Probing the Proteome with Capillary Isoelectric Focusing-ESI-FTICR Mass Spectrometry

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Bacterial strains such as *Shewanella putrefaciens* strain MR-1 are key organisms in the bioremediation of metals due to their ability to enzymatically reduce and precipitate a diverse range of heavy metals and radionuclides. Important in these processes is the need to develop improved enzymatic pathways in these organisms. As a first step, the proteome of the organism must be completely characterized. The proteome is defined as the entire protein complement of the cell expressed under a given set of conditions. A single genome can exhibit many different proteomes depending on stage in cell cycle, cell differentiation, response to environmental conditions (nutrients, temperature, stress, etc.), or the manifestation of disease states. While the availability of full genomic reference sequences provides a set of road maps as to what is possible, and measurements of the expressed RNAs tells us what might happen, the proteome is the key that tells us what really happens. Therefore, the study of proteomes under well-defined conditions can provide a better understanding of complex biological processes, which requires faster and more sensitive capabilities for the characterization of cellular constituents.

We are currently developing technologies that will allow the visualization of the protein complement by obtaining comparative displays for the expression of many proteins simultaneously, based upon stable-isotope labeling. Two versions of each protein are generated and analyzed simultaneously, to precisely establish changes in expression. Capillary isoelectric focusing on-line with Fourier transform ion cyclotron resonance mass spectrometry provides a powerful tool to study the changes in expression (i.e., repression or induction) for hundreds of proteins simultaneously. Further characterization of the proteome can be accomplished by characterization of the proteolytic fragments of the proteins in the organism. For many proteins, these proteolytic fragments can be used as unique mass markers for the identification of the proteins in question. Additionally, the sequence of the peptides can be determined as another identification technique. These combined technologies will enable ultra-sensitive proteome-wide expression profiling to evaluate changes in the complete proteome of the iron-reducing bacterium *Shewanella putreficiens* strain MR-1 induced by switching from aerobic to anaerobic respiration with heavy metals and radionuclides.