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Engineering *Deinococcus radiodurans* for Metal Remediation in Radioactive Mixed Waste Sites

Final Report for Project Period 1997-2004 (18 papers, see below)

Institutional Endorsement: **Henry M. Jackson Foundation for the Advancement of Military Medicine (HMJFAMM),** Rockville, MD 20852

Products:

Work funded by the existing grant contributed to 10 papers in the period 1997-2000 on the fundamental biology of *D. radiodurans* and its design for bioremediation of radioactive waste environments. The final project period (2000-2004) has generated an additional 8 papers currently in press or under review as indicated below.

Summary of Publications: NABIR Grant DE-FG02-97ER62492: 2001-2004 (8), and 1997-2000 (10).

<u>2001-Present</u>

1 J. K. Fredrickson, J. M. Zachara, D. L. Balkwill, D. Kennedy, S. W. Li, H. M. Kostandarithes, M. J. Daly, M. F. Romine, and F. J. Brockman (2004) Geomicrobiology of high level nuclear waste contaminated vadose sediments at the Hanford Site, Washington state. *Appl. Environ. Microbiol*. **70**, 4230-4241.

2 H. Brim, A. Venkateswaran, H. M. Kostandarithes, J. K. Fredrickson, and M. J. Daly (2003) Genetic development of *D. geothermalis* for bioremediation of high temperature radioactive waste environments. *Appl. Environ. Microbiol*., **69**, 4575-4582.

3 Y. Liu, J. Zhou, A. Beliaev, J. Stair, L. Wu, D.K. Thompson, D. Xu, A. Venkateswaran, M. Omelchenko, M. Zhai, E. K. Gaidamakova, K. S. Makarova, E. Koonin, and M. J. Daly (2003) Transcriptome dynamics of *Deinococcus*

radiodurans recovering from ionizing radiation. *Proc. Natl. Acad. Sci. USA*, **100**, 4191-4196.

4 M. Sandigursky, S. Sandigursky, P. Sonati, **MICHAEL J. DALY**, and W. A. Franklin (2004) Multiple uracil-DNA glycosylase activities in *D. radiodurans. DNA Repair*, **3**, 163-169.

5 K. S. Makarova, L. Aravind, Y. I. Wolf, R. L.Tatusov, K. Minton, E. V. Koonin, and M. J. Daly (2001) The genome of the extremely radiation resistant bacterium *Deinococcus radiodurans* viewed from the perspective of comparative genomics. *Microbiology and Molecular Biology Reviews* 65, 44-79.

6 M. S. Lipton, L. Pasa-Tolic, G. A. Anderson, D. J. Anderson, D. Auberry, M. J. Daly, J. K. Fredrickson, R. D. Smith *et al.* (2002) Global analysis of the *Deinococcus radiodurans* R1 proteome using accurate mass tags. *Proc. Natl. Acad. Sci. USA*. 99, 11049-11054.

7 J-I Kim, A. K. Sharma, S. N. Abbot, E. A Wood, D. Dwyer, A. Jambura, K. W. Minton, R B. Inman, M. J. Daly, and M. M. Cox (2002) RecA protein from the extremely radioresistant bacterium *Deinococcus radiodurans*: Expression, purification, and characterization. *J. Bacteriol*. 184, 1649-1660.

8 J. R. Battista, M. M. Cox, **MICHAEL J. DALY**, I. Narumi, M. Radman, and S. Sommer (2003) The structure of *Deinococcus radiodurans*. *Science*, **302**, 567-568.

<u>1997-2000</u>

1. H. Brim, S. McFarlan, L. Wackett, K. W. Minton, M. Zhai, J. Fredrickson, and M. J. Daly (2000) Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. *Nature Biotechnology* 18, 85-90.
2. C. Lange, L. P. Wackett, K. W. Minton and M. J. Daly (1998) Engineering a recombinant *Deinococcus radiodurans* for organopollutant degradation in radioactive mixed waste environments. *Nature Biotechnology* 16, 929-933.
3. M. J. Daly (2000) Engineering radiation-resistant bacteria for environmental biotechnology. *Current Opinion in Biotechnology* 11, 280-285.
4. J. K. Fredrickson, H. M. Kostandarithes, A. W. Li, A. E. Pyle, and M. J. Daly (2000) Reduction of Fe(III), Cr(VI), U(VI), and Tc(VII) by *Deinococcus radiodurans*. *Appl. Environ. Microbiol*. 66, 2006-2011.
5. A. Venkateswaran, S. C. McFarlan, D. Ghosal, K. W. Minton, A.Vasilenko, K. Makarova, L. P. Wackett, and M. J. Daly (2000) Physiologic determinants of radiation resistance in *Deinococcus radiodurans*. *Appl. Environ. Mircobiol*. 66, 2620-2626.
6. K. S. Makarova, L. Aravind, M. J. Daly and E. Koonin (2000) Specific expansion of protein families in the radioresistant bacterium *Deinococcus radiodurans*. *Genetica* 108, 25-34.
7. K. S. Makarova, Y. I Wolf, K. W. Minton, O. White, and M. J. Daly (1999) Short

repeats and insertional elements in *Deinococcus radiodurans* and comparison to other bacterial species. *Res. Microbiol*. 150, 1-14.

