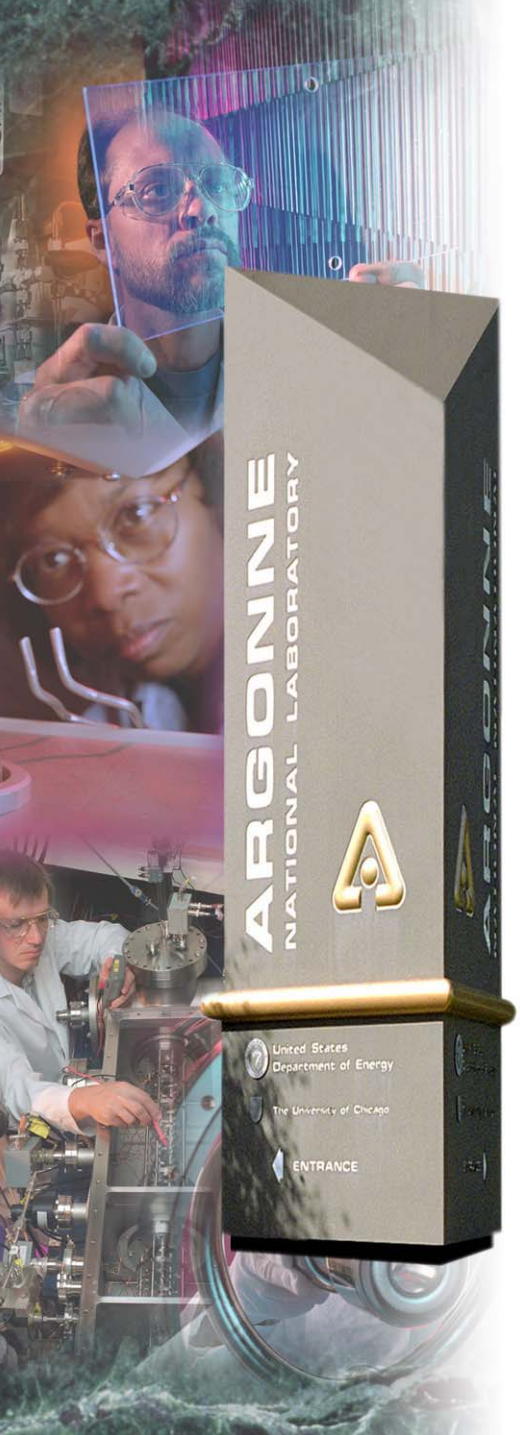


Protein Expression in *Shewanella oneidensis* MR-1

Carol S. Giometti
ANL Biosciences Division
March 16, 2004

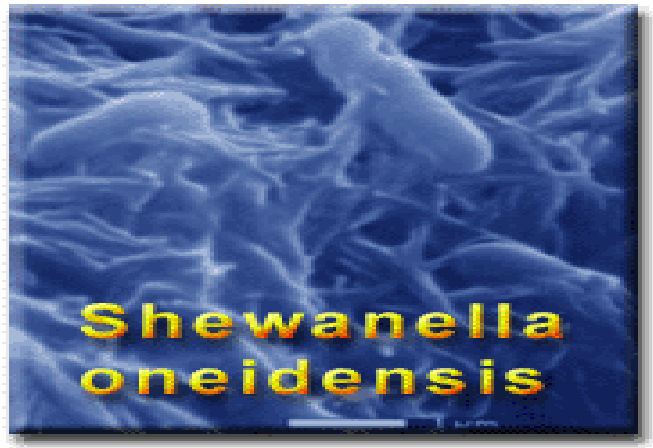
Argonne National Laboratory

Office of Science
U.S. Department of Energy

A U.S. Department of Energy
Office of Science Laboratory
Operated by The University of Chicago

MR-1: A Microbe with Metal Reducing Capability

- **Gram negative**
- **Facultative anaerobe capable of using multiple electron acceptors**
  - **Oxygen**
  - **Fumarate**
  - **Nitrate**
  - **Iron**
  - **Manganese**
  - **Uranium**
  - **Chromium**
- **Links carbon cycling to reduction of metal oxides**



How is this activity regulated??

Is gene expression the sole determinant of metal reduction activity?

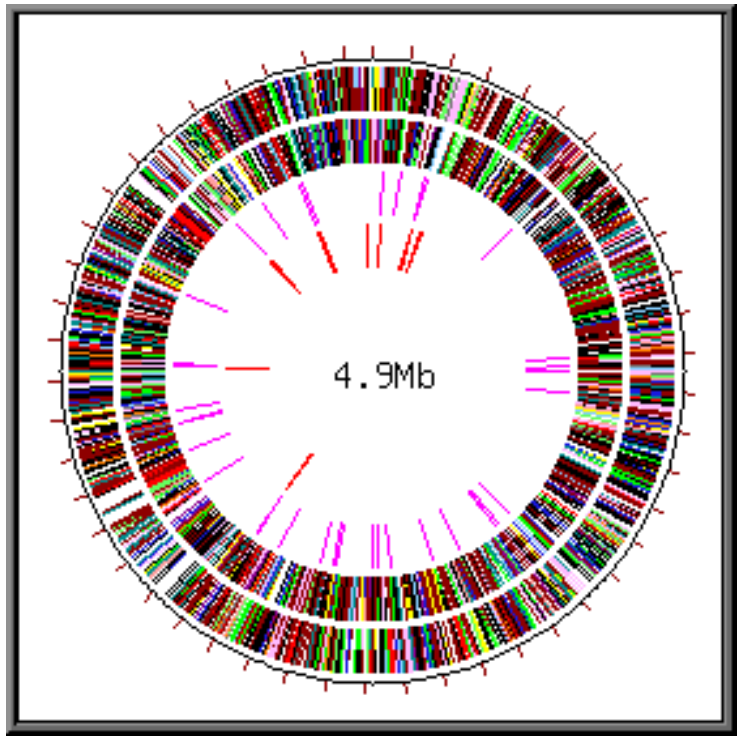
OR

Are the proteins involved in metal reduction reactions regulated by cofactors or post-translational modifications?

