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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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RESULTS TO DATE: This is the first annual report submitted for NABIR grant ER63918, which was awarded to Mike Daly (USUHS), Jim Fredrickson (PNNL) and Larry Wackett (UMN) in September 2004.

Natural selection in highly radioactive waste sites may yield bacteria with favorable bioremediating characteristics. However, until recently the microbial ecology of such environments has remained unexplored because of the high costs and technical complexities associated with extracting and characterizing samples from such sites. We have examined the bacterial ecology within radioactive sediments from a high-level nuclear waste plume in the vadose zone on the DOE?s Hanford Site in south-central Washington state (Fredrickson et al, 2004). Manganese-dependent, radiation resistant bacteria have been isolated from this contaminated site including the highly Mn-dependent Deinococcus and Arthrobacter spp.

Several environmentally relevant bacteria show a relationship between Mn accumulation, radiation resistance and metal reduction. Whereas Mn(II) salts are soluble, Mn(III,IV) oxides are relatively insoluble at circaneutral pH, and both forms are widely distributed in the environment. Mn-dependent microorganisms such as Deinococcus, Arthrobacter, Bacillus, Streptococcus and cyanobacteria spp. have been implicated in the deposition of manganese oxides in dark manganiferric rock varnish coatings on desert rocks. Organisms that belong to those groups are known for their radiation and desiccation resistance, and our recent work has established a link between the role of Mn(II) and environments known to be enriched with these organisms (Ghosal et al., 2005). We have also shown that D. radiodurans and Deinococcus geothermalis are able to utilize colloidal Mn(IV) oxides for growth in defined minimal medium, indicating that they possess the ability to reductively mobilize solid-phase Mn(IV). Metal reductase activities in these organisms might facilitate the reductive assimilation of environmental sources of Mn(III,IV) oxide, and we are characterizing the metal-reducing pathways of D. radiodurans and other radioresistant bacteria isolated from the Hanford site (Ghosal et al, 2005). Previously, we have shown that D. radiodurans is able to reduce Fe(III), Mn(III,IV), Cr(VI), U(VI) and Tc(VII). As a first step, we are purifying the Cr(VI)-reducing enzyme(s) of D. radiodurans. This is being carried out using conventional biochemical procedures such as cell fractionation and fast performance liquid chromatography (FPLC).

Ionizing radiation (IR) resistance in the manganese(II)-accumulating bacterium D. radiodurans exhibits a concentration-dependent response to manganous chloride (Daly et al, 2004). Importantly, we have recently shown that chronic IR in culture conditions where oxygen is limited induces growth of the obligate aerobic D. radiodurans. During water radiolysis, Mn(II) reacts with superoxide to produce Mn(III) and hydrogen peroxide (H2O2), and Mn(III) reacts with H2O2 to form Mn(II) and oxygen. We propose that Mn(II)-Mn(III) cycling in D. radiodurans scavenges superoxide, and that superoxide is a major protagonist in radiation toxicity. The ability of D. radiodurans to grow under chronic radiation without ambient O2 is highly relevant to the prospective use of this organism and other Mn-accumulating bacteria for bioremediation of radioactive waste sites, many of which are anaerobic. The mechanism by which high levels of intracellular Mn(II) scavenge superoxide and related reactive oxygen species (ROS) in the absence of superoxide dismutase (SOD) and catalase is not fully characterized. However, Archibald and Fridovich (1982) showed that at high concentrations, Mn(II) acts as a true catalyst of the dismutase of superoxide, with Mn cycling between the divalent and trivalent states. We believe efficient Mn-cycling occurs in D. radiodurans, where the ratio of Mn and Fe in a cell might determine the relative abundance of different ROS induced by IR since Mn-cycling favors superoxide-scavenging and O2 production without

intermediate hydroxyl radical release, whereas Fe-cycling favors the production of hydroxyl radicals and O2 without superoxide-scavenging.

Consistent with Mn-cycling, we have shown that: (i) abiotic exposure of manganous chloride solutions to acute ionizing radiation, aerobically or anaerobically, yields copious Mn oxides and O2 gas; and (ii) under a static argon (Ar) atmosphere, growth of the obligate aerobic D. radiodurans is induced by chronic IR. However, under continuous Ar flow, which removes gasses arising during irradiation, growth of D. radiodurans under chronic IR dose not occur. Since D. radiodurans is able to sustain growth under pure Ar and 50 Gy/hour, we conclude that efficient Mn-cycling scavenges substantial amounts of superoxide, generating O2 in irradiated cells, even under anaerobic conditions.

