

Evolution of Deep Subsurface Microbial Life by Horizontal Gene Transfer Processes Mediated by Plasmids and Phages

DUSEL White Paper by Patricia Sobecky (School of Biology; Georgia Institute of Technology)

Analysis of completed microbial genomes unequivocally demonstrates that horizontal gene transfer continues to be an important factor contributing to the innovation of microbial genomes (Beiko et al., 2005; Gogarten and Townsend, 2005; Nakamura et al. 2004). Horizontal gene transfer (HGT) mediated by mobile genetic elements such as plasmids, insertion sequences, integrons, transposons, and phages has been shown to provide microbes with a wide variety of adaptive traits for microbial survival and proliferation (e.g., antibiotic and heavy metal resistance, diverse metabolic capabilities including xenobiotic degradation and virulence). While point mutations contribute to microbial adaptation, the horizontal dissemination of genes has proven to be more critical in promoting rapid genomic flexibility and microbial evolution (Thomas and Nielsen, 2005). However, HGT among some subsurface microbial populations, particularly those present in the deep subsurface, has been postulated to be unlikely to occur owing to the low cell densities and the low permeability of the soil strata (van Waasbergen et al. 2000).

Recently, detectable HGT of bacterial P_{IB} -type ATPases in bacterial isolates from a deep subsurface environment free of heavy metal contamination has been reported (Coombs and Barkay, 2004). The P -type ATPases represent a chromosomally-encoded superfamily of ion translocating proteins present in all three domains of life. The prokaryotic heavy metal translocating P_{IB} -type ATPases detoxify the cell cytoplasm by effluxing the divalent ions of cadmium, cobalt, lead, nickel and zinc. The P_{IB} -type ATPases represent one of three mechanisms for promoting microbial heavy metal resistance or tolerance: 1) metal reduction, 2) metal complexation and 3) ATP-dependent metal efflux. Previous studies have also identified the presence of P_{IB} -type ATPase genes encoded on mobile genetic elements (i.e., plasmids and transposons) present in both Gram-positive (Nucifora et al., 1989) and Gram-negative bacteria (Mergeay et al., 2003). Although the HGT of P_{IB} -type ATPases was detected in only a few isolates, the extent of HGT may have been underestimated due to the close relatedness of the bacterial lineages studied (Coombs and Barkay, 2004). My research group recently examined the extent of horizontally transferred P_{IB} -type ATPases among bacteria cultured from subsurface soils with a history of radionuclide and heavy metal contamination (Martinez et al., 2006). The isolates were obtained from soils sampled from the DOE Field Research Center (FRC) located within the Oak Ridge National Laboratory Reservation (Oak Ridge, TN). The FRC subsurface represents an extreme geochemical environment that provides a number of stresses on the extant microbial community, which include low pH (e.g., < 4), nitrate concentrations that can exceed 100 mM, as well as co-occurring heavy metals and radionuclides (U and other actinides; <http://www.esd.ornl.gov/nabirfrc>). Our main objective was to examine the role of HGT in the evolution of metal homeostasis using phylogenetic analyses of sequences of *zntA/cadA/copA*-like genes amplified from the genomes of 50 lead resistant (Pb^r) subsurface bacteria. Our results indicate that the dissemination of P_{IB} -type ATPases by HGT has occurred recently among isolates representing the *Actinobacteria*, *Firmicutes* and *Proteobacteria* phyla present in metal and radionuclide contaminated soils of the FRC (Martinez et al., 2006).