S. oneidensis MR-1 Genome

- **5.2 Mbp**
  - Chromosome: 4.9 Mbp
  - Megaplasmid: 150 Kbp
  - Phage: 50 Kbp
  - Plasmid: Kbp
- **4748 Open Reading Frames**
  - 54.4% with predicted function
  - 22.2% conserved hypothetical
  - 23.4% hypothetical

(Nature Biotechnology 20:1119 (2003)



How many of these genes are expressed and when??

Comparison of Gene and Protein Expression

S. oneidensis **MR-1 cells (wild type or mutant) were grown at ORNL in batch culture with different electron acceptors (e.g., fumarate, iron)**

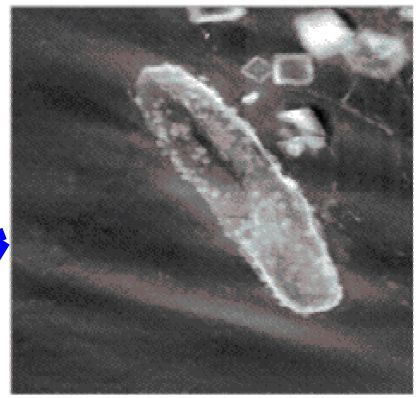
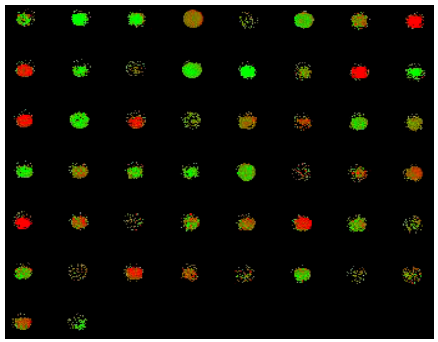


Photo from PNNL (J. Fredrickson)

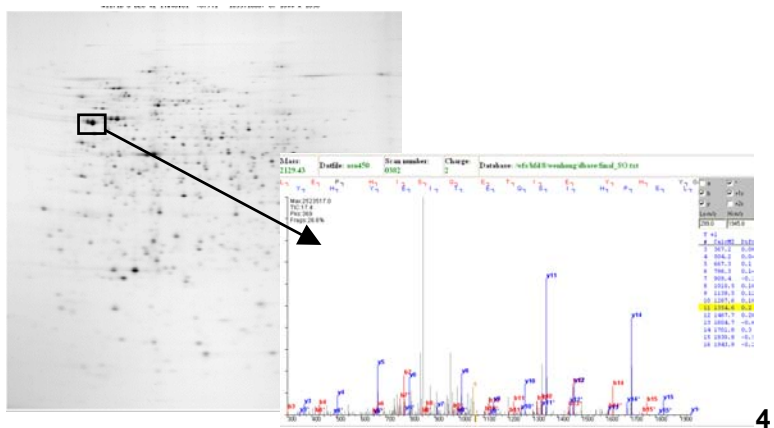


Array image from ORNL

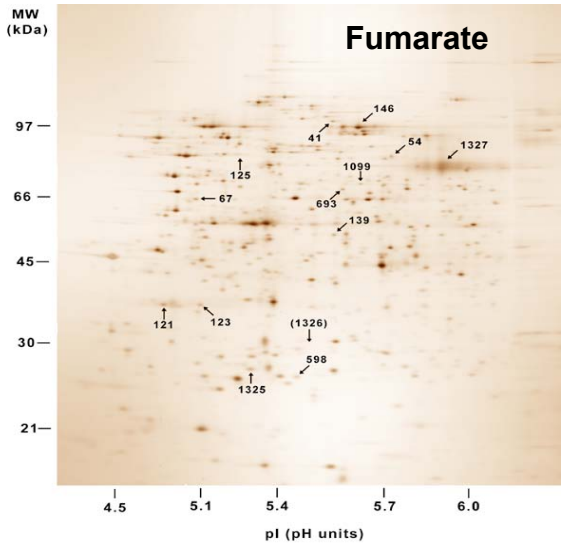
Gene expression monitored using microarrays (J. Zhou, ORNL)

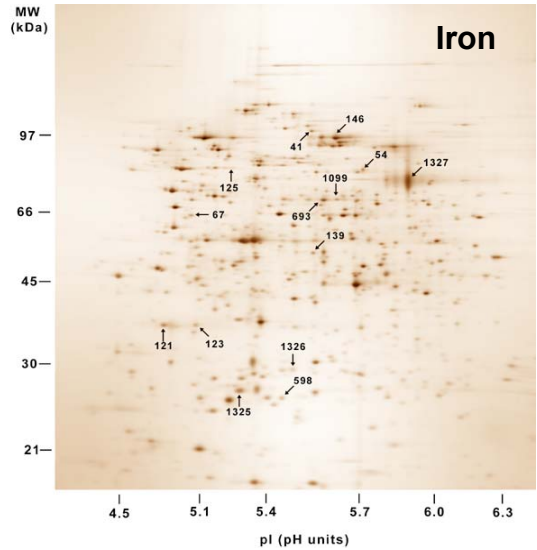
Protein expression monitored using two-dimensional gel electrophoresis and mass spectrometry

