

Links between anaerobiosis and carbon source selection in *Shewanella*

or
The Amazing Coping Strategies of a Metal-Respiring Bacterium

Amolya Singh

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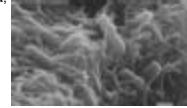
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Shewanella oneidensis and its many talents

- Widespread soil and marine bacterium, Gram negative, rod-shaped, 2-3µ, motile, non-spore-former
- Close relatives are *V. cholerae*, *E. coli* (gamma-proteobacteria)
- 65 years of ecological, morphological and biochemical studies
 - (Re-)Named after JM Shewan, a Scottish microbiologist who observed fish spoilage
 - Isolated from Oneida Lake, NY; Black Sea; Southern Ocean; Great Lakes, ...
- Genome sequenced in 2002
- Can grow on metals, respire with or without oxygen
- Candidate for bioremediation efforts



S. oneidensis growing on hematite (image courtesy PHNL)



S. oneidensis growing on crystals of magnetite (image courtesy I.C.H. Neelson)

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Bioremediation

"...use of microorganisms, plants, or their enzymes to detoxify pollutants in water and soil." (Skipper & Turco, 1995)

US produces 300m metric tons of hazardous waste / year

- 1.3 metric tons per capita
- Food, textile, lumber, petroleum, chemical, transportation industries
- 10,000 lbs of carcinogens released to US lands
- >1500 EPA superfund sites

US DoE is sitting on much of that

- 1.7 trillion gallons of contaminated groundwater
- 40 million cubic meters of contaminated soil
- 50-60% of DoE facilities have groundwater & soil contaminated with metals or radionuclides

Spend >\$1m / year on cleaning it up

Strategies

Stimulation: enhance activities of indigenous microbes by adding nutrients, changing pH, or removing other limiting conditions

Inoculation: introduce specific microbes into polluted sites

Enzyme Treatment: application of immobilized enzymes for specific pollutants



US Superfund sites in California, Washington, and New Jersey (images courtesy US-EPA, epa.gov)

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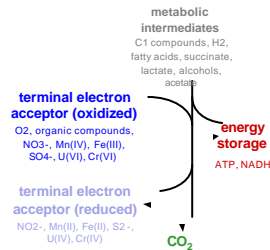
Respiration (recap)

electron donor (energy source)

light, chemical (organic, inorganic)

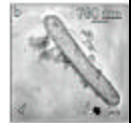
carbon source

organic, inorganic



S. oneidensis can use

- nitrate
- nitrite
- fumarate
- trimethylamine N-oxide
- dimethyl sulfoxide
- insoluble oxides of Fe(III), Mn(IV), U(VI), Cr(VI)



S. oneidensis respiring crystals of Cr(VI) (Daulton et al. 2001)

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Bacterial regulation of anaerobiosis

- Oxygen sensing in *E. coli* done by *fnr* (fumarate & nitrate reduction)
 - + glycerol and formate dehydrogenases
 - + nitrate, fumarate and dimethylsulfoxide (DMSO) reductases
 - + proteins for transport of carbon sources or electron acceptors
 - enzymes required for aerobic respiration, such as NADH dehydrogenase II
- Functional homolog of *fnr* found in *S. oneidensis*: *etrA* (Saffarini & Neelson, 1993)
- Regulatory mechanisms of anaerobic respiration in *Shewanella* not fully understood
- crp* mutants somewhat defective in anaerobic respiration (Saffarini et al. 2003)
 - could not respire Fe, Mn, nitrate, fumarate, DMSO
 - but could respire nitrite, TMAO
- Interesting link between anaerobic respiration and carbon source selection? Or pleiotropy of *crp*?

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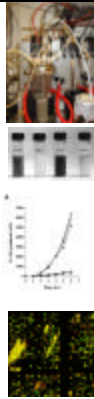
Research Questions

- What does *crp* regulate, and how?
- How is it regulated?
- What other networks is it cross-linked to?
- How has it evolved?
- What are the dynamics and time-frames of reactions?

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Approaches

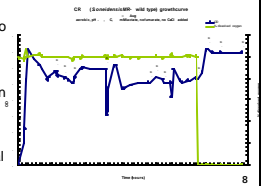
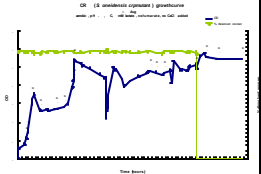
- Culture in chemostat under various growth conditions
- Perform continuous biochemical assays to measure levels of key metabolites
- Electron microscopy
- Mutagenesis
- Expression profiling of mutant vs. wt
 - rtPCR
 - microarrays
 - proteome analysis
- Comparative genomics



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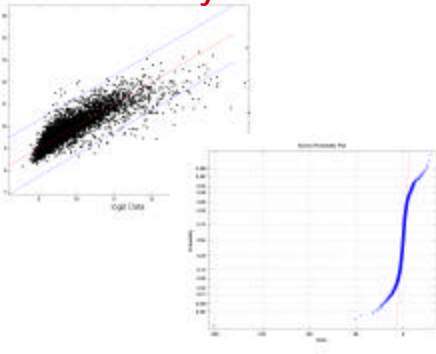
Experimental Procedure

