EMSL Research and Capability Development Proposals

3-Dimensional Reconstruction and Modeling of Bacterial Extracellular Polymeric Substances (EPS)

Project start date: Spring 2007

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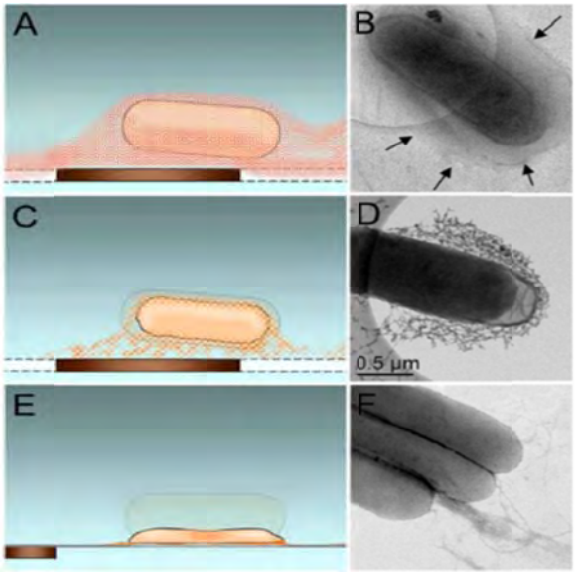
Co-investigators:
Matthew Marshall
Environmental Microbiology, FCSD, PNNL

Bacterial extracellular polymeric substances (EPS) play essential role in a wide range of cellular processes such as biofilm formation, cell infection, and bacterial attachment to mineral substrates. In biogeochemistry, EPS's chemical reactivity can most likely directly influence the fate and transport of heavy metals and radionuclides. The very fragile, highly hydrated intricate networks of macromolecules presents a great challenge in elucidation of its structure because of its immediate collapse during the dehydration process that is a prerequisite for all vacuum-based, high resolution imaging methods, such as electron microscopy. We applied the method of Cryo-electron micrscopy for fine scale imaging of the EPS for obtaining near-to-native structure preservation, without artifact caused by physical structural collapse after losing water during chemical processing and dehydration.

EMSL acquired a devoted biological TEM Tecnai T-12 (FEI) with the cryostage in 2006. We built upon its capacity of room temperature imaging by developing the capability of Cryo-electron microscopy (CryoTEM) imaging for observation of material in its closest-to-natural state (CET).



Model and correlated EM images of cells and associated EPS structural alteration resulting from different methods of sample processing. (A,B) CryoTEM of frozen-hydrated cells vitrified in amorphous ice imaged at -178ºC. Arrows indicate the outline of the EPS. (C,D) RT TEM of initially vitrified cells that have been gently dried in low vacuum of the DPS. (E,F) RT TEM of air dried cells, stained with a negative stain prior to imaging.(D,F) Notice the significant morphological difference induced by dehydration.

By studies of *Shewanella* in precisely controlled conditions, we visualized for the first time the closest-to-natural state state of hydrated bacterial networks, and we brought the evidence that extracellular polymers have a much greater function than being just the building blocks of microbial communities.

Products and Output

New Capability for EMSL Users

The results from this project provide a pathway to addressing scientific challenges in areas of environmental microbiology, and will bring high demand for applying these means to the variety of high profile research in the biogeochemistry and structural biology areas.

Publications

Dohnalkova AC, Marshal MJ, Arey BW, Williams KH, Buck EC and Fredrickson JK." Imaging Hydrated Microbial Extracellular Polymers: Comparative Analysis by Electron Microscopy". Appl Environ Microb, 2011, Vol. 77, No. 4, p. 1254–1262.

Presentations

Invited talk: Dohnalkova AC, "Bacterial Interactions with Minerals and Metals – Imaging and Analysis Using Novel Methods in Electron Microscopy". Center for Biofilm Engineering at the Montana State University, Bozeman, MT, Nov 6, 2008

Invited talk: Dohnalkova AC, Arey BW, Williams KH and Marshall MJ."Strategies for Visualization of Extracellular Polymeric Substances (ExPS) in Biofilms by Electron Microscopy". Microscopy Society of America national meeting , Richmond, VA, 2009, Biofilms session.

Dohnalkova AC, Marshall MJ, Buck EC, Jarisch WR Fredrickson JK. "CryoTEM Tomography Provides a New Perspective to the Bacterial-Mineral Association in Biogeochemistry Studies. 5th International Congress on Electron Tomography (ICET), Brisbane, Australia, January 2009.

A. Dohnalkova chaired a session on Biominerals at the national meeting of the Microscopy Society of America, Richmond, VA, Aug 09. Marshall, JM presented our work as an invited talk at this conference.