

Probing Marine Ecosystems for Novel Polycyclic Aromatic Hydrocarbon Degraders

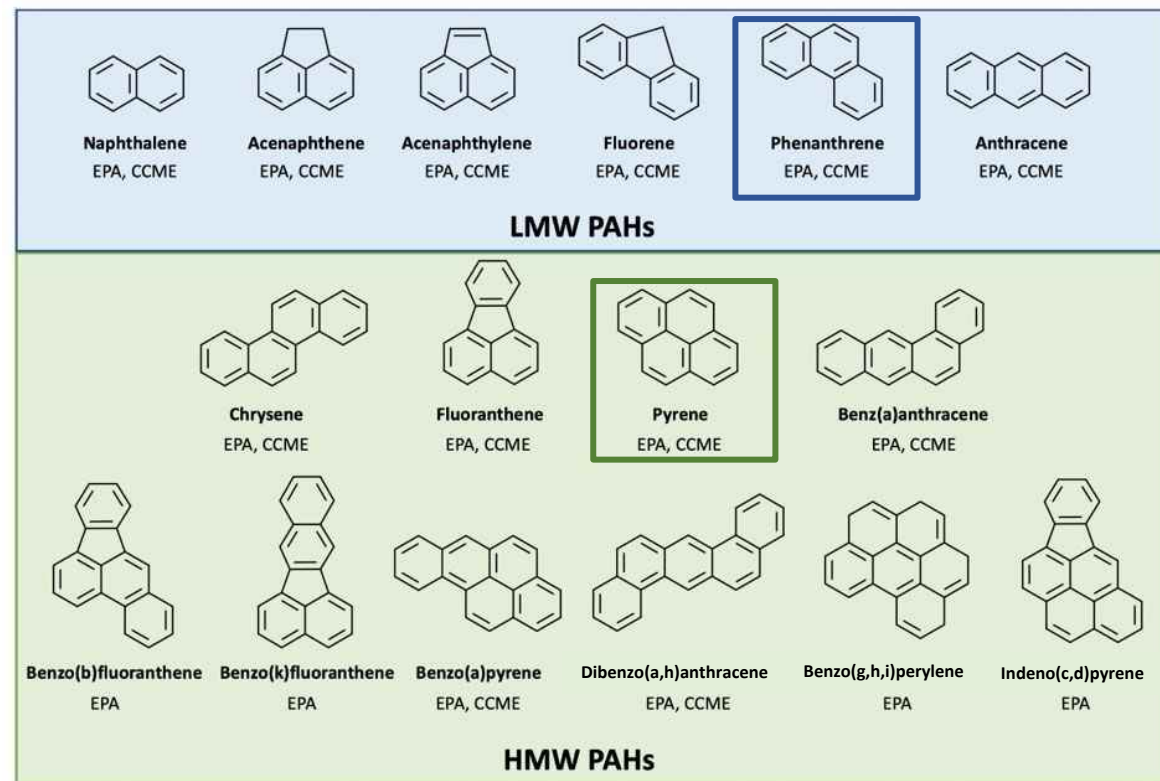
Jillian Walton, Ellen Bobo, & Dr. Alison Buchan

Microbiology Department

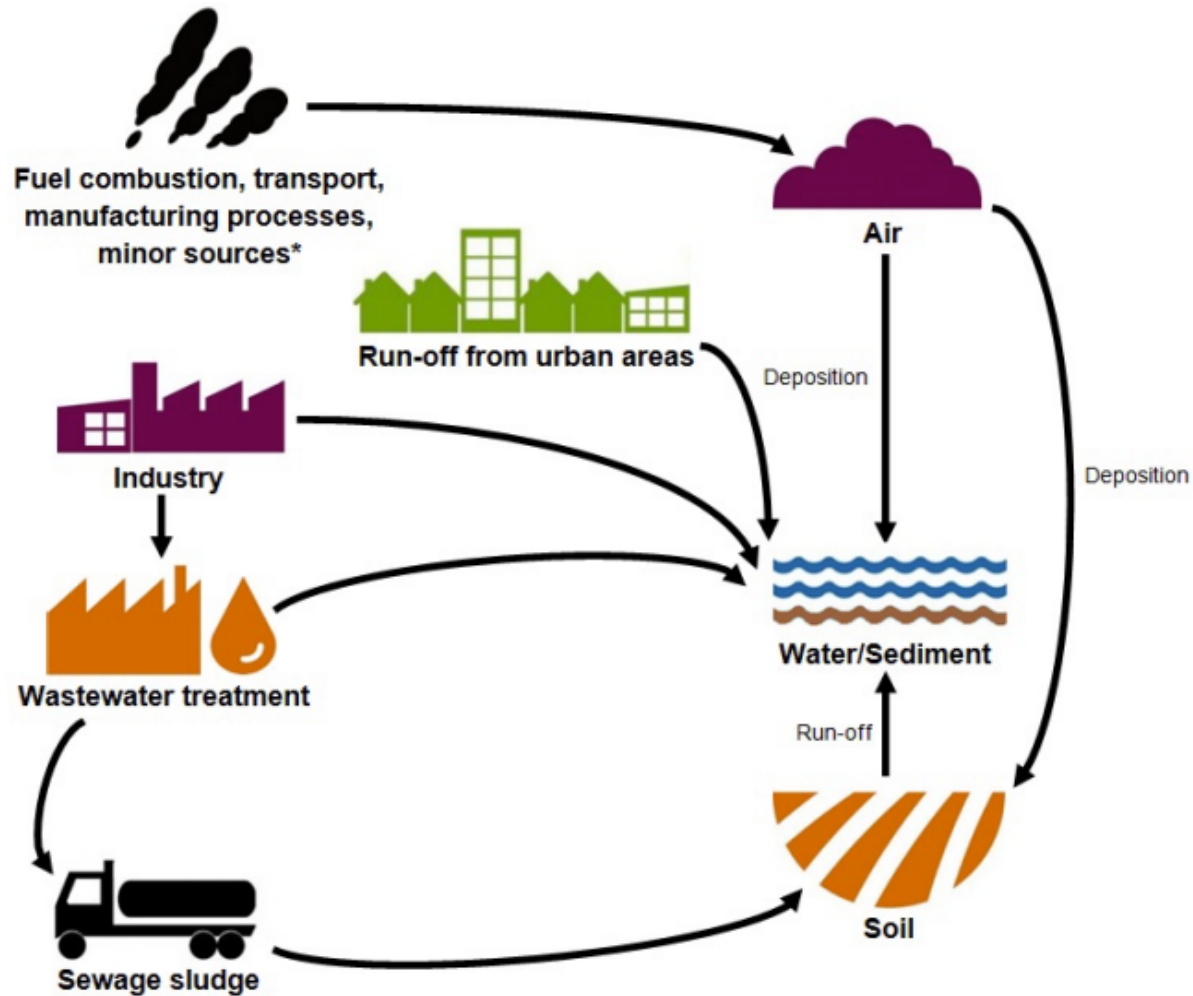
University of Tennessee, Knoxville, TN

Polycyclic Aromatic Hydrocarbons (PAHs)

- Toxic, carcinogenic pollutants
- Hydrophobic, low bioavailability, and high stability
 - Recalcitrant to degradation
- Ubiquitous throughout the environment
- EPA listed 16 as priority pollutants
- CCME listed 12 under Canadian Environmental Quality Guidelines

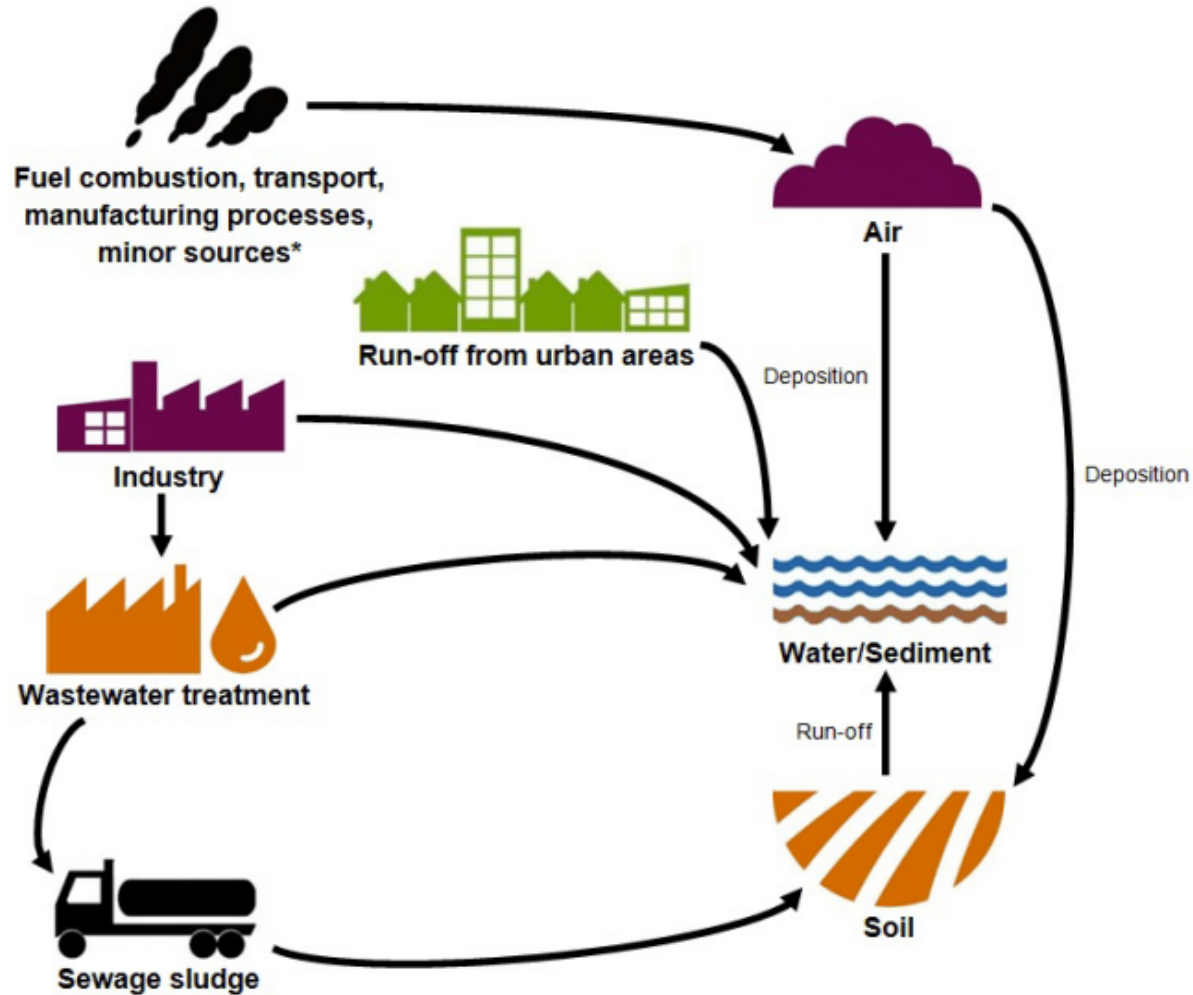


How do PAHs enter the environment?

