1. Cover Sheet

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Project Title: Design and Construction of *Deinococcus radiodurans* for Biodegradation of Organic Toxins at Radioactive DOE Waste Sites

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3. Executive Summary

Seventy million cubic meters of ground and three trillion liters of groundwater have been contaminated by leaking radioactive waste generated in the United States during the Cold War. A cleanup technology is being developed based on the extremely radiation resistant bacterium *Deinococcus radiodurans* that is being engineered to express bioremediating functions. Research aimed at developing *D. radiodurans* for organic toxin degradation in highly radioactive waste sites containing radionuclides and heavy metals was started by this group in September 1997 with support from DOE EMSP grant DE-FG07-97ER20293. Work funded by the existing grant has already contributed to eleven papers (**Section 7**) on the fundamental biology of *D. radiodurans* and its design for bioremediation of highly radioactive waste environments - several of the research papers received extensive coverage by national and international news agencies (**Section 11**).

Our progress since September 1997 closely matches the Aims proposed in our previous EMSP application (**Section 7**) and is summarized as follows. We have demonstrated that *D. radiodurans* can be genetically engineered for degradation of toxic organic compounds using expression systems that were tested during growth of engineered *D. radiodurans* at 6,000 rad/hour. While *D. radiodurans* is naturally tolerant to the concentrations of Uranium at most radioactive DOE waste sites, its growth will likely be inhibited by the concentrations of heavy metals at those sites. Therefore, we are cloning metal resistance functions into toxin-degrading *D. radiodurans*. For example, we have cloned the *Escherichia coli merA* locus into engineered *D. radiodurans* strains capable of degrading chlorobenzene, 3,4-dichloro-1-butene, and toluene. These *merA*-containing strains were resistant to highly toxic Hg(II), and could reduce Hg(II) to much less toxic and volatile Hg(0). An assortment of organic toxin-degrading genes is now being tested

in *D. radiodurans* in combination with genes encoding resistance to Hg(II), Pb(II), and Cr(VI). Characterization of bioremediating strains was aided by our development of a synthetic minimal medium for *D. radiodurans.* In combination with our genomic informatic analyses, this medium has enabled us to identify the minimum nutrient requirements necessary to support growth in nutrient poor radioactive environments, and to explain how radiation resistance relates to this organism's metabolic repertoire. Together, these studies have facilitated our ongoing experimental efforts to engineer *D. radiodurans* for growth on toxic organic compounds in radioactive environments. It now appears likely that we will be able to engineer *D. radiodurans* to use chlorinated hydrocarbons as carbon/energy sources that could provide electron donors for growth of toxin-degrading *Deinococcus* and, possibly, metal reduction; we have shown that anaerobic cultures of wildtype *D. radiodurans* can reduce U(VI), Tc(VII), and Cr(VI).

4. Research Objectives

Immense volumes of radioactive waste, generated from the production of over 46,000 nuclear weapons in the United States between 1945 and 1986 [1], were disposed directly to the ground; this is a period when national security priorities often surmounted concerns over the environment. These wastes contain inorganic and organic contaminants that include radionuclides, heavy metals, acids/bases, and solvents [1]. In the United States, buried radioactive wastes (3×10^6 M^3) have contaminated about 7×10^7 M^3 of surface and subsurface soils and about 3×10^{12} dM^3 of groundwater [2]. Environmental nuclear contamination is believed to be even more severe in the former Soviet Union [3]. With the end of the Cold War in the early 1990's, The United States Department of Energy (DOE) shifted its emphasis from nuclear weapons production to stabilization and cleanup of these waste environments. This remediation effort is now the largest program of its kind ever undertaken by the United States [2].

