Characterization of Diverse Naphthalene Degradative Pathways Among Soil Actinobacteria

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Worldwide consumption of fossil fuels has led to increased contamination of soils with polycyclic aromatic hydrocarbons (PAHs). These substances bioaccumulate in natural food webs and are potential carcinogens in higher animals including humans. The Actinobacteria constitute a phylum of the Bacteria characterized by a positive Gram-reaction, a high G+C content in their DNA, and have been shown to effectively metabolize a variety of PAHs. Actinobacteria may be more important in decontamination of PAH-contaminated sites than are the more well-studied *Pseudomonas* sp. The overall objective of this study is to investigate the diversity of naphthalene degrading pathways among soil Actinobacteria. Toward this end, we are isolating diverse naphthalene-degrading bacteria from soils, and will investigate the diversity of these pathways via culture-dependent and -independent approaches. Efforts are currently underway to isolate PAH-degrading Actinobacteria from a variety of soils using naphthalene as a sole carbon substrate/selective compound. Serial batch enrichments were performed on fourteen soils in minimal salts media using solid naphthalene added, either directly to liquid media or indirectly via test tube, as the only added carbon substrate to isolate a broad range of soil bacteria capable of metabolizing naphthalene. Periodic transfers of these cultures to fresh media were performed along with spread plating of serial dilutions on minimal medium with purified agar fumigated with solid naphthalene in a desiccator. To date, 158 strains have been isolated from the indirect (fumigation) approach and 204 from the direct addition. The pathways and metabolic range of these isolates will be investigated. For culture-independent investigation, gene sequences for naphthalene dioxygenase (NDO), phthalate dioxygenase (phtDO), gentisate dioxygenase (GenDO), and salicylate monoxygenase (SalMO) for various Actinomycetes were obtained and aligned based on sequence similarity to the NDO sequence of *Rhodococcus opacus* (GB|AF082663.3). These enzymes function in the systematic breakdown of naphthalene to carbon dioxide through two identified pathways in *R. opacus*: via salicylate and catechol/gentisate; or via phthalate and protocatechuate. Phylogenetic trees were developed that revealed the evolutionary relatedness of individual Actinobacteria to each other with respect to enzyme function as well as the relationship between different enzymes. These associations will be used in the development of a suite of PCR primers which will target specific sequences for enzyme functions within individual groupings of Actinomycetes. Gene sequencing of the resulting PCR products, as well as PCR and sequencing for 16S r RNA, will be used to identify individual isolates with a focus on identifying which isolates belong to the Actinomycetes.