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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 4-1/2 years of a 6-year project. In the first 3 years of the project, we developed a suite of genetic tools for *D. radiodurans*, analyzed *D. radiodurans* promoters, and developed stable expression systems for broad host-range oxygenases. In the most recent year of the project we have focused on systems necessary for effective application of treatment strains. First, we have studied stress response systems, because treatment processes will involve chemical and physical stress. The *D. radiodurans* genome does not appear to contain known stress response regulatory genes, and so it is of central importance to understand basic stress response in this bacterium, in order to monitor and manipulate stress response for treatment. Second, we have studied the phosphate regulon, in collaboration with Prof. Jay Keasling at UC-Berkeley. The Keasling group has a DOE-funded project to assess the feasibility of using their system for precipitating metal phosphates on the surface of cells in *D. radiodurans*, to not only provide broad spectrum metal resistance, but to also potentially remove toxic metals and radionuclides. This system involves manipulation of polyphosphate metabolism, such that high concentrations of phosphate are released at the surface of the cell. We have joined forces to achieve this goal in our treatment strain, with the idea of simultaneously removing metals and detoxifying solvents. Our part of this project involves characterizing the phosphate regulon of *D. radiodurans*.

1. Analyze Stress Response Systems

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. We have shown that both of these sigma factors function in regulation of heat shock response, and that the heat shock response overlaps with solvent response but not with response to other stresses. A paper has been published describing this work (see below). In addition, we have discovered that *D. radiodurans* is remarkably resistant to a variety of chemical stresses, including acid, solvent, and oxidative agents. We are currently investigating genes involved in this broad resistance, but regardless of the mechanism this finding suggests that *D. radiodurans* has inherent characteristics that lend itself well to the treatment of mixed wastes.

2. Analyze Phosphate Regulon

We have analyzed the putative phosphate regulon genes in the genome of *D. radiodurans*, and have shown that a complete regulon appears to be present. We have cloned genes for the production and degradation of polyphosphate, and have generated mutants in these. We are in the process of characterizing the mutants and promoters of these genes.

Implications

We are analyzing stress response and phosphate release systems in *D. radiodurans*, for the purpose of manipulating specific stress response and phosphate release to increase resistance to harsh conditions and remove heavy metals. 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 and remove heavy metals.

Planned Activities:

1. Continue to assess stress response promoters and regulatory elements.
2. Continue to analyze the phosphate regulon

Information Access:

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

Schmid AK, Lidstrom ME. 2002. Involvement of two putative alternative sigma factors in stress response of the radioresistant bacterium *Deinococcus radiodurans*. J Bacteriol. 184(22):6182-9.