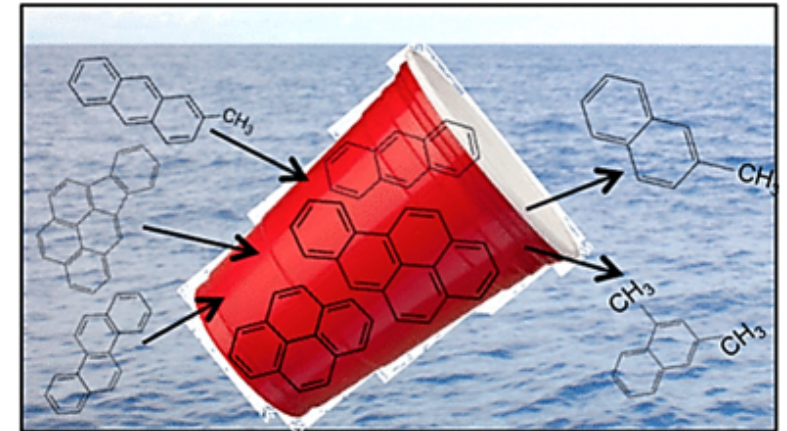


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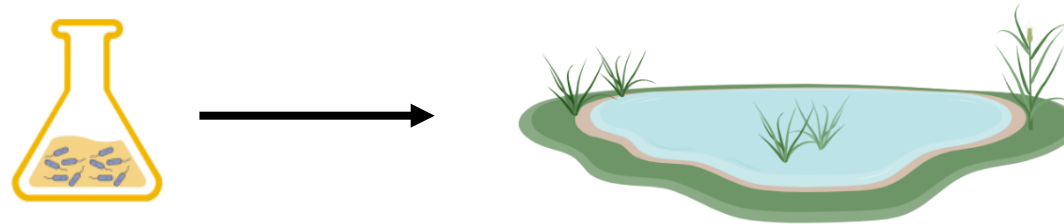


Microbial Biodegradation of PAHs

- Most effective remediation strategy for PAH contaminated environments

Microbial Biodegradation of PAHs

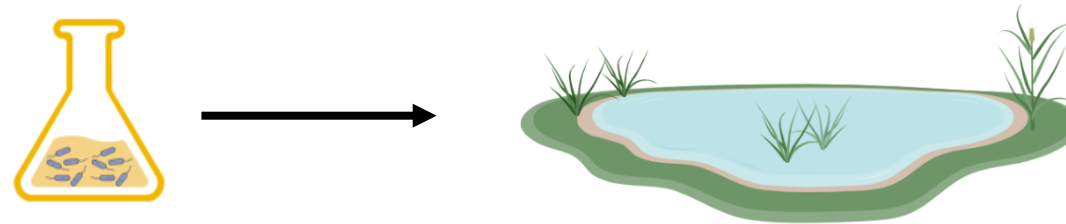
- Most effective remediation strategy for PAH contaminated environments
- Bioaugmentation



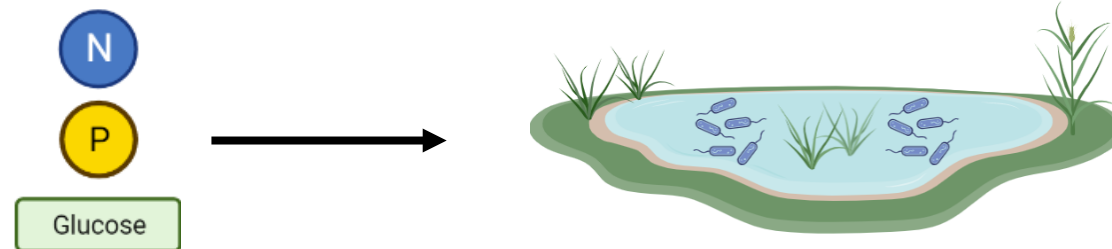
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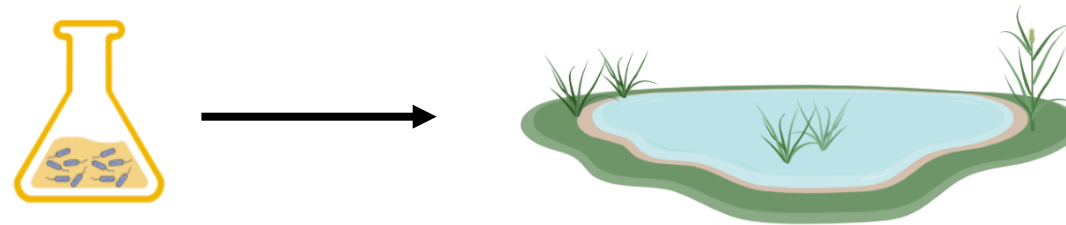
- Biostimulation



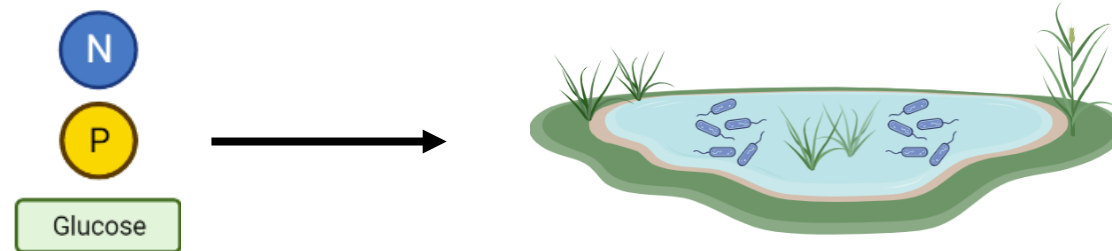
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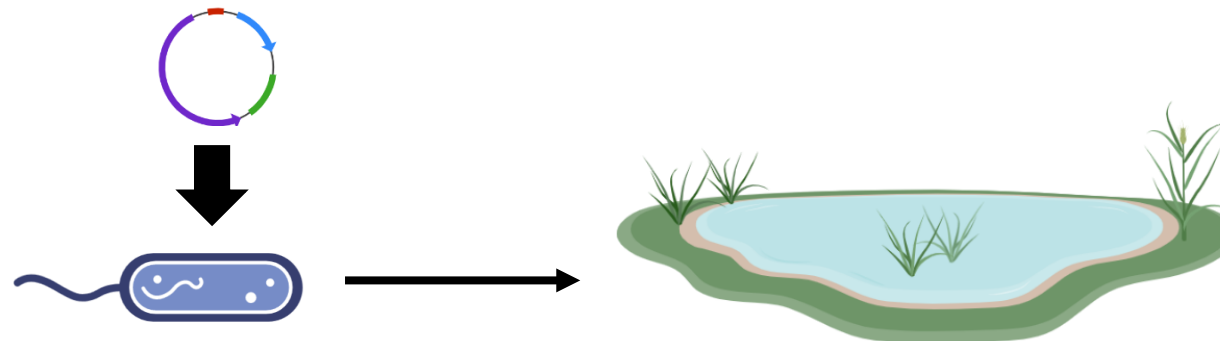
- Bioaugmentation



- Biostimulation



- Synthetic biology



Microbial Biodegradation of PAHs

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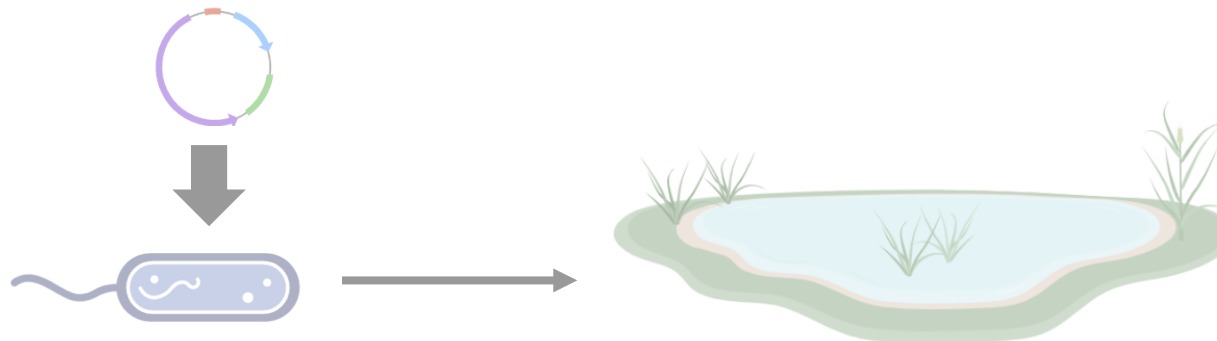


Limited knowledge of natural attenuation of PAHs in marine systems

- Bi

Glucose

- Synthetic biology

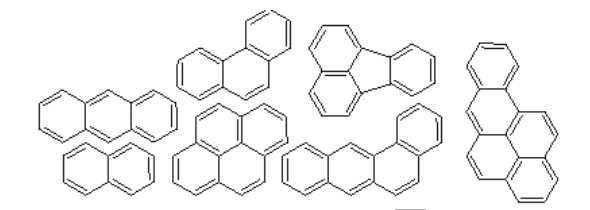


Current Knowledge of PAH Biodegradation

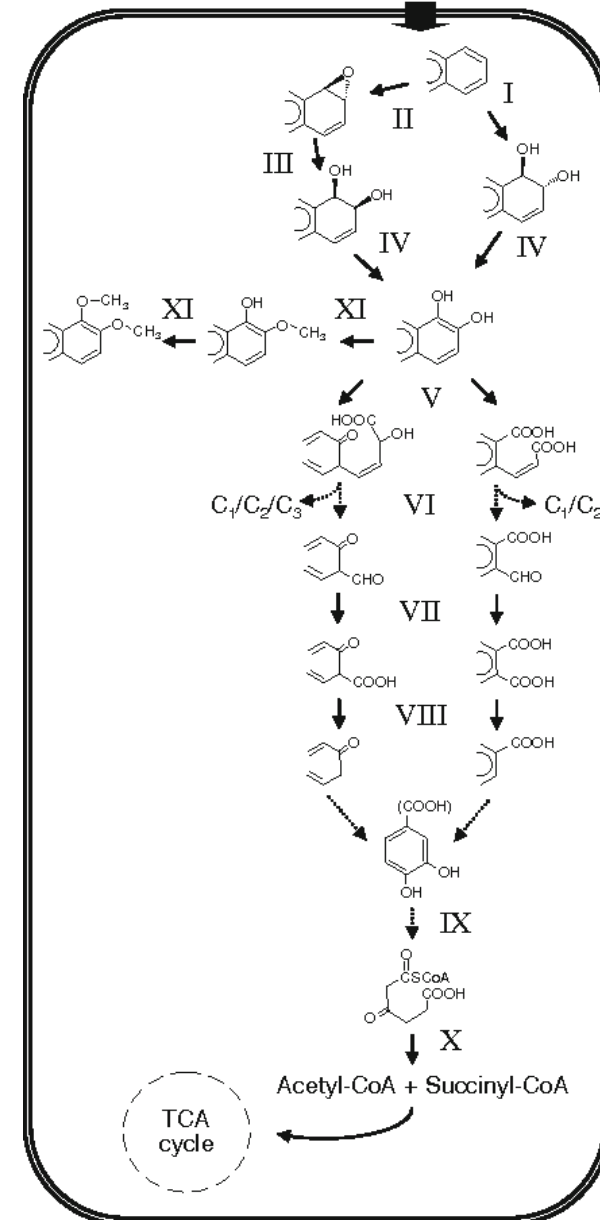
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 - *Mycobacterium* spp.,
Pseudomonas spp., and
Sphingomonas spp.
- Limited number of characterized pathways
 - Limited diversity of genetic biomarkers

