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Genetic Analysis of Stress Responses in Soil Bacteria for Enhanced Bioremediation of Mixed Contaminants

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Research Objective

To realize the full potential of bioremediation, the individual bacterial responses to the stresses (lack of nutrients or oxygen; mixed pollutants) encountered at contaminated sites must be understood. This information can then be extrapolated to field applications using indigenous bacteria or genetically engineered micro-organisms. Studying bacterial response to stresses presents an opportunity for improving bioremediation strategies, both with indigenous populations and genetically engineered microbes, and should contribute to environmental management and restoration goals.

Enhancing in-situ removal of hazardous wastes by stimulating the growth of indigenous bacteria with nutrients has been demonstrated. But how much and how often to apply these supplements has been difficult to determine, and controlled and reproducible degradation of pollutants in the environment has not yet been achieved.

Research Progress and Implications

As of May 31st 1998, this report summarizes work after 17 months of a 36 month project.

The starvation responses of Sphingomonas aromaticivorans F199 were analyzed. Using S. aromaticivorans as a model, the effects of adding nutrient to starved cells were investigated in terms of protein and gene expression. Cells were starved in reconstituted well water without carbon and nitrogen sources for nine months. Nutrient (TGY medium) was added to the starved cell, and proteins were extracted from the cell culture after 4, 8, and 24 hours. Only a few major proteins were present and few changes were detectable four hours after the nutrient was added, but several prominent proteins appeared after eight hours, suggesting that there is a lag time before the starved cells respond to the nutrient. These identified proteins may be useful for monitoring the bioremediation process during biostimulation with nutrient supplements.

Twenty-four hours after nutrient was added, the size of rRNA molecules of recovery cells had increased. The rRNA may have undergone fragmentation under starvation conditions. Efforts are focused on determining the sequences of the rRNA from starved and recovery cells. Comparing these sequences may enable scientists to develop oligonucleotide probes to monitor the number of recovery cells that are metabolically active during bioremediation.

Few genes on the catabolic plasmid pNL1, which encodes genes for the degradation of compounds such as naphthalene, biphenyl and xylene, are still active in the starved cells. These genes responded more robustly to the nutrient. Apparently, most of the catabolic genes were not activated by the nutrient, suggesting that, although the nutrient supplement stimulates the growth of the bacteria, it does not necessarily stimulate the biodegradative activities of the bacteria.

The stress responses of Deinococcus radiodurans R1 were analyzed. RNA was harvested from cells grown in starvation media. RAP-PCR was performed, and 12 tentative differentially expressed genes were identified. Sequence analysis revealed that one of the identified genes has a significant sequence identity with transposase. Experiments are in progress to confirm stress inducibility of that gene. The promoter of that gene may be useful in engineering D. radiodurans for the degradation of TCE under starvation or environmental stress conditions.

Genetic engineering methods are being developed for S. aromaticivorans F199, which has become tetracycline-resistant for better performance in bioremediation. It was successfully transformed using the electroporation method. Using this tetracycline-resistant strain, transposon elements were delivered into the chromosome via conjugation.

A modification of duplication insertion has been developed to create insertional mutations in the D. radiodurans genome, called duplication inactivation. Several approaches have been pursued to identify appropriate promoters for expression of the tbu gene in D. radiodurans. The first gene identified, sigma factor, encodes an important regulatory protein. RNA polymerase, the enzyme complex responsible for transcription, is composed of multiple subunits, including the sigma factor. Experiments have been initiated to determine the expression pattern of the other four putative stress factors. Promoters from these genes will be used to express toluene 3-monooxygenase enzyme complex in D. radiodurans for degradation of TCE under starvation or environmental stress conditions.

Investigations have also been focused on the katA gene, which encodes the major catalase enzyme. We investigated whether the katA promoter is good for the expression of Tbu under nutrient-limiting conditions. The role of katA in radiation and peroxide resistance was investigated. The findings suggest that the major catalase is involved in the extreme hydrogen peroxide resistance of D. radiodurans and to a lesser extent is involved in ionizing radiation resistance. Thus, expressing heterologous genes useful for bioremediation with katA promoter might be ideal for bioremediation at the mixed-contaminant site with radionuclides.

Planned Activities

1. Determine the complete sequences of rRNA of starved cells and recovery cells to develop oligonucleotide probes for monitoring the physiological state of soil bacteria.
2. Study stress responses to trichloroethylene, a major contaminant at DOE sites.
3. Because over-expressing robA or pspA genes in Escherichia coli can enhance its resistance to metal and organic solvent, we will attempt to over-express these genes in both S. aromaticivorans and D. radiodurans.
4. Further analyze all Deinococcus strains expressing Tbu, including Northern analysis, timing of expression, and analysis of these strains' degradative ability for trichloroethylene and toluene in pure culture studies.