Impact of Hydrogen Peroxide on Horizontal Transfer of Naphthalene-Degrading Genes

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Background/Objectives. Bioremediation can be a slow process when the necessary bacteria cannot grow or compete at a contaminated site. The genes required to degrade contaminants are often contained within a narrow subset of bacteria, which may be present at sites in low numbers. One approach to address this limitation is to promote horizontal transfer of genes related to bioremediation to alternative species and genera. This would increase the number of cells capable of bioremediation, which would allow bacteria more common to the site to degrade contaminants of concern. However, horizontal gene transfer (HGT) occurs at low frequency under environmental conditions, limiting its role in site cleanup. Previous studies suggest that reactive oxygen species increase the transfer rates of plasmid-based antimicrobial resistance genes, but it is unknown whether reactive oxygen can promote the transfer of plasmid-based catabolic genes related to contaminant degradation.

The goal of this study is to determine whether low concentrations of hydrogen peroxide, a form of reactive oxygen, can increase the transfer frequency of genes associated with biodegradation. NAH7 (which encodes for the aerobic degradation of naphthalene) has been selected as a model catabolic plasmid. This plasmid occurs naturally in *Pseudomonas putida* but is uncommon outside of the genera *Pseudomonae*. The specific aims of this work are to: i) demonstrate horizontal transfer of NAH7 from *P. putida to Escherichia coli* (a model, non-*Pseudomonae* cell line); ii) investigate the impact of varying hydrogen peroxide concentrations upon HGT; and iii) relate the changes in horizontal transfer to the degradation of naphthalene.

Approach/Activities. Experiments using *Pseudomonas putida* strain *G7* (which possesses the NAH7 plasmid) and *Escherichia coli* strain *S17-1* are currently underway. Pure cultures of *G7* and *S17-1* are maintained in minimal media containing only one substrate. Optical density and DNA extractions will determine cellular concentrations before mating experiments. Experiments will be conducted in phosphate buffered solutions containing naphthalene and hydrogen peroxide, which will provide selective pressures for horizontal transfer of NAH7. Copies of NAH7 plasmids will be quantified using qPCR after mating and will be compared to the values prior to mating. To confirm transfer to *E. coli*, cells will be plated on selective media to determine the total copies of NAH7 and the number of *E. coli* cells that grow on naphthalene. After mating experiments, cells will be amended into media containing naphthalene as the only substrate. Concentrations of naphthalene over time will be monitored and compared for varying hydrogen peroxide concentrations.

Results/Lessons Learned. The theoretical framework supporting these experiments and preliminary findings from ongoing experiments will be presented. Specifically, data demonstrating horizontal transfer of NAH7 and the influence of hydrogen peroxide will be discussed. At this time, we hypothesize that metabolic plasmids will react similarly to antibiotic resistant plasmids to environmental stressors such as hydrogen peroxide. Forthcoming results will determine the extent to which NAH7 is transferred and the increased rate of contaminant degradation due to recipient cells. This work is the first step towards promoting HGT as a bioremediation strategy, which may result in decreased clean up times and more efficient bioremediation of contaminated sites.