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Project Detail	ID: ---/ER61680/0000173 Principal Investigator: Robert V. Miller 405-744-7180 Co-PIs: Institution: Oklahoma State University Title: Comparative Evolution of the recA Gene of Surface and Deep Subsurface Microorganisms (An Evolutionary Clock of Intermediate Rate) ER Division: ER-74 Program Manager: Frank J. Wobber (301)903-5549 Research Areas: Cleanup
	Project Progress Most recent report of results to date: FINAL REPORT Demonstration of the Effects of Mutation in the recA gene of <i>Pseudomonas aeruginosa</i>. Identification of the Nucleotide Base Changes in Two recA Alleles with Mutant Phenotypes. Because of the ability of the RecA protein product to maintain both DNA integrity and increase genetic diversity, this gene may be essential to the survival of microorganisms following the damaging effects of numerous environmental stresses such as exposure to solar UV radiation, exposure to gamma radiation, starvation, and changing environments. While the various activities and amino-acid sequence of RecA have been highly conserved among the eubacteria and archaea, little is known as to whether a strict structure-function relationship has been conserved. In other words, are the same regions of this highly plastic, functionally heterogeneous protein involved in the same catalytic capacities throughout the bacterial kingdom? While it is reasonable to assume that this type of conservation has also occurred, we felt it necessary to test the assumption by demonstrating that mutations in different genera of bacteria which eliminate similar functions (i.e., lead to similar phenotypes) are caused by changes in the amino-acid sequence in the same regions of their RecA proteins. Therefore, we located the changes in nucleotide sequence in two recA mutants of <i>P. aeruginosa</i> which displayed mutant phenotypes in recombination and UV resistance. Our assumption was that if structure-function relationships held, these mutations would be found in areas already identified as essential for the function of the <i>E. coli</i> RecA protein.
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The sequence changes found in each of the *P. aeruginosa* mutants led to a single amino acid change. In strain RM8 (recA908), the change was Ser-69 to Phe and in strain RM265 (recA102) the change was Gly-27 to Asp. These changes were computer modeled using the crystal structure of *E. coli* RecA as a template. In both cases the changes were found to be incompatible with the wild-type structure. The amino acid change in RM8 is within a site believed to be involved in nucleotide binding and hydrolysis. The putative protease constitutive nature of this mutant would be explained if this mutant protein had not lost the ability to bind ATP but had lost the ability to hydrolyze it. The change in RM265 occurred near the site believed to serve as contacts between RecA polymers causing bundling and inhibition of RecA activity. The change at this site could serve to stabilize the polymer interactions, thus inhibiting their functions. This would explain the apparently null phenotype of recA102. These data are consistent with the assumption of structure-function conservation in RecA.

Effects of UVA and UVB Irradiation on the Expression and Function of the recA Gene.

Solar radiation contains UVA and UVB wavelengths. UVC is currently filtered from sun light by the ozone layer before it reaches the earth's surface. Thus UVA and UVB radiation have greater potential for altering survival in natural environments than does UVC. Unfortunately, little is known about the role of the RecA protein in protection from and repair of damage caused by UVA and UVB. Most studies have been done using UVC wavelengths because these are known to be absorbed directly by DNA and have been important for sterilization of medical devices and spaces. To evaluate the importance of the recA gene in environmental survival of microbes, we wished to determine the role of this gene's protein product in UVA-UVB survival. To do this, we used as our model organism *P. aeruginosa* and followed the physiological and molecular consequences of inactivation of the recA gene on survival following exposure to solar UV wavelengths. *P. aeruginosa* was chosen as a model because it is an autochthonous soil and aquatic organism whose recA gene we have studied in great detail.

P. aeruginosa recA mutants (both RM8 or RM265) were found to be more sensitive to both UVA and UVB radiation than were their RecA⁺ isogenic parents. Introduction of a low-copy-number plasmid containing the cloned wild-type *P. aeruginosa* recA allele restored UVA and UVB resistance to these mutants. These data demonstrate the involvement of the RecA protein in some, as yet undefined, way in protection from UV wavelengths found in sun light. We also found that the concentration of RecA protein increased two-fold, 120 minutes after exposure to either UVA or UVB radiation suggesting these wavelengths capable of inducing expression of the recA gene. A functional RecA protein was found to be required for activation of bacteriophage prophages from lysogenic cells following exposure to UVB radiation. Prophage were not induced by exposure of their hosts to UVA radiation. Induction of damage-inducible (din) genes in response to UVA or UVB radiation was also shown to be RecA dependent. These data indicate that the recA gene plays a role in the

regulation of genes and functions associated with the SOS Global Regulon following exposure of this environmentally significant species to UVA and UVB radiation. (These results have been published in Microbiology.)

