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Project Title: **Genetic Engineering of a Radiation-Resistant Bacterium for Biodegradation of Mixed Wastes**

Lead Principal Investigator:

Dr. Mary E. Lidstrom
Professor
Department of Chemical Engineering
University of Washington
Box 351750
Seattle, Washington 98195
Telephone: 206-543-8388
e-mail: lidstrom@u.washington.edu

Genetic Engineering of a Radiation-Resistant Bacterium for Biodegradation of Mixed Wastes

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Lead Principal Investigator: Prof. Mary E. Lidstrom
University of Washington
Departments of Chemical Engineering and Microbiology
Box 351750
Seattle WA 98195-1750
(206)616-5282
lidstrom@u.washington.edu

Co-Investigators: Heather Rothfuss (graduate student)
Lindy Gewin (graduate student)
Amy Schmid (graduate student)
Rob Meima (post-doc)

Research Objective: The mixture of toxic chemicals, heavy metals, halogenated solvents and radionuclides in many DOE waste materials presents a challenging problem for separating the different species and disposing of individual contaminants. One approach for dealing with mixed wastes is to genetically engineer the radiation-resistant bacterium, *Deinococcus radiodurans* to survive in and detoxify DOE's mixed waste streams, and to develop process parameters for treating mixed wastes with such constructed strains. The goal for this project is to develop a suite of genetic tools for *Deinococcus radiodurans* and to use these tools to construct and test stable strains for detoxification of haloorganics in mixed wastes.

Research Progress and Implications:

This report summarizes work after 1-1/2 years of a 3-year project, during which we have developed a suite of genetic tools for *D. radiodurans* and demonstrated their use in analyzing *D. radiodurans* promoters..

1. Develop Genetic Tools

Three types of genetic tools have been developed, integration vectors, replicating vectors, and enhanced transformation systems. All of these systems are needed to broaden the range of genetic capabilities for manipulating *D. radiodurans* strains and to increase the convenience of working with these strains.

A. Integration vectors

A series of vectors have been developed that target integration of expressed genes to non-essential sites in the chromosome via double crossover recombination. Such inserted genes are stable in the absence of selection, a prerequisite for process strains. The sites that have been chosen and found to be successful are genes encoding amylase and pullulanase. The vectors include derivatives for cloning and expression and for analysis of promoter activity using the two reporters, catechol dioxygenase (XylE) and beta-galactosidase (LacZ).

B. pI3-based replicating vectors

A second series of replicating vectors have been developed based on the previously reported *D. radiodurans* plasmid, pI3. The plasmid has been completely sequenced and potential replication functions identified by deletion analysis. A minimal replicon has been cloned and used to generate a suite of small and convenient shuttle vectors, including vectors for general cloning, expression, and promoter analysis with the same two reporters, catechol dioxygenase (XylE) and beta-galactosidase (LacZ).

C. Enhanced transformation systems

Although *D. radiodurans* is naturally transformable at high frequencies with its own DNA, the transformation frequencies for DNA passed through *E. coli* are low. For convenience of routine genetic manipulations, we have optimized transformation of *D. radiodurans* with DNA passed through *E. coli*, by using *E. coli* strains defective in DNA methylases and by changing the transformation protocol to optimize it for this system. Transformation frequencies with this system are increased orders of magnitude over standard protocols.

2. Clone and Characterize Promoters

In order to construct process strains for biodegradation, we need a suite of expression systems, preferably regulated and capable of being modulated at different promoter strengths. Since virtually nothing is known about promoters in this strain, we have cloned and characterized a variety of promoters from *D. radiodurans*.

Two different approaches were used to isolate *D. radiodurans* promoters. First, random *D. radiodurans* clone banks were generated in our new promoter screening vector and tested for activity in *E. coli*. Those showing activity were then tested in *D. radiodurans*. This screen resulted in several fragments with promoter activity in both strains. Second, genes were chosen from the genome sequence that were expected to contain strong promoters, and upstream regions were cloned into the promoter screening vector and tested in both *D. radiodurans* and *E. coli*. A

number of promoters showing activity in *D. radiodurans* were isolated in this way, and a subset also showed activity in *E. coli*.

We are in the process of mapping transcriptional start sites for several of these promoters, and our preliminary data suggest a tentative consensus promoter sequence. Once we have mapped a larger number of these start sites, it will be possible to develop a more robust consensus sequence.

A subset of these promoters have been used to develop expression systems for both types of vectors noted above.

Planned Activities:

1. Finish promoter characterization and determine dynamic range of promoter activity in chromosomal and free-replicating constructions.
2. Use expression vectors to construct a suite of strains with biodegradative capabilities and test the stability and expression properties of such strains.
3. Prepare a manuscript describing the analysis of pI3 and the development of pI3-based vectors.
4. Prepare a manuscript describing the promoter analysis.

Information Access:

R. Meima, H. Rothfuss, L. Gewin, and M.E. Lidstrom, "Genetic Engineering of a Radiation-Resistant Bacterium for Biodegradation of Mixed Wastes" Poster presentation at the DOE Environmental Management Science Program Workshop, Chicago, Ill., July 27-30, 1998.