Proteome Profiling of *Shewanella oneidensis* MR-1 *fur*, *etrA*, and *fur*/*etrA* Mutants

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Shewanella oneidensis MR-1 is a facultative aerobic Gram-negative bacterium. It is unique in its capability of using a wide variety of oxidized metals, fumarate, nitrate, and other chemical species as well as oxygen as terminal electron acceptors during respiration. During this process, heavy metals are reduced by the microbe rendering them insoluble thus making this microbe a primary candidate for bioremediation. While the exact mechanism of this microbe's ability to reduce these chemicals is currently widely studied, the regulation of metal transport and reduction as well as the switch between aerobic and anaerobic growth are not well understood. A putative ferric uptake regulator (*fur*) gene and a putative electron transport regulator (*etrA*) gene have been identified in *S. oneidensis* by sequence annotation. Recently, the *S. oneidensis* genome was fully sequenced and fully annotated (Heidelberg et al. Nature Biotechnology 2002) allowing for whole transcriptome and whole proteome analysis (figure 1). Studying gene knockout strains of both of these potential global regulators by whole transcriptome and whole proteome methods should greatly increase our understanding of these regulatory mechanisms. The goal of this study was to make a preliminary comparison of semi-quantitative proteomic data and microarray data using *Shewanella oneidensis* MR-1 *fur*, *etrA*, and *etrA*/*fur* knockouts with wild type strain.

To accomplish this goal *S. oneidensis* MR-1 WT, *fur*, *etrA*, and *fur*/*etrA* mutants were grown to mid-log phase under conditions of aerobic respiration**.** Transcriptome profiles of the mutant strains were compared to those of the wild-type reference using DNA microarrays covering ~99% of the total predicted protein-encoding open reading frames in *S. oneidensis*. The entire proteome from each growth states was denatured, reduced and digested with sequencing grade trypsin. The resultant peptide mixtures were reduced a final time and desalted/concentrated with a C18 solid phase extraction cartridge. Protein fractions from WT and mutant samples were analyzed by 1D-LC-MS/MS employing 4 or 5 mass ranges or by an automated nano switching 2D LC-MS/MS (SCX-RP C18) system with an Thermo-Finnigan LCQ-Deca ES-ion trap employing multiple mass range scans. All MS/MS data were obtained in data-dependent mode with dynamic exclusion enabled. The resultant MS/MS spectra from each sample were searched with SEQUEST against all predicted ORFs from *S. oneidensis* MR-1 (ORNL annotation). The raw output files were filtered and sorted with DTASelect and growth states were compared with Contrast.

Triplicate whole proteome analysis of WT and *fur* mutant by nano switching 2D LC-MS/MS and 1D-LC-MS/MS resulted in the identification of 837 proteins from WT, 963 from *fur*, for a total of 1102 proteins from both. A qualitative comparison between WT and *fur* was made using the Contrast software. Proteins showing dramatic change in identification level between the two samples (4 or more unique peptides and/or 30% sequence coverage) were compared with microarray data. Thirty-one proteins from proteome data showed significant change between WT and *fur* of these 17 agreed with microarray data, 10 did not, and 4 have no microarray data (figure 2). Thirteen of these genes are thought to be involved in iron uptake or utilization from the annotation database. A follow up experiment involved a small-scale analysis of WT, *fur/etrA* and *etrA* by 1D-LC-MS/MS with multiple mass range scanning. From duplicate analysis a total of 605 proteins were identified in the *etrA/fur* mutant, 595 in *etrA mutant*, and 654 in WT. The total proteins identification from all three samples was 842. Preliminary analysis of this sample shows that only the *etrA/fur* mutant shows any significant change with many of the same iron-uptake and utilization genes observed in the *fur* mutant showing dramatic change in the proteome data leading to the initial conclusion that the *etrA mutant* does not effect dramatic change in the overall proteome. Microarray analysis of these mutants is in progress.