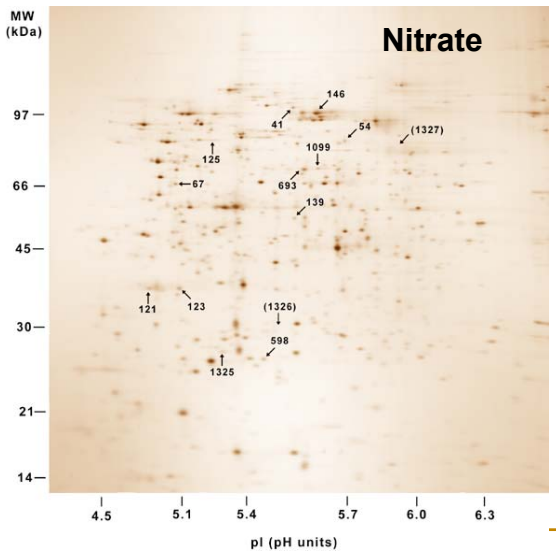
(C. Giometti, ANL; J. Yates III, Scripps)

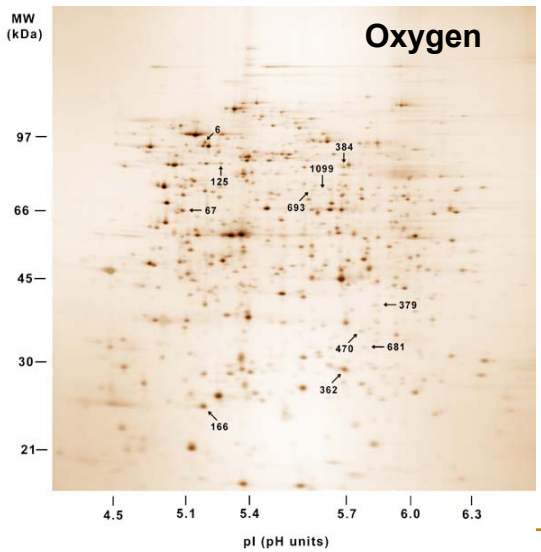


MR-1 Whole Cell Lysate Proteins with Different Electron Acceptors







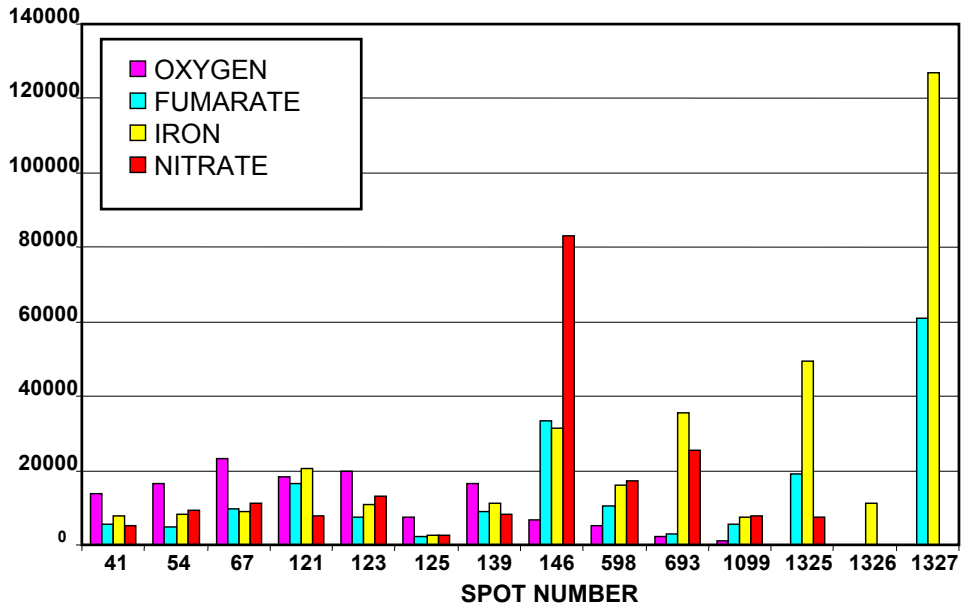


Omics (2002) 6: 39-59;
Belieav et al.

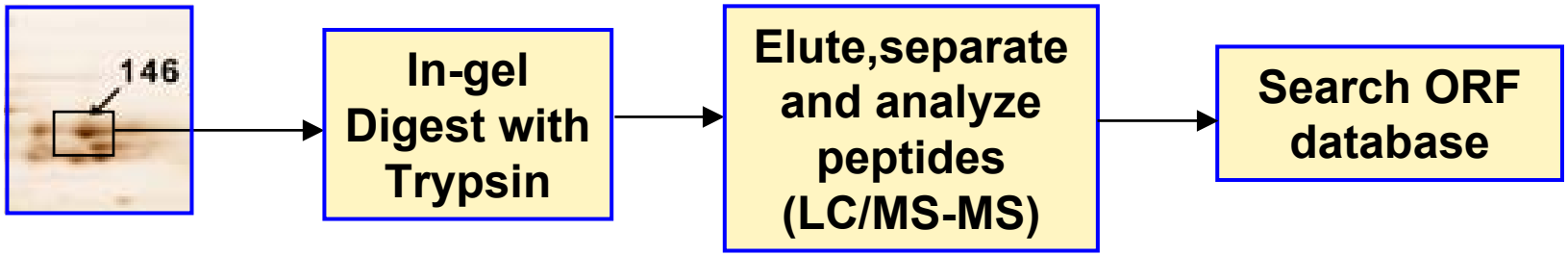
Quantitative Analysis of Protein Expression

