2006 ERSD Annual Report

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Characterizing the Catalytic Potential of *Deinococcus*, *Arthrobacter* and other Robust Bacteria in Contaminated Subsurface Environments of the Hanford Site

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Most Recent Report of Results to Date:

Summary of Progress for NABIR Grant DE-FG02-04ER63918 (May 2005-May 2006): In the last year, we have published five papers, and a sixth paper is under review. The following is a summary of those publications and how they relate to our NABIR goals. The new papers are listed at the end of this annual report:

Ionizing Radiation (IR) Resistance in Bacteria

Until recently, there have been no clear physiologic predictors of a cell's ability to recover from ionizing radiation (IR), desiccation, and other DOE-relevant oxidative stress conditions. In general, the most resistant bacteria have been Gram-positive (e.g., *Deinococcus*, *Arthrobacter*, *Lactobacillus* & *Enterococcus* spp.) and the most sensitive have been Gram-negative (e.g., *Pseudomonas*, *Shewanella* & *Neisseria* spp.). However, there are several reported exceptions to this paradigm, the Gram-negative cyanobacterium *Chroococcidiopsis* is extremely resistant to IR, whereas the Gram-positive *Micrococcus luteus* is sensitive. We have identified biomolecular signatures for radiation sensitivity and resistance which are independent of phylogeny, where very high and very low intracellular Mn/Fe concentration ratios correlated with very high and very low resistances, respectively; and restricting Mn(II) in the famously resistant *Deinococcus radiodurans* sensitized this eubacterium to IR (http://cfyn.ifas.ufl.edu/radiation.pdf). For example, *D. radiodurans* (Mn/Fe ratio: 0.24) accumulates >300 times more Mn than the extremely IR sensitive *Pseudomonas putida* (Mn/Fe ratio: <0.0001), and *P. putida* accumulates 4.6 times more Fe than *D. radiodurans*; consistently, for the moderately resistant *Escherichia coli* and *Thermus thermophilus*, the intracellular Mn/Fe ratios are 0.007 and 0.04, respectively (B.2.A) (see list of papers below B.2.A-B.2.G). However, the mechanism by which Mn(II) facilitates IR resistance was undefined. In summary, for eight phylogenetically distinct bacterial species, we have shown a strong relationship between oxidative stress resistance and intracellular Mn/Fe concentration ratios. These model bacteria were selected for investigation because they have been subjected to whole genome sequencing and annotation, revealing that they encode a similarly complex set of DNA repair and protection functions. Most recently, we showed that unlike radiation-induced DNA damaged in vivo, there is a strong correlation between bacterial IR resistance, Mn/Fe concentration ratios and radiation-induced oxidative protein damage (B.2.B).

A New Hypothesis

In one of the most cited textbooks on radiation biology, Clemens von Sonntag states 'In the

hierarchy of targets for IR-induced reproductive cell death, DNA must surely be placed at the top.' The field of radiobiology is built on such assertions, found or inferred in virtually all publications that deal with this subject. Yet, the pathway connecting IR with endpoint biological damage is far from clear, largely because the identity of the first critical molecular targets is still not established. Much evidence has accumulated that is not readily explained by classical radiation toxicity models. Among these heretical results for prokaryotes are extreme radiation sensitivities observed in bacteria which encode and express a complement of DNA repair and protection systems (Daly et al., 2004); for eukaryotes, IR-induced bystander effects. Our current results support that protein is the main cellular target of the biological action of IR in bacteria.

We have reported four surprising and novel experimental results, which are framed within the context of a new view of IR resistance emerging for bacterial cells (B.2.B): (i) Whereas a given dose of IR causes very similar DNA damage in different bacteria, this is not the case for proteins. We have shown a strong correlation between IR-induced protein damage and bacterial IR resistance; (ii) Under anaerobic conditions, we have identified and quantified a Mn(II)-dependent mechanism of IR-driven dioxygen (O_2) and hydrogen peroxide (H_2O_2) generation, and showed that radioresistant Mn-accumulating bacteria display the hallmarks of Mn(II,III) redox-cycling observed in vitro; (iii) Our findings support that Mn redox-cycling protects proteins from superoxide during *in vivo* irradiation, where inhibition of Mn-cycling leads to IR sensitivity and high levels of oxidative protein damage; and (iv) using a recently developed micro(spectro)scopic approach, we revealed an unusual distribution of Fe and Mn in *D. radiodurans* cells, which could forestall the generation of reactive oxygen species (ROS) during irradiation. In summary, the possibility that DNA is not the first major class of molecules damaged by IR and other oxidative stress conditions warrants careful investigation, especially as it may come to affect estimates of risk, models of IR-induced toxicity, and approaches to modulating IR resistance in prokaryotes. A review discussing the possibility of modulating Mn and Fe homeostasis as a mechanism to increase the resistance of bacteria and eukaryotes has been published (B.2.C).

