

# Identification of Protein Complexes in *Rhodospseudomonas palustris*

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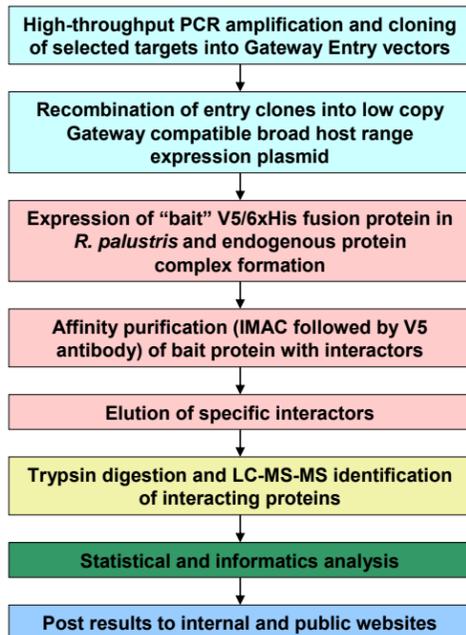
## OVERVIEW

- We are elucidating protein-protein interaction networks in *Rhodospseudomonas palustris* by affinity isolation and mass spectrometry
- Results are available via the Microbial Protein-Protein Interaction Database at [mippi.ornl.gov](http://mippi.ornl.gov)

## INTRODUCTION

- Genome-scale elucidation of protein-protein interactions is
  - a component of systems biology
  - a tool for generating hypotheses regarding protein function
- The current focus of the Genomics:GTL Center for Molecular and Cellular Systems (CMCS) is to identify the set of protein complexes used by *Rhodospseudomonas palustris*, and to determine changes in these protein-protein interactions under different metabolic conditions.
- Affinity tags can be introduced into proteins of most gram negative bacteria using a cloning system that combines a *convenient commercial system* with a *broad host range plasmid*.
- R. palustris* is a ubiquitous, metabolically diverse anoxygenic phototrophic bacterium that
  - fixes nitrogen
  - performs photosynthesis (carbon sequestration)
  - degrades aromatics (bioremediation)
  - produces hydrogen gas (energy production)
- We have identified protein-protein interactions from cultures of *R. palustris* grown under anaerobic photoheterotrophic growth conditions, in the presence or absence of fixed nitrogen. Interactors identified by this approach include homologues of a number of well-characterized protein complexes as well as numerous putative novel interactions.

## EXPERIMENTAL



- Primers are designed for each gene of interest, and PCR amplification is performed. A modified commercial system (Gateway, Invitrogen) is used to prepare clones encoding a dual affinity tag (hexahistidine and V5 epitope) at the position corresponding to the C terminus of each protein. A broad host range plasmid allows expression of affinity-tagged proteins in a variety of gram negative bacteria. Proteins are expressed in separate 800 mL *R. palustris* cultures, followed by cell harvest and lysis. A two-stage affinity purification isolates the tagged protein, and other proteins in the complex. Complexes are digested with trypsin, and analyzed by reverse-phase nano-scale HPLC (LCPackings Famos/Switchos/Ultimate system) coupled via electrospray with a quadrupole ion trap mass spectrometer (ThermoFinnigan Deca XP+). Tandem mass spectra are identified using Sequest, and results compiled using DTASelect

(Pelletier, D.A., Hurst, G.B., Foote, L.J., Lankford, P.K., McKeown, C.K., Lu, T.-Y., Schmoyer, D.D., Shah, M.B., Hervey, W.J. IV, McDonald, W.H., Hooker, B.S., Cannon, W.R., Daly, D.S., Gilmore, J.M., Wiley, H.S., Auberry, D.L., Wang, Y., Larimer, F.W., Kennel, S.J., Doktycz, M.J., Morrell-Falvey, J.L., Owens, E.T., Buchanan, M.V., "A general system for studying protein-protein interactions in gram-negative bacteria," J. Proteome Research 2008 - in press).

Further details of methods are available at <http://mippi.ornl.gov/methods/index.shtml>

## Cloning and Strain Production



Product ( <i>R. palustris</i> )	PCR Amplified gene	Entry Clone	Expression Clone	Host Transformation
Success Rate	96%	89%	100%	97%
Number Successful	1289	1151	1135	1058
Number Attempted	1334	1289	1135	1080

## Strain Growth

## Affinity Isolations

	Distinct Baits	Total (with controls, reps, QC)
Production Cultures	850	1294
Affinity Isolations	571	1055
LC-MS-MS Analyses	568	4427

## Protein Identification

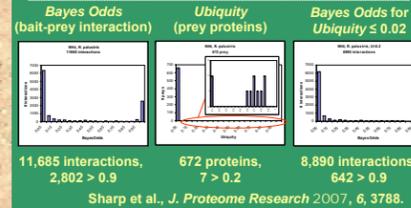
Identifications	In Tagged Pulldowns	In Wild Type Controls	Total (with QC samples)
Distinct Proteins	2858	248	2965
All Proteins	122645	538	135136
Proteins above Background	58640	0	67332
Peptides	244388	949	279982

## RESULTS AND DISCUSSION

## Data Analysis and Informatics

(See poster 651)

## Statistical Analysis of Interactions: BePro3



## Network Inference and Storage: SEBINI



## Bioinformatics Information: CABIN, Bioverse



## CONCLUSIONS

- The Center for Molecular and Cellular Systems is currently focusing on the completion of the characterization of soluble protein-protein interactions in *Rhodospseudomonas palustris*. The CMCS approach combines expression of affinity tagged proteins, affinity purification of interacting proteins, and tandem mass spectrometric identification of these proteins. Our goal is to provide a capability for generating high quality protein-protein interaction data from a variety of energy- and environment-relevant microbial species. This poster provides a status report of the CMCS "pipeline" for measuring protein-protein interactions in *R. palustris*, which is of high relevance to DOE missions due to its ability to produce hydrogen, to degrade lignin monomers, and for its exceptional metabolic versatility.
- These protein-protein interactions are disseminated through the publicly accessible Microbial Protein-Protein Interaction Database (MiPPI.ornl.gov). MiPPI is updated every 6 months (May and November). MiPPI provides tables of observed protein-protein interactions, as well as background information on CMCS measurement and analysis techniques. Various results (mass spectrometry results, corresponding metadata, and identified protein-protein interactions, including the statistical analysis scores) are also available for download in various formats, including Cytoscape-compatible files.

## ACKNOWLEDGMENTS

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