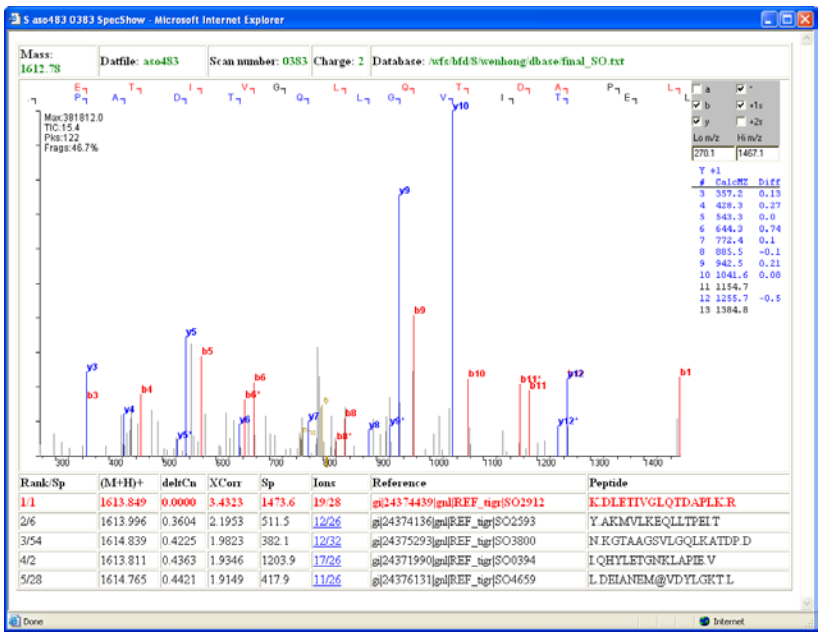
- **Multiple gels per sample (e.g., 4-6)**
- **All patterns matched to a reference pattern**
- **Integrated densities normalized**
- **Integrated densities averaged within groups**
- **Student t test applied**
- **Proteins showing statistically significant differences identified**

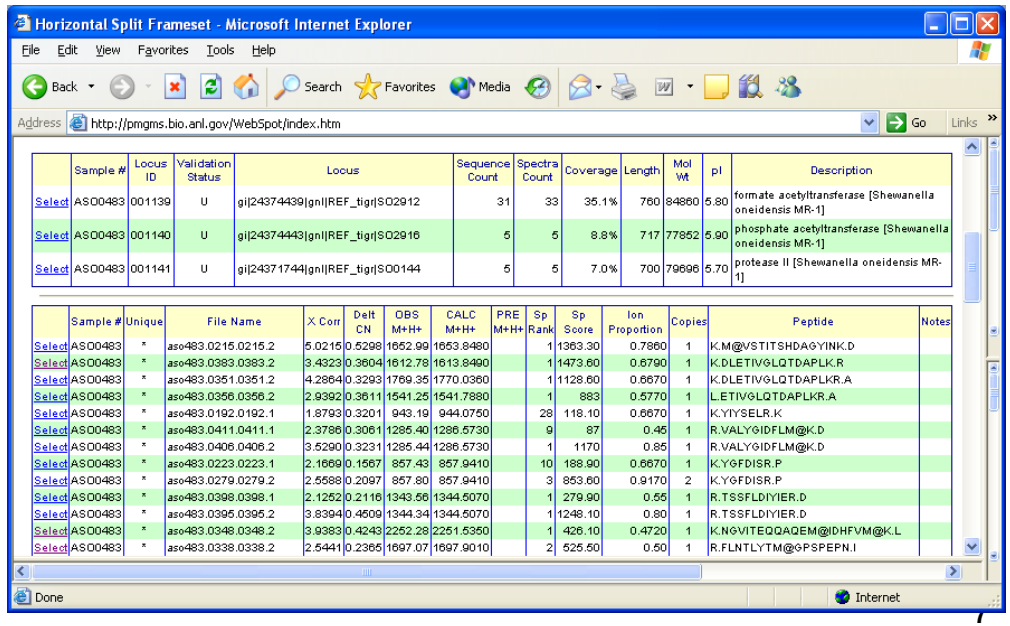
QUANTITATIVE PROTEIN DIFFERENCES IN *Shewanella oneidensis* MR1 GROWN WITH DIFFERENT ELECTRON ACCEPTORS



Protein Identification







S. oneidensis Proteins

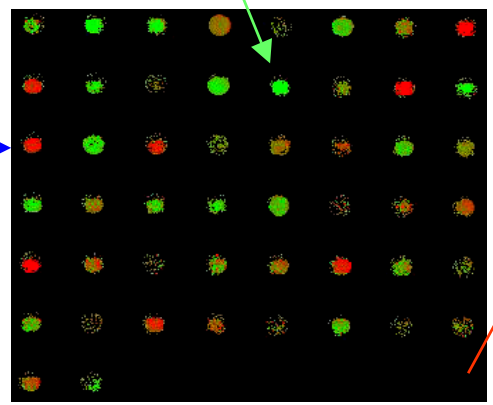
Anaerobic < Aerobic

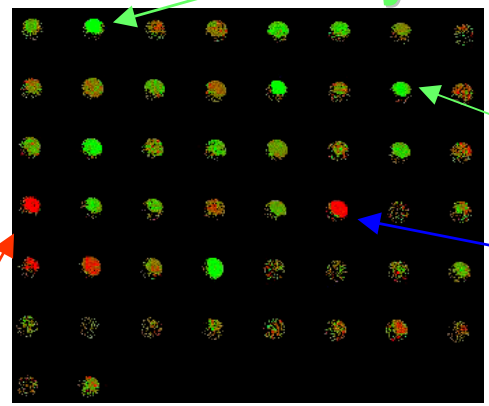
MSN	F/I/N	ANNOTATION
41	+/+/+	DNA TOPOISOMERASE II, B
54	+/+/+	ALCC PROTEIN
67	+/+/+	AGGLUTINATION PROTEIN
123	+/+/+	ELECTRON TRANSFER FLAVOPROTEIN, ALPHA
125	+/+/+	CONSERVED HYPOTHETICAL PROTEIN
139	+/+/+	DIHYDROLIPOAMIDE SUCCINYL TRANSFERASE
121	-/-/+	OPRF

