Lateral gene transfer in the subsurface

Final report

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Lateral gene transfer (LGT) is an important adaptive mechanism among prokaryotic organisms. This mechanism is particularly important for the response of microorganisms to changing environmental conditions because it facilitates the transfer of a large number of genes and their rapid expression. Together the transferred genes promote rapid genetic and metabolic changes that may enhance survival to newly established and sometimes hostile environmental conditions.

The goal of our project was to examine if and how LGT enhances microbial adaptation to toxic heavy metals in subsurface environments that had been contaminated by mixed wastes due to activities associated with the production of nuclear energy and weapons. This task has been accomplished by dividing the project to several sub-tasks. Thus, we:

1. Determined the level of resistance of subsurface bacterial isolates to several toxic metals, all identified as pollutants of concern in subsurface environments
2. Designed, tested, and applied, a molecular approach that determined whether metal resistance genes had evolved by LGT among subsurface bacteria
3. Developed a DNA hybridization array for the identification of broad host range plasmids and of metal resistance plasmids

The results are briefly summarized below with references to published papers and manuscripts in preparation where details about our research can be found. Additional information may be found in copies of our published manuscripts and conference proceedings, and our yearly reports that were submitted through the RIMS system.

Metal and radionuclide resistance among subsurface bacteria: Three collections of subsurface bacterial isolates were examined for their resistance to mercury, lead, and chromate. These were from the DOE sites in Savannah River (SRS) and Hanford (Benyehuda, Coombs et al. 2003) and from the Field Research Center (FRC) in Oak Ridge, TN (Martinez, Wang et al. 2006). The FRC collection was also tested for resistance to cadmium. Results showed that resistances were broadly distributed among the tested strains and that the distribution varied among the collections. Metal resistance levels and distribution were related to both the taxa composition of the collections and to the prevailing environmental conditions of the site. Some of the FRC isolates were also shown to be highly resistant to uranate (UO_2^{-2}) at pH 4.0 (Martinez, Wang et al. 2006). In all collections, *Arthrobacter* spp., common soil bacteria belonging to the actinobacteria phylum, stood out for their high level of resistance. **Together, the data showed a high level of metal resistance among subsurface bacteria suggesting that the toxicity of**

the tested metals may be of little concern when remedial treatments, which are based on microbial activities, are stimulated in the subsurface.

Lateral transfer of lead resistance among subsurface bacteria: We have developed an experimental approach that allowed us to assess the role of LGT in the evolution of resistance to lead among bacterial isolates. The approach is based on the identification of molecular signatures implicating LGT in the evolution of lead resistance genes of subsurface bacteria (Coombs and Barkay 2004). Briefly, we developed a nested PCR approach for the amplification of the gene that encodes for lead resistance (*pbrA*), obtained these sequences from the genomes of lead resistant subsurface bacteria, and then compared the phylogeny of these genes to the phylogeny of the 16S rRNA gene obtained from the same strains. Incongruence between the position in the *pbrA* phylogenetic tree and the position of the cognate rRNA gene in the rRNA gene phylogenetic tree suggested evolution by LGT, a suggestion that was then tested by considering the G+C mol percent of *pbrA* relative to that of the organism's genome. This approach has identified four instances of LGT among 40 lead resistant SRS strains (Coombs and Barkay 2004) and 10 among 50 FRC strains (Martinez, Wang et al. 2006). In comparison, when LGT of the *pbrA* gene was examined in complete microbial genomes that are available from databases, only 13 out of close to 300 genes contained evidence for evolution by LGT (Coombs and Barkay 2005). **Thus, our results show that lead resistance is horizontally transferred among bacteria that reside in metal contaminated subsurface soils. Lateral gene transfer may therefore be a process that enhances competence of microorganism in metal and radionuclide contaminated subsurface environments.**

The genetic elements that facilitate LGT among subsurface bacteria: To further study LGT and its mechanisms in subsurface microbial communities we designed and optimized a gene transfer hybridization array (Coombs, Chatziefthimiou et al. in preparation). The array consists of 261 70-mer probes, 170 of which target origin of replication regions in broad host range (BHR) plasmids, and the remaining 91 target metal resistance genes. Plasmid DNA is labeled with Cy3 or Cy5 and hybridized to the array. Positive hybridization signals identify the origin of replication of BHR plasmids from metal resistant subsurface bacteria and indicate whether metal resistance genes are found on the plasmids. To date, several plasmids from FRC and SRS metal resistant strains have been characterized by the hybridization array (Coombs, Chatziefthimiou et al. in preparation). The hybridization array is therefore a tool for the classification of BHR plasmids and their identification as metal resistance plasmids. Such plasmids may be involved in the spread of metal resistance in microbial communities in subsurface soils. **The gene transfer array will be an important tool in future studies on the genetic elements that facilitate LGT in the subsurface and other environments.**

References

Benyehuda, G., J. Coombs, et al. (2003). "Metal resistance among aerobic chemoheterotrophic bacteria from the deep terrestrial subsurface." Canadian Journal of Microbiology 49(2): 151-156.