In 1992, the DOE surveyed 91 out of 3,000 contaminated sites at 18 US research facilities [1]. The most common contaminants from DOE wastes that have been found in ground and groundwaters include the radionuclides ^{235}Uranium $(\gamma, \alpha)^E$, ^{238}Plutonium $(\alpha)^E$, ^{99}Technetium $(\beta^-)^E$, ^{90}Strontium $(\beta^-)^E$, and ^{137}Cesium $(\gamma, \beta^-)^E$; and the metals, Chromium, Lead and Mercury; and a myriad of toxic organic compounds (*e.g.*, toluene and trichloroethylene (TCE)) [1]. One third of the 91 characterized sites are radioactive with some reported radiation levels as high as 10 mCi/L, within or close to the contaminating sources [1]. These high radiation levels in combination with the chemical hazards are extremely damaging to living organisms over extended periods, often resulting in cell death.

Of the 3,000 waste sites disclosed by the DOE, the total cleanup cost, by methods that utilize costly pump and treat technologies and/or soil excavation and incineration, was estimated recently between $189 and $265 billion (Internet: http://www.em.doe.gov/bmr96; The 1996 baseline management report). DOE budget projections for cleanup activities for the next ten years exceed $60 billion [2]. These vast waste sites are, thus, potential targets for less expensive *in situ* bioremediation technologies utilizing specialized microorganisms that can detoxify both metallic and organic contaminants. However, the utility of microbiological methods for the primary treatment of highly radioactive environmental wastes will largely be determined by 1) the ability of microorganisms catalyzing the desired function(s) to survive and function under radiation stress; and 2) the ability of basic research to produce bioremediation systems that do not cause undesired secondary effects that threaten the general public or further damage the environment.

Numerous bacteria (including *Shewanella* and *Pseudomonas* spp.) have been described and studied in detail for their ability to transform, detoxify, or immobilize a variety of metallic and organic pollutants [4-11]. Like most organisms, however, these bacteria are sensitive to the damaging effects of radiation [12], and their use in bioremediation likely will be limited to environments where radiation levels are very low. By developing microbiological techniques suitable for intervention in

areas close to or within the leaking sources where radiation levels are highest, stabilization/decontamination efforts could begin before the pollutants disseminate into the environment. Therefore, radiation resistant microorganisms that can be used for environmental cleanup need to be found in nature or engineered in the laboratory to address this problem. Remarkably, highly radioactive DOE waste environments (*e.g.*, beneath the leaking single-shell tanks at Hanford) have not yet been surveyed for their microbial ecology, where natural selection may have already yielded bacteria with favorable bioremediating characteristics. The isolation of radiation resistant bacteria is easy, even from non-extreme environments; one highly effective method is to select for growth on solid nutrient-rich medium, incubated in the presence of chronic gamma radiation (6,000 rad/hour). Numerous novel bacteria have been isolated this way including several from the Washington DC area.

Most radiation resistant bacteria that have been reported are spore-formers and are not remarkably radiation resistant when growing vegetatively; many of them are pathogens and most lack a developed system for genetic manipulation [12,13]. A few vegetative bacteria, like *Enterococcus faecium* and *Alcaligenes* spp., show high resistance to irradiation, but often these too are pathogenic [13] and, therefore, would not be suitable for bioremediation. Bacteria belonging to the family *Deinococcaceae* are distinctly unusual [14,15]. Not only are they the most radiation resistant organisms discovered, but they are vegetative, easily cultured, and nonpathogenic. Despite ubiquitous distribution and ancient derivation, only seven species of the family *Deinococcaceae* have been described [15,16]. Of these species, *Deinococcus radiodurans* is the only one for which a system of genetic transformation and manipulation has been developed [17-20].

The bacterium *D. radiodurans* is likely the first polyextremophile to be identified, showing remarkable resistance to a range of damage caused by ionizing radiation, dessication, ultraviolet radiation, oxidizing agents, and electrophilic mutagens [15,21,22]. It is an aerobic, large tetrad-forming soil bacterium [14] that is most famous for its extreme resistance to ionizing radiation; it not only can survive acute exposures to gamma radiation that exceed 1,500,000 rads without lethality or induced mutation, but it can also grow continuously in the presence of chronic radiation (6,000 rad/hour) without any effect on its growth rate or ability to express cloned genes [23]. For comparison, an acute exposure of just 500-1,000 rads is lethal to the average human [12]. Adding to the growing resource of genetic technologies available for *D. radiodurans* is the recent whole-scale sequencing, annotation and analysis of its genome [24, 25, 26]. This combination of factors has positioned *D. radiodurans* as one of the most promising candidates for the development of microbiological treatments of radioactive environments.

