THE DISTRIBUTION AND DIVERSITY OF POLYCYCLIC AROMATIC COMPOUND-DEGRADING BACTERIA AND KEY DEGRADATIVE GENES

Submitted by

Rachel May Long

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Abstract

Petroleum hydrocarbons are the most widespread contaminants in the environment. Interest in the biodegradation of polycyclic aromatic hydrocarbons and compounds (PAHs/PACs) is motivated by their ubiquitous distribution, their low bioavailability, high persistence in soils and their potentially deleterious effects to human health. Identifying the diversity of microorganisms that degrade PAHs/PACs can be utilised in the development of bioremediation techniques. Understanding the mechanisms of bacterial populations to adapt to the presence of pollutants and the extent that lateral transfer of key functional genes occurs, will allow the exploitation of microbial PAC/PAH-degradative capabilities and therefore enhance the successful application of bioremediation strategies.

A key aim of this study was to isolate and identify PAC-degrading bacteria for potential use in future bioremediation programmes. A series of PAC enrichments were established under the same experimental conditions from a single sediment sample taken from a highly polluted estuarine site. Distinct microbial community shifts were directly attributable to enrichment with different PAC substrates. The findings of this study demonstrate that five divisions of the *Proteobacteria* and *Actinobacteria* can degrade PACs. By determining the precise identity of the PAC-degrading bacteria isolated, and by comparing these with previously published research, this study showed how bacteria with similar PAC degrading capabilities and 16S rRNA signatures are found in similarly polluted environments in geographically very distant locations e.g. China, Italy, Japan and Hawaii. Such a finding suggests that geographical barriers do not limit the distribution of key PAC-degrading bacteria. This is significant when considering the diversity and global distribution of microbes with PAC-degradative capabilities and the potential for utilising these microbial populations in future bioremediation strategies.

In the laboratory, enrichment of bacteria able to utilise PAHs has commonly been performed in liquid media, with the PAH dissolved in a carrier solvent. This study found the presence of a carrier solvent significantly affects the resultant microbial population. Although the same sediment sample was used as the bacterial source in all enrichments, different bacterial strains were obtained depending upon the presence of the carrier solvent and the PAH. This is important when considering appropriate methodology for the isolation of PAH-degrading bacteria for future bioremediation programmes. Additionally, the species comprising the resultant population of the enrichment when a carrier solvent was present were similar to previously reported PAH-degrading species. Such a finding necessitates review of previously reported PAH-degrading bacterial species that have been isolated and identified from enrichments using a carrier solvent.

Understanding how bacteria acclimatise to environmental pollutants is vital for exploiting these mechanisms within clear up strategies of contaminated sites. Two major lineages of the α subunit of PAH dioxygenases were identified: *Actinobacteria* and *Proteobacteria.* Comparison of the α subunit phylogeny with the 16S rRNA phylogeny implies that the PAH-dioxygenases evolved prior to the separation of these phyla or that lateral transfer occurred in the very distant past. No evidence for lateral transfer of the α subunit between the *Actinobacteria* and *Proteobacteria* was found in the phylogenetic analyses of this research. Multiple lateral transfer events were inferred between the species of the *Actinobacteria* and between the classes of the *Proteobacteria*. The clustering of the taxa within the α subunit phylogeny indicates that lateral transfer of the α subunit gene occurred after the separation of the classes of *Proteobacteria* and also after the speciation of the γ-*Proteobacteria.* These findings reveal how bacteria have acclimatised to PAH pollutants through multiple lateral transfer events of a key PAH-degradative gene. This knowledge of the transfer of genetic material will broaden our prospects of exploiting microbial populations.

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Abbreviations

ANT	Anthracene
API gravity	American Petroleum Institute gravity
CTAB	Ceylrimethylammonium bromide
DBT	Dibenzothiophene
DGGE	Denaturing gradient gel electrophoresis
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
GC-FID	Gas chromatography-Flame ionisation detector
HGT	Horizontal gene transfer
ISP	Iron-sulphur protein
HMW PAHs	High molecular weight PAHs
LMW PAHs	Low molecular weight PAHs
MGE	Mobile genetic element
MSM	Minimal salts medium
nahAc/ndoB/nidA	Encodes the large iron-sulphur (Fe-S) subunit of naphthalene dioxygenase
NDO	Naphthalene dioxygenase
PAC	Polycyclic aromatic compound
PAH	Polycyclic aromatic hydrocarbon
pahAc	Encodes the large Fe-S subunit of phenanthrene dioxygenase
PCR	Polymerase chain reaction
PEG	Poly ethylene Glycol
PHE	Phenanthrene
phnAc	Encodes the large Fe-S subunit of phenanthrene dioxygenase
*Pp*9816-4	*Pseudomonas putida* strain 9816-4
*Pp*G7	*Pseudomonas putida* strain G7
*Pp*OUS82	*Pseudomonas putida* strain OUS82
RBF	Round bottom flask
TAE	Tris-acetate-EDTA