Isolation and Characterization of the *recA* Gene from *Arthrobacter*, an Environmentally Significant Bacterial Species -- Placement in the Phylogenetic Array using *recA* Sequence Characteristics.

We have carried out an examination of a subcollection from the Subsurface Microbiology Culture Collection (SMCC) in order to validate the potential of the *recA* gene for use as a tool in estimating evolutionary uniqueness of environmental populations of microorganisms. Following initial discussions with Dr. David Balkwill, curator of the SMCC, we decided to direct our preliminary assessment to the relatively well-characterized collection of isolates from the Savanna River Site (SRS). The most prevalent group of aerobic organisms isolated from this site was *Arthrobacter* spp. We began our studies with the characterization of the *recA* gene from these isolates. No *recA* gene had been identified in an *Arthrobacter* species when we began our studies. We chose to investigate the *recA* gene from the ATCC type strains of various *Arthrobacter* species in preparation for our analysis of the deep subsurface isolates. These studies were carried out for two reasons. First, and most obviously, the sequences of the type strains of these species were needed as an index in order to allow clade analysis of the subsurface isolates and their comparison to surface organisms. Second, by determining whether we could use our *recA* sequence data to place these species in phylograms was important to insure that our future analysis of the subsurface isolates would be valid. We felt it important to see if our phylograms would reflect those produced by use of 16S rRNA sequence analysis since rRNA sequence would be useful in establishing the species of environmental isolates. Our analysis of the phylogenetic tree based on analysis of the *Arthrobacter recA* gene placed this group in the same phylogenetic relationship to other Gram(+) species as does 16S rRNA analysis. Thus, our assumption that *recA* analysis can be used to determine evolutionary relationships among both Gram(+) and Gram(-) autochthonous soil organisms was validated.

Analysis of *Arthrobacter* spp. Isolates from the Savanna River and Hanford Sub-Collection.

Our analysis of deep subsurface isolates was restricted to selected isolates from the SRS and Hanford sub-collections that had been previously classified as *Arthrobacter* sp. By 16S rRNA sequence analysis. These organisms were monitored for resistance to UV irradiation and their *recA* genes were amplified and sequenced.

UV resistance of Deep Subsurface Isolates.

One important question that relates to the conservation of the *recA* gene in deep-subsurface populations is whether there has been an evolutionary selection for the retention of an active *recA* gene and whether increased *recA*-gene activity can lead to increased survivorability and rates of genetic change. As a first indication of the

functionality of the *recA* genes in SRS isolates, we measured the survival capacity of these organisms following UVC irradiation. While not the most appropriate wavelengths for in situ correlation, UVC survival rates are more simply determined than are UVA or UVB curves and can be most easily correlated with the available scientific literature. From ours and other investigators' studies, UVC survival rates correlate well with the activity level of RecA protein.

UVC-survival curves were run on all of the SRS *Arthrobacter* isolates obtained from the SRS collection. These consisted of two isolates from the top soil, three from the Black Creek, and five from the Middendorf. The UV sensitivity of these isolates was compared to type strains. All isolates were supplied to us by Dr. Balkwill, curator of the SMCC collection. In general, we found that surface strains had sensitivities comparable to the type strains. Deep-subsurface strains were as UV resistant as the surface strains and even appeared to be very slightly (but consistently) more resistant than the surface strains. These data support the hypothesis that the *recA* genes of deep-subsurface isolates are functional.

Clade Analysis of *Arthrobacter* Isolates from SRS and Hanford.

The *recA* alleles from the SRS and Hanford *Arthrobacter* spp. isolates were sequenced and the data used to obtain a cladogram of the isolates. The trees produced were "rooted" with the *Streptomyces ambiofiaciens recA* gene sequence as an outgroup. The trees were generated using the PHYLIP Software Package (Felsenstein J. 1989. *Cladistics* 5:164-166). Alternative trees based on maximum parsimony (MPM) and distance matrix methods (CDMM) gave similar results.

A clade is a group of organisms (or in the case of this study, alleles) which share a common ancestor which is not shared by any other group outside the clade. Cladograms have often been used to determine relatedness between groups of subspecies. One such study by Avise and Nelson (1989. *Science* 243:646-648) used cladograms derived by the MOM analysis of mitochondrial DNA sequences to demonstrate that the subspecies of dusky seaside sparrows from the Atlantic Coast were more closely related to each other than to any of the subspecies dwelling on the Gulf Coast. The Gulf Coast sparrows were likewise more closely related to each other evolutionarily than to any of the Atlantic Coast Subspecies.