5. **Methods and Results** of Project (While reading this section, the reader is directed to our published data listed in **Section 7, Refs. 1-11**)

This final report summarizes work after 2.8 years of a 3-year project dedicated to engineering the radiation resistant bacterium *Deinococcus radiodurans* for toxic organic compound degradation in radioactive DOE waste sites. Our research aimed at developing *D. radiodurans* for toxin degradation in radioactive environments began in 1997 with the demonstration that this bacterium

can grow in the presence of ionizing radiation at 6,000 rads/hour (**Ref. 1**, **2**), comparable to the most radioactive DOE waste sites. In fact, all reported members of the *Deinococcaceae* can grow at this dose rate and are poised to contribute their individual characteristics to this developing technology. For example, *D. geothermalis* grows optimally at about 50ºC, and we have recently shown that the expression systems developed for *D. radiodurans* work in this thermophile and that its growth is superior to *D. radiodurans*' in radioactive environments (**Ref. 3, 4**). As such, it is likely that the genetic technology being developed for *D. radiodurans* will be readily transferable to *D. geothermalis*, and could be useful in thermally insulated radioactive environments (*e.g.*, within or beneath leaking tanks at Hanford) where temperatures can be elevated due to radioactive decay. Initially, growth of the *Deinococcaceae* during high-level chronic irradiation exposure was thought to be unlikely since it had been reported that DNA replication in *D. radiodurans* ceases upon DNA damage and RecA expression (**Ref. 8**). However, as manifested by their growth in a ^{137}Cs irradiator, these bacteria are proficient at simultaneous semi-conservative DNA replication and homologous recombination (**Ref. 4**), as modeled previously. We have tested *D. radiodurans* with a variety of cloned bioremediating gene functions that can be expressed during growth at 6,000 rads/hour; and these engineered strains are now being used in the design of more complex organic compound degradation systems that exploit our recent progress.

Growth on Toxic Organic Compounds During Metal/Radionuclide Remediation:

It now appears likely that we will be able to engineer *D. radiodurans* to use chlorinated hydrocarbons as carbon/energy sources that could provide electron donors for growth of toxin-degrading *Deinococcus* and, possibly, metal reduction; we have shown that anaerobic cultures of wildtype *D. radiodurans* can reduce U(VI), Tc(VII), and Cr(VI) (**Ref. 5**). With respect to DOE facilities, there has been no adequate method for microbiological treatment of contaminant waste sites containing both hazardous radioactive metal and organic components since organisms like *Pseudomonas* spp. are very radiation sensitive (**Ref. 3**). High concentrations of toxic organic compounds (*e.g.*, toluene) in mixed radioactive wastes pose as viable carbon/energy sources for engineered *Deinococcus* (**Ref. 2, 3**). Such metabolic capabilities would not only potentially provide electron donors for growth of metal-remediating *Deinococcus* (**Ref. 3**), but would provide an effective alternative to conventional physicochemical treatments of toxic organic compounds.

In the presence of toluene, *D. radiodurans* expressing *todC1C2BA* (TDO) produces toluene-*cis*-dihydrodiol that is further metabolized to 3-methylcatechol by a native non-specific dehydrogenase (**Ref. 1, 2**). Once formed, catechols readily polymerize to form insoluble polymers and this has been observed in *tod*-engineered *D. radiodurans* strains, that turn dark brown in the presence of toluene over the course of several days to weeks. Other *Pseudomonas* catabolic genes that convert 3-methylcatechol to pyruvate have been introduced into *todC1C2BA*-containing *D. radiodurans*, that may yield a strain that is able to mineralize toluene and related chlorinated compounds.

