

Characterization of Protein Complexes in *Rhodopseudomonas palustris* by Mass Spectrometry

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The Genomes to Life (GTL) program of the U.S. Department of Energy (DOE) extends the information available from large-scale DNA sequencing efforts by cataloging and understanding all the protein “machines” that are present in a particular organism, with initial emphasis on microbial species that have relevance to missions of the DOE, including bioremediation, fuel production, and carbon sequestration. The Center for Molecular and Cellular Systems is a multi-institutional effort, led by Oak Ridge National Laboratory, to identify the protein components of molecular machines in support of GTL. At ORNL, we are focusing on the bacterial species *Rhodopseudomonas palustris*, which is of interest because of its ability to produce hydrogen, to degrade lignin monomers, and to survive under a variety of conditions (aerobic/anaerobic, light/dark). We are culturing *R. palustris* under a variety of growth conditions, and identifying the expressed proteins¹ and protein complexes using mass spectrometry-based proteomics techniques. Clones are being produced that express a selected protein as a fusion with affinity tags, such as His6, GST, and the V5 epitope. Affinity isolation of the tagged protein will also yield the proteins with which the tagged protein interacts strongly. Analysis of these “pulldowns” by mass spectrometry will provide identities of proteins that interact with the tagged protein. Eventually, the goal is to perform the entire process in high throughput, to allow rapid and accurate characterization of the entire complement of protein complexes in a microbial species. This information will be a valuable resource for biological researchers elucidating biochemical pathways.

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1. Verberkmoes, N.C.; Strader, M.B.; Lankford, P.; Pelletier, D.A.; Hauser, L.; Land, M.; Hurst, G.B.; Kennel, S.J.; Harwood, C.S.; Hettich, R.L.; Larimer, F.W. “Proteome analysis of the anoxygenic photobacterium *Rhodopseudomonas palustris*.” Proceedings of the 51st ASMS Conference on Mass Spectrometry and Allied Topics, Montreal, Quebec, Canada, June 8-12, 2003.