- Comparison of genome-wide mRNA expression levels between wt and *crp* mutant
- Grown in continuous culture chemostat, ~700 hours, under oxygen-limiting conditions, constant pH and temperature, with different e-acceptors:
 - Fe(II)
 - Fumarate
 - Nitrate
 - Dimethyl sulfoxide (DMSO)
- Total RNA extracted from 10mL samples, purified, fluorescently labeled, hybridized to cDNA genomic microarrays (ORNL) in triplicate
- Microarray images scanned, spots identified, data extracted, normalized within slides (global constant) and between replicates (geometric mean)
- Significance assessed by deviation of t-statistic of log expression ratio from normal distribution



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Preliminary Results



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crp Mutant Gene Expression for each e-acceptor NIT

SO		membrane protein, <i>outative</i>	Cell envelope
SO	[cAMP]	cyclic nucleotide binding protein, <i>putative</i>	Unknown function
SO		nifU-related protein (only similar at N-terminus)	Unknown function
SO	<i>alcA</i>	alkaloin biosynthesis enzyme <i>AlcA</i>	Transport and binding proteins
SO		sensor histidine kinase	Signal transduction
SO	[<i>alcB</i>]	alkaloin biosynthesis protein <i>AlcB</i> <i>putative</i>	Transport and binding proteins
SO		heme transport protein	Transport and binding proteins
SO		universal stress protein family	Cellular processes
SO		lipoprotein, <i>putative</i>	Cell envelope
SO	[<i>ISSod - 1</i>]	[<i>ISSod - 1</i>] <i>ISSod - 1</i> <i>transposase</i>	Other categories
SO		S-adenosylmethionine- <i>demethylmenaquinone</i>	Biosynthesis of cofactors, prosthetic groups, and carriers
SO	<i>menG</i>	methyltransferase	Energy metabolism
SO	[<i>nifA</i>]	cytochrome c nitrite reductase (<i>hemes</i>)	Energy metabolism
SO		sensor histidine kinase	Signal transduction
SO	[<i>fimL</i>]	type IV pilus biosynthesis protein, <i>putative</i>	Cell envelope
SO		- <i>oxoacyl</i> acyl-carrier protein I synthase III, <i>outative</i>	Fatty acid and cholesterol metabolism
SO		SIM domain protein	Unknown function
SO		transporter, <i>putative</i>	Transport and binding proteins
SO		site-specific recombinase phage integrase family	Unknown function
SO		(interrupted by <i>ISSod</i>)	DNA metabolism
SO		conserved hypothetical protein	Conserved hypothetical
SO		ParB family protein	Cellular processes
SO	<i>cspD</i>	cold shock domain family protein	Cellular processes

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crp Mutant Gene Expression for each e-acceptor FE

SO		ornithine decarboxylase cytochrome B subunit	Energy metabolism
SO	<i>BA</i>	lipoic acyltransferase protein A (NAC family)	Regulatory functions, Cellular processes
SO	<i>acpP</i>	<i>ac</i> -acyl carrier protein	Transport and binding proteins
SO	<i>acpD</i>	lipid protein	Unknown function
SO		acyltransferase, GNAT family	Unknown function
SO	<i>nifH</i>	Nif-H enzyme	Transport and binding proteins
SO		conserved domain protein	Unknown function
SO	<i>psrA</i>	hydroxylase reductase/oxidase A	Energy metabolism
SO		amA protein	Unknown function
SO	<i>dhxA</i>	<i>dh</i> -adenosylmethionine- <i>RNA</i> biosynthase, <i>isomerase</i>	Protein synthesis
SO	<i>fadR</i>	fatty acid metabolism regulator protein	Regulatory functions, Fatty acid and phospholipid metabolism
SO		ProB family protein	Unknown function
SO	[<i>IR</i> - 1]	inorganic Molybdo- <i>F</i> protein, <i>putative</i>	Other categories
SO	<i>acpE</i>	acyl- <i>RNA</i> synthetase	Protein synthesis
SO	<i>mrhE</i>	MSHA pilin protein <i>MrhE</i> (moss pilin)	Cellular processes
SO	<i>acpD</i>	acyl carrier protein phosphodiesterase	Fatty acid and phospholipid metabolism
SO	<i>acpB</i>	<i>acpB</i> 1 transfer phosphodiesterase beta subunit	Energy metabolism
SO	<i>acpB</i>	highly conserved	Energy metabolism
SO		iron-sulfur cluster binding protein	Unknown function
SO		hydroxase, alpha-beta fold family	Unknown function
SO		PAF family protein	Unknown function
SO		(on Williams) factor type A domain protein	Unknown function
SO		serine-threonine kinase (PAS domain)	Regulatory functions
SO		phosphoethanolamine protein A	Unknown function

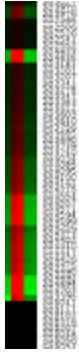
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crp Mutant Gene Expression for each e-acceptor FUM