Engineering *D. radiodurans* for Resistance to Common Metallic Waste Constituents:

While *D. radiodurans* is naturally tolerant to the uranium and plutonium concentrations at most DOE

sites, growth of these bacteria will likely be inhibited by the concentrations of heavy metals prevalent at those sites (mercury, chromium, and lead). A series of genetic vectors that encode resistance to these metals have been constructed and are being examined in *D. radiodurans* strains also expressing organic toxin degrading genes (**Ref. 2**). For example, the highly characterized *merA* locus from *Escherichia coli* has been cloned into *D. radiodurans* also expressing genes encoding toluene dioxygenase (*tod*) (**Ref. 1, 2**). *tod*-containing *D. radiodurans* expressing *merA* were resistant to the bacteriocidal effects of highly toxic, thiol-reactive mercuric ion, Hg(II), at concentrations (50 μM) well above the highest concentration reported for mercury-contaminated DOE waste sites (10 μM). These strains were also very effective at reducing Hg(II) to much less toxic and nearly inert elemental and volatile Hg(0). We have very carefully characterized these *D. radiodurans* strains expressing both organic-degrading and metal-reducing functions and have shown how expression of these genes can be regulated. These studies have formed the foundation of our ongoing efforts to expand the remediating capabilities of this bacterium and to ensure survival of these constructs in environments co-contaminated with radionuclides, heavy metals and toxic organic compounds.

Physiology, Radiation Resistance, and Degradation of Organic Toxins:

Adding to the challenge of surviving the harsh radioactive, metallic, and organic properties of DOE waste sites is the likelihood that *D. radiodurans* may be limited by several inherent physiologic constraints. For example, genomic informatics shows that the amino acid biosynthetic pathways for serine, cysteine and lysine are incomplete in wildtype *D. radiodurans*. One major thrust of our research involves characterization of its physiology and optimization of the external parameters for growth and survival in adverse radioactive environments (**Ref. 4**). For example, under optimal growth conditions *D. radiodurans*' DNA repair capabilities are extremely well suited to survive either acute (**Ref. 10**) or chronic irradiating exposures (**Ref. 1, 4**). However, *D. radiodurans* is unable to grow and is rapidly killed in certain nutrient poor radioactive environments that support luxuriant *D. radiodurans* growth when radiation is absent (**Ref. 4**). This phenotypic reversal from radiation resistance to sensitivity is of great interest and concern to us since it questions the suitability of *D. radiodurans* as a bioremediation host in radioactive waste sites. A combination of growth studies (**Ref. 4**) and analysis of the complete *D. radiodurans* genomic sequence (**Refs. 6, 7, 9, 11**) has identified several defects in *D. radiodurans*' global metabolic regulation that limit carbon, nitrogen and DNA metabolism. In nutrient-restricted conditions, DNA repair was found to be limited by this organism's metabolic capabilities and not by any nutritionally induced defect in genetic repair; and, this information has been used successfully as a guide to identify key nutritional constituents that restore luxuriant growth of *D. radiodurans* in nutritionally restricted radioactive environments (**Ref. 4**). Analyses like these, coupled to the possible integration of native and cloned metabolic pathways in *Deinococcus*, will facilitate the design of *in situ* remediation protocols for this organism.

Genome Flexibility and its Impact on Constructing Bioremediating *D. radiodurans*:

The preeminent factors that are allowing rapid progress in the area of genetic engineering of *D. radiodurans* are: 1) *D. radiodurans* is highly transformable using DNA with homology to its genome (**Ref. 1, 2**); 2) it is prolific in its ability to amplify DNA sequences that are flanked by direct repeats

(**Ref. 2, 6**); 3) a large variety of *D. radiodurans* vectors has already been developed (**Ref. 2**); and 4) the entire genomic sequence of *D. radiodurans* is now available and is being subjected to analysis (**Ref. 6, 7, 9, 11**).

Upon transformation, if a gene is integrated between direct repeats of *D. radiodurans* genomic DNA, selection pressure can yield recombinant strains with about 20 duplicated copies per chromosome, 8-10 identical chromosomes per cell. We have used this strategy to amplify vectors as large as 20kb, encoding organic toxin degradation in *D. radiodurans*, resulting in a genome that contains about 4 Mbp more DNA than wildtype (**Ref. 2**); these expansions are stable and readily maintained for many generations, even without selection. We have exploited this organism's ability to amplify genes and have engineered a strain that can detoxify both toluene (and related compounds) and Hg(II) ions efficiently during growth in the presence of chronic radiation (**Ref. 1**, **2, 3**).

