

# **Environmental Management Science Program**

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

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### Research Objective

Because of their tolerance to very high levels of ionizing radiation, members of the genus *Deinococcus* have received considerable attention over the past years. The type species of the genus, *Deinococcus radiodurans*, has been studied extensively in several labs. Although researchers are only beginning to understand the mechanisms by which this Gram-positive bacterium is able to repair massive DNA damage after radiation dosages as high as 5 Mrad, it has become evident that its recombination machinery has several unique characteristics (1-4).

The aim of the present studies is to engineer *D. radiodurans* into a detoxifier for bioremediation of complex waste mixtures, containing heavy metals, halo-organics and radionuclides, making use of its ability to be biologically active in environments where they will be exposed to high levels of radiation. For that purpose, we aim to clone and express several broad spectrum oxygenases and heavy metal resistance determinants, and test survival and activities of these strains in artificial mixtures of contaminants, designed to simulate DOE mixed waste streams.

- 1) Daly, M.J., et al. (1996) An alternative pathway of recombination of chromosomal fragments precedes recA-dependent recombination in the radioresistant bacterium *Deinococcus radiodurans*. *J Bacteriol* 178: 4461-4471
- 2) Daly, M.J., and Minton, K.W. (1995) Resistance to radiation. *Science* 270: 1318
- 3) Minton, K.W., and Daly M.J. (1995) A model for repair of radiation-induced DNA double-strand breaks in the extreme radiophile *Deinococcus radiodurans*. *Bioessays* 17: 457-464
- 4) Battista, J.R. (1997) Against all odds: The survival strategies of *Deinococcus radiodurans*. *Annu Rev Microbiol.* 51: 203-224

### Research Progress and Implications

#### Progress Report

This report summarizes work after 0.5 year of a 3-year project. The initial studies have focused on the development of an insertional expression system for *D. radiodurans* R1. This effort has involved two parts, namely: (1) promoter analysis, and (2) development of insertion systems.

Several studies have shown that the expression signals used by *D. radiodurans* differ considerably from those found in other bacteria. Although *D. radiodurans* contains a typical eubacterial RNA polymerase core enzyme (based on TBLASTN searches on the genome sequence), *Escherichia coli* promoters are not recognized in *D. radiodurans* and vice versa (5). To expand our basic understanding of the requirements for transcription, and to optimize expression of (heterologous) genes, we will follow two strategies. First, a promoter-probe vector is being developed for the selection of promoter sequences from the *D. radiodurans* R1 genome. This system, which uses either lacZ or gfp as a reporter for expression, is based on single-copy replacement recombination (DCO) in either the thyA or dfrA (folA) gene. From numerous studies in both Gram-positive and Gram-negative organisms it is known that mutations in these genes, encoding thymidilate synthase and dihydrofolate reductase, respectively, render the host resistant to trimethoprim (e.g., 6). This obviates the need of an efficiently expressed antibiotic resistance marker for the initial selection of transformants. This system will then be used for the construction of a shotgun-library of promoter fragments and subsequent screening of *D. radiodurans* transformants for expression of b-galactosidase or fluorescence. The second strategy involves primer extension studies of a number of genes which are expected to be transcribed at a substantial level. This will enable us to map transcription start sites and identify possible -35 and -10 sequences.

Two potential integration systems have been developed, one based on insertion into amyE (α-amylase), and a second based on insertion into amyX (pullulanase) genes. A kanamycine-resistance marker has been used to test both systems and both appear to be good candidates for an insertion vector. In both cases, double cross-over recombinants were obtained that showed both kanamycin-resistance and loss of function, i.e. failure to grow on the appropriate substrate as sole carbon and energy source (starch or pullulan).

5) Smith, M.D., et al. (1991) Gene expression in *Deinococcus radiodurans*. *Gene* 98:45-52

6) Stacey, K.A., and Simson, E. (1965) Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. *J Bacteriol* 90: 554-555

### **Planned Activities**

In the next 6-12 months we will characterize promoters, generate an expression system for the insertion vector, and optimize expression of cloned broad-range oxygenases.