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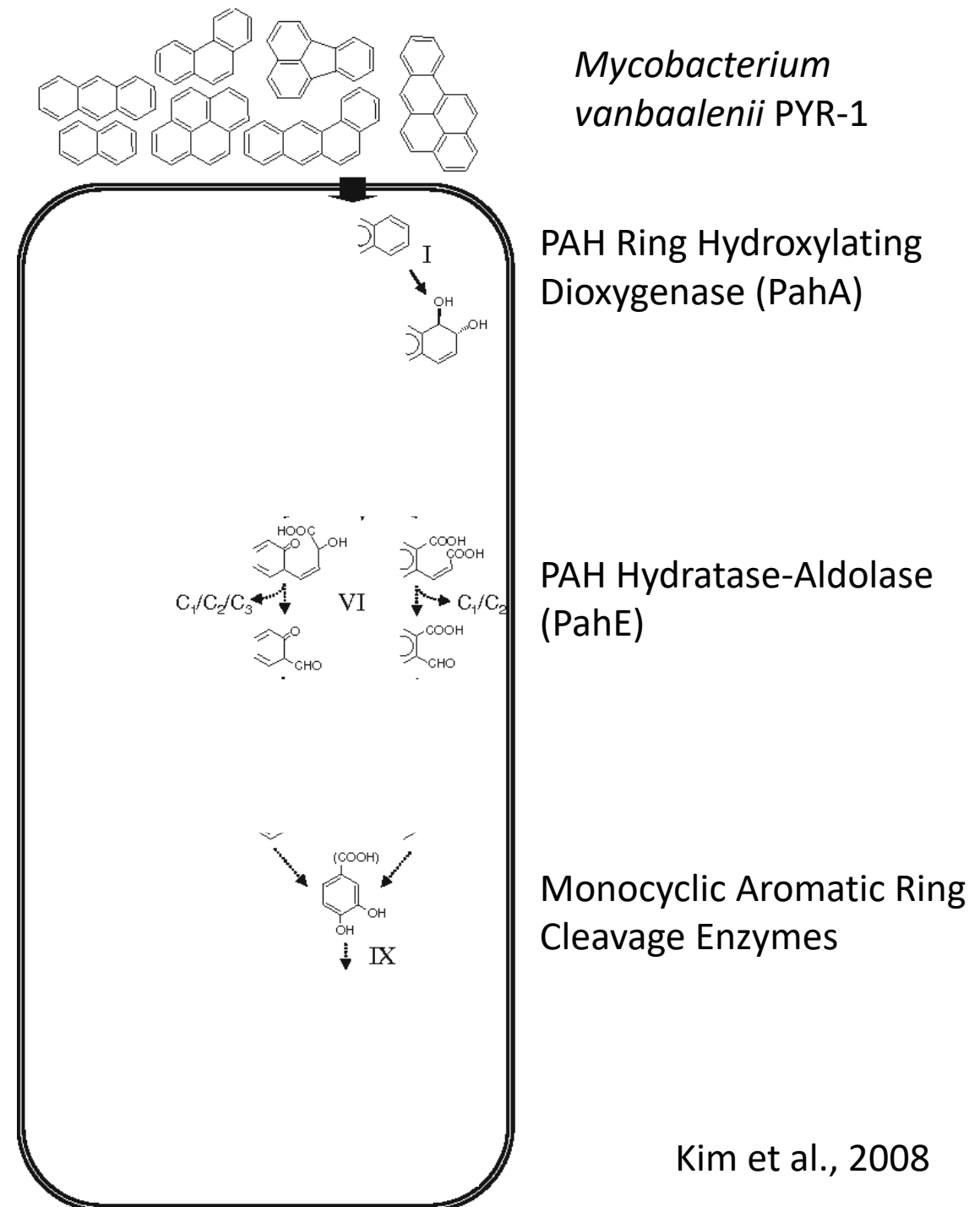
Mycobacterium
vanbaalenii PYR-1



Kim et al., 2008

Current Knowledge of PAH Biodegradation

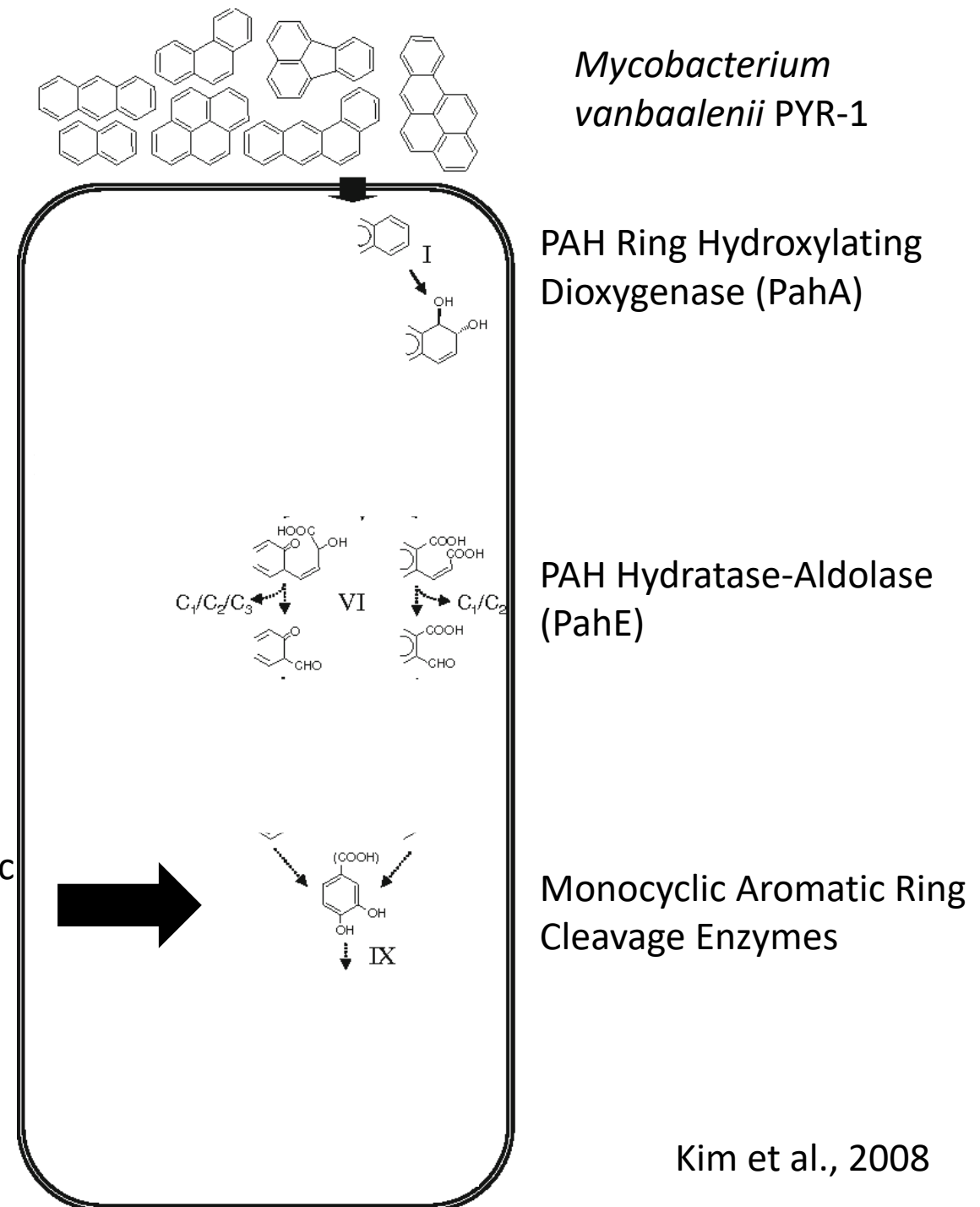
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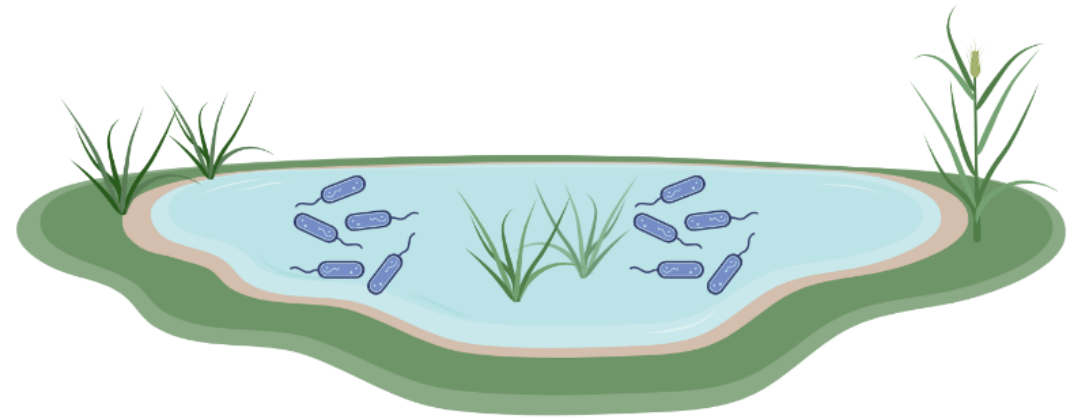
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Lignin Derived Monocyclic Aromatic Compounds

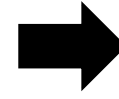
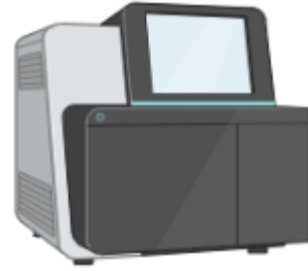
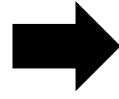
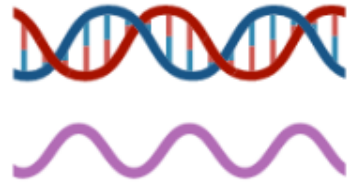
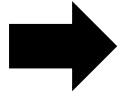
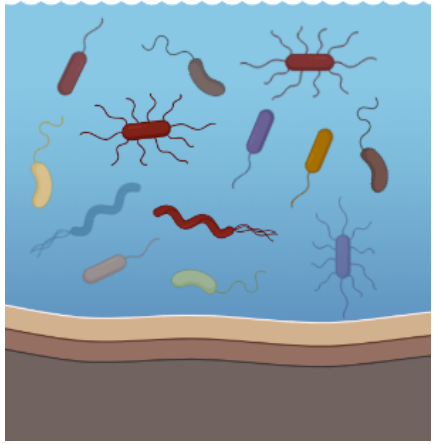


Natural Attenuation in Marine Ecosystems

- Provides a more sustainable bioremediation strategy
 - May also decrease recovery times of polluted sites
- Halotolerant/halophilic bacteria
 - Can be applied to high salinity wastewaters
- Marine ecosystems are a sink for PAHs

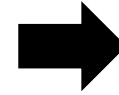
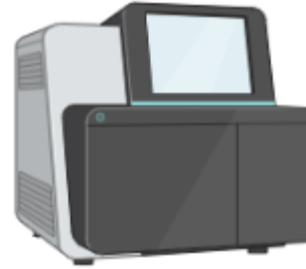
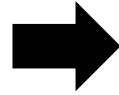
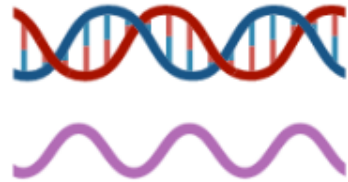
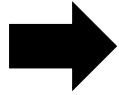
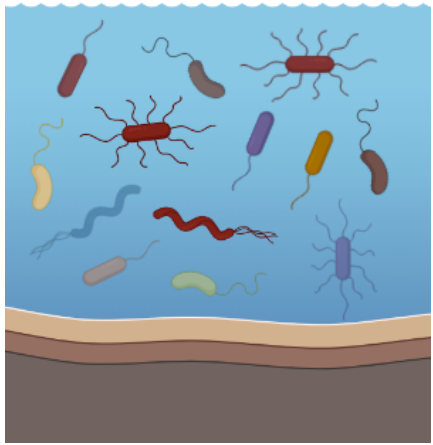