6. Relevance, Impact and Technology Transfer

a. How does this new scientific knowledge focus on critical DOE environmental management problems?

The technology under development promises to yield a cost-effective mechanism to reduce the threat to the environment by released actinides, heavy metals and organic toxins. For example, about half of the older single-shell million-gallon storage tanks at Hanford have leaked into the environment. The contents of these tanks are highly radioactive. Bioremediation using extremely radiation resistant *Deinococcus* beneath the tanks could reduce the migration of metals into the environment as well as detoxify the organic component of these wastes.

b. How will the new scientific knowledge that is generated by this project improve technologies and cleanup approaches to significantly reduce future costs, schedules, and risks and meet DOE compliance requirements?

Current DOE plans call for emptying the storage tanks, reducing the waste volume with evaporators, followed by vitrification and storage of concentrated waste. This is distinct from the task of cleaning the millions of cubic yards of soils and sediments contaminated by progressive leaks or controlled releases between 1944 and the end of the Cold War. Highly radioactive contaminated soils beneath leaking tanks, pits or trenches will have to be treated separately. If no efficient method for stabilizing sediment waste is developed, then tens of billions of dollars will have to be spent on excavating these, followed by relocation and storage. Many fear that such a solution could become tomorrow's new problem. The use of *Deinococcus* or related bioremediating systems could help stabilize these soil/sediment contaminants. If successful, such soil/sediment waste environments could be left intact, and wastes could be allowed to decay *in situ* without relocation or fear of it spreading. If the technology only slows the dissemination of contaminants, this would still give the DOE more time to develop other cleanup technologies. Either way, it seems an appropriate approach.

c. To what extent does the new scientific knowledge bridge the gap between broad

fundamental research that has wide-ranging applications and the timeliness to meet needs-driven applied technology development?

Numerous organisms capable of degrading toxic organic compounds or immobilizing toxic metals have been the subject of intense research over the last decade; *e.g.*, *Pseudomonas* spp (organic toxins) and more recently *Shewanella* spp. (metal immobilization). While these bacteria show great promise, they will not be useful in DOE waste environments that are radioactive since these organisms are very sensitive to radiation. Bioremediating systems that use *Deinococcus* to express cloned bioremediating gene functions is an important bridge to the ultimate goal of a biological solution to the sediment/soil/groundwater problem. Our research efforts have demonstrated that complex genetic bioremediating gene systems from a variety of organisms can be expressed in *Deinococcus*. For example, we have successfully engineered *Deinococcus* to degrade chlorinated aromatic compounds while at the same time decontaminating mercury (II). The recombinant strain is capable of expressing these functions during growth in the presence of 6,000 rads per hour; this dose-rate is comparable to some of the most heavily contaminated DOE waste environments.

d. What is the project's impact on individuals, laboratories, departments, and institutions? Will results be used? If so, how will they be used, by whom, and when?

The results of our work since 1997 have had a large impact on other laboratories as well as institutions like the DOE. In part, the results of our collaborative group were impetus for arranging the first meeting on *Deinococcus* and its applications, held at the EMSL 2000 conference at PNNL in June 2000. Several previously distinct areas of Deinococcal research have now joined yielding collaborations committed to the further development of this organism for bioremediation (*e.g.*, with Anne Summers University of GA; Hg(II) remediation). The Institute for Genomic Research has successfully sequenced the genome of *D. radiodurans*, and we have demonstrated expression of bioremediating genes in this strain. Using the sequence as a guide to manipulation we are further developing this bacterium and making our results readily available to other research groups through publications, but also at an informal level (*e.g.*, with Richard Smith, chief scientist, PNNL, whole genome-scale proteomics for *Deinococcus*). The DOE has cited our work in several of its FY2000 'call for proposals' and we have pledged support to a variety of DOE-sponsored groups interested in studying *Deinococcus*.

e. Are larger scale trials warranted? What difference has the project made? Now that the project is complete, what new capacity, equipment or expertise has been developed?

