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Bacterial and plant proteome analysis by 1D and 2D LC-MS

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Abstract:

A main research objective within the U.S. Department of Energy Office of Biological and Environmental research is a system biology approach to “characterize the molecular machines of life” for microbes and plants. The goal of this work is to gain better insight into carbon sequestration and carbon cycling as well as develop better microbial/plant agents for bioremediation. To achieve these goals DOE has initiated the Genomes to Life program in attempt to use systems biology to understand entire living organisms and their interactions with the environment. We are addressing this challenge with the use of liquid chromatography interfaced with mass spectrometers for the study of whole proteomes and protein complexes from model bacterial and plant species capable of bioremediation or involved in carbon sequestration and carbon cycling.

Our methodology has focused on the use of high performance liquid chromatography coupled to electrospray mass spectrometry for the direct analysis of complex protein mixtures from cell/organelle preps or protein complex isolations. We have employed a dual approach of “top-down” and “bottom-up” proteomics for comprehensive protein characterization. The “top-down” approach relies on measurements of intact proteins by 1D or 2D HPLC coupled with a 9.4 Tesla Fourier Transform mass spectrometer (ES-FTMS). The data from these experiments are then correlated with “bottom-up” proteome analysis by tryptic digestion followed by 1D or 2D LC-MS/MS on electrospray ion traps (ES-QIT). For comprehensive “shotgun” proteome analysis we are also investigating combining data acquisition from LC-MS/MS on ES-QIT and LC-MS on the ES-FTMS for a more comprehensive analysis of complex protein mixtures.

Our work over the last year has focused on proteome analysis of four separate species. *Shewanella oneidensis* MR-1 is a metal reducing microbe of potential importance to the field of bioremediation. This microbe has been shown to be capable of reducing a wide variety of heavy metals and organic compounds. The exact mechanism of this microbe’s ability to reduce these chemicals is only now beginning to be understood. Our work has focused on a comprehensive analysis of the wild-type strain under aerobic growth conditions as well as qualitative comparisons between the wild-type strain and a *fur* knockout mutant. *Rhodospseudomonas palustris* is a purple nonsulfur phototrophic bacterium that is ubiquitous in soil and water samples. *R. palustris* is of great interest due to its high metabolic diversity and ability to degraded complex aromatic hydrocarbons (lignin). Currently we are analyzing the whole proteome of this microbe under multiple growth conditions as well as preparing protein complexes for analysis. *Arabidopsis thaliana* is a model plant organism that has recently been fully sequenced and annotated. Our work in this organism has focused on isolation and characterization of the chloroplast proteome. This project ties in with our analysis of cyanobacteria and marine cyanophage viral communities, which along with the purple nonsulfur phototrophic bacterium represent a significant fraction of the organisms that participate in and affect the carbon cycle.

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