8. J. Lin, R. Qi, C. Aston, J. Jing, T. S. Anantharaman, B. Mishra, O. White, K. W. Minton, M. J. Daly, J. C. Venter, and D. C. Schwarzt (1999) Whole genome shotgun optical mapping of *Deinococcus radiodurans* using genomic DNA molecules. *Science* 284, 1558-1561.
9. O. White, J. A. Eisen, J. F. Heidelberg, E. K. Hickey, J.D. Peterson, R. J. Dodson, D. H. Haft, M. L. Gwinn, K. S. Makarova, L. Aravind, M. J. Daly, C. M. Fraser *et al.* (1999) Sequencing and functional analysis of the *Deinococcus radiodurans* genome. *Science* 286, 1571-1577.
10. R. C. Richmond, R. Sridhar, and M. J. Daly (1999) Physiocochemical survival pattern of the radiophile *D. radiodurans*. *SPIE*, 3755, 210-222.

Final Technical Report:

A comprehensive progress report for this project period was submitted with our 2003 NABIR renewal application and additionally is being submitted as a full final technical report via the DOE Energy Link (E-Link) https://www.osti.gov/elink/index_2413.html.

Introduction: Immense volumes of radioactive waste were generated from the production of 46,000 nuclear weapons in the United States between 1945 and 1986. This is a period when national security priorities often surmounted concerns over the environment. Most wastes included mixtures of inorganic and organic contaminants such as radionuclides, heavy metals, and solvents, which were disposed directly to the ground or stored in subterranean tanks at 120 sites across the United States. Low levels of widespread contamination originating from waste sites have contaminated over 40 million cubic meters of surface and subsurface soils and over 1 trillion liters of groundwater. As a result of the chemical reprocessing of 110,000 tons of nuclear fuel at the Hanford Site, near Richland, Washington, 55 million gallons of radioactive waste were produced at nine reactors and stored in 177 single-shell underground tanks. These tanks have been used since 1943, but had a design-life of only 10-20 years, and leaks were confirmed in 1961. The amount and distribution of waste leakage from the Hanford tanks is unknown, but current estimates range from 0.6 to 1.0 million gallons. The wastes leaked from these tanks have been migrating into the surrounding soils and vadose sediments for decades. The most common contaminants at DOE sites, including the Hanford Site, that have been found in ground and groundwaters include the radionuclides uranium and technetium, the heavy metals chromium and mercury, and a variety of toxic chlorinated hydrocarbons.

Results: Our NABIR-supported research aimed at developing *D. radiodurans* for metal remediation in radioactive environments began in 1997 with the demonstration that

the organism can grow and functionally express cloned genes in the presence of chronic ionizing radiation (60 Gy/hour), comparable to the most radioactive DOE waste sites. The 1997-2000 NABIR grant was renewed for the period 2000-2003. Our progress since September 1997 is summarized as follows. We have further refined expression vectors for *D. radiodurans* and successfully tested engineered strains in natural DOE sediment and groundwater samples. Further, we have shown that *D. geothermalis* is transformable with plasmids and integration vectors designed for *D. radiodurans.* This was demonstrated by engineering Hg(II)-resistant *D. geothermalis* strains capable of reducing Hg(II) at elevated temperatures and under chronic irradiation. Additionally, we showed that *D. geothermalis*, like *D. radiodurans*, is naturally capable of reducing U(VI), Cr(VI), and Fe(III). These characteristics support the prospective development of this thermophilic radiophile for bioremediation of radioactive mixed waste environments with temperatures as high as 55°C, of which there are many examples.

Our annotation of the *D. radiodurans* genome has been an important guide throughout this project period and continues to be a source of inspiration in the development of new genetic technologies dedicated to this bacterium. For example, our genome analyses have enabled us to achieve engineering goals that were unattainable in our first NABIR project period (1997-2000), where uncertainties relating to its metabolic configuration prevented efforts to expand its metabolic capabilities. As just one example, we showed that *D. radiodurans* has a functioning tricarboxylic acid (TCA) cycle glyoxylate bypass which could be integrated with toluene oxidation. And, we successfully engineered *D. radiodurans* to derive carbon and energy from complete toluene mineralization and showed that toluene oxidation can be coupled to cellular biosynthesis, survival, as well as its native and engineered metal reducing capabilities. In collaboration with ORNL, we have also constructed a whole genome microarray for *D. radiodurans* covering ~94% of its predicted genes and have successfully used the array to examine the response of cells to radiation and other DOE relevant conditions. Similarly, we have used high throughput proteomic approaches to examine how *D. radiodurans* responds to a variety of stress conditions. These studies have validated our annotation and are facilitating analysis of its metabolism, resistance, and metal reduction pathways.