Project #1026700

Title: Molecular Mechanism of Bacterial Attachment to Fe(III)-Oxide Surfaces

Principal Investigator: Neal, Andrew

Organization: Savannah River Ecology Laboratory

Results To Date: To date, our studies have concentrated upon two aspects of the chemistry and architecture of the Shewanella outer membrane surface: the influence of terminal electron acceptor during anaerobic respiration, and the role that protein secretion systems play in determining the presence and chemistry of exopolymers. Using Shewanella oneidensis strain MR-1 and S. putrefaciens strain 200R as model organisms we have developed a microelectrophoresis approach to estimating cell mobility in solution over a range of ionic strengths. From this data, and applying Ohshima's soft particle theory, we have successfully estimated the net electrostatic charge and relative thickness of any capsular material of the two model strains under various terminal electron acceptor availabilities. Additionally, by employing state-of-the-art cryo-electron microscopy techniques we have been able to not only confirm presence or absence of capsular material but also visualise the outer cell surface architecture in a completely hydrated state.

Effect of electron acceptor availability upon cell surface architecture of wild type S. oneidensis MR-1 and S. putrefaciens 200R

Results indicate that wild type Shewanella oneidensis MR-1 and S. putrefaciens 200R present cell surfaces largely free of exopolymers when grown on fumarate or nitrate as terminal electron acceptor. Different terminal electron acceptors have a significant affect upon exopolymer production: cells grown on nitrate as terminal electron acceptor have a fixed charge density (Rhofix) of -7.7 plus/minus 1.2 mM (estimate plus/minus 95% confidence interval of the estimate) and an apparent electrophoretic softness (1/lambda) of 0.4 plus/minus 0.6 nm. For fumarate-grown cells the estimates are not significantly different from the nitrate-grown estimates (Rhofix = -8.7 plus/minus 1.7 mM; 1/lambda= 0.4 plus/minus 0.6 nm). Electrophoretic softness estimate for both sets of cells is not significantly different from zero. In contrast, the fitted estimates for the trimethylamine N-oxide (TMAO)-grown cells are Rhofix = -8.6 plus/minus 1.5 mM and 1/lambda= 2.6 plus/minus 0.4 nm suggesting that while the fixed charge potential for the cells grown on the three terminal electron acceptors are not significantly different from each other, electrophoretic softness estimates are. This suggests that MR-1 cells respiring on TMAO as terminal electron acceptor produce a more extensive layer of extracellular material than cells respiring on either fumarate or nitrate, but that this does not affect the overall net charge at the cell surface. Cryo-high resolution SEM confirms increased production of capsular material on the surface of cells in with TMAO. A similar analysis of S. putrefaciens 200R indicates a similar trend in mobility (and consequently Rhofix and 1/lambda) is observed for the three terminal electron acceptors. For S. putrefaciens, fumarate-grown cells have a fixed charge potential of -43.0 plus/minus 38.8 mM and an electrophoretic softness of 1.6 plus/minus 0.9 nm. These estimates are similar to those for nitrate-grown cells (Rhofix = -43.0 plus/minus 32.7 mM and 1/lambda= 1.8 plus/minus 0.9 nm). Unlike S. oneidensis, cells grown on TMAO as terminal electron acceptor exhibit a reduced fixed charge potential of -20.8 plus/minus 0.9 mM: the increased electrophoretic softness

(1/lambda = 3.0 plus/minus 0.7 nm). The trend is clearly similar to that observed for S. oneidensis cells. Again, cryo-high resolution SEM confirms increased production of capsular material on the surface of cells in with TMAO.

Effect of outer membrane proteins upon cell surface architecture of S. oneidensis MR-1 and S. putrefaciens 200R

A contiguous cluster of twelve Gsp genes (gspC to N) has been described in Shewanella oneidensis MR-1. The Gsp family of proteins is found in type II protein secretion systems in a wide variety of gram negative bacteria. Insertional mutants of gspD in S. oneidensis and gspE in S. putrefaciens are unable to respire on solid-phase Fe(III) or Mn(IV) oxides but retain the ability to reduce soluble electron acceptors. Since membrane-mineral interactions are involved in direct electron transfer to solid phase acceptors we are studying the influence of these two proteins upon outer membrane physicochemistry and cell attachment.