The ultimate aim of our EMSP project is to construct *Deinococcus* strains that can survive and effectively compete with resident bacteria while degrading (metabolizing) the organic contaminants. This ultimate aim will be contingent on renewed funding of our work by the EMSP program. On the basis of their natural resistance to radiation, desiccation and to high concentrations of toluene and TCE, *Deinococcus* GEMs are likely to have high natural survival rates and will likely

be competitive with indigenous soil and subsurface microorganisms. However, this must be thoroughly evaluated in laboratory experiments before field experimentation and applications. If these strains prove to be efficacious for organic contaminant degradation and utilization as expected, they would be excellent candidates for future field experiments conducted as part of the EMSP program.

The priority should be on experiments utilizing natural materials (soils, sediments, and groundwater) from DOE sites including Hanford and ORNL. As the research being described herein was proposed as a companion to a funded DOE-OBER NABIR proposal focused on metal and radionuclide reduction, we would coordinate the use of field samples from both projects if our FY2000 EMSP application is successful. One option is to utilize materials from the NABIR Field Research Center (FRC) (Bear Creek Valley FRC in Oak Ridge, TN). FRC site descriptions will be examined and specific materials will be requested. We will also investigate the possibility of obtaining vadose zone sediments from beneath leaking Hanford waste tanks as it is our understanding there are tentative plans for coring such materials in FY2000. However, given the uncertainties in these efforts, an alternative plan is to utilize uncontaminated materials from adjacent, uncontaminated areas. Such materials are readily accessible from outcrops along the Columbia River north of the Hanford Sites 300 Area. Our understanding is that the FRC is to include both contaminated and control sites where the controls will consist of uncontaminated regions in the same aquifer or formation with similar geological, geochemical and hydrologic properties, save for differences due to contamination. If necessary, these materials can be spiked with known concentrations of organic contaminants (TCE and/or toluene, for example), metals (Hg(II)), and/or radionuclides (uranium, cesium) and used in microcosm studies to evaluate the survival and function of *Deinococcus* strains engineered for degradation of specific organic contaminants.

f. How have the scientific capabilities of collaborating scientists been improved?

In the United States, only two laboratories have been studying *D. radiodurans* as their sole research focus over the last ten years; John Battista's lab at LSU, and Daly's group at USUHS. As such, both groups have worked hard at disseminating information and strains, and teaching other labs how to work with this peculiar organism. In the case of the Daly lab, formal collaborations have been established with the following groups: Jim Fredrickson (PNNL), Larry Wackett (University of MN), Anne Summers (University of GA), Jonathan Trent (NASA Ames Research Center), Owen White (TIGR), Eugene Koonin (NCBI), Jay Keasling (University of CA), Bob Richmond (Marshall Space Flight Center), and several other groups.

g. How has this research advanced our understanding in the area?

The research has advanced a major field in biotechnology: We have developed the only system capable of expressing cloned gene functions in radioactive environments. This has implications not only for cleanup of DOE wastes, but potentially also for decontamination of radioactive medical waste, and the production of radiolabeled pharmaceuticals.

h. What additional scientific or other hurdles must be overcome before the results of

this project can be successfully applied to DOE Environmental Management problems?

DOE wastes are highly complex and for the most part are not characterized. We will need to introduce many more bioremediating gene functions into *Deinococcus* in order to adequately match the spectrum of toxins that will be encountered at DOE facilities. In addition to this genetic engineering, the *Deinococcus* GEMS will have to be evaluated for their effectiveness in simulated contaminated environments before applying this technology to large-scale environmental management problems (see also **Section 6e**).

i. Have any other government agencies or private enterprises expressed interest in the project? Please provide contact information.

Our work on developing *Deinococcus* has stimulated a lot of interest. Greater than 100 articles on this work have been printed in the national and international press; from The NY Times to large articles in US News and World Report. Mostly the inquiries have been from non-governmental environmental biotechnology companies asking if we have applied for patents. The answer is yes. We have a patent application under review covering the development of *Deinococcus* for bioremediation of radioactive waste sites. The patent application is being handled by Morgan, Lewis & Bockius LLP, Washington DC.