Bioinformatic Approaches



Metagenomics
Metatranscriptomics
16S rRNA Amplicon Sequencing

Bioinformatic Approaches



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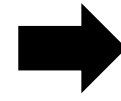
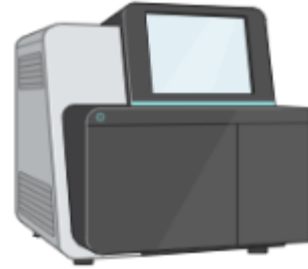
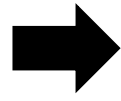
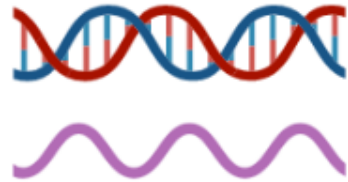
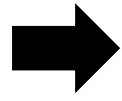
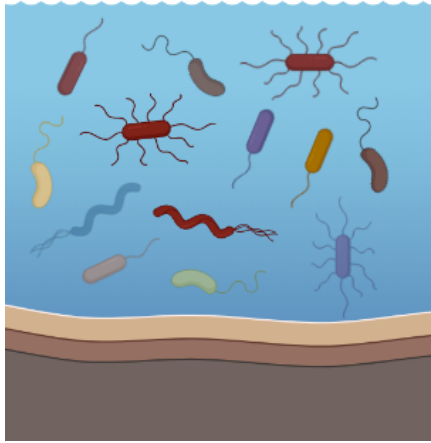
Pros

Assess abundances and activity in environment

Provides data on multiple genera of bacteria at once

Investigate non-culturable isolates

Bioinformatic Approaches



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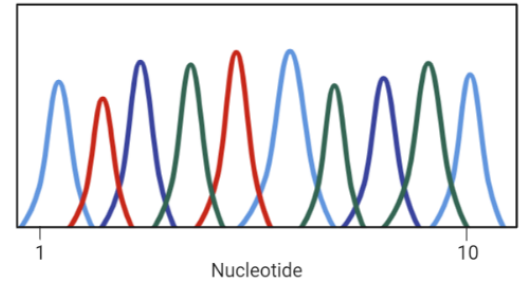
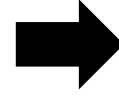
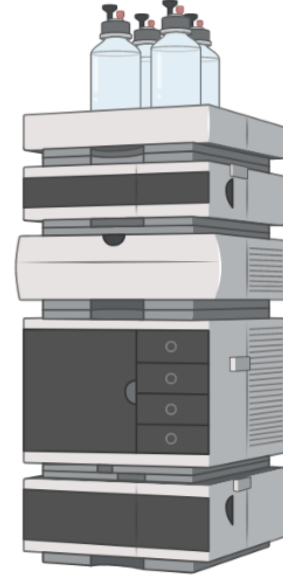
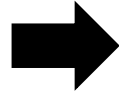
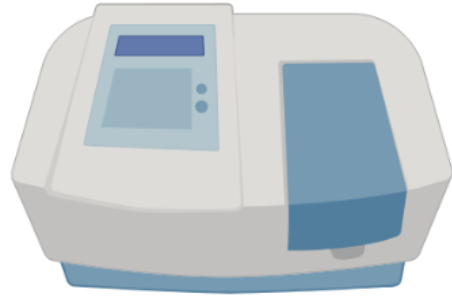
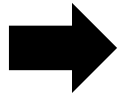
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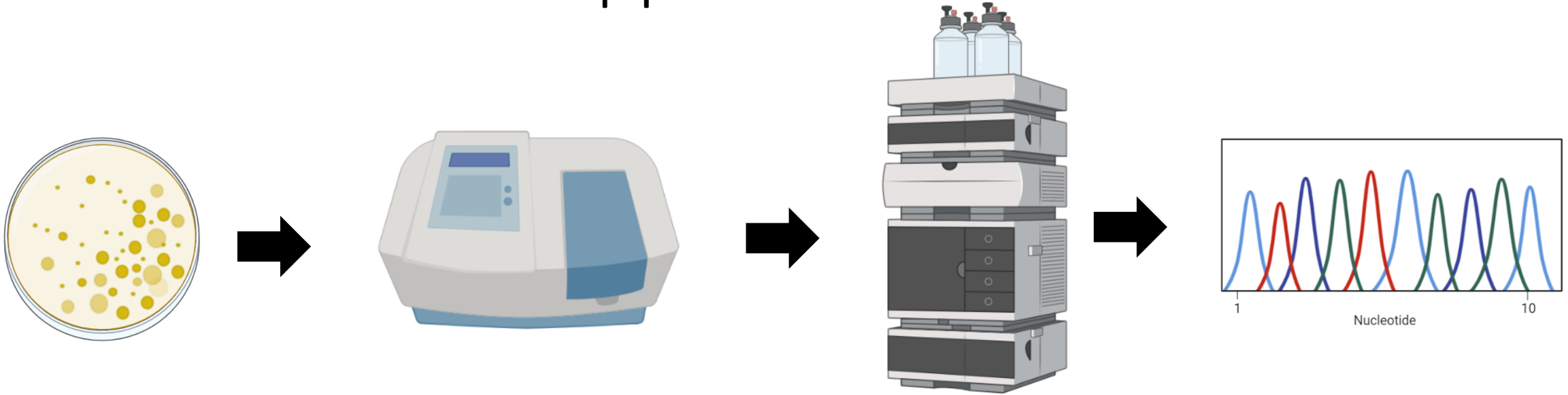
Cons

- Dependent on prior knowledge
- Cannot directly connect function to a species
- Cannot detect novel pathways

Culture-Based Approaches



Culture-Based Approaches



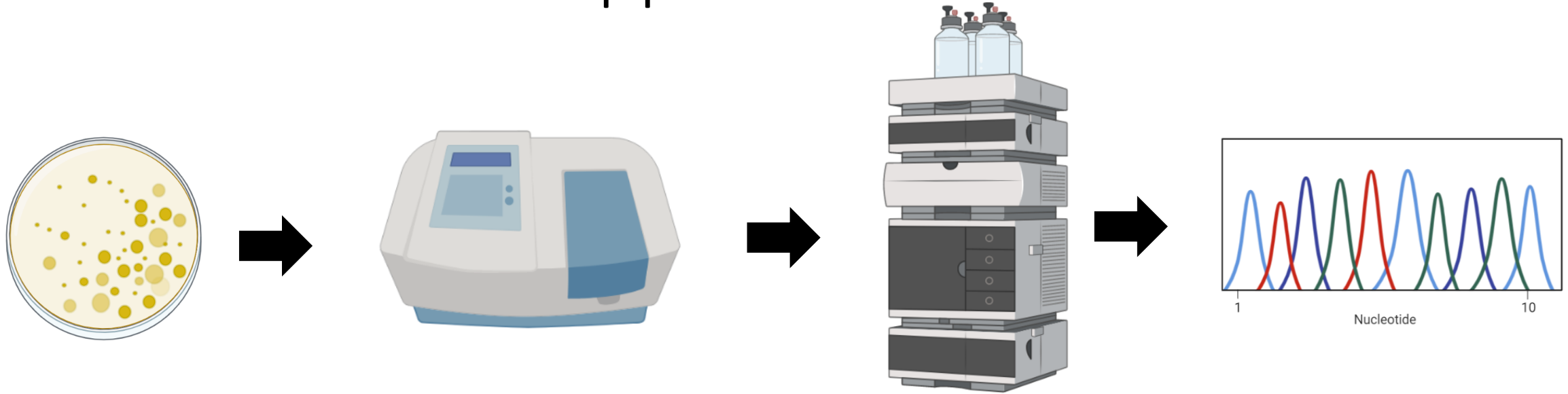
Pros

Describe novel pathways

Directly connect function to a species

Test under what conditions biodegradation occurs

Culture-Based Approaches



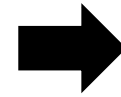
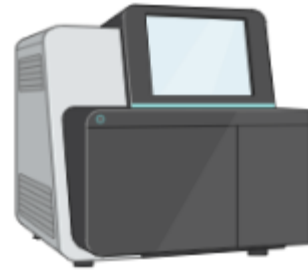
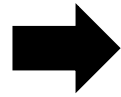
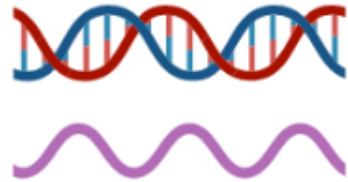
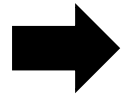
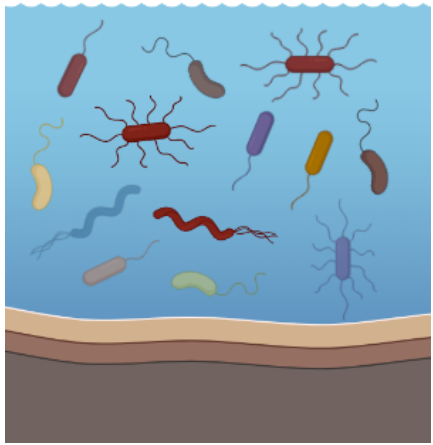
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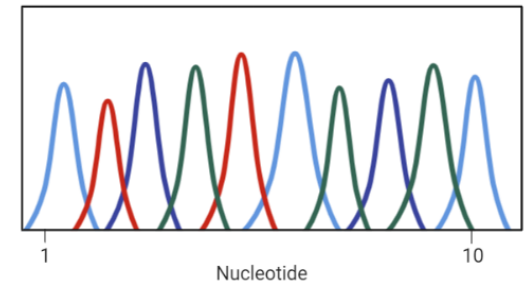
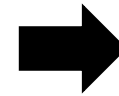
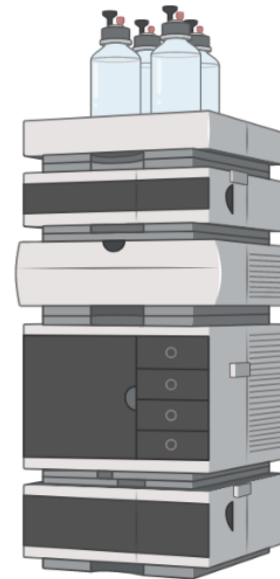
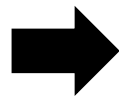
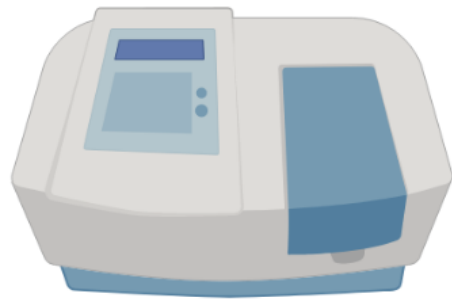
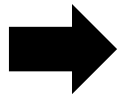
Cons

- Biodegradation may not occur in the environment
- Limited to culturable isolates
- Narrow scope of biodegradation

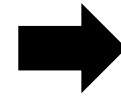
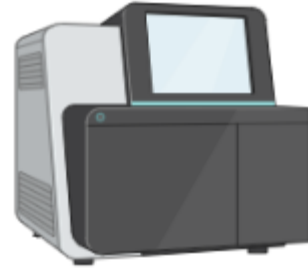
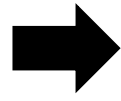
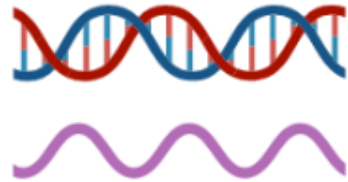
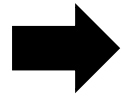
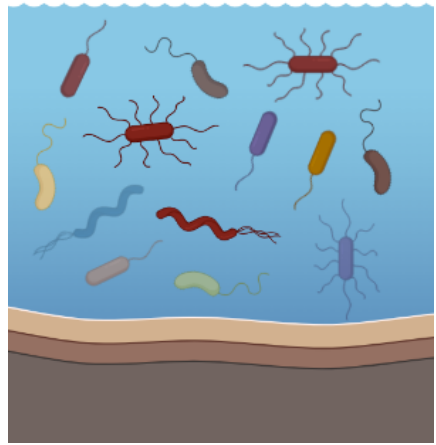
Paired Culture and Bioinformatic Approaches