The fundamental processes of HGT that shape the diversity of microbial assemblages in the deep subsurface are largely unknown. The scientific objectives that could be addressed in regard to subsurface microbial evolution in the deep subsurface include the following. What is the extent of HGT as a source or mechanism for genomic, evolutionary and ecological innovation in the deep subsurface? How relevant is HGT if deep subsurface microbes "live isolated existences"? Are the mobile genetic elements (plasmids, phages etc.) molecularly distinct from their shallow subsurface counterparts? Is there selection for or against (metabolic) specialization in the deep subsurface? The experimental design to address some of these fundamental questions would require a multi-phasic approach. A retrospective and prospective

experimental methodology needs to be conducted to 1) determine the extant (distribution) and molecular signatures of plasmids and phages; 2) targeting of key metabolic genes/pathways to determine their evolutionary histories; 3) 'real-time' HGT experiments designed to quantify the contributions of HGT mediated process in the deep subsurface preferably using endogenous mobile genetic elements that would be obtained during studies to determine their distribution and molecular diversity. The anticipated outcomes are several-fold. It is expected that mobile genetic elements characterized from deep subsurface microbial communities will be unique from their shallow subsurface and aquatic counterparts. My group has previously demonstrated that plasmids from marine microbial populations are unique and unrelated to the well-characterized broad- and narrow-host range replicons from the *Enterobacteriaceae* (Sobecky et al., 1997; 1998; Cook et al. 2001; Hazen et al. in press). We expect that deep subsurface MGEs will exhibit novel and as yet unknown lineages. We also expect that HGT-mediated processes will significantly contribute to genome diversification and innovation in potentially surprising ways.

Literature Cited:

- Beiko, R. G., T. J. Harlow, and M. A. Ragan.** 2005. Highways of gene sharing in prokaryotes. *Proc. Natl. Acad. Sci. USA* **102**:14332-14337.
- Cook, M.A., A.M. Osborn, J.A. Bettendorff, and P.A. Sobecky.** 2001. Endogenous isolation of replicon probes for assessing plasmid ecology of marine sediment microbial communities. *Microbiology* **147**:2089-2101.
- 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. *Appl. Environ. Microbiol.* **70**:1698-1707.
- Gogarten, J. P., and J. P. Townsend.** 2005. Horizontal gene transfer, genome innovation and evolution. *Nat. Rev. Microbiol.* **3**:679-687.
- Hazen, T.H., Wu, D. Eisen, J.A., and P.A. Sobecky.** 2007. Sequence characterization and comparative analysis of three plasmids isolated from environmental *Vibrio* spp. (in press). *Applied and Environmental Microbiology*.
- Martinez, R.J., Wang, Y., Raimondo, M.A., Coombs, J.M., Barkay, T., and P.A. Sobecky.** 2006. Horizontal gene transfer of P_{IB}-type ATPases among bacteria isolated from radionuclide- and metal-contaminated subsurface soils. *Applied and Environmental Microbiology*. **72**:3111-3118.
- Mergeay, M., S. Monchy, T. Vallaey, V. Auquier, A. Benotmane, P. Bertin, S. Taghavi, J. Dunn, D. van der Lelie, and R. Wattiez.** 2003. *Ralstonia metallidurans*, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes. *FEMS Microbiol. Rev.* **27**:385-410.
- Nakamura, Y., T. Itoh, H. Matsuda, and T. Gojobori.** 2004. Biased biological functions of horizontally transferred genes in prokaryotic genomes. *Nature Genetics* **36**:760-766.
- Sobecky, P.A., T.J. Mincer, M.C. Chang, and D.R. Helinski.** 1997. Plasmids isolated from marine sediment microbial communities contain replication and incompatibility regions unrelated to those of known plasmid groups. *Applied and Environmental Microbiology* **63**:888-895.
- Sobecky, P.A., T.J. Mincer, M.C. Chang, A. Toukdarian, and D.R. Helinski.** 1998. Isolation of broad-host-range replicons from marine sediment bacteria. *Applied and Environmental Microbiology* **64**:2822-2830.
- van Waasbergen, L. G., D. L. Balkwill, F. H. Crocker, B. N. Bjornstad, and R. V. Miller.** 2000. Genetic diversity among *Arthrobacter* species collected across a heterogeneous series of terrestrial deep-subsurface sediments as determined on the basis of 16S rRNA and *recA* gene sequences. *Appl. Environ. Microbiol.* **66**:3454-3463.