7. **Project Productivity and Publications: Did the project accomplish all of the proposed goals?**

Yes, the project accomplished the goals described in our original 1997 EMSP proposal.

The following four Aims were described in our 1997 EMSP grant. Following each Aim are papers generated (see list below) addressing aspects of the specific Aim. Note that the underlined numerals refer to the papers listed below. Some papers apply to several Aims, such as #11- *Science*, **286**, 1571-1577, reporting the *D. radiodurans* genomic sequence and its preliminary analysis.

1997-AIM 1) Cloning and expressing genes from other organisms in *D. radiodurans* that can degrade toxic organic compounds and resist heavy metals.
Papers: 1, 2, 3, 11

1997-AIM 2) Assaying toxin degrading and metal remediating gene expression in *D. radiodurans* in the presence and absence of radiation.
Papers: 1, 2, 3, 4, 6

1997-AIM 3) Development of *D. radiodurans* strains capable of using toxic organic compounds as carbon and energy sources in nutritionally limited radioactive environments.
Papers: 1, 2, 3, 4, 7, 11

1997-AIM 4) Laboratory evaluations of wildtype and engineered *D. radiodurans* strains for effectiveness at degrading organic toxins and reducing select metals. Assessing the effects of radioactive environments on *D. radiodurans*.
Papers: 4, 5, 8, 9, 10, 11

1 C. Lange, L. P. Wackett, K. W. Minton and **MICHAEL J. DALY** (1998) Engineering a recombinant *Deinococcus radiodurans* for organopollutant degradation in radioactive mixed waste environments. *Nature Biotechnology*, **16**, 929-933.

2 H. Brim, S. McFarlan, L. Wackett, K. W. Minton, M. Zhai, J. Fredrickson, and **MICHAEL J. DALY** (2000) Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. *Nature Biotechnology*, **18**, 85-90.

3 **MICHAEL J. DALY** (2000) Engineering radiation-resistant bacteria for environmental biotechnology. *Current Opinion in Biotechnology* **11**, 280-285.

4 A. Venkateswaran, S. C. McFarlan, D. Ghosal, K. W. Minton, A. Vasilenko, K. Makarova, L. P. Wackett, and **MICHAEL J. DALY** (2000) Physiologic determinants of radiation resistance in *Deinococcus radiodurans*. *Appl. Environ. Mirobiol*. **66**, 2620-2626.

5 J. K. Fredrickson, H. M. Kostandarithes, A. W. Li, A. E. Pyle, and **MICHAEL J. DALY** (2000) Reduction of Fe(III), Cr(VI), U(VI), and Tc(VIII) by *Deinococcus radiodurans*. *Appl. Environ. Microbiol.* **66**, 2006-2011.

6 K. S. Makarova, Y. I Wolf, K. W. Minton, O. White, and **MICHAEL J. DALY** (1999) Short repeats and insertional elements in *Deinococcus radiodurans* and comparison to other bacterial species. *Res. Microbiol.*, **150**, 711-724.

7 K. S. Makarova, L. Aravind, **MICHAEL J. DALY**, and E. Koonin (2000) Specific expansion of protein families in the radioresistant bacterium *Deinococcus radiodurans*. *Genetica*, In Press.

8 A. K. Sharma, A. Jambura, M. M. Cox, R. B. Inman, K. W. Minton, and **MICHAEL J. DALY** (2000) Expression and characterization of the RecA protein from the extremely radioresistant bacterium *Deinococcus radiodurans*. Submitted to *J. Biol. Chem*.

9 K. S. Makarova, E. V. Koonin, L. Aravind, L. Tatusov, Y. I. Wolf, O. White, J. R. Battista, and **MICHAEL J. DALY** (2000) The genome of the extremely radioresistant bacterium *Deinococcus radiodurans*: Comparative genomics and applications. *Microbiology and Molecular Biology Reviews*. Submitted.