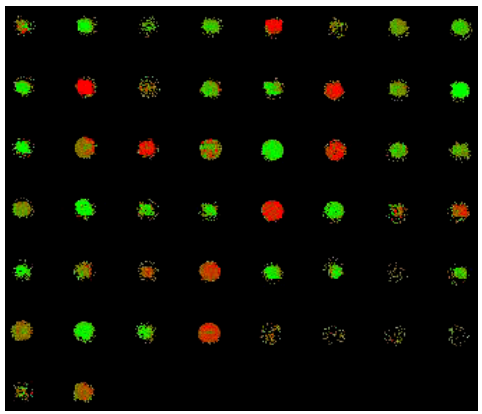
S. oneidensis Proteins
Anaerobic > Aerobic

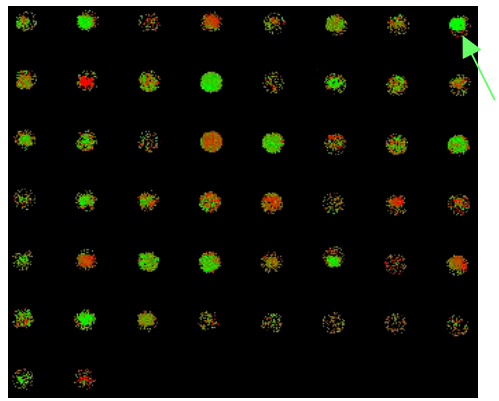
MSN	F/I/N	ANNOTATION
146	+/+/+	FORMATE ACETYLTRANSFERASE
598	+/+/+	CONSERVED HYPOTHETICAL
1099	+/+/+	PHOSPHOMANNOMUTASE
1325	+/+/+	3,4-DIHYDROXY-2-BUTANONE 4-PHOSPHATE SYNTHASE
693	-/+/+	PRISMANE
1326	-/+/-	CONSERVED HYPOTHETICAL
1327	+/+/-	FUMARATE REDUCTASE

Shewanella oneidensis Partial Microarray









200 full length open reading frames

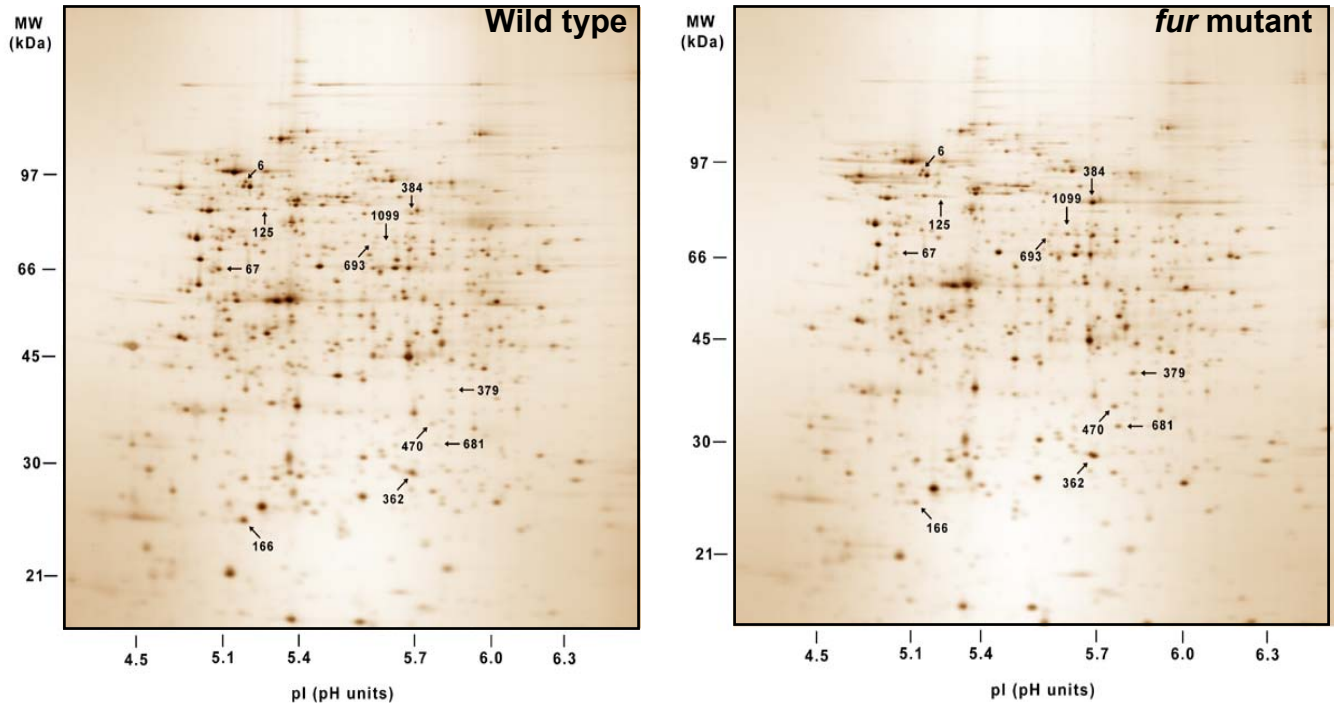
- Green = cy3 label = aerobically expressed.
- Red = cy5 label = anaerobically expressed, nitrate as a terminal electron acceptor