The role superoxide in our model of radiation toxicity might help explain why: (i) many organisms are killed at radiation doses that cause relatively little DNA damage; (ii) the non-metal superoxide-scavenger Tempol (4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl) is an effective radioprotector; and (iii) in eukaryotic cells, toxicity can follow irradiation of the cytoplasmic compartment of cells, where the nucleus has received no direct exposure to IR (the bystander effect), perhaps mediated by peroxynitrite produced intracellularly by the reaction of superoxide with nitric oxide. Whereas superoxide is membrane non-permeable, peroxynitrite is membrane permeable, not a substrate of superoxide dismutase and can inactivate [4Fe-4S]-containing proteins. Interestingly, the induction of growth of the obligate aerobe D. radiodurans under anaerobic, chronic radiation conditions also raises the possibility of radiation-driven ecosystems. Our finding that abiotic radiolytic systems containing Mn(II) or Fe(II) generate substantial amounts of O2 will be taken into consideration when modelling the redox chemistry of radionuclides and other metals at radioactive waste sites. Mn and Fe are widely distributed in contaminated DOE sediments, and the level of oxygenation within sediments can profoundly impact the migration of contaminants.

Our collaborative NABIR-supported papers currently under review:

1. Hg Sequestration and Protection by the MerR Metal Binding Domain (MBD)

MerR, the metalloregulator of the bacterial mercury resistance (mer) operon binds Hg(II) with high affinity. We previously engineered a small protein embodying in a single polypeptide the metal binding domain (MBD) ordinarily formed between two monomers of MerR. Here we examine the ability of MBD expressed on the cell surface of Escherichia coli or in the cytosol of Deinococcus radiodurans to sequester Hg(II), and both approaches enhanced survival of cells after Hg(II) exposure. Over 20,000 surface copies of MBD were expressed per cell with metal stoichiometries just 1.0 Hg(II) per MBD monomer. Cells expressing MBD on their surface bound 610% more Hg(II) than those not expressing the MBD. They also survived Hg(II) challenge and recovered more quickly than cells without MBD. Cell-surface expressed MBD bound Hg(II) preferentially even in the presence of a 22-fold molar excess of Zn(II) and also when exposed to equimolar Cd(II). Cytosolic expression of MBD also increased Hg(II) resistance in the radiation resistant bacterium Deinococcus radiodurans, which has been proposed for bioremediation of metal-contaminated waste sites.

2. Comparative Genomics of Thermus thermophilus HB27 and Deinococcus radiodurans R1: Divergent Paths of Adaptation to Thermophily and Radiation Resistance:

BACKGROUND: Thermus thermophilus and Deinococcus radiodurans belong to a distinct bacterial clade. However, these organisms have remarkably different phenotypes. T. thermophilus is a thermophile, which is relatively sensitive to ionizing radiation and desiccation, whereas D. radiodurans is a mesophile, which is highly radiation- and desiccation-resistant. Here we present an in-depth comparison of the genomes of these two bacteria and analyze the genetic features that are likely to be important for their survival under different stress conditions. RESULTS: We delineate the common genomic core of Thermus and Deinococcus, consisting of approximately 1,300 gene clusters, and demonstrate a high level of after-divergence gene flux in both lineages. We present an analysis of the

genome basis of distinct adaptive traits and identify the likely source of genes which are responsible for differences in the Thermus and Deinococcus physiologies. A significant bias is identified in the amino acid composition of Thermus proteins, a feature that is likely to be linked to thermostability. Various aspects of the adaptation to high temperature in Thermus can be attributed to horizontal gene transfer from archaea and thermophilic bacteria; many of these, apparently, horizontally transferred genes are located on the single megaplasmid of Thermus. In contrast, Deinococcus seems to have acquired numerous genes related to stress response systems from various bacteria. A comparison of the distribution of orthologous genes among the four partitions of the Deinococcus genome and the two partitions of the Thermus genome reveals homology between the Thermus megaplasmid and Deinococcus megaplasmid B (DR412) (Note discrepancy between here and MMBR, which refers to 2 chromosomes, 1 megaplasmid and 1 plasmid: DR_Main, DR412, one megaplasmid (DR177) and one plasmid 46 kbp). CONCLUSIONS: The comparative-genomic analysis of Thermus and Deinococcus revealed a unique, common core of ~1300 genes, which strongly supports the idea that these bacteria belong to a distinct phylogenetic lineage. However, each of the genomes also has numerous genes that apparently have been acquired via horizontal gene transfer after the divergence from the common ancestor. Some of these genes can be linked to the distinct adaptations evolved by Thermus and Deinococcus.