10 J. Lin, R. Qi, C. Aston, J. Jing, T. S. Anantharaman, B. Mishra, O. White, K. W. Minton, **MICHAEL 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.

11 O. White, J. A. Eisen, J. F. Heidelberg, E. K. Hickey, J.D. Peterson, R. J. Dodson, D. H. Haft, M. L. Gwinn, W. C. Nelson, D. L. Richardson, K. S. Moffat, H. Qin, L. Jiang, W. Pamphile, M. Crosby, M. Shen, J. J. Vamathevan, P. Lam, L. McDonald, T. Utterback, C. Zalewski, K. S. Makarova, L. Aravind, **MICHAEL J. DALY**, K. W. Minton, R. D. Fleischmann, K. A. Ketchum, K. E. Nelson, S. Salzberg, H. O. Smith, J. C. Venter, C. M. Fraser (1999) Sequencing and functional analysis of the *Deinococcus radiodurans* genome.

Science, **286**, 1571-1577.

8. Personnel Supported

Daly Lab (USUHS)	Postdoc:	Dr. Hassan Brim
	Technician:	Min Zhai
	Ph.D. Student:	Amudhan Venkateswaran
Wackett Lab (UMN)	Postdoc:	Dr. Cleston Lange
	MA student:	Sarah McFarlan
Fredickson Lab (PNNL)	Technician:	Heather Kostandarithes

9. Patents

We have a patent application under review, covering the use of *Deinococcus* for bioremediation of radioactive waste environments. The patent application is being handled by Morgan, Lewis & Bockius LLP, Washington DC. Contact: Michael S. Tuscan, Ph.D. 202-467-7176.

10. Future Work

Our planned future work has been outlined in a grant submitted to the EMSP program (FY2000). The major aims of this future work are summarized below:

The proposed research will build on a wealth of results generated by our research group since 1997. Our ultimate aim is to develop organisms and approaches that will be useful for degrading toxic organic compounds present in radioactive DOE waste sites. The goal of our FY2000 EMSP proposal is to continue to develop strains of *D. radiodurans* and other radiation resistant bacteria that can degrade chlorinated hydrocarbons, while at the same time resisting the toxic effects of co-contaminating radionuclides and heavy metals. To achieve this goal, we have proposed four specific research aims:

1) Continue to develop radiation resistant bacteria for toxic organic compound degradation, with primary emphasis on *D. radiodurans*, but also on *Deinococcus geothermalis* that may yield strains more suitable for bioremediation in nutritionally restricted radioactive environments. As an extension of this research we will continue to introduce and evaluate metal resistance

genes in organic toxin degrading *Deinococcus* strains.

2) Develop *D. radiodurans* strains capable of utilizing chlorinated hydrocarbons and related compounds as carbon and energy sources for growth and, possibly, metal reduction. Metabolic engineering of *D. radiodurans* will be guided using a combination of genomic informatics and a novel system to evaluate gene expression patterns developed specifically for *D. radiodurans*.

3) Exploiting *D. radiodurans*' prolific genetic recombination capabilities for *in vivo* gene shuffling to generate novel catabolic genes and pathways. *D. radiodurans* containing different, but related, catabolic genes will be irradiated to extremely high levels and allowed to recover. Recombinants will be selected and studied for novel catabolic functions.

4) Evaluate engineered *D. radiodurans* strains for effectiveness at degrading chlorinated hydrocarbons in natural subsurface materials from DOE sites containing organic contaminants and toxic metals.

11. News Publications (From >100) on *D. radiodurans* and Bioremediation.

US News & World Report	01-03-00	**A Tenacious Bug: A Tonic for Toxic Waste**
New York Times	11-19-99	**Decoding a Radiation Resistant Bug**
USA Today	11-19-99	**Microbe Can Resist Radiation**
News Day (NY)	07-27-99	**Superbug to the Rescue**
Frankfurter Allgemeine	11-04-98	**Robuste Bakterien zur Sanierung von Boden**
The Economist	10-10-98	**Radioactive Taste**
US News & World Report	10-19-98	**World's Toughest Bugs**
The Sciences (NYAS)	07-01-98	**Conan the Bacterium**

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