Figure 1 Comparison of Microarray and mass spectrometry

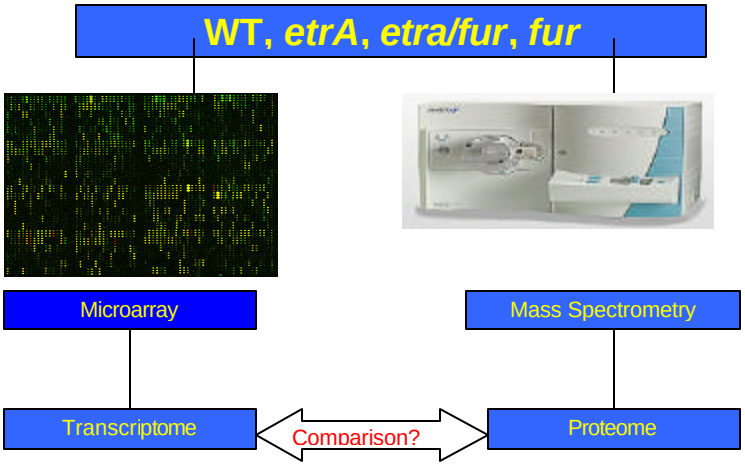

Figure 2

***S. oneidensis Fur* vs WT comparison by Proteome and Whole Genome Microarray data**

RULES for acceptance: At least a 30% change in sequence coverage or 4 or more unique peptides difference
Microarrays: If the MEAN is above 1 then it is up regulated if below 1 then down regulated

Up Regulated in FUR

	Proteome Data								Array Data	
ORNL Gene #	FUR_5mz % Cov.	FUR_1mz % Cov.	FUR_2mz % Cov.	WT_5mz % Cov.	WT_1mz % Cov.	WT_2mz % Cov.	Fur_pept Tot pept	WT_pept Tot pept	MEAN (Fur/WT)	ORNL Annotation
264	37.9	34.8	26.5	0	0	0	21	0	26.81	ferrichrome-iron receptor
525	30.2	36.5	37	0	0	0	7	0	0.50	alkyl hydroperoxide reductase c22 protein
613	10.4	18.4	18	0	0	0	9	0	0.39	TonB-dependent receptor
775	775	16.8	19.5	4.6	4.6	0	9	1	0.92	formyltransferase/IMP cyclohydrolase
798	58.7	63.1	54	0	0	0	12	0	N/A	probable oxidoreductase
844	36.5	18.5	23.5	16.1	4.7	4.7	21	8	0.41	ornithine decarboxylase, inducible
989	27.1	32.3	40.5	0	6.2	6.2	19	1	26.20	Ferrichrome-iron receptor
1106	69.2	56.9	54.6	7.8	4.2	6.8	33	4	5.61	Fe-regulated outer mem. virulence protein, TonB receptor family
1109	10.7	10.7	25	0	0	0	8	0	8.21	ferric vibriobactin receptor
1320	41.1	42.2	42.2	6.6	20.9	20.9	6	3	0.75	uridine phosphorylase
1462	19.5	10.9	24	10.4	0	0	15	4	2.83	probable outer membrane receptor for iron transport
1594	53.5	41.6	43.8	15.4	9.8	9.8	30	7	25.45	probable tonB-dependent receptor HI0262 precursor
1595	17.2	18.3	18.3	0	0	0	2	0	37.50	conserved hypothetical protein
1596	37.3	42.7	31.4	7	0	0	9	1	59.66	conserved hypothetical protein
2429	25.9	31.7	25.9	11.6	0	0	9	2	N/A	alanine dehydrogenase
3789	44.6	42.5	35.5	0	13	13	11	2	N/A	TonB-dependent receptor, iron-siderophore receptor
3790	35	11.3	15.8	0	0	0	8	0	N/A	Similar to ferric aerobactin receptor
4265	0	11.7	42.3	0	0	0	4	0	1.17	FKBP-type peptidyl-prolyl cis-trans isomerases
4308	2.2	9.9	12.7	2.2	0	0	6	1	0.77	Cation efflux system protein: AcrB/AcrD/AcrF family
4719	37.6	58	58	0	20	5.1	13	2	15.47	conserved hypothetical protein
4960	14.7	12.9	25.8	0	3.1	0	12	1	2.48	heme-hemopexin utilization protein C precursor
5234	13.2	16	14.1	4.2	4.2	4.2	8	1	1.11	UDP-sugar hydrolase

Up Regulated in WT

125	0	0	0	4.2	12.5	13.6	0	5	0.17	deca-heme type cytochromes
126	0	0	0	7.5	15.7	17.7	0	10	0.19	deca-heme type cytochromes
1552	0	0	0	21.5	8	13.1	0	12	0.94	Hypothetical
2240	0	0	0	3.6	25.8	25.8	0	3	1.92	hypothetical protein
2404	0	8.7	19.7	22.1	37	39.4	2	8	0.94	cytochrome c oxidase, subunit CcoO
3043	4.5	10.5	4.3	25.6	37.1	40.2	4	28	1.32	TonB-dependent (outer membrane) receptor, mostly iron transport
3608	0	0	0	11.8	15.3	17.3	0	8	0.97	oprC; outer membrane protein c
4840	0	0	0	23.3	26.7	25.5	0	16	1.47	putative membrane protein, Membrane protease subunits
75a	0	0	0	6.3	10.6	6	0	7	1.24	probable acylamino-acid-releasing enzyme

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