3. Transcriptome Analysis Applied to Survival of Shewanella oneidensis MR-1 Exposed to Ionizing Radiation.

The ionizing radiation (IR) doses that yield 17% cell survival of Escherichia coli and Deinococcus radiodurans are higher by factors of 20 and 200, respectively, than those for Shewanella oneidensis MR-1. Whole transcriptome analyses were used to identify the genes of S. oneidensis responding to 40 Gy. We observed the induction of 170 genes and repression of 87 genes in MR-1 during a 1h recovery period after irradiation. The genomic response of MR-1 to IR is very similar to ultraviolet radiation (254 nm), which included induction of systems involved in DNA repair and prophage synthesis. In contrast to the radiation resistant D. radiodurans, differential regulation of tricarboxylic acid cycle activity in MR-1 after IR was not observed and the cells strongly induced antioxidant enzymes during recovery. Given the very limited DNA damage induced by 40 Gy of IR and the large induction of its DNA repair and protection systems following irradiation, DNA damage might not be the primary cause of cell death in irradiated MR-1. Instead, protein damage produced during IR, oxidative stress after irradiation, and activation of prophages may underlie this response.

4. Radiation-Driven Oxygenic Mn-Cycling in Deinococcus radiodurans

Extreme ionizing radiation (IR) resistance in the manganese(II)-accumulating bacterium Deinococcus radiodurans exhibits a concentration-dependent response to manganous chloride. Here we show that chronic IR in anaerobic culture conditions induces growth of the obligate aerobe D. radiodurans. During water radiolysis, Mn(II) reacts with superoxide to produce Mn(III) and hydrogen peroxide (H2O2), and Mn(III) reacts with H2O2 to form Mn(II) and oxygen. We propose that Mn(II)-Mn(III) cycling in irradiated D. radiodurans scavenges superoxide and generates oxygen, and that superoxide is a major protagonist in radiation toxicity, mediated by protein damage before DNA is significantly affected.

DELIVERABLES: Published and submitted papers:

In Press:

1. D. Ghosal, M. V. Omelchenko, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, A. Venkateswaran, H. M. Kostandarithes, H. Brim, K. S. Makarova, L. P. Wackett, J. K. Fredrickson and M. J. Daly (2005) How radiation kills cells: Survival of Deinococcus radiodurans and Shewanella oneidensis under oxidative stress. FEMS Microbiology Reviews 29, 361-375.

2. M. J. Daly, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, A. Venkateswaran, M. Hess, M. V. Omelchenko, H. M. Kostandarithes, K. S. Makarova, L. P. Wackett, J. K. Fredrickson and D. Ghosal

(2004) Accumulation of Mn(II) in Deinococcus radiodurans facilitates gamma-radiation resistance. Science 306, 925-1084.

3. 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.

Submitted:

1. J. Qin, L. Song, Hassan Brim, M. J. Daly and A. O. Summers (2005) Sequestration and protection by the MerR metal binding domain (MBD). Mol. Microbiol., Submitted.

2. M. V. Omelchenko, Y. I. Wolf, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. J. Daly, E. V. Koonin and K. S. Makarova (2005) Comparative genomics of Thermus thermophilus HB27 and Deinococcus radiodurans R1: Divergent paths of adaptation to thermophily and radiation resistance. Genome Biology, Submitted.

3. X. Qiu, M. J. Daly, A. Vasilenko, M. V. Omelchenko, L. Wu, J. Zhou, G. W. Sundin and J. M. Tiedje (2005) Transcriptome analysis applied to survival of Shewanella oneidensis MR-1 exposed to ionizing radiation. J. Bacteriol., Submitted.

4. H. Brim, J. P. Osborne, A Venkateswaran, M. Zhai, J. K. Fredrickson, L. P. Wackett and M. J. Daly(2005) Facilitated chromate reduction by Deinococcus radiodurans engineered for growth on toluene. Appl. Environ. Microbiol., Submitted.

5. M. J. Daly, E. K. Gaidamakova, A. Vasilenko, V. Y. Matrosva, M. Zhai, L. P. Wackett and J. K. Fredrickson (2005) Mn-Cycling in Deinococcus radiodurans. On schedule to be submitted in June, 2005.

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