

## ***Acidovorax* species reveal potential for oxidation of H<sub>2</sub> coupled to reduction of O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, and radionuclides across a subsurface redox gradient**

A.E. PLYMALE<sup>1\*</sup>, J-H. LEE<sup>1,2</sup>, J.K. FREDRICKSON<sup>1</sup>,  
A.C. DOHNALKOVA<sup>1</sup>, J.J. MORAN<sup>1</sup>, C.T. RESCH<sup>1</sup>,  
J.P. MCKINLEY<sup>1</sup>, L. SHI<sup>1</sup>, E.E. RODEN<sup>3</sup>,  
AND B.J. CONVERSE<sup>3</sup>

<sup>1</sup>Pacific Northwest National Laboratory, Richland, WA, USA  
(\*correspondance: plymale@pnl.gov)

<sup>2</sup>Korea Institute of Geoscience and Mineral Resources,  
Daejeon, Republic of Korea

<sup>3</sup>University of Wisconsin, Madison, WI, USA

In the aquifer at the 300 Area of the Hanford Site in south-central Washington, we observed increasing H<sub>2</sub> at depth, along with decreasing O<sub>2</sub> and NO<sub>3</sub><sup>-</sup>, within the fine grained Ringold formation, near its interface with the overlying coarse-grained Hanford formation. To characterize the microorganisms influencing this chemical gradient, we incubated sterile sand, in-well, across the redox transition.

We detected H<sub>2</sub>-driven <sup>99</sup>TcO<sub>4</sub><sup>-</sup> reduction in laboratory microcosms constructed from colonized sand from all depths, with a reduction maximum just below an observed interface between oxidized and reduced sediments within the Ringold formation (17 m bgs). From this depth, which was coincident with the highest in-situ NO<sub>2</sub><sup>-</sup> concentration, we isolated 11 bacterial strains, including 5 *Acidovorax* species, from H<sub>2</sub> + NO<sub>3</sub><sup>-</sup> enrichments.

A representative *Acidovorax* strain, JHL-9, was selected for further experiments and genome sequencing. Under denitrifying conditions, JHL-9 could couple the oxidation of H<sub>2</sub> to the reduction of pertechnetate (<sup>99</sup>TcO<sub>4</sub><sup>-</sup>), consistent with the presence of two NiFe hydrogenase genes in the genome [1]. Strain JHL-9, whose genome contains Rubisco CO<sub>2</sub> fixation genes, also grew lithoautotrophically on bicarbonate and H<sub>2</sub>, using either NO<sub>3</sub><sup>-</sup> or O<sub>2</sub> as an electron acceptor, and was shown to harbor the genes necessary for full reduction of NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>.

The results suggested that H<sub>2</sub>-oxidizing bacteria such as *Acidovorax* could contribute to the maintenance of the redox gradient in the Hanford/Ringold transition zone and influence the migration of contaminants in the Hanford subsurface [2].

[1] Shi *et al* (2011) *Appl. Environ. Microbiol.* **77**, 5584-5590.

[2] Lin *et al* (2012) *Environ. Microbiol.* **14**, 414-425.