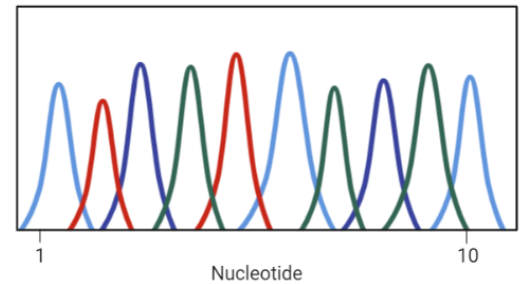
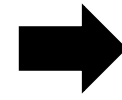
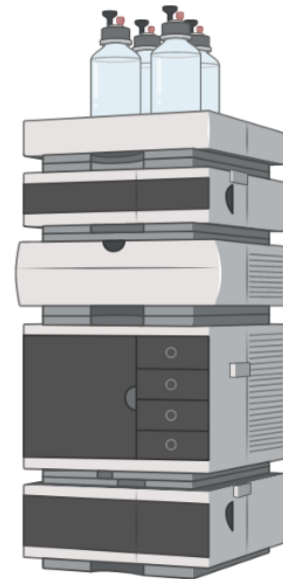
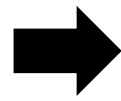
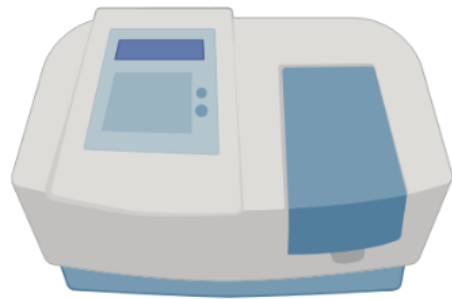
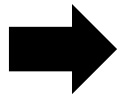
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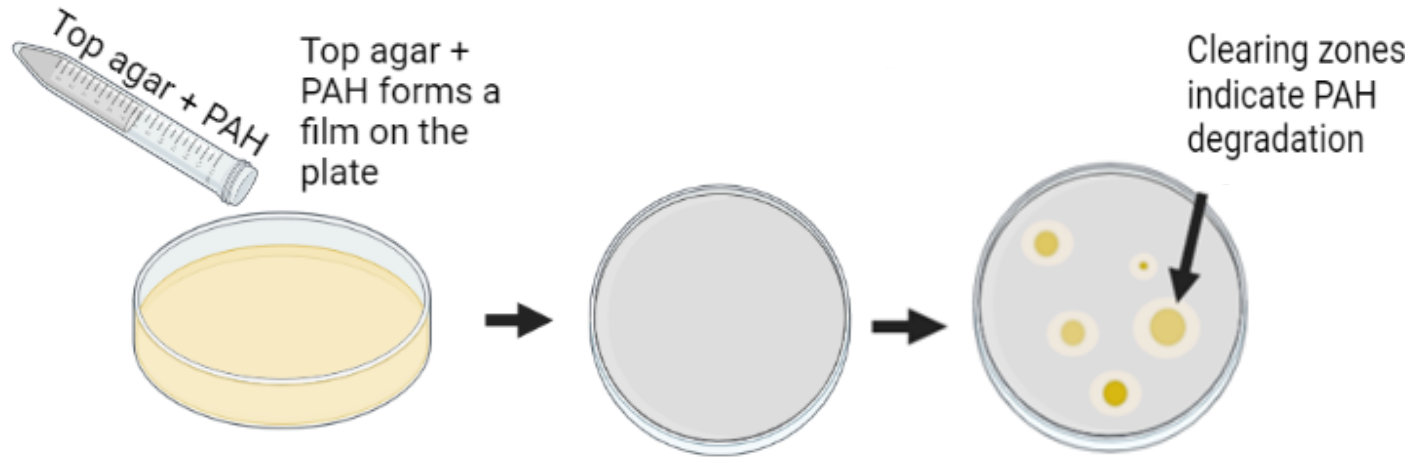
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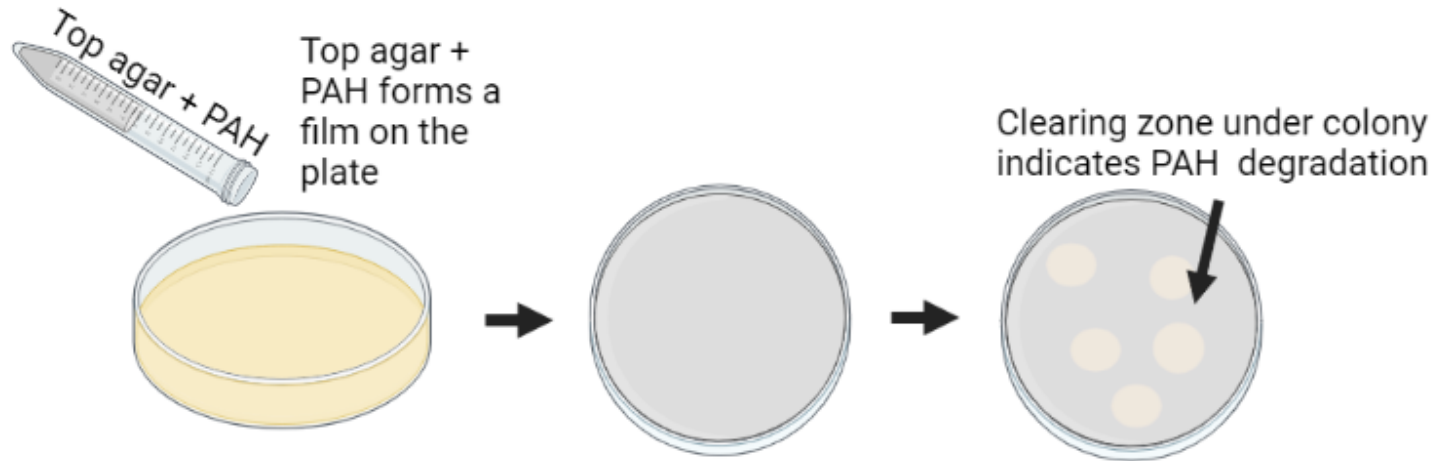
Research Objectives

- Investigating novel PAH degraders in marine environments
 - Culture collections – existing bacterial isolates
 - Natural seawater – newly isolated bacteria

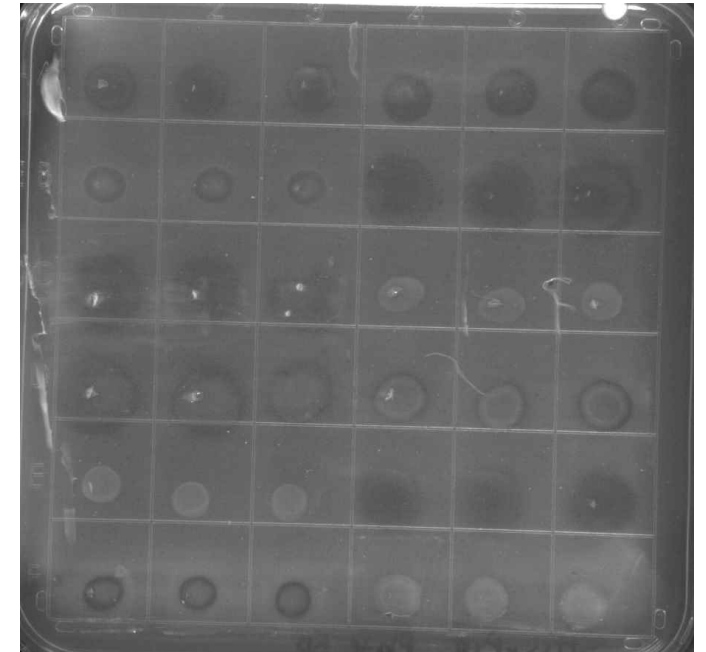
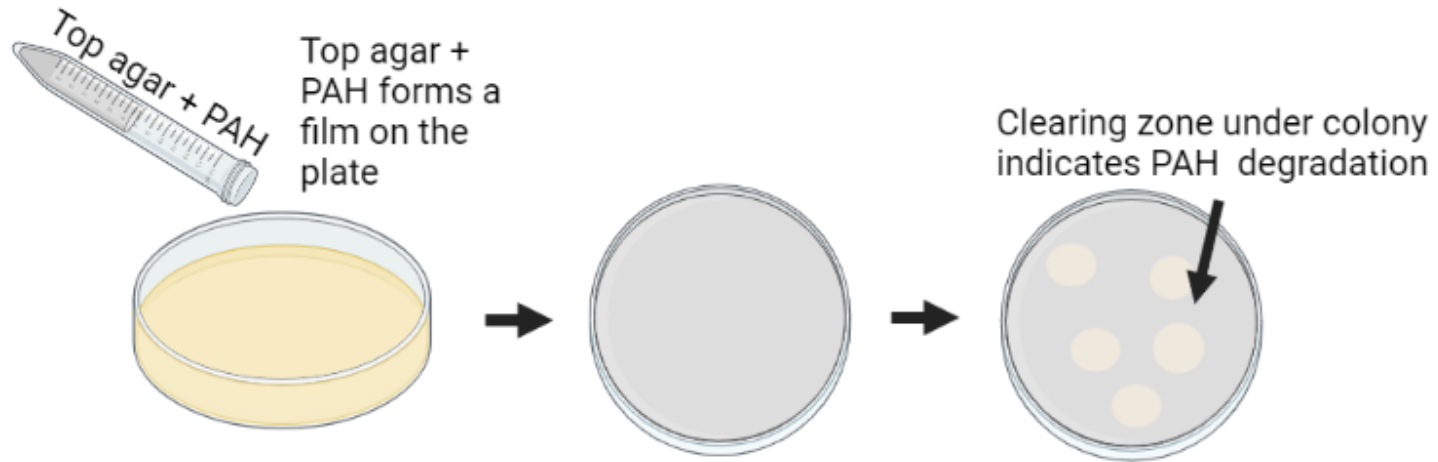
Screening Bacteria for PAH Degradation



