location	Type (Urban/Forested)	Soil Series	Direct Exposure to PAHS	Received Burn Treatment	No. of Total ARD-like Sequences Retrieved
MS	Michigan	Urban	ND	Yes	No	4
EV	Tennessee	Forested	ND	Yes	No	38
AS	Gainesville	Urban	ND	Yes	N/A	26
R14	Gainesville	Urban	ND	Yes	N/A	25
MW	University of Florida	Forested	Millhopper	Unknown	No	31
MUMC	University of Florida	Forested	Arredondo	No	No	26
MBAA	University of Florida	Forested	Arredondo	No	Yes	36
MBMB	University of Florida	Forested	Millhopper	No	Yes	22
JUAC	University of Florida	Forested	Arredondo	No	No	39
JBAA	University of Florida	Forested	Arredondo	No	Yes	37

Figure 3. Sample neighbor-joining tree showing the phylogenetic associations of soil R14 clones among published Actinobacterial ARD and ARD-like genes.



Results

Figure 4. P-test showing the significance of library differences among our experimental soils.

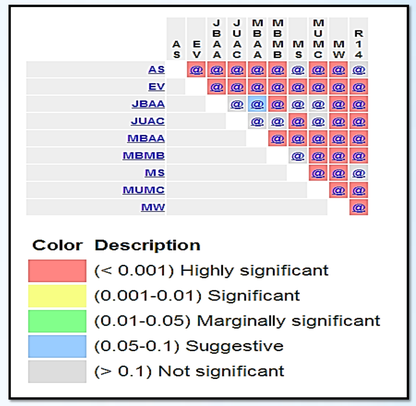
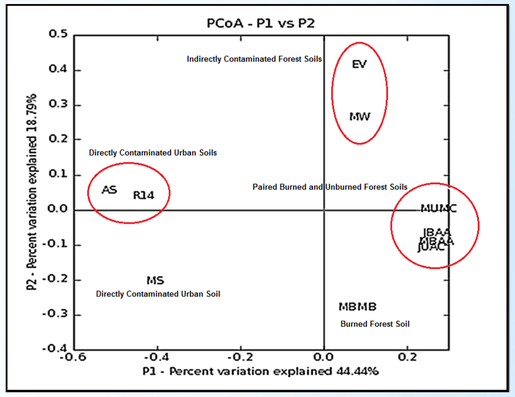


Figure 5. PCoA analysis of the sequence diversity of Actinobacterial-like ARD and ARD-like genes obtained from the soils listed in Table 1.



Conclusions

- Direct exposure of soils to PAHs is the primary driver of Actinobacterial ARD gene distribution in our soils.
- Preliminary data indicate that soil series may play a secondary role in the diversity and distribution of Actinobacterial ARD genes in our soils.
- Differences in vegetation and burn treatment appear to have no impact in the diversity or distribution of ARD and ARD-like genes.

References

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