MPM was developed by Eck and Dayhoff (1966. *Atlas of Protein Sequence and Structure*. National Biomedical Research Foundation, Silver Spring, MD) for analysis of amino-acid sequences and later modified for use with nucleotide sequences by Fitch (1977. *Am. Nat.* 11:223-257). It compares sequences site by site and utilizes "informative sites" (i.e., ones in which a nucleotide base change has taken place) to determine phylogenetic gene trees. Each possible tree of alleles is developed and the minimum number of substitutions at each informative site necessary to construct that tree is calculated. Finally, the total number of substitutions is summed over all informative sites for each tree, and the tree associated with the smallest number of substitutions is chosen as the most likely. This is done on the assumption that the most likely evolutionary path to the array of alleles observed is the one which requires the smallest number of sequence

changes. To increase the probability that there have been no topological errors in construction of a tree, the largest possible data set should be used to develop the cladogram.

DMM uses the unweighted pair method with arithmetic mean (UPGMA) developed by Sokal and Michener (1958. Univ. Kansas. Sci. Bull. 28:1409-1438) as modified by Nei (1975. Molecular Population Genetics and Evolution. North Holland, Amsterdam). This is the simplest method for the construction of gene trees. It produces gene trees that emphasize the similarities between alleles as a measure of evolutionary relatedness. As such, these trees are usually more appropriate for development of phylograms which measure phylogenetic relationships and distances.

What conclusions can be made from our preliminary study of the SRS *Arthrobacter* spp. isolates? First, it appears that while 16S rRNA sequences are very informative in determining inter-species relationships, they are less informative in developing clade analysis of isolates showing intra-species relationships (i.e., strains). This observation supports the conclusions that 16S is too slow a clock to be able to robustly differentiate relationships among deep subsurface isolates of the same species. The *recA* sequence, on the other hand, is evolving too rapidly to be greatly informative above the intra-species level. However, *recA* analysis is very useful in developing clade relationships between the closely-related subsurface isolates. Second, our specific conclusions include the observation that the deep-subsurface isolates seem most closely related to *Arthrobacter* oxidans and that clades show grouping by geographical formation suggesting that evolution in place may have taken place.

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Journal Articles

van Waasbergen, L. G., Saranga P. Kidambi, and R. V. Miller. 1997. Construction of a *recA* mutant of *Burkholderia* (formerly *Pseudomonas*) *cepacia*. Applied Microbiology and Biotechnology (in press).

Kidambi, S. P., M. G. Booth, Tyler A. Kokjohn, and R. V. Miller. 1996. *recA*-dependence of the response of *Pseudomonas aeruginosa* to UVA and UVB irradiation. Microbiology 142:1033-1040.

In Preparation

van Waasbergen, L. G. And R. V. Miller. Genetic analysis of two mutations of the *recA* gene of *Pseudomonas aeruginosa*. In preparation.

van Waasbergen, L. G., D. Balkwill, and R. V. Miller. Cladistic analysis of *Arthrobacter* spp. isolated for the deep subsurface at a Savanna River Site. In preparation.

van Waasbergen, L. G. D. Balksill, and R. V. Miller. Evolutionary relationships of *Arthrobacter* spp. isolated from the deep subsurface at a site in Northwestern United States. In preparation.

Abstracts

Miller, R. V., and L. G. van Waasbergen. 1996. Deducing evolutionary relationships among surface and deep subsurface *Arthrobacter* species by measuring gene-sequence divergence of *recA* DNA sequences, p. 83. Proceeding of the Fifth International Symposium on Bacterial Genetics and Ecology. Nauplion, Greece.

van Waasbergen, L. G. A. El-Masri, and R. V. Miller. 1996. Deducing the phylogenetic relationships of deep-subsurface *Arthrobacter* species through evolution of the *recA* gene, p. 404. Abstracts of the Annual Meeting of the American Society for Microbiology.

van Waasbergen, Lorraine G., and R. V. Miller. 1995. Structure-function analysis of *recA* in *Pseudomonas aeruginosa*, p. 560. Abstracts of the General Meeting of the American Society for Microbiology.

El-Masri, A., S. D. Walls, L. G. van Waasbergen, J. T. Brown, and R. V. Miller. 1995. Analysis of *recA* expression and evolution in deep-subsurface *Arthrobacter* species, p. 443. Abstracts of the General Meeting of the American Society for Microbiology.

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