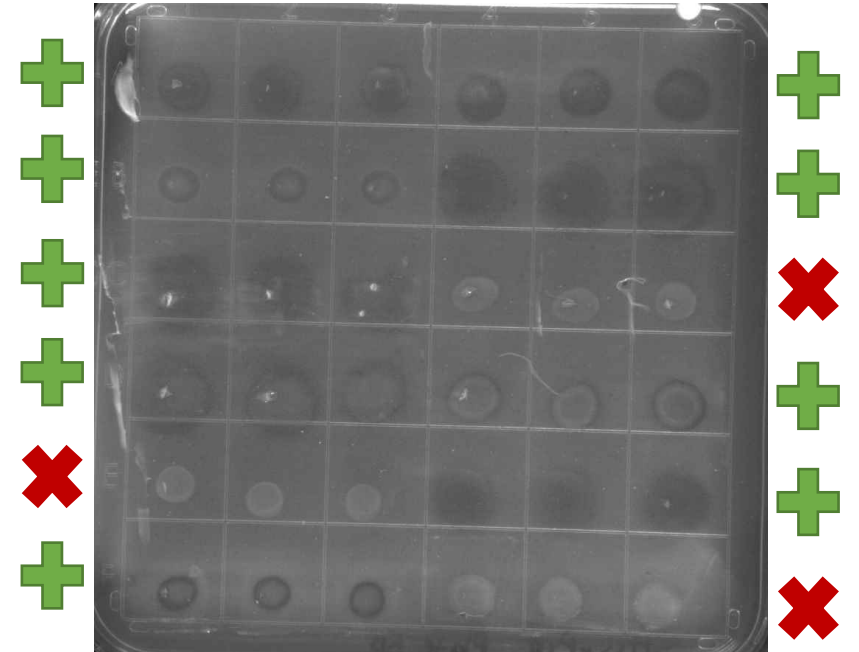
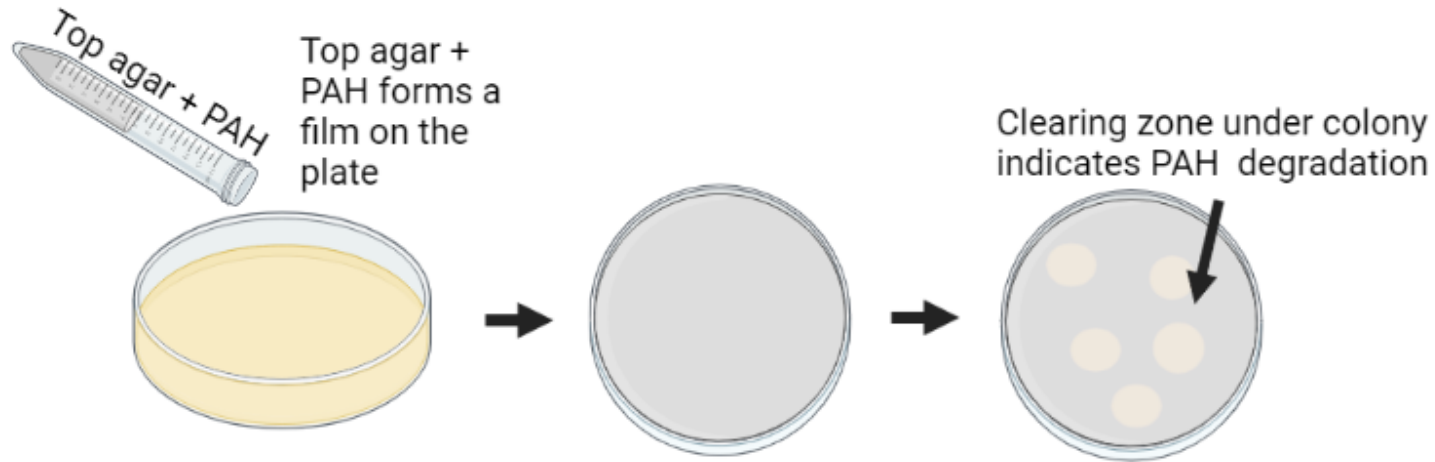
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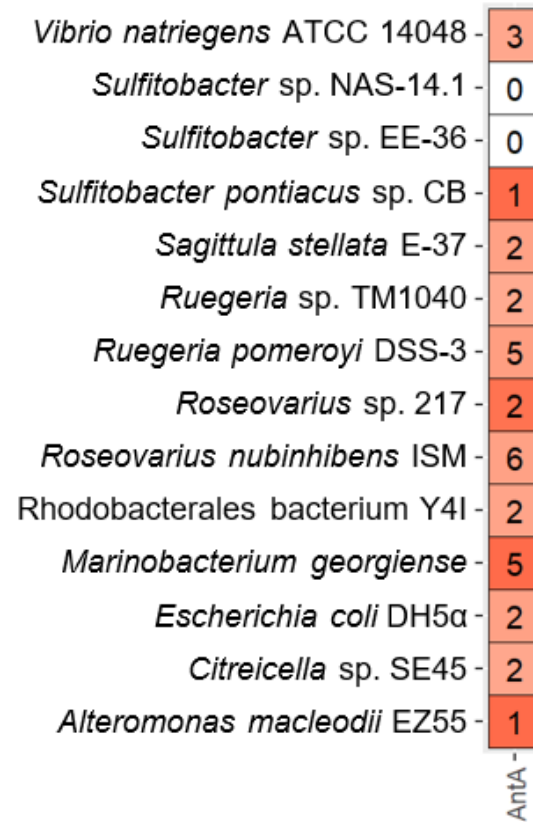


Culture Collection Strain	Taxonomic Phyla/Class	Pyrene Degradation	Phenanthrene Degradation
<i>Marinobacterium georgiense</i>	Gammaproteobacteria	-	-
<i>Bacillus-Clostridium</i> strain SE165	Firmicutes	+	+
<i>Bacillus-Clostridium</i> strain SE98	Firmicutes	+	+
<i>Alteromonas macleodii</i> strain EZ55	Gammaproteobacteria	+	+
<i>Vibrio natriegens</i> ATCC 14048	Gammaproteobacteria	+	+
<i>Rhodospirillaceae</i> strain EZ35	Alphaproteobacteria	+	+
<i>Flavobacteriaceae</i> strain EZ40	Bacteroidetes	+	+/-
<i>Alcanivorax</i> sp. strain EZ46	Gammaproteobacteria	-	-
<i>Ruegeria pomeroyi</i> DSS-3	Alphaproteobacteria	+	+
<i>Citreicella</i> sp. SE45	Alphaproteobacteria	+	+
<i>Sagittula stellata</i> E-37	Alphaproteobacteria	+	+
<i>Ruegeria</i> sp. TM1040	Alphaproteobacteria	+	+
<i>Sulfitobacter</i> sp. EE-36	Alphaproteobacteria	+	+
<i>Sulfitobacter</i> sp. NAS-14.1	Alphaproteobacteria	+	+
<i>Sulfitobacter</i> sp. CB-D	Alphaproteobacteria	+	+
<i>Roseovarius nubinhibens</i> ISM	Alphaproteobacteria	+	+/-
<i>Roseovarius</i> sp. 217	Alphaproteobacteria	+	+
Rhodobacterales strain Y4I	Alphaproteobacteria	+	+
<i>Escherichia coli</i> DH5 α	Gammaproteobacteria	-	-

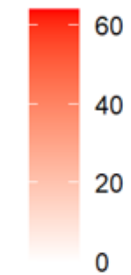
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<i>Bacillus-Clostridium</i> strain SE98	Firmicutes	+	+
<i>Alteromonas macleodii</i> strain EZ55	Gammaproteobacteria	+	+
<i>Vibrio natriegens</i> ATCC 14048	Gammaproteobacteria	+	+
<i>Rhodospirillaceae</i> strain EZ35	Alphaproteobacteria	+	+
<i>Flavobacteriaceae</i> strain EZ40	Bacteroidetes	+	+/-
<i>Alcanivorax</i> sp. strain EZ46	Gammaproteobacteria	-	-
<i>Ruegeria pomeroyi</i> DSS-3	Alphaproteobacteria	+	+
<i>Citreicella</i> sp. SE45	Alphaproteobacteria	+	+
<i>Sagittula stellata</i> E-37	Alphaproteobacteria	+	+
<i>Ruegeria</i> sp. TM1040	Alphaproteobacteria	+	+
<i>Sulfitobacter</i> sp. EE-36	Alphaproteobacteria	+	+
<i>Sulfitobacter</i> sp. NAS-14.1	Alphaproteobacteria	+	+
<i>Sulfitobacter</i> sp. CB-D	Alphaproteobacteria	+	+
<i>Roseovarius nubinhibens</i> ISM	Alphaproteobacteria	+	+/-
<i>Roseovarius</i> sp. 217	Alphaproteobacteria	+	+
Rhodobacterales strain Y4I	Alphaproteobacteria	+	+
<i>Escherichia coli</i> DH5 α	Gammaproteobacteria	-	-

Culture Collection Strain	Taxonomic Phyla/Class	Pyrene Degradation	Phenanthrene Degradation
<i>Marinobacterium georgiense</i>	Gammaproteobacteria	-	-
<i>Bacillus-Clostridium</i> strain SE165	Firmicutes	+	+
<i>Bacillus-Clostridium</i> strain SE98	Firmicutes	+	+
<i>Alteromonas macleodii</i> strain EZ55	Gammaproteobacteria	+	+
<i>Vibrio natriegens</i> ATCC 14048	Gammaproteobacteria	+	+
<i>Rhodospirillaceae</i> strain EZ35	Alphaproteobacteria	+	+
<i>Flavobacteriaceae</i> strain EZ40	Bacteroidetes	+	+/-
<i>Alcanivorax</i> sp. strain EZ46	Gammaproteobacteria	-	-
 <i>Ruegeria pomeroyi</i> DSS-3	Alphaproteobacteria	+	+
<i>Citricella</i> sp. SE45	Alphaproteobacteria	+	+
<i>Sagittula stellata</i> E-37	Alphaproteobacteria	+	+
<i>Ruegeria</i> sp. TM1040	Alphaproteobacteria	+	+
<i>Sulfitobacter</i> sp. EE-36	Alphaproteobacteria	+	+
<i>Sulfitobacter</i> sp. NAS-14.1	Alphaproteobacteria	+	+
<i>Sulfitobacter</i> sp. CB-D	Alphaproteobacteria	+	+
<i>Roseovarius nubinhibens</i> ISM	Alphaproteobacteria	+	+/-
<i>Roseovarius</i> sp. 217	Alphaproteobacteria	+	+
Rhodobacterales strain Y4I	Alphaproteobacteria	+	+
<i>Escherichia coli</i> DH5α	Gammaproteobacteria	-	-