Protein/mRNA Abundance Under Anaerobic Conditions (relative to O2)

MSN	ANNOTATION	FUM	FE	NO3
41	DNA topoisomerase II	0.4/0.8	0.6/1.4	0.4/0.9
54	AlcC protein	0.3/0.1	0.5/0.01	0.6/0.5
123	Electron transfer flavoprotein	0.4/0.4	0.5/0.1	0.7/0.5
139	Dihydrolipoamide succinyltransferase	0.6/0.3	0.7/0.4	0.5/0.4
146	Formate acetyltransferase	5/0.9	5/1.5	12/0.9
693	Prismane	1.4/1.8	17/2	12/56
1099	Phosphomannomutase	5/0.4	7/0.3	7/0.45
1327	Fumarate reductase	50/4.8	100/1.4	nd/1.6

Good correlation observed between mRNA and protein expression in some but not all cases.

Protein Expression in *fur (ferric uptake regulator)* Mutant

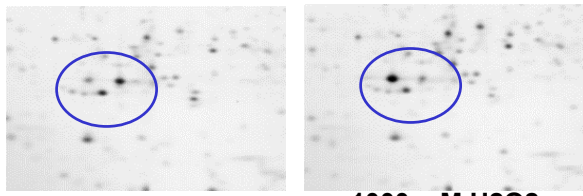


Spot Number	Annotation	FUR1/WT ratio (Protein)	FUR1/WT ratio (mRNA)
166	Superoxide dismutase	0.35	0.5
384	Alcaligin siderophore biosynthesis protein	5.2	137
681	Hemin binding protein	ND (not on partial array)	4.4

App. Environ. Microbiol. (2002) 68: 881-892; D.K. Thompson et al.

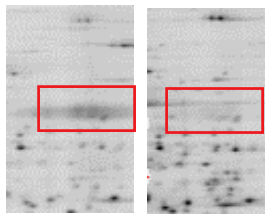
Other Whole Cell Analyses in Progress

- **Hydrogen peroxide effects**
  - Post-translational modification???



Ting Li, ORNL

- **Oxy R mutant**
  - Fumarate reductase differential expression??



Ting Li, ORNL

Analysis of MR-1 Gene and Protein Expression in Whole Cells

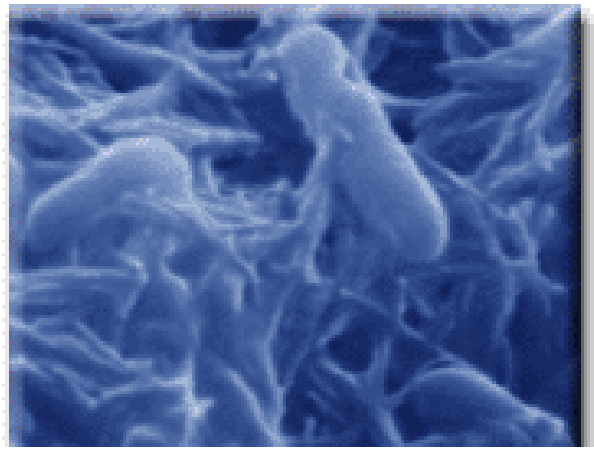
Conclusions

- In experiments with different soluble electron acceptors, major gene and protein expression differences observed were indicative of cells adjusting their metabolism to the availability of varying electron acceptors.
- Correlation was found between some mRNAs and their corresponding proteins, but not all. Regulation at the protein translation/degradation level?
- An example of post-translational modification observed after growth with hydrogen peroxide.

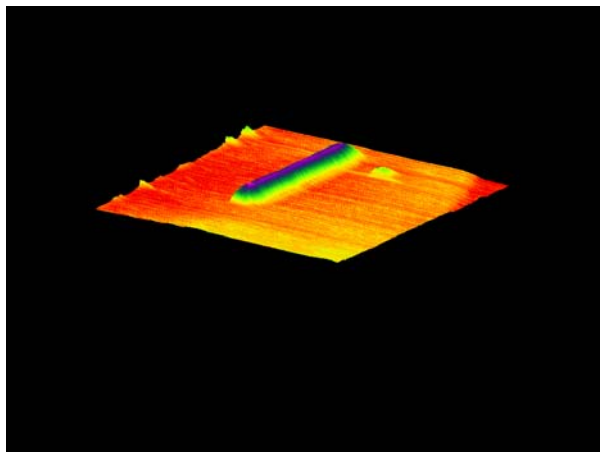
Response to electron acceptor availability involves regulatory mechanisms at both the gene and protein levels.

Shewanella oneidensis MR-1 Makes Contact with the Environment and with Neighboring Cells

Shewanella on hematite
(PNNL)



Shewanella biofilm
(PNNL)



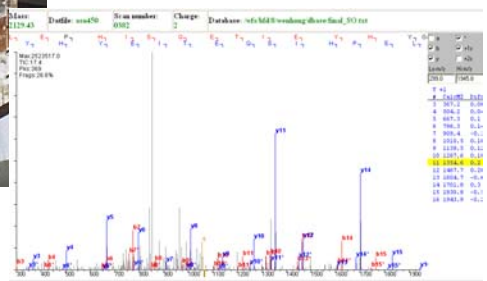
Shewanella on goethite
(Virginia Tech; Atomic Force Microscopy)

