

## Probing Marine Ecosystems for Novel Polycyclic Aromatic Hydrocarbon Degraders

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**Background/Objectives.** Polycyclic aromatic hydrocarbons (PAHs) are toxic, carcinogenic pollutants that commonly enter marine ecosystems through industrial site discharge, pipeline leakage, and petroleum well spills. Removal of PAHs is challenging due to their low bioavailability, hydrophobicity, and thermodynamic stability. Whereas physical and chemical remediation of PAHs is costly and invasive, microbial transformation of PAHs offers an effective and cost-efficient method for the remediation of these hazardous pollutants. While bacterial PAH degraders have been thoroughly investigated in terrestrial environments, less is known about marine bacterial PAH degraders and their degradation pathways. Exploring the landscape of marine microbial PAH degraders is not only crucial for environmental bioremediation practices but also that of PAH-contaminated wastewaters. PAHs are common wastewater contaminants but due to the high salinity of wastewater many non-salinity adapted PAH degraders are unable to degrade PAHs under these conditions. To identify novel PAH degraders in marine environments, we investigated culturable marine isolates originating from natural seawater and PAH enrichment culture isolates for their ability to degrade PAHs as well as genetic PAH degradation biomarkers.

**Approach/Activities.** Previous marine isolates from both open ocean and costal ecosystems were screened for their ability to degrade pyrene (4-ring PAH) and phenanthrene (3-ring PAH) using a PAH screening plate assay. Degradation efficiency was investigated using HPLC to quantify PAH disappearance. We also investigated the genomes of these isolates for PAH degradation biomarkers, such as PahE and PahAc. Additionally, PAH (either pyrene or phenanthrene) enrichment cultures were conducted with Gulf Coast of Florida seawater. Over the course of the month, bacterial strains were isolated weekly. Purified isolates were screened on pyrene- and phenanthrene-containing screening plates and those able to degrade PAHs were identified via 16S rDNA gene sequence analysis. Publicly available genomes for closely related strains were investigated for PAH degradation biomarkers.

**Results/Lessons Learned.** Screening an existing collection of marine bacterial isolates revealed that 95% of screened marine isolates showed some ability to degrade PAHs and that all strains were missing biomarkers for PAH degradation, suggestive of novel PAH degradation pathways. Previously undescribed PAH degraders were also isolated from PAH enrichment cultures, further displaying that the landscape of marine PAH degraders is relatively unexplored. Currently, all bacterial isolates from the enrichment cultures are Alphaproteobacteria. While Alphaproteobacteria are generally found in enrichment cultures of this type, Gammaproteobacteria are generally the dominant phyla and many of the Alphaproteobacteria isolated do not display PAH degradation. It is evident that marine ecosystems contain many previously undescribed PAH degraders and that these marine bacteria may not contain PAH degradation genetic biomarkers. Thus, investigating marine environments for novel PAH degraders can help us create inclusive bioinformatic tools to discover non-culturable PAH degraders and further identify bacterial PAH degrading communities. Marine PAH degradation isolates can also be applied to contaminated wastewater bioaugmentation practices, showing their application to both natural and applied bioremediation.