SO	<i>emh</i>	ribosomal protein L	Protein synthesis
SO		methyltransferase domain protein	Unknown function
SO	<i>nHL</i>	hemim-mono-phosphate kinase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO		ToxB domain protein	Unknown function
SO		cytochrome c family protein (<i>hemex</i>)	Energy metabolism
SO		transcriptional regulator, LysR family	Regulatory functions
SO		transporter, <i>putative</i>	Transport and binding proteins
SO	<i>pitT</i>	switching mobility protein <i>PitT</i>	Cellular processes
SO		(fused domain protein (PAS domain)	Unknown function
SO		iron sulfur cluster binding protein	Energy metabolism
SO	<i>ubG</i>	<i>ubG</i> methyltransferase	Biosynthesis of cofactors, prosthetic groups, and carriers
SO	<i>acp</i>	C- <i>dicarboxylate</i> -binding periplasmic protein	Transport and binding proteins
SO		membrane protein, <i>putative</i>	Cell envelope
SO	<i>mvn</i>	MVN protein	Cellular processes
SO		polyketide <i>ABC</i> transporter, periplasmic	Transport and binding proteins
SO		melanin-binding protein	Transport and binding proteins
SO		prophage Lambda/S ϕ 10, hypothetical protein	Hypothetical
SO	<i>ugd</i>	UDP-glucose 4-epimerase	Cell envelope
SO	<i>nRE</i>	nitrogen regulator protein (<i>nRE</i>)	Signal transduction
SO		lysD family secretion protein	Transport and binding proteins
SO		cyt <i>CDK</i> acetyltransferase family protein	Fatty acid and phospholipid metabolism
SO	<i>uA</i>	uric uracil-binding receptor	Transport and binding proteins
SO	[<i>MG</i>]	polyacrylamide biosynthesis protein	Cell envelope
SO		response regulator	Signal transduction
SO		sensor histidine kinase	Signal transduction
SO	<i>psrA</i>	serine kinase <i>F</i>	Energy metabolism
SO		D- <i>alanyl</i> D-alanine carbonylpeptidase - related protein (propeptide)	Unknown function
SO	[<i>ISSod - a</i>]	[<i>ISSod - a</i>] <i>ISSod - a</i> <i>transposase</i> <i>DNA</i>	Other categories
SO	<i>katG</i>	catalase/peroxidase <i>Hp</i>	Cellular processes

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crp Mutant Gene Expression for all e-acceptors



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Genomic Analysis

Gene	Accession	Protein
crp	U00002	CRP
crpA	U00003	CRP
crpB	U00004	CRP
crpC	U00005	CRP
crpD	U00006	CRP
crpE	U00007	CRP
crpF	U00008	CRP
crpG	U00009	CRP
crpH	U00010	CRP
crpI	U00011	CRP
crpJ	U00012	CRP
crpK	U00013	CRP
crpL	U00014	CRP
crpM	U00015	CRP
crpN	U00016	CRP
crpO	U00017	CRP
crpP	U00018	CRP
crpQ	U00019	CRP
crpR	U00020	CRP
crpS	U00021	CRP
crpT	U00022	CRP
crpU	U00023	CRP
crpV	U00024	CRP
crpW	U00025	CRP
crpX	U00026	CRP
crpY	U00027	CRP
crpZ	U00028	CRP
crpAA	U00029	CRP
crpAB	U00030	CRP
crpAC	U00031	CRP
crpAD	U00032	CRP
crpAE	U00033	CRP
crpAF	U00034	CRP
crpAG	U00035	CRP
crpAH	U00036	CRP
crpAI	U00037	CRP
crpAJ	U00038	CRP
crpAK	U00039	CRP
crpAL	U00040	CRP
crpAM	U00041	CRP
crpAN	U00042	CRP
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crpAQ	U00045	CRP
crpAR	U00046	CRP
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crpAW	U00051	CRP
crpAX	U00052	CRP
crpAY	U00053	CRP
crpAZ	U00054	CRP
crpBA	U00055	CRP
crpBB	U00056	CRP
crpBC	U00057	CRP
crpBD	U00058	CRP
crpBE	U00059	CRP
crpBF	U00060	CRP
crpBG	U00061	CRP
crpBH	U00062	CRP
crpBI	U00063	CRP
crpBJ	U00064	CRP
crpBK	U00065	CRP
crpBL	U00066	CRP
crpBM	U00067	CRP
crpBN	U00068	CRP
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crpBQ	U00071	CRP
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crpCA	U00081	CRP
crpCB	U00082	CRP
crpCC	U00083	CRP
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crpCE	U00085	CRP
crpCF	U00086	CRP
crpCG	U00087	CRP
crpCH	U00088	CRP
crpCI	U00089	CRP
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crpCK	U00091	CRP
crpCL	U00092	CRP
crpCM	U00093	CRP
crpCN	U00094	CRP
crpCO	U00095	CRP
crpCP	U00096	CRP
crpCQ	U00097	CRP
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crpCU	U00101	CRP
crpCV	U00102	CRP
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crpDM	U00119	CRP
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crpLL	U00326	CRP
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crpLN	U00328	CRP
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