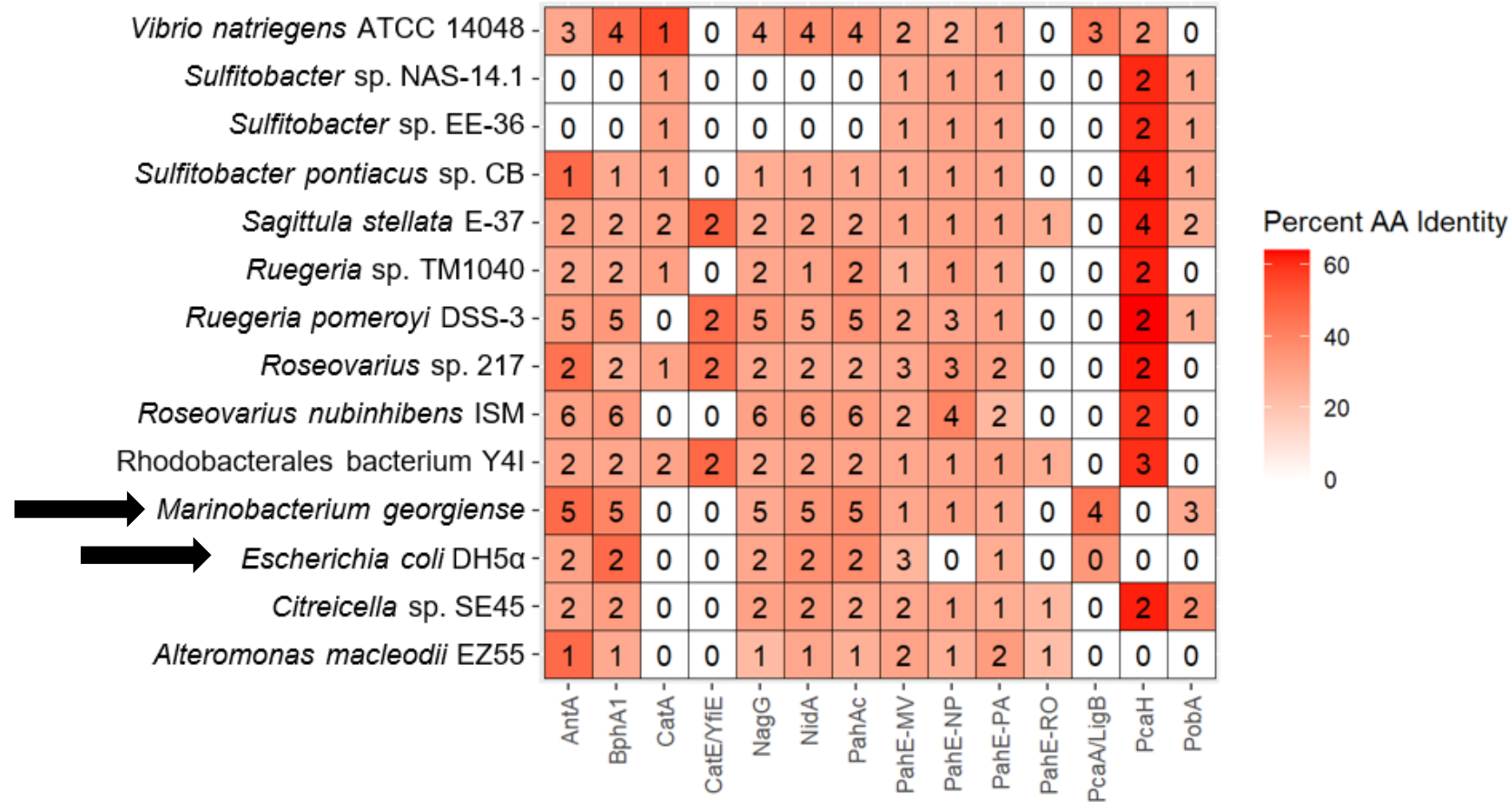
PAH Biodegradation Biomarkers



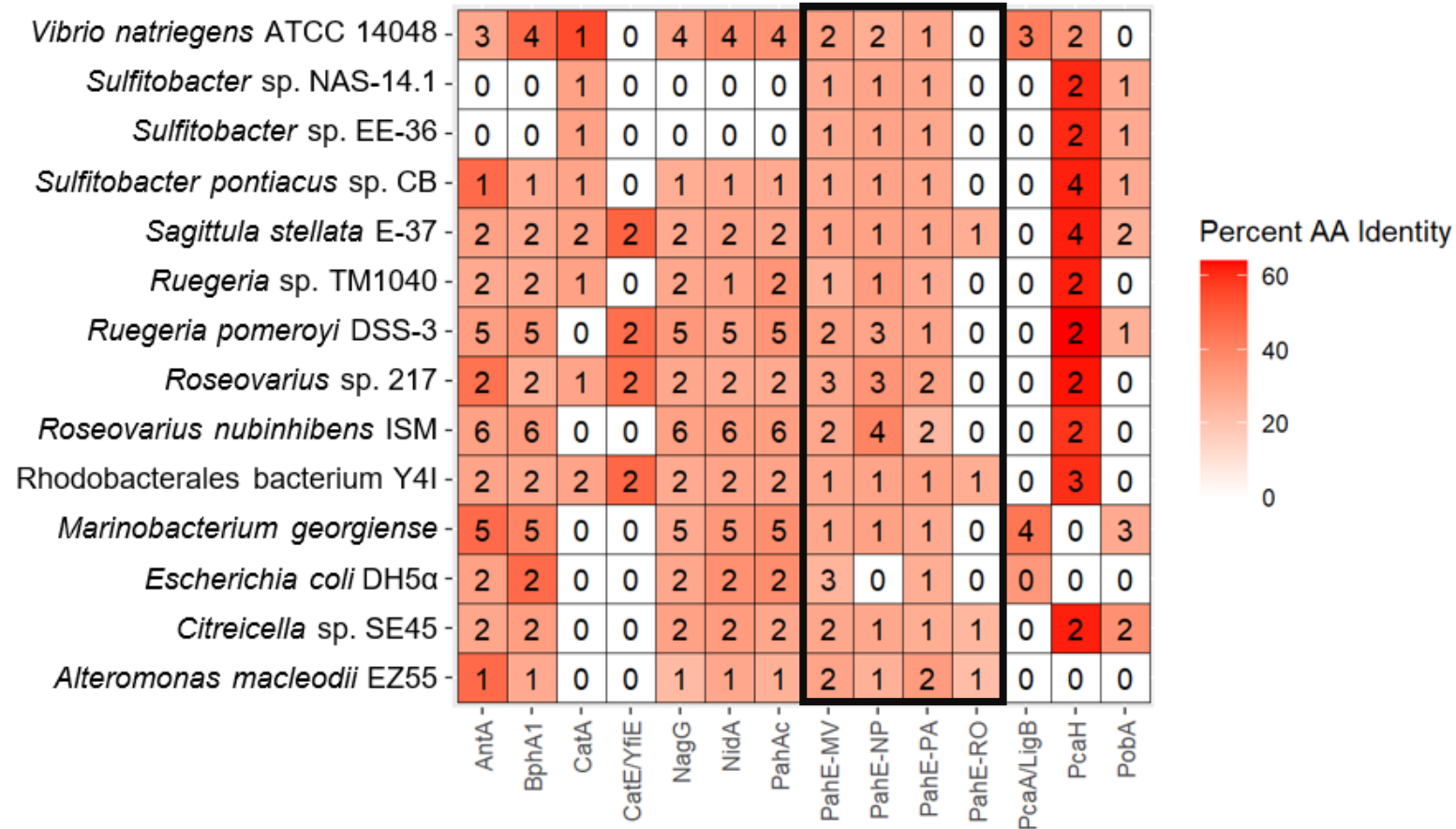
Percent AA Identity



PAH Biodegradation Biomarkers

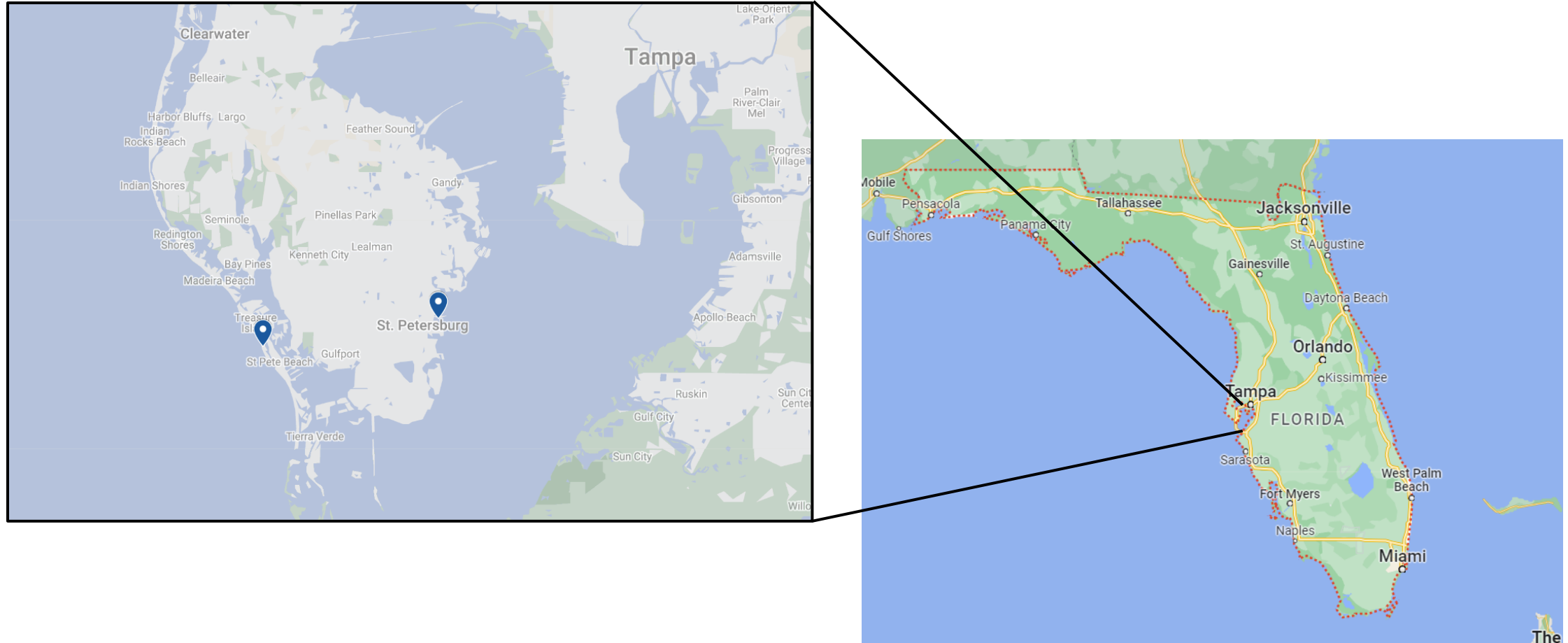


PAH Biodegradation Biomarkers



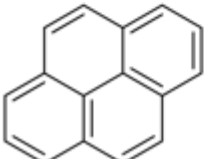
Alphaproteobacteria may play a greater role in PAH bioremediation than previously thought.

Natural Seawater Enrichment Cultures

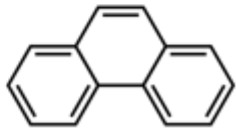


Natural Seawater Enrichment Cultures

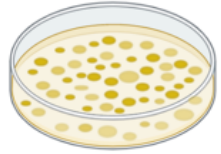
Complex Medium



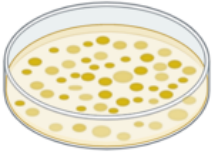
Pyrene



Phenanthrene

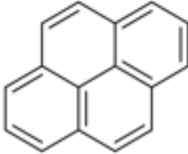


Freezer stocks Isolated Colonies

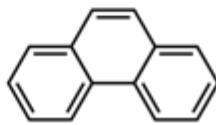


Freezer stocks Isolated Colonies

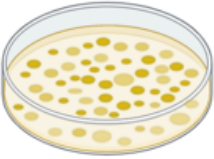
Minimal Medium



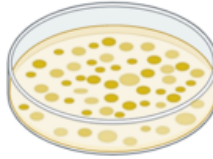
Pyrene



Phenanthrene



Freezer stocks Isolated Colonies

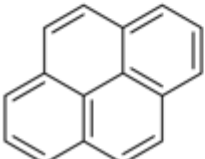


Freezer stocks Isolated Colonies

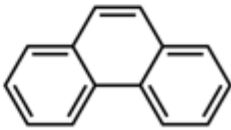
Natural Seawater Enrichment Cultures

Complex Medium

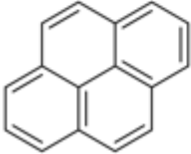
Minimal Medium



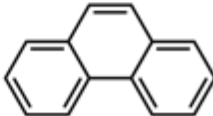
Pyrene



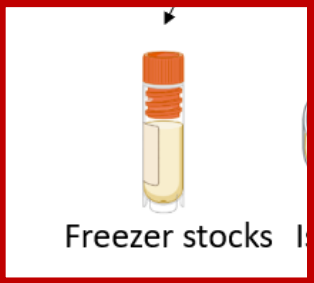
Phenanthrene



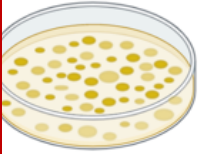
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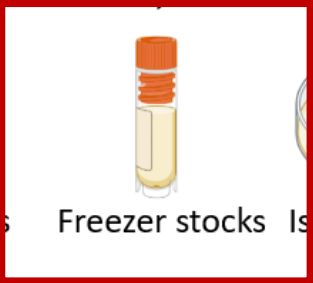
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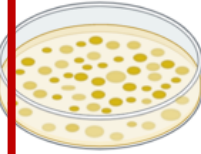
Freezer stocks