Transcriptome Analyses of Irradiated D. radiodurans and Shewanella oneidensis

We previously investigated the possibility that extreme IR resistance in *D. radiodurans* is determined by novel genes. At least 20 predicted genes of *D. radiodurans*, which were identified by transcriptional profiling following IR as the most highly induced, have been disrupted and the corresponding mutants have been characterized for IR resistance (http://www.usuhs.mil/pat/deinococcus/index_20.htm). Remarkably, the resistances of these novel mutants remained very high, indicating that survival of irradiated *D. radiodurans* might depend on a relatively conventional set of repair functions. Additionally, the transcriptome studies indicated that following irradiation, additional cellular damage might be prevented by attendant cellular responses that minimize the production of metabolism-induced ROS.

To identify cellular determinants of radiation sensitivity, we subjected *S. oneidensis* to transcriptome analyses following IR and compared the expression profiles to those of irradiated *D. radiodurans* (B.2.D). In summary, approximately 80% of *S. oneidensis* cells were killed following exposure to just 40 Gy, which causes less than 1 DNA double stranded break (DSB) per genome (5.1 Mbp) and about 40 DNA single stranded (SSB) breaks per genome. In light of the strong induction of DNA repair and protection systems in irradiated *S. oneidensis*, the relatively minor DNA damage did not explain the high levels of cell-killing. As a respiratory generalist *S. oneidensis,* in contrast to *D. radiodurans*, is rich in iron containing proteins required

for anaerobic respiration, (B.2.C). We concluded that a sudden increase of free iron due to protein damage could have proliferated ROS in *S. oneidensis* during and after irradiation, thereby predisposing *S. oneidensis* cells to a burst of oxidative stress at the onset of recovery. Furthermore, we showed the induction of genes for lytic phages in S. oneidensis, indicating that viral-induced cell death might have contributed to radiation toxicity. However, the analysis of two other *Shewanella* species, now shown not to encode prophages or other known viruses (unpublished data), were similarly sensitive to IR (B.2.D). Thus, virus-induction in *S. oneidensis* likely has only a small affect on survival following irradiation to IR or UV radiation (B.2.D).

The Role of Metal Reduction in Mn-Dependent Deinococcal Species

D. radiodurans and *Deinococcus geothermalis* are able to reduce colloidal Mn(IV), presumably as a mechanism to acquire Mn(II) from the environment (Ghosal et al., 2005). We are currently testing if activities involved in Mn reduction are also responsible for the reduction of U(VI), Tc(VII) and Cr(VI) by *D. radiodurans* and *D. geothermalis*. Genetic disruption of the predicted metal reduction (e.g., cytochrome C-related protein, DR01936) and Mn transport systems (e.g., Nramp, DR1709) of *D. radiodurans* is underway. However, with the exception of a putative Mn uptake regulator TroR (DR2539), gene knockouts have not yet yielded pure mutants, indicating that Mn-homeostasis genes are very important to *D. radiodurans*. As part of this work, we are in the final stages of annotation and analysis of the *D. geothermalis* genome, which represents the forth major sequencing/annotation project supported by members of the USUHS group (*D. radiodurans, Clostridium acetobutylicum, Thermus thermophilus* (B.2.A) and *D. geothermalis* (B.2.E). DOE's Joint Genome Institute (JGI) informed us in April 2006 that full assembly of the whole genome sequence of *D. geothermalis* (DSM11300) is now complete (B.2.E). Our whole genome comparisons between *D. radiodurans* and *D. geothermalis* have helped delineate the genes involved in metal reduction and Mn assimilation, with our findings to be submitted for publication in August, 2006.

Engineered Deinococcus Strains as Models for Bioremediation

Our collection of >110 aerobic heterotrophic bacteria isolated from beneath tank SX-108 (Fredrickson et al., 2004) has expanded to include other interesting isolates from surface soil environments at the Hanford Site. To date, they include *D. radiodurans*, *D. murrayi*, *S. sonorensis, D. maricopensis* and *D. proteolyticus*, as well as numerous highly resistant non-deinococcal bacteria. Most interestingly, a strain of *Kocuria* rosea displays luxuriant growth on toluate (m-methylbenzoate) and related compounds as the sole carbon source under chronic IR (50 Gy/hour). As part of this project, we will continue to examine the metal-reducing and aromatic compound-degrading abilities of these Hanford Site strains. While no Hanford Site microorganism has yet been isolated which can couple metal-reduction with toxic organic compound degradation under radioactive conditions, we have succeeded in genetically engineering such *Deinococcus* strains (B.2.F), and others which reduce Hg(II) (B.2.G). Importantly, these engineered strains are serving as models for comparison with native Hanford Site bacterial isolates now under investigation.

