Characterization of Microbial Metaproteomes Following Exposure to Naphthalene

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Introduction

- Polycyclic Aromatic Hydrocarbons (PAHs) are hazardous byproducts of anthropogenic activities, mainly the incomplete combustion of fossil fuels[1].
- High levels of PAHs are carcinogenic, mutagenic, and teratogenic to humans and animals. These high levels are emerging more frequently in both rural and urban environments[2].
- PAHs are comprised of at least two fused aromatic rings and double bonds placed throughout the structures. This makes them difficult to degrade[3].
- Research shows that bacteria *Pseudomonas aeruginosa* degrades PAHs in low concentrations[4].
- Naphthalene is one of the simplest PAHs, comprised of two aromatic rings. Because it is one of the most volatile and least toxic PAH, scientists often use it to model PAH degradation[4].
- MALDI-TOF mass spectrometry characterizes bacterial growth phases by detecting proteomic shifts[5].
- We investigated the metaproteomes of *Escherichia coli* and *P. fluorescens* in the presence of naphthalene during different stages of growth.

Materials and Methods

- Researchers inoculated flasks containing 100 mL of media with 1 mL inoculum of *P. fluorescens* and *E. coli* with optical density (OD) at 1.0 and 0.883 respectively.
- Researchers added naphthalene to bacterial cultures in early log, mid-to-late log, and stationary phases followed by measuring OD. Bacterial pellets with OD 1.0 were collected following naphthalene exposure.
- BioNumerics v7.1 was used to analyze the spectra using multi-dimensional and jackknife analysis.

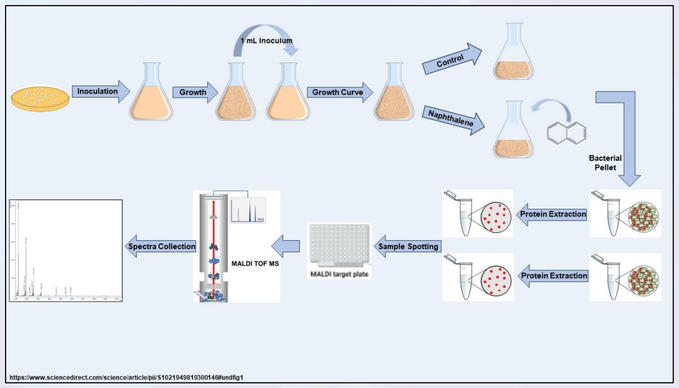

Figure 1. Experimental workflow for naphthalene exposure

Results

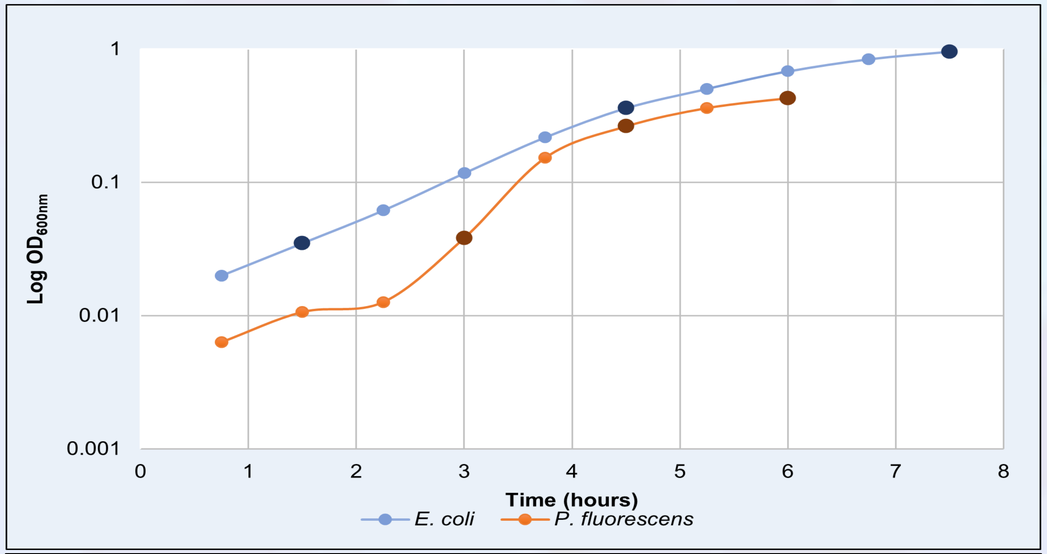

Figure 1. Growth curve of *P. fluorescens and E. coli.* Highlighted data points indicate time points where bacteria cultures were exposed to naphthalene