Effect of GspD upon cell surface architecture of S. oneidensis MR-1 = MR-1::ΔGspD cells present a significantly softer surface (1/lambda = 3.5 plus/minus 0.1 nm compared to 0.4 plus/minus 0.4 nm) than wild type cells = Wild type cell surfaces appears free of extensive exopolymers = Cryoetch-HRSEM confirms the presence of copious exopolymeric material on the MR-1::ΔGspD surface = ATR-FTIR difference spectra indicate this extracellular material is predominantly carbohydrate in nature, a reduction in protein is also evident. = XPS indicates an increase in O/C, and reduction in N/C ratios for the MR-1::ΔGspD cell surface compared to wild type cells supporting ATR-FTIR observations = Rhofix is significantly reduced from -8.7 plus/minus 1.7 mM for wild type cells to -1.9 plus/minus 0.6 mM for MR-1::ΔGspD cells

Effect of GspE upon cell surface architecture of S. putrefaciens 200R = 200R::ΔGspE cells present a softer surface than wild type cells, although poor agreement with Ohshima?s model precludes statistical comparison. = Cryoetch-HRSEM confirms the increased secretion of exopolymeric material on the 200R::ΔGspE cell surface = ATR-FTIR difference spectra indicate this to be predominantly carbohydrate, a reduction in protein is also evident. = XPS again indicates increased O/C, and reduced N/C ratios for the 200R::ΔGspE cell surface compared to wild type cells. = Deletion of GspE has little apparent effect upon Rhofix in 200R (-43 mM)

The autotransporter family of proteins consists of in excess of 700 proteins with a wide variety of functions such as proteolysis, cytotoxicity, serum resistance, host invasion and adhesion. Many autotransporter adhesins play a role in autoaggregation and biofilm formation. The presence of a well defined capsule may be detrimental to autotransporter adhesin and autoaggregation activity. Given the potential role of autotransporter proteins in adhesion and biofilm formation, we are currently investigating the influence of SO3800 upon the chemistry and architecture of the S. oneidensis MR-1 outer membrane surface. An SO3800 mutant of MR-1 retains the ability to reduce solid phase Fe(III)-oxides.

= MR-1::ΔSO3800 cells present a significantly softer cell surface (1/lambda = 2.0 plus/minus 0.5 nm compared to 0.4 plus/minus 0.4 nm) than the exopolymer-free wild type = ATR-FTIR difference spectra indicate this extracellular material is predominantly proteinaceous in nature, a reduction in carbohydrate is also evident. = XPS indicates an increase in the C-O/C-N contribution to the C 1s photopeak from 22.4 at. % at the MR-1 wild type cell surface to 29.5 at. % at the MR-1::ΔSO3800

cell surface supporting ATR-FTIR observations = Rhofix is significantly reduced from -8.7 plus/minus 1.7 mM for wild type cells to -1.9 plus/minus 0.4 mM for MR-1::ΔSO3800 cells

Publications

Apkarian R.P., Shamsi S.A., Rizvi S.A., Benian G., Neal A.L., Taylor J.V. and Dublin S.N. Cryoetch and cryo-planing for low temperature HRSEM: SE-I imaging of hydrated multicellular, microbial and bioorganic systems. Microscopy and Microanalysis in press

DiChristina, T., D. Bates, J. Burns, J. Dale and A. Payne. 2006. Microbial metal reduction by members of the genus Shewanella: novel strategies for anaerobic respiration. In L. Neretin (ed.), Biogeochemistry of Anoxic Marine Basins. Kluwer Publ. Co., Dordrecht, NL.

DiChristina, T., J. Fredrickson and J. Zachara. 2005. Enzymology of electron transport: Energy generation with geochemical consequences. Reviews in Mineralogy and Geochemistry, 59: 27-52.

Neal A.L., Bank T.L., Hochella M.F. and Rosso K.M. (2005) Cell adhesion of Shewanella oneidensis to iron oxide minerals: effect of different single crystal faces. Geochemical Transactions 6(4)

Invited Presentations

DiChristina T. Molecular mechanism of microbial metal respiration. 106th General Meeting, American Society for Microbiology. Orlando FL May 2006.

DiChristina T. Molecular mechanism of microbial metal respiration. University of Texas Pan American, Edinburg, TX, May 2006.

DiChristina T. Molecular mechanism of microbial metal respiration. DOE-ERSD PI meeting April 2006.

DiChristina T. Molecular mechanism of microbial metal respiration. Biogeochemistry Grand Challenge, PNNL, Richland, WA, February 2006.

DiChristina T. Molecular mechanism of microbial metal respiration. Peking University, Beijing, China, January 2006.

DiChristina, T. Molecular mechanism of microbial metal respiration. Mineralogical Society of America, Short Course on Molecular Geomicrobiology, Berkeley, CA, December 2005.

DiChristina T. Molecular mechanism of microbial metal respiration. Allegheny College, Meadville, PA, November 2005.

Contributed Presentations/Posters

Neal. A.L. Life at the rock face: surface structural controls on bacterial activity. North

Carolina State University, Raleigh NC September 27-28

Neal A.L., Bates D., Burns J., DiChristina T. J. Molecular determinants of cell surface physicochemistry in Shewanella. Session entitled Recent Advances in Geomicrobial Processes. 231st American Chemical Society National Meeting, Atlanta GA

Neal A.L., Bates D., Burnes J., DiChristina T.J. Physicochemical description of the outer membrane surface of Shewanella spp. 106th General Meeting, American Society for Microbiology. Orlando FL May 2006.

Neal A.L., Burns J., Bates D. and DiChristina T.J. Physicochemical description of the outer membrane surface of Shewanella spp. DOE-ERSD PI meeting April 2006.