Papers Funded by Grant DE-FG02-04ER63918 (May 2004-May 2006):

B.2.A
Omelchenko, M. V., Y. I. Wolf, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, M. J. Daly, E. V. Koonin, and K. S. Makarova. 2005. Comparative genomics of Thermus thermophilus and Deinococcus radiodurans: divergent routes of adaptation to thermophily and radiation resistance. *BMC Evol. Biol.* 20:57-78.

B.2.B
Daly, M. J., E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, K. M. Kemner, B. Lai, B. Ravel, L. P. Wackett, S.-M. W. Li, and J. K. Fredrickson. 2006. Bacterial radioresistance: determined by oxidative protein damage. (Submitted).

B.2.C
Daly, M. J. 2006. Modulating radiation resistance: insights based on defenses against reactive oxygen species (ROS) in the radioresistant bacterium Deinococcus radiodurans. *Clin. Lab. Med.* 27:1-14.

B.2.D
Qiu, X., M. J. Daly, A. Vasilenko, M. V. Omelchenko, E. K. Gaidamakova, L. Wu, J. Zhou, G. W. Sundin, and J. M. Tiedje. 2006. Transcriptome analysis applied to survival of Shewanella oneidensis MR-1 exposed to ionizing radiation. *J. Bacteriol.* 188:1199-1204.

B.2.E
Makarova, K. S., M. V. Omelchenko, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, J. K. Fredrickson, K. M. Kemner, B. Lai, B. Ravel, M. J. Daly and 4 contributors at the Joint Genome Institute. 2006. Whole genome sequence of Deinococcus geothermalis: viewed from the perspective of comparative genomics and environmental biotechnology. (To be submitted in August).

B.2.F
Brim, H., J. P. Osborne, H. M. Kostandarithes, J. K. Fredrickson, L. P. Wackett, and M. J. Daly. 2006. Deinococcus radiodurans engineered for complete toluene degradation facilitates Cr(VI) reduction. *Microbiology* (In Press).

B.2.G
Qin, J., L. Song, H. Brim, M. J. Daly, and A. O. Summers. 2006. Hg(II) sequestration and protection by the MerR metal-binding domain (MBD). *Microbiology* 152:709-719.

Most Recent Products Delivered:

Omelchenko, M. V., Y. I. Wolf, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, M. J. Daly, E. V. Koonin, and K. S. Makarova. 2005. Comparative genomics of Thermus thermophilus and Deinococcus radiodurans: divergent routes of adaptation to thermophily and radiation resistance. *BMC Evol. Biol.* 20:57-78.

Brim, H., J. P. Osborne, H. M. Kostandarithes, J. K. Fredrickson, L. P. Wackett, and M. J. Daly. 2006. Deinococcus radiodurans engineered for complete toluene degradation facilitates Cr(VI) reduction. *Microbiology* (In Press).

Daly, M. J., E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, K. M. Kemner, B. Lai, B. Ravel, L. P. Wackett, S.-M. W. Li, and J. K. Fredrickson. 2006. Bacterial radioresistance: determined by oxidative protein damage. (Submitted).

Daly, M. J. 2006. Modulating radiation resistance: insights based on defenses against reactive oxygen species (ROS) in the radioresistant bacterium Deinococcus radiodurans. *Clin. Lab. Med.* 27:1-14.

Qin, J., L. Song, H. Brim, M. J. Daly, and A. O. Summers. 2006. Hg(II) sequestration and protection by the MerR metal-binding domain (MBD). *Microbiology* 152:709-719.

Qiu, X, M. J. Daly, A. Vasilenko, M. V. Omelchenko, E. K. Gaidamakova, L. Wu, J. Zhou, G. W. Sundin, and J. M. Tiedje. 2006. Transcriptome analysis applied to survival of Shewanella oneidensis MR-1 exposed to ionizing radiation. *J. Bacteriol.* 188:1199-1204.

Other Project Information Sources:

Project URL: http://www.usuhs.mil/pat/deinococcus/index_20.htm

PNNL Contributions:

PNNL's role in this project has been to contribute Hanford-relevant organisms and the physiology and phylogeny of such organisms. PNNL also contributes specific analyses of cellular Fe and Mn contents of various organisms for comparison in relation to IR and desiccation tolerance.