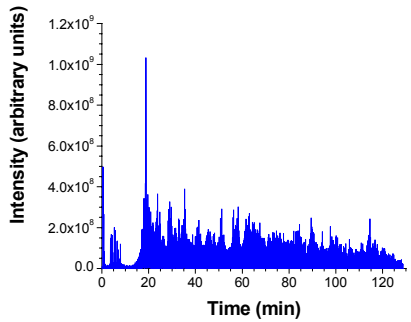
New NABIR Project FY04

Analysis of *Shewanella oneidensis* Membrane Protein Expression in Response to Electron Acceptor Availability

- To identify of all of the proteins expressed in wild type *S. oneidensis* membrane fractions from cells grown with a variety of terminal electron acceptors (including solid-phase as well as soluble metals, e.g., iron and manganese oxides)
- To quantify the differences in the membrane proteins expressed by wild type *S. oneidensis* cells after growth with different terminal electron acceptors
- To use mutants of *S. oneidensis* (e.g., *mtrB* and *mtrC*) to dissect the outer membrane electron transport pathways by comparing the membrane protein expression in the mutant cells with wild type cells

Two Complementary Proteomics Approaches

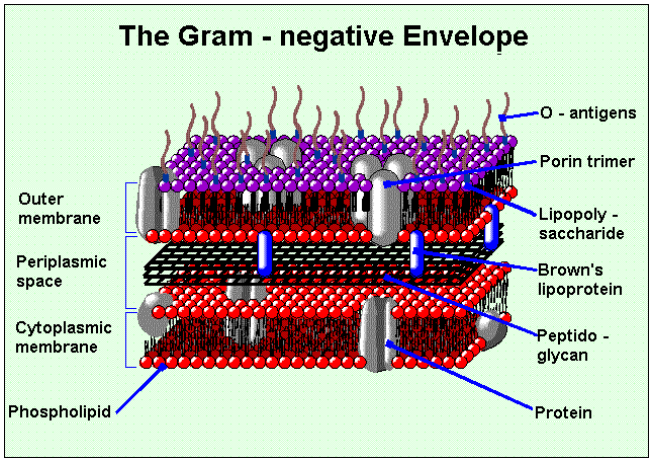


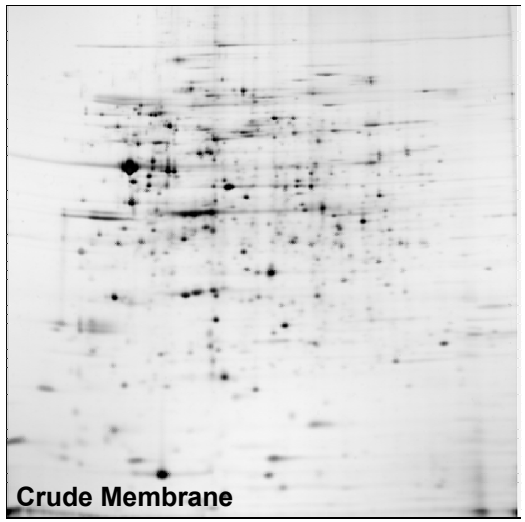


2DE with LC/MS-MS of particular proteins for the assessment of relative abundance and the identification of specific proteins showing differential expression (ANL)

"Shotgun" Proteomics with LC/MS-MS for the identification of ALL proteins present in membrane preparations (ORNL – R. Hettich and N. VerBerkmoes)

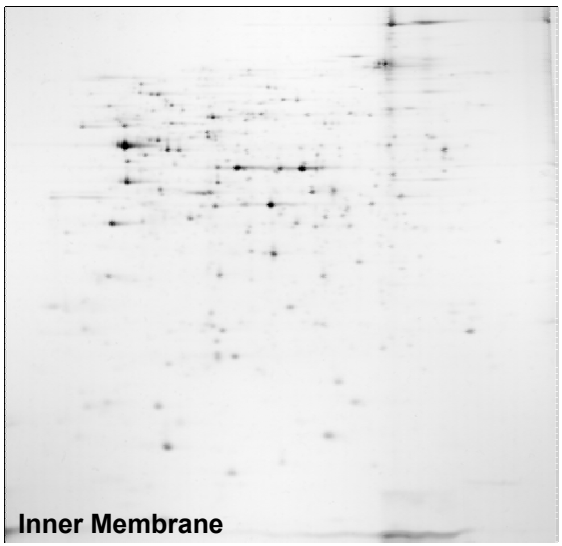
First Challenge: Isolate Membranes

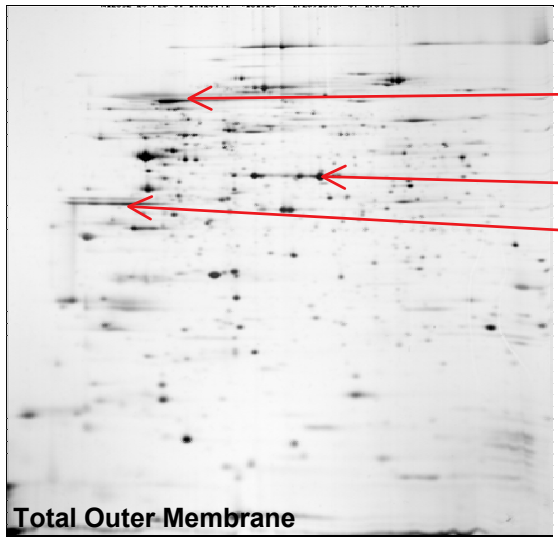


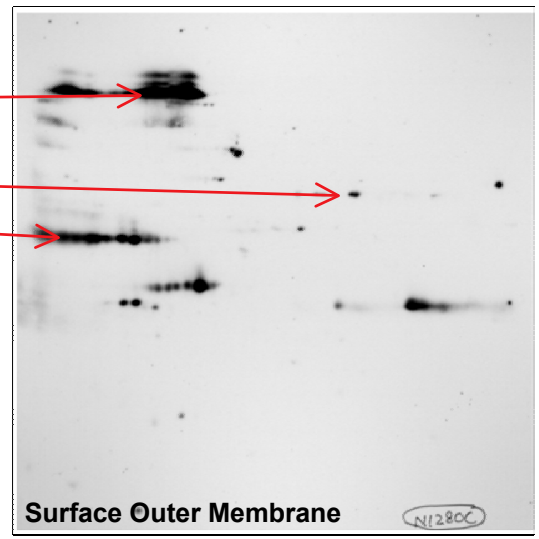


Cells grown aerobically in batch culture were lysed, treated with a detergent solution and the inner and outer membranes were separated by density gradient centrifugation.

