



Influence of Bacterial Pathogen Condition on Cell Transport in Groundwater Environments

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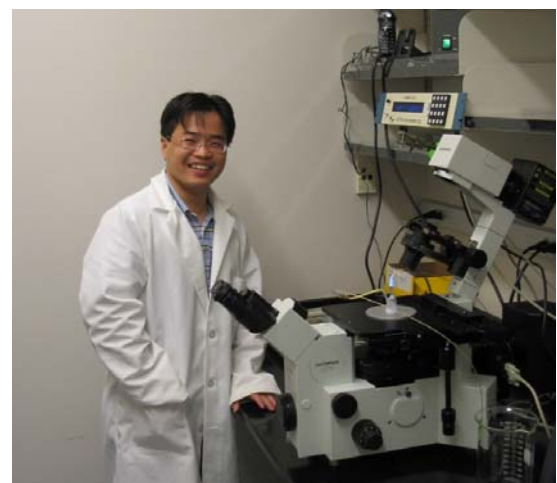
A systematic and extensive examination of the physiological and environmental factors controlling bacterial adhesion and transport is actively being pursued. Currently we are investigating the role that extracellular polymeric substances (EPS) exuded by cells plays in controlling the fate of pathogenic bacteria in groundwater environments.

Due to California's continuing population growth driving demand for an increased municipal water supply and political pressures for decreased reliance on Colorado River water, there is a vital need to ensure protection and quality of groundwater. In the 2,800 square miles covered by the Santa Ana Regional Water Quality Control Board, with a population approaching 6 million people, reclaimed wastewater is increasingly utilized for groundwater recharge. Additionally, non-point source pathogen pollution is a mounting problem due to sources such as dense dairy farming and urban runoff. The ability to predict the fate of human pathogens in the environment is critical, and a mechanistic understanding of bacterial transport in the subsurface environment is imperative for assessing the environmental impact of groundwater contamination from sources including urban runoff, septic tank/leach field systems, and animal manure from agricultural operations. Such information is also vital for effective design of water quality technologies such as riverbank filtration, wastewater reclamation, and recharge into aquifers.

A systematic and extensive examination of the *physiological and environmental factors* controlling bacterial adhesion and transport in subsurface environments is actively being pursued. Currently we are investigating the role that extracellular polymeric substances (EPS) exuded by bacterial pathogens plays in controlling the adhesive nature of the cells – expressly analyzing the composition of the EPS and adhesion trends in a flowing

environment as a coupled phenomenon. This novel approach of investigating the dynamic surface chemistry of bacterial cells and the extent of adhesion will provide a more complete understanding of bacterial pathogen transport mechanisms.

The project is conducted in two core areas. The first is the development of methodologies to extract and analyze the composition of the EPS. These methods were developed in the past; however, the various methods have limitations of efficiency, detection, and reproducibility that need to be worked out and optimized for analyzing EPS of groundwater-borne pathogens. To date, work has involved testing extraction methods involving ethanol, lyophilization (or freeze-drying), and sonication. The compositional analysis involves traditional



Graduate student Gexin Chen with the microscopic RSPF system through which bacterial adhesion can be observed and studied.

spectroscopic quantification techniques; however new methods using HPLC and gas chromatography are currently being explored. The EPS extraction and analysis methods have been tested on a variety of relevant organisms in the lab including *E. coli* (strains including O157:H7, D21g, XL1, and numerous natural isolates), *Salmonella enterica* serovar pullorum *Burkholderia cepacia* G4g, and *Halomonas pacifica* g. The ability to extract and analyze the EPS of *E. coli* isolates from dairy cattle and humans, as well as the *Salmonella* strain, is being tested for cells that have been stressed through starvation (0, 6, 12, and 18 hours). Additionally, an ongoing study is looking into the influence of solution chemistry (artificial groundwater at varying ionic strengths) and exposure time on *Salmonella* EPS production.

The second area of research involves the investigation of cell adhesion and transport in groundwater environments. This work is conducted in two experimental systems: 1) a packed bed column, and 2) a radial stagnation point flow (RSPF) cell. Both systems simulate transport of bacteria within porous media. The packed bed column is a macroscopic approach of quantifying cell transport; whereas, the RSPF system is a microscopic method of observing cell adhesion to a surface. Experiments in both systems are conducted under solution chemistry and hydrodynamic conditions simulating the subsurface environment. Experiments in the packed bed have been conducted utilizing *E. coli* (strains including O157:H7, D21g, XL1, and numerous natural isolates) and *Salmonella enterica* serovar pullorum. *Burkholderia cepacia* G4g and *Halomonas pacifica* g have been used in the RSPF system. To fully analyze the trends in cell transport, the surface chemistry of the bacterium requires consideration. The EPS composition and content provides considerable insight into this surface chemistry. Hence, EPS analysis has been compared to the transport data. The trend observed, regardless of cell type or environmental condition, is that the charge on the cell surface and the ratio of sugars to proteins

within the EPS provides an indication of the type of interaction forces that will result between the cells and the surfaces. Ongoing work will provide further insight into the applicability of this trend and whether this may provide a future predictive tool.

The overall goal for this project is that this fundamental research will provide a greater understanding of how environmental conditions influence cell fate in groundwater environments; and hence, lead to more effective water management and re-use practices in the future.

Publications

Chen, G. and Walker, S.L., The role of solution chemistry and ion valence on the adhesion kinetics of groundwater and marine bacteria. *Langmuir*, 2007, 23 (13) 7162-7169.

Selected Professional Presentations

Kim, H.J. and S. L. Walker, *Escherichia coli* Deposition and Transport in Porous Media: The coupled role of temperature and solution chemistry, American Chemical Society 81st Colloid & Surface Science Symposium, Newark, DE, June 2007.

Walker, S.L, Mechanisms of Bacterial Adhesion and Transport in Aquatic Systems, University of Sheffield, Sheffield, England, April 2007; University of California, Davis, CA, February 2007; and University of California, Berkeley, CA, October 2006.

Walker, S.L, What's in Your Water? UCR Life Program at the UCR Extension, Riverside, CA, February 2007.

Kim, H., S. ShojaeTazehkand , and S. L. Walker, *E. coli* deposition and transport in porous media: Influence of solution chemistry and bacterial surface polymers, American Institute of Chemical Engineers National Meeting, San Francisco, CA, November 2006.

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