Isolated Colonies



Freezer stocks



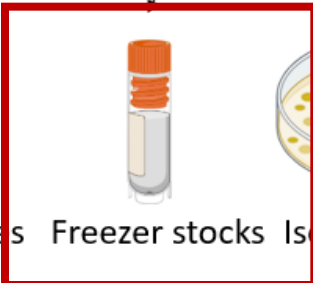
Isolated Colonies



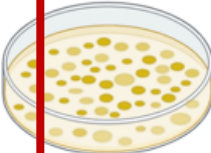
Freezer stocks



Isolated Colonies



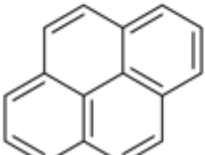
Freezer stocks



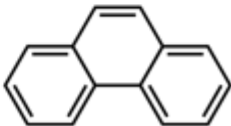
Isolated Colonies

Natural Seawater Enrichment Cultures

Complex Medium



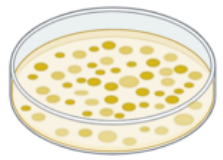
Pyrene



Phenanthrene



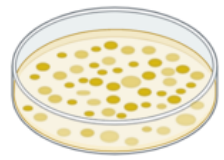
Freezer stocks



Isolated Colonies

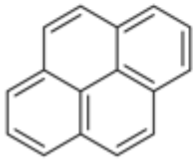


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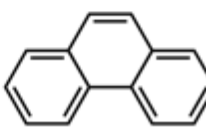


Isolated Colonies

Minimal Medium



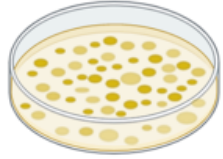
Pyrene



Phenanthrene



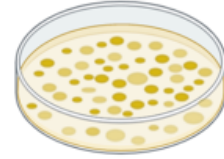
Freezer stocks



Isolated Colonies



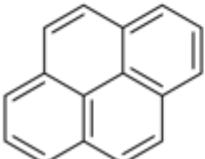
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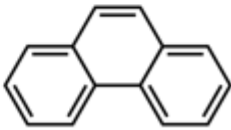
Isolated Colonies

Natural Seawater Enrichment Cultures

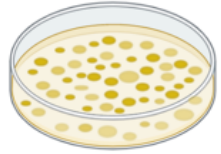
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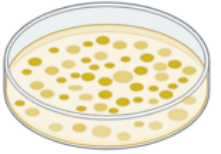
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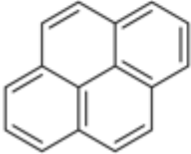


Freezer stocks Isolated Colonies

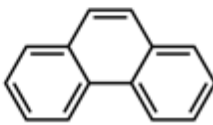


Freezer stocks Isolated Colonies

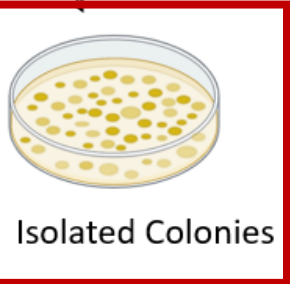
Minimal Medium



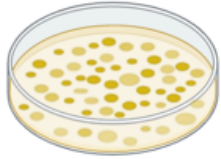
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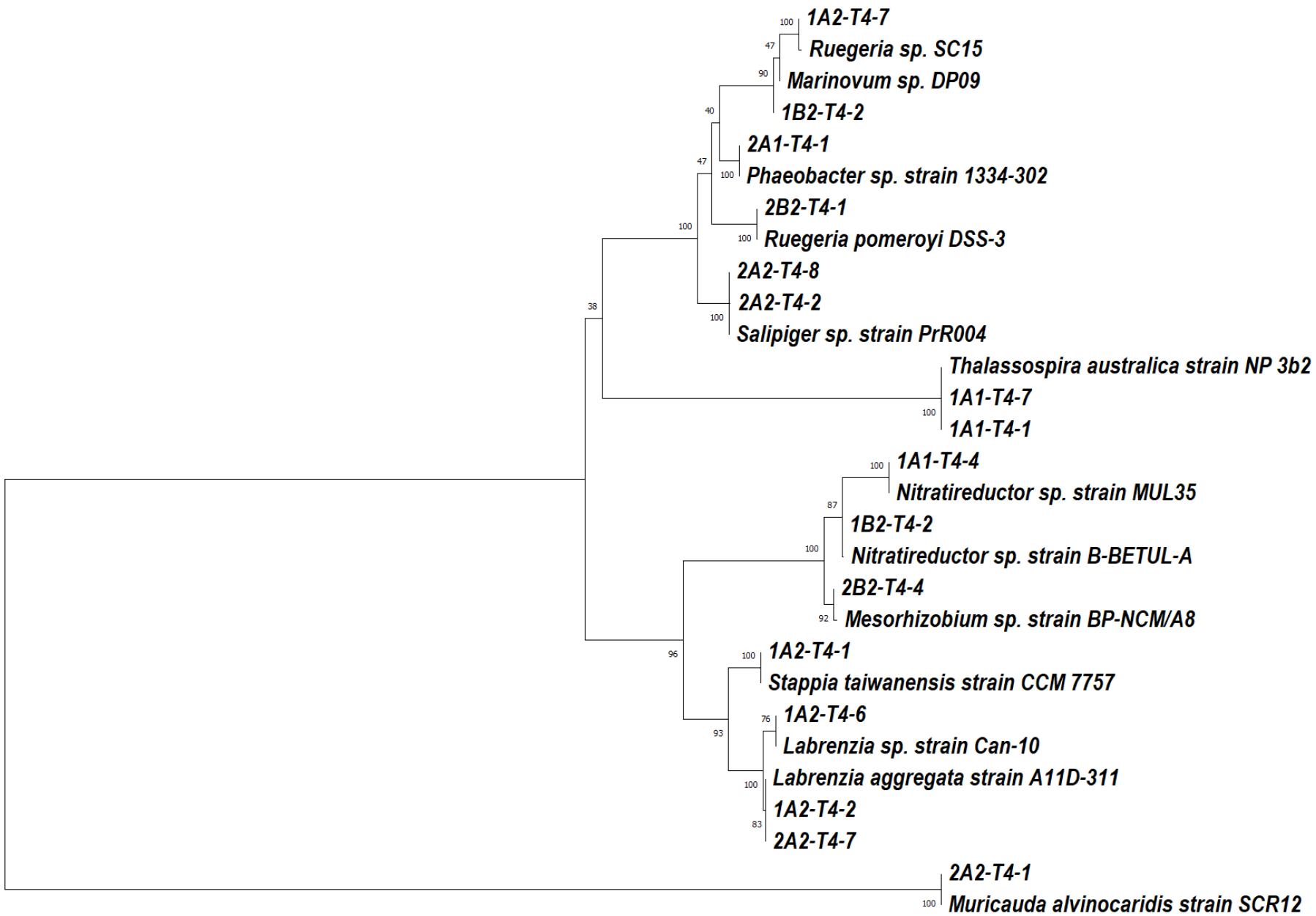
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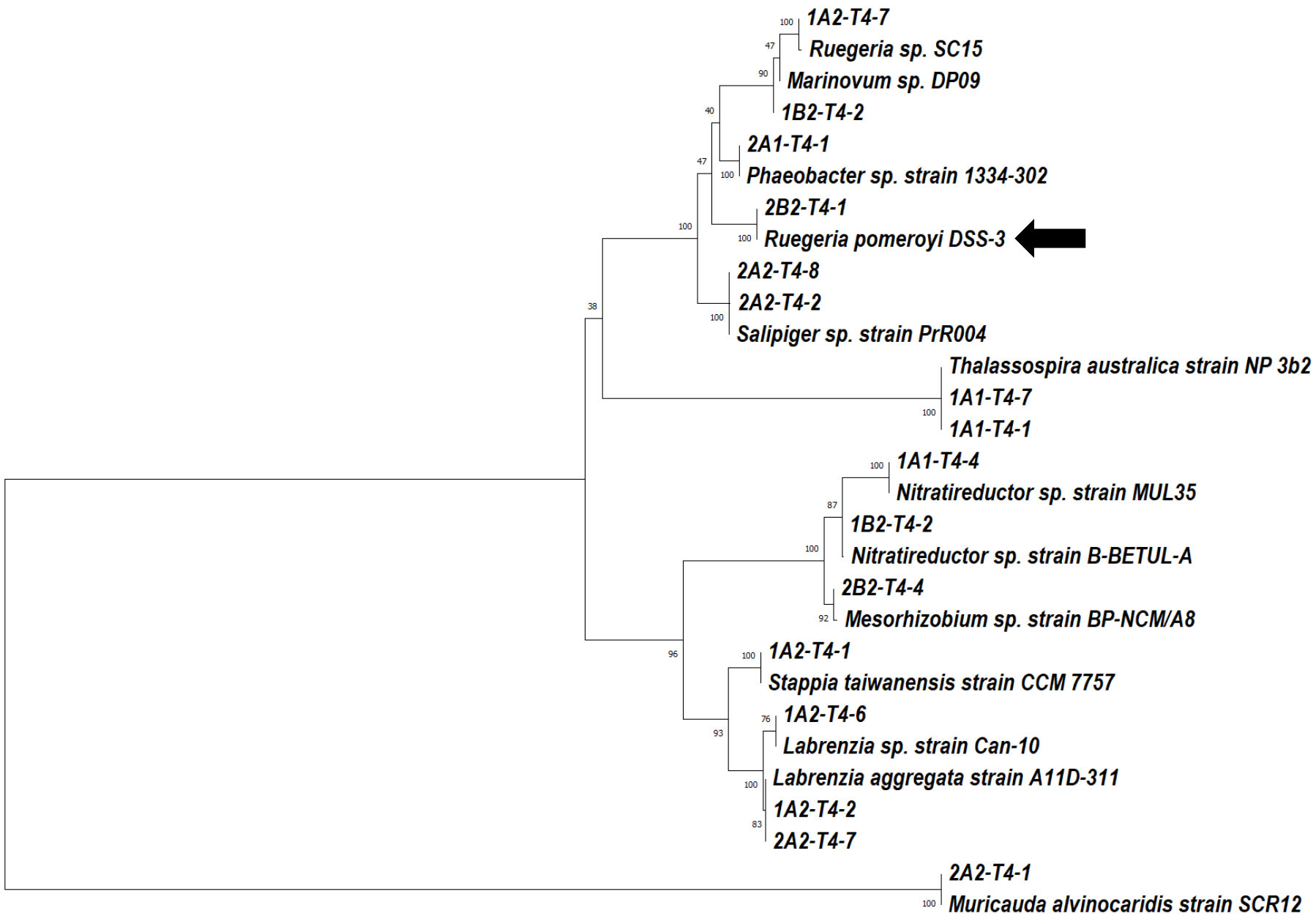
Freezer stocks Isolated Colonies



Freezer stocks Isolated Colonies



0.20



0.20

Conclusion

- Alphaproteobacteria may play a larger role in PAH bioremediation than previously thought
 - Novel pathways – genetic biomarkers can't identify
 - Co-metabolism – need another carbon source present for degradation to occur
- Culture-based methods allow us to expand known genetic biomarkers and connect individual bacteria to degradation potential

Acknowledgements

- Buchan Lab
 - Dr. Alison Buchan
 - Frank May
 - Cameron Jackson
 - Taylor Smith
 - **Ellen Bobo**
 - Hannah Edwards
 - Anna-Stuart Britt
 - Dr. Mustafa Guzel
 - **Dr. Zachary Burcham**



Questions?



@JillianLWalton



[linkedin.com/in/jillianlwalton](https://www.linkedin.com/in/jillianlwalton)