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Genetic Engineering of a Radiation-Resistant Bacterium for Biodegradation of Mixed Wastes
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DOE Problems: above-ground bioremediation treatment systems to remove halogenated organics from mixed waste

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 3-1/2 years of a 6-year project, during which we have developed a suite of genetic tools for *D. radiodurans*, analyzed *D. radiodurans* promoters, and developed stable expression systems for broad host-range oxygenases.

1. Develop Genetic Tools

Two types of genetic tools have been developed, integration vectors and replicating vectors. These systems were needed to broaden the range of genetic capabilities for manipulating *D. radiodurans* strains, to increase the convenience of working with these strains, and to provide tools to generate constructions that are stable in the absence of selective pressure, a prerequisite for generating process 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. We have shown that such inserted genes are stable in the absence of selection. 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 diooxygenase (XylE) and beta-galactosidase (LacZ). The vectors have also been tested with green fluorescent protein.

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 LacZ as a reporter. These vectors are useful cloning and screening tools, but are not stable in the absence of antibiotic selection and will not be used for strain construction. A manuscript describing this work has been published.

2. Clone and Characterize Promoters

In order to optimize the construction of process strains for biodegradation, a suite of expression systems are needed, 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 and the pI3 plasmid 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 have mapped transcriptional start sites for several of these promoters and have developed a consensus promoter sequence. Surprisingly, this sequence is similar to the standard *E. coli* σ^{70} promoter sequence, with modifications to the –10 region. A manuscript describing this work has been published.

3. Develop Stable Expression Constructs

A subset of these promoters has been used to develop a suite of expression systems for both types of vectors noted above. A series of broad spectrum oxygenases have been cloned into these vectors and are undergoing analysis to determine the range and extent of expression. However, our preliminary data show high activities in some cases, with high stability in the absence of selection for the insertion systems.

4. Analyze Stress Response Systems

We have cloned a number of regions upstream of putative stress response genes into our new promoter screening vector, and have shown that some of these increase expression in response to specific stress conditions. In addition, we have generated insertion mutants in the two genes predicted to encode RpoE-type sigma factors, subunits of RNA polymerase that in other strains direct transcription to promoters of genes involved in extracytoplasmic functions and stress response. Preliminary information suggests that one of these is involved in transcription of heat shock genes. We are now testing the expression of the other cloned stress promoters in these mutants.

Implications

We have developed and tested a suite of tools for genetic manipulation of *D. radiodurans*, including stable expression systems with dynamic promoter ranges, to allow the construction and testing of optimized process strains for biotreatment of mixed wastes. In addition, we are analyzing stress response systems in *D. radiodurans,* for the purpose of manipulating specific stress response to increase resistance to harsh conditions. The strains we develop should be amenable to above-ground treatment scenarios, since they have high level biodegradative capabilities with stable expression, and should have increased ability to withstand harsh environmental conditions.

Planned Activities:

1. Complete expression analysis of strains with biodegradative capabilities.
2. Continue to assess stress response promoters and regulatory elements.

Information Access:

M. Lidstrom home page: http://faculty.washington.edu/lidstrom
Publications:

Meima, R. and M.E. Lidstrom. 2000. Characterization of the minimal replicon of a cryptic *Deinococcus radiodurans* SARK plasmid and development of versatile *Escherichia coli-D. radiodurans* shuttle vectors. Appl. Env. Microbiol. 66:3856-67.

Meima, R., H.M. Rothfuss, L. Gewin, and M.E. Lidstrom. 2001. Promoters in the radioresistant bacterium *Deinococcus radiodurans*. J. Bacteriol., 183:3169-3175.