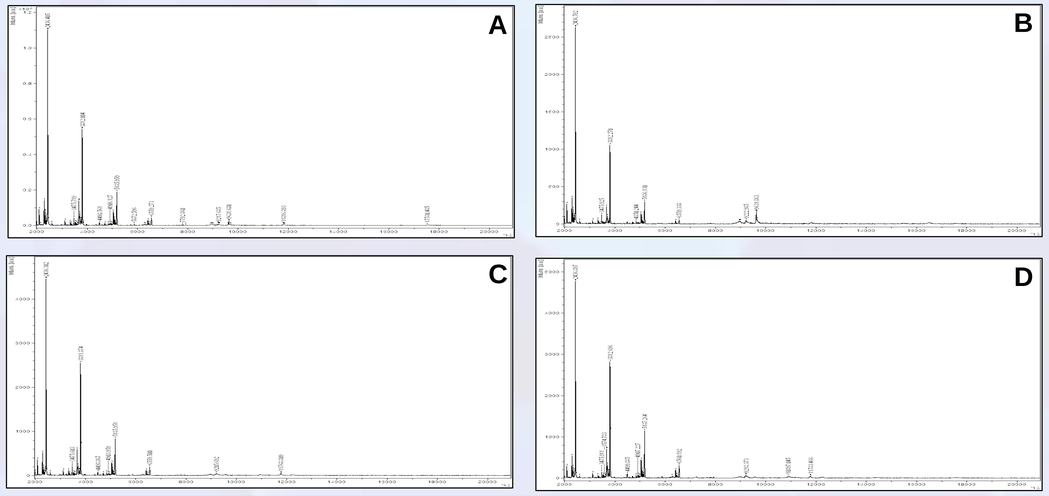

Figure 2. Replicate mass spectra of *P. fluorescens* control (A), *P. fluorescens* naphthalene (B), *E. coli* control (C) and *E. coli* naphthalene (D)

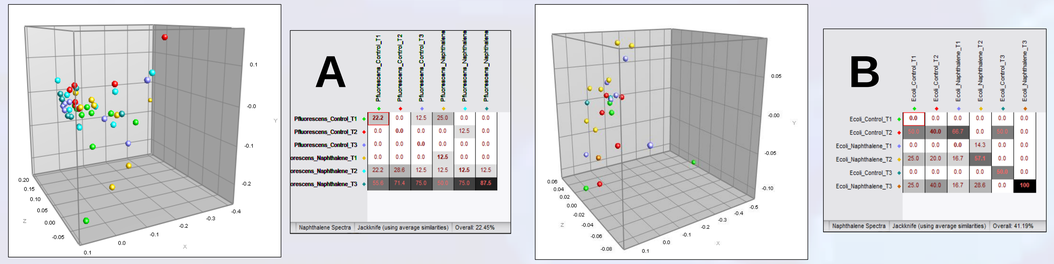

Figure 3. Multi-dimensional and jackknife analysis of *P. fluorescens* (A) and *E.coli* (B)

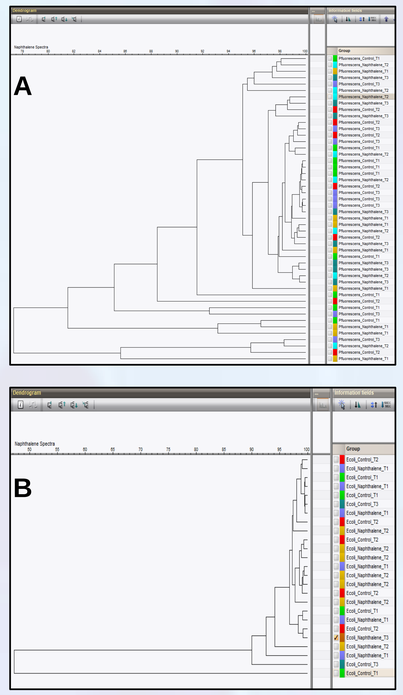

Figure 4. Dendrograms of *P. fluorescens* (A) and *E. coli* (B). Dendrograms were constructed with replicates totaling 1000 shots while excluding insufficient samples on MALDI target plate. Analysis was run using curve-based Pearson correlation following baseline subtraction, curve-smoothing, and signal-noise ratio thresholds set to 2. Data point colors correspond to experimental group.

Conclusions and Future Research

- Multi-dimensional and jackknife analysis revealed there is no difference between metaproteomes of the naphthalene and control groups of both species.
- The analysis also revealed that comparison of control *E. coli* and *P. fluorescens* showed no species differentiation. Since MALDI-TOF is commonly used to distinguish species, this suggests experimental error [6].
- Experimental results suggest that additional replicate testing and continued experimentation are necessary for further analysis.
  - Refinement of naphthalene exposure protocol may enable detection of metaproteomic shifts due to PAH exposure.

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