To identify proteins exposed on the surface, MR-1 cells were isolated by using a biotin tag and detected using fluorescent avidin.







(See T. Khare poster for details)

Flow Chart for Dual CNBr/Trypsin for LC/LC-MS/MS for Full Protein Inventory

Membrane Fraction

↓

Acetone Precipitate

↓

Solubilize in 70% Formic Acid

↓

Digest with Excess CNBr (24hrs)

↓

Neutralize solution to pH 7.5

↓

Digest with Trypsin (24hrs)

↓

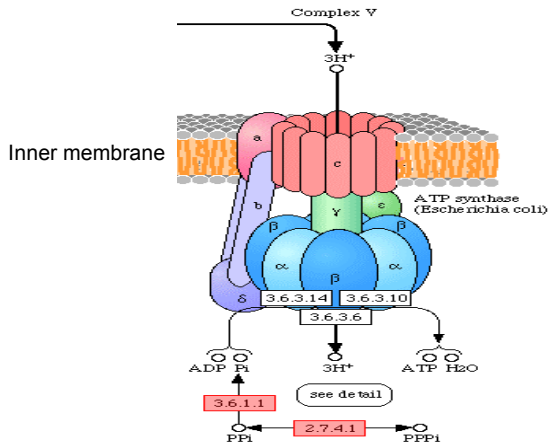
Analyze sample with LC/LC-MS/MS

Membrane prep at ANL → ORNL for LC/LC-MS/MS

But What About Function?

Classical 2DE requires denaturation of proteins into their polypeptide subunits, in most cases causing total inactivation of biochemical function and loss of noncovalently-bonded ligands/cofactors.

An additional aspect of proteomics is the analysis of protein function and identification of the components of protein complexes.



Non-Denaturing 2DE

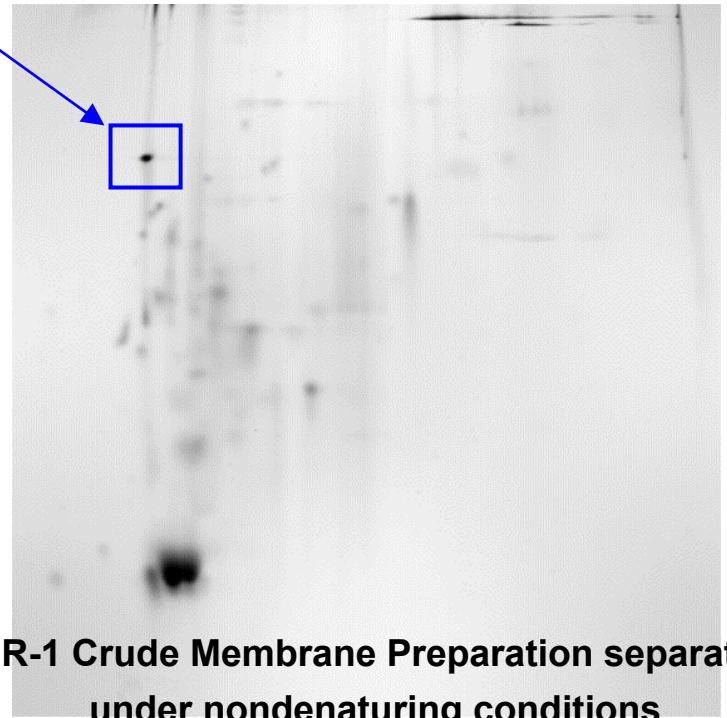
- Cell lysis by sonication at pH 8.0 in isotonic buffer containing protease inhibitors
- First-dimension isoelectric focusing with carrier ampholytes – no denaturants
- Second-dimension electrophoresis (charge-to-mass ratio) – no denaturants

Membrane proteins require a low concentration of zwitterionic detergent (CHAPS) in the gels.

Membrane Proteins Under Nondenaturing Conditions

- ATP synthase F1, alpha (33 peptides)
- ATP synthase F1, beta (38 peptides)
- ATP synthase F1, gamma (14 peptides)
- ATP synthase F1, delta (3 peptides)
- ATP synthase F1, epsilon (4 peptides)

(ASO0210)



MR-1 Crude Membrane Preparation separated under nondenaturing conditions

Differences with growth conditions???

Proteomics 3: 777 (2003) Giometti et al.

Exploration of the MR-1 Membrane Proteome

- **Comparison of membrane proteins from cells grown with different electron acceptors (quantitative and qualitative)**
  - Soluble (Fe, Cr, Ur)
  - Solid (e.g., birnesite, goethite, hematite): K. Nealson @ USC
- **Comparison of membrane protein expression in wildtype MR-1 and specific mutants with genetic deficiencies in specific membrane proteins**:
  - MtrB
  - MtrC → A. Belieav @ PNNL

Goal: To elucidate the specific membrane proteins involved in reduction of different metals with environmental relevance.

Acknowledgments

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Ting Li, ORNL
Ken Nealson, USC