

Proposal Title

Whole-genome sequence of the bacterium *Deinococcus grandis*: A deeply branching representative of the *Deinococcus* group that is extremely radiation-resistant and the only known representative which is a facultative anaerobe.

Sequencing Description

Please briefly describe sequencing needs for your proposal. Please include estimated genome size for each whole genome shotgun organism, or estimated total sequence needs for other sequencing projects.

The complete genome of *Deinococcus grandis* is proposed for sequencing at JGI using a combination of 3 kb-, 8 kb- and fosmid- (40 kb) libraries. Library construction, sequencing, finishing, and automated annotation steps to be carried out as for *Deinococcus geothermalis*, which was successfully finished and published in collaboration with Daly's group in 2007.

Makarova K. S., M.V. Omelchenko, E.K. Gaidamakova, V.Y. Matrosova, A. Vasilenko, M. Zhai, A. Lapidus, A. Copeland, E. Kim, M. Land, K. Mavrommatis, S. Pitluck, P. Richardson, J.C. Detter, T. Brettin, E. Saunders, B. Lai, B. Ravel, K.M. Kemner, Y. I. Wolf, A. Sorokin, A. V. Gerasimova, M. S. Gelfand, J.K. Fredrickson, E. V. Koonin, **MICHAEL J. DALY** (2007) *Deinococcus geothermalis*: The Pool of Extreme Radiation Resistance Genes Shrinks, PLoS ONE, 9, e955.

The genome size of *Deinococcus grandis* is estimated to be 3.5 Mbp.

Sequencing Justification

Please briefly describe the scientific rationale for performing this sequencing.

To date, only two extremely radiation resistant bacteria have been subjected to whole-genome sequencing: *Deinococcus radiodurans* and *Deinococcus geothermalis*. In the context of understanding the genomic basis of extreme resistance phenotypes and the nature of the common ancestor of the *Deinococcus-Thermus* group, we consider *D. grandis* an appropriate next candidate for whole-genome sequencing. *D. grandis* is a deeply branching representative of *Deinococcus* that is both facultatively anaerobic and extremely resistant to ionizing radiation. Our recent finding that *D. grandis* can grow very well under strict anaerobic chronic irradiation (60 Gy/hour) conditions is a breakthrough. *D. radiodurans* and *D. geothermalis* have been proposed for bioremediation of radioactive DOE waste sites but they are obligate aerobes, a serious drawback since the most radioactive DOE waste environments are anaerobic. One approach to delineating a minimal set of genes involved in extreme resistance is to compare the whole-genome sequences of phylogenetically related but distinct species that are equally resistant, whereby genes that are unique to both organisms are ruled out, whereas shared genes are pooled as candidates for involvement in resistance. We believe

the whole-genome sequence of *D. grandis* will help further define the minimum gene set needed for extreme radiation resistance in *Deinococcus* bacteria, and for radioanaerobiosis. Such work could subsequently be complemented by whole-transcriptome and whole-proteome studies as we have done for *D. radiodurans*.

Sequence Utilization

Please briefly describe how the sequence produced will be used.

We will use the whole-genome sequence of *Deinococcus grandis* for genome comparisons with *D. radiodurans*, *D. geothermalis*, two *Thermus* species, and any other newly sequenced species belonging to this clade. Specifically, we propose to examine: genome partitioning; repeats and prophages; energy metabolism, evolutionary provenance of the genomic features previously implicated in the radiation resistance of *Deinococcus* species; further delineate the *Deinococcus* 'radiation response regulon'; reassess the genetic determinants of radiation resistance and the *Deinococcus* lineage; and evaluate the trends of gene-gain and gene-loss, and the impact of the comparative-genomic analysis of the three *Deinococcus* genomes on extreme resistance models.

Genomics Community Interest

Please briefly describe the scientific community that will utilize this sequencing product and how it stands to benefit from this work.

Since 2000, several groups (including ours) have led comprehensive bioinformatics effort aimed at deciphering the complex, multi-gene phenotype of extreme radiation resistance in the bacterial family *Deinococcus*. This group of bacteria has been engineered by our group for cleanup of radioactive DOE waste sites. Recent bioinformatic work on *Deinococcus* underscores the constraints on the degree to which functional inferences can be made from whole-genome transcriptome analyses based on a single organism. For example, two independent analyses of gene induction in *D. radiodurans* recovering from different radiation doses revealed numerous genes that are upregulated during the post-irradiation recovery, many of which were viewed as plausible candidates for a significant role in resistance. The hierarchy of induced genes in both transcriptome analyses was very similar, however, most of the highly induced *D. radiodurans* genes have no orthologs in *Deinococcus geothermalis*, and knockout of many of the uncharacterized unique *D. radiodurans* genes that were strongly induced by radiation had little effect on radiation resistance. A similar paradigm is emerging from the analysis of other systems, where the cellular transcriptional response to stress was largely stochastic, and frequently involving genes known to be unrelated to the mechanisms under investigation. Thus, it stands to reason that any comprehensive bioinformatics effort aimed at deciphering a complex, multi-gene phenotype using whole-genome, transcriptome and proteome approaches should aim to study at least three closely-related species. The whole-genome sequence of *Deinococcus grandis* would be used by DOE-, NIH, and NSF-funded investigators now dedicated to fundamental and applied studies on extreme resistance mechanisms.

DOE Mission Relevance

Please briefly describe the relevance of this research to DOE Missions and goals.

Based on the ability of *Deinococcus* bacteria to reduce a variety of metals including U(VI), Cr(VI), Hg(II), Tc(VII), Fe(III) and Mn(III,IV), this group of extremely radiation resistant bacteria (including *D. grandis*) have been viewed as prospective candidates for bioremediation of radioactive waste sites maintained by DOE. These characteristics were the impetus for whole-genome sequencing of *D. radiodurans* and *D. geothermalis* by DOE. The justification for sequencing *Deinococcus grandis* would build on this investment. Our recent finding that *D. grandis* can grow exceptionally well under strict anaerobic chronic irradiation (60 Gy/hour) conditions is a breakthrough. In contrast, *D. radiodurans* and *D. geothermalis* have been proposed for bioremediation of radioactive DOE waste sites but they are obligate aerobes, a serious drawback since the most radioactive DOE waste environments are anaerobic.

The USUHS *Deinococcus grandis* strain: The USUHS *D. grandis* strain was originally obtained by the Minton Laboratory in 1989, directly from the Oyaizu lab (Oyaizu *et al.*, *Int. J. Sys. Bacteriol* **37**, 62-67, 1987). Over the years, we have published radiation resistance of this strain, its cell morphology by TEM, and growth under high-level chronic irradiation. Most recently, we showed it can grow slowly on solid medium under strictly anaerobic conditions. In the original paper, Oyaizu described several *D. grandis* strains and it is not clear if the USUHS *D. grandis* strain is the one which was deposited at DSMZ (DSM 3963). The USUHS *D. grandis* strain was sent for microbial ID (MIDI Labs), and it came back as most closely related (99%) to *Deinococcus ficus*, which almost overlaps *D. grandis* on phylogenetic trees based on 16S rRNA gene sequences (Lai, W-A, *et al.*, *Int. J. Sys. Evo. Microbiol.* **56**, 787-791 (2006)).