

Enhanced Characterization of the Membrane Proteome from the Anoxygenic Phototrophic Bacterium *Rhodospseudomonas palustris* under all Major Metabolic States

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Introduction:

Rhodospseudomonas palustris is a purple nonsulfur anoxygenic phototrophic bacterium that is ubiquitous in the natural environment. *R. palustris* is of great interest due to its extraordinary metabolic diversity and its ability to degrade complex aromatic hydrocarbons. Furthermore, this microbe can fix nitrogen and carbon dioxide and produces hydrogen gas. This microbe is also known to have a complicated invaginated membrane system, which presumably houses many of the proteins and protein complexes necessary for the diverse metabolic pathways encoded by the microbe's genome. We are developing proteomic methodologies to explore the proteome and membrane subproteome of all the major metabolic modes for this microbe in attempt to determine those proteins and protein complexes critical to this microbe's metabolic diversity.

Methods:

R. palustris cultures were grown under seven conditions representing all major growth states for this bacteria. All growth conditions were harvested from 2L batches, cells were disrupted by sonication and the samples were split into four crude fractions by high-speed centrifugation. All four fractions from the seven growth states were digested with trypsin and analyzed in duplicate by fully automated 1D LC-MS/MS with multiple mass range scanning (MMS) on an ion trap ES-MS (LCQ-DECAXPplus). The crude membrane fraction was digested by a combined CNBr/trypsin digest and duplicate analysis by a fully automated 2D nano-LC-MS/MS methodology is on going. All MS/MS spectra obtained from LC-MS/MS runs were processed by SEQUEST, filtered with DTASelect and compared with Contrast.

Preliminary results:

Our goal of this study is to develop proteomic methodologies to obtain a greater understanding of the diverse metabolic states of *R. palustris*. We have characterized *R. palustris* baseline proteomes in duplicate for seven growth conditions that define the major metabolic modes of this microbe. This analysis involved a tryptic digestion of the fractionated samples followed by analysis by 1D LC-MS/MS with MMS. This analysis resulted in the identification of 1,646 proteins with conservative filtering constraints. Qualitative analyses of these growth conditions have revealed over 400 proteins exhibiting large-scale differences between conditions, including many unknown or conserved unknown protein species. While these preliminary results were very useful in initial mapping of the proteomes it was clear that the methodology was limited in the analysis of integral membrane proteins, especially protein known or thought to be imbedded deep inside membrane bound protein complexes. We inspected the entire dataset for percent sequence coverage for known proteins complexes imbedded in the membrane. This study indicated that the common tryptic method was clearly identifying those proteins found on the outside of known membrane complexes but barely identifying or missing completely known integral membrane proteins from the same complexes. These findings lead us to test a combined CNBr/trypsin digestion for the analysis of the crude membrane fractions. This digestion method was directly compared to the tryptic method of the same sample and revealed superior results in the total number of proteins found and the total coverage of known integral membrane proteins from membrane bound protein complexes. We have further optimized the method and are using 2D-LC-MS/MS to analyze in duplicate all crude membrane fractions from the seven metabolic states. We have identified several advantageous and disadvantageous of this method for a high throughput study of proteins and protein complexes associated with the membrane.

Novel Aspect:

First demonstration of complete analysis of membrane subproteome across numerous growth states for the microbe *R. palustris*.