Coombs, J. M. and T. Barkay (2004). "Molecular evidence for the evolution of metal homeostasis genes by lateral gene transfer in bacteria from the deep terrestrial subsurface." Applied and Environmental Microbiology 70(3): 1698-1707.

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Martinez, R. J., Y. Wang, et al. (2006). "Horizontal gene transfer of PIB-type ATPases among bacteria isolated from radionuclide- and metal-contaminated subsurface soils." Applied and Environmental Microbiology 72(5): 3111-3118.

Publications and conference presentations that have resulted of this project

(Note: Efforts to upload copies of published manuscripts to the STI system have failed. The listed manuscripts and conference abstracts are available from the PI's upon request)

I. *Published papers and submitted manuscripts*:

Benyehuda, G., J. Coombs, P.M. Ward, D. Balkwill, and T. Barkay. 2003. Metal resistance among aerobic chemoheterotrophic bacteria from the deep terrestrial subsurface. Can. J. Microbiol. 49:151-156.

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Barkay, T., and B.F. Smets. 2005. Horizontal gene flow in microbial communities. ASM News 71:412-419

Smets B.F., and T. Barkay. 2005. Horizontal gene transfer: Perspectives at a crossroads of scientific disciplines. Nat. Rev. Microbiol. 3:675-678

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Coombs, J.M., and T. Barkay. 2005. New findings on evolution of metal homeostasis genes: Evidence from comparative genome analysis of bacteria and archaea. Appl. Environ. Microbial. 71:7083-7091

Martinez, R.J. Y. Wang, M.A. Raimondo, J.M. Coombs, T. Barkay, and P.A. Sobecky. 2006. Horizontal gene transfer of P_{IB}-type ATPases among bacteria isolated from radionuclide- and metal contaminated subsurface soils. Appl. Environ. Microbiol. 72:3111-3118

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Coombs, J.M., A. Chatziefthimiou, P.A. Sobecky, and T. Barkay. A DNA hybridization array for the analysis of the genetic linkage of metal resistance genes on bacterial plasmids. In preparation

Sobecky, P.A. and J. M. Coombs. Horizontal Gene Transfer in Metal and Radionuclide Contaminated Soils. Invited Book Chapter, in preparation.

II. *Conference presentations*:

Barkay, T., G. Benyehuda, and D. Balkwill. Metal resistance among bacteria isolated from subsurface cores. DOE-NABIR PI Workshop. Warrenton, VA. March 12-14, 2001.

Coombs J.M., G. Benyehuda, and T. Barkay. A nested PCR approach to examine the molecular basis of resistance via heavy metal efflux pumps among bacteria of the deep terrestrial subsurface. 102th Annu. Meet. Am. Soc. Microbiol. Salt Lake City, May 19–23, 2002, and Bioremediation and Biodegradation: Current Advances in Reducing Toxicity, Exposure and Environmental Consequences, Asilomar Conference Center, Pacific Grove, California, June 1 –12, 2002.

Coombs J.M., G. Benyehuda, J. de Lipthay, S. Sørensen, and T. Barkay. Lateral gene transfer of genes encoding heavy metal efflux pumps in bacteria of the deep terrestrial subsurface. International Symposium on Subsurface Microbiology, Copenhagen, Denmark, Sept. 8–13, 2002, and The 34th Mid-Atlantic Industrial & Hazardous Waste Conference, Rutgers University, New Brunswick, NJ, Sept. 20-21, 2002.

Coombs, J.M., and T.Barkay. Lateral transfer of metal homeostasis genes: a comparison between surface bacteria and isolates from the deep terrestrial subsurface. 103th Annu. Meet. Am. Soc. Microbiol. Washington DC, May 18–22, 2003.

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Raimondo, M., M. Humphrys, J.M. Coombs, T. Barkay and P.A. Sobecky. 2005. Heavy Metal Resistance of Aerobic Subsurface Chemoheterotrophs Obtained from the Field Research Center, Oak Ridge, Tennessee. Poster presentation. Annual American Society for Microbiology Meeting, Atlanta, GA.

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Sobecky, P.A. 2007. Horizontal Gene Transfer of P_{IB}-type ATPases among Bacteria Isolated from Radionuclide and Metal Contaminated Subsurface Soils. Invited Talk presented at the 9th Symposium on Bacterial Genetics and Ecology (BAGECO 9), Wernigeroden, Germany.

Martinez, R.M. and P.A. Sobecky. 2007. Microbial Ecology of Extreme Environments. Invited Oral Presentation, Clayton State University.