

**Nitrate Enhanced Chromium Reduction in Three
Model Organisms: *Geobacter metallireducens*,
Sulfurospirillum barnesii, and *Desulfovibrio
desulfuricans* 27774**

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Chromium contaminates ground water and sediments at many DOE sites (reactor operations, irradiated fuel production, fuel recovery)

Also used in wood preservation (CCA) and industrial applications



D Reactor Building, Hanford

Microbial Cr(VI) reduction:

Several microbes known to readily reduce Cr(VI) to Cr(III)

Rhodobacter sphaeroides (Moore and Kaplan, 1992)

Pseudomonas ambigua (Susuki et al., 1992)

Pseudomonas putida (Park et al., 2000)

Geobacter metallireducens (Lovley et al., 1993)

Shewanella oneidensis (Viamajala et al., 2002)

Desulfovibrio vulgaris (Clark et al., 2008)

Several enzymes shown to have Cr(VI) reductase activity

FADH₂-dependent metal reductase

nitroreductase

c-type cytochrome

hydrogenase

Field trials using lactate stimulated bioreduction of Cr(VI)

BUT:

Chromium can be inhibitory to growth and activity

Cr(VI) reduction can be inhibited by co-contaminants

NO₃ Nitric acid and nitrate salts in nuclear fuel processing and fabrication has resulted in nitrate being the most commonly reported anion

Nitrate reduction to ammonia generates more energy than chromate reduction

So: Need to understand dynamics of Cr(VI) reduction under nitrate reducing conditions

Nitrate Enhanced Chromium Reduction

The central hypothesis of this project is that the presence of nitrate can impact the biotransformation of Cr(VI) in three ways:

1) as a competitive alternative electron acceptor inhibiting transformation

Geobacter metallireducens

2) as a co-metabolite resulting in concomitant reduction, stimulating transformation

Sulfurospirillum barnesii

3) as an inducer of specific proteins and pathways involved in oxidation/reduction reactions stimulating transformation

Desulfovibrio desulfuricans strain 27774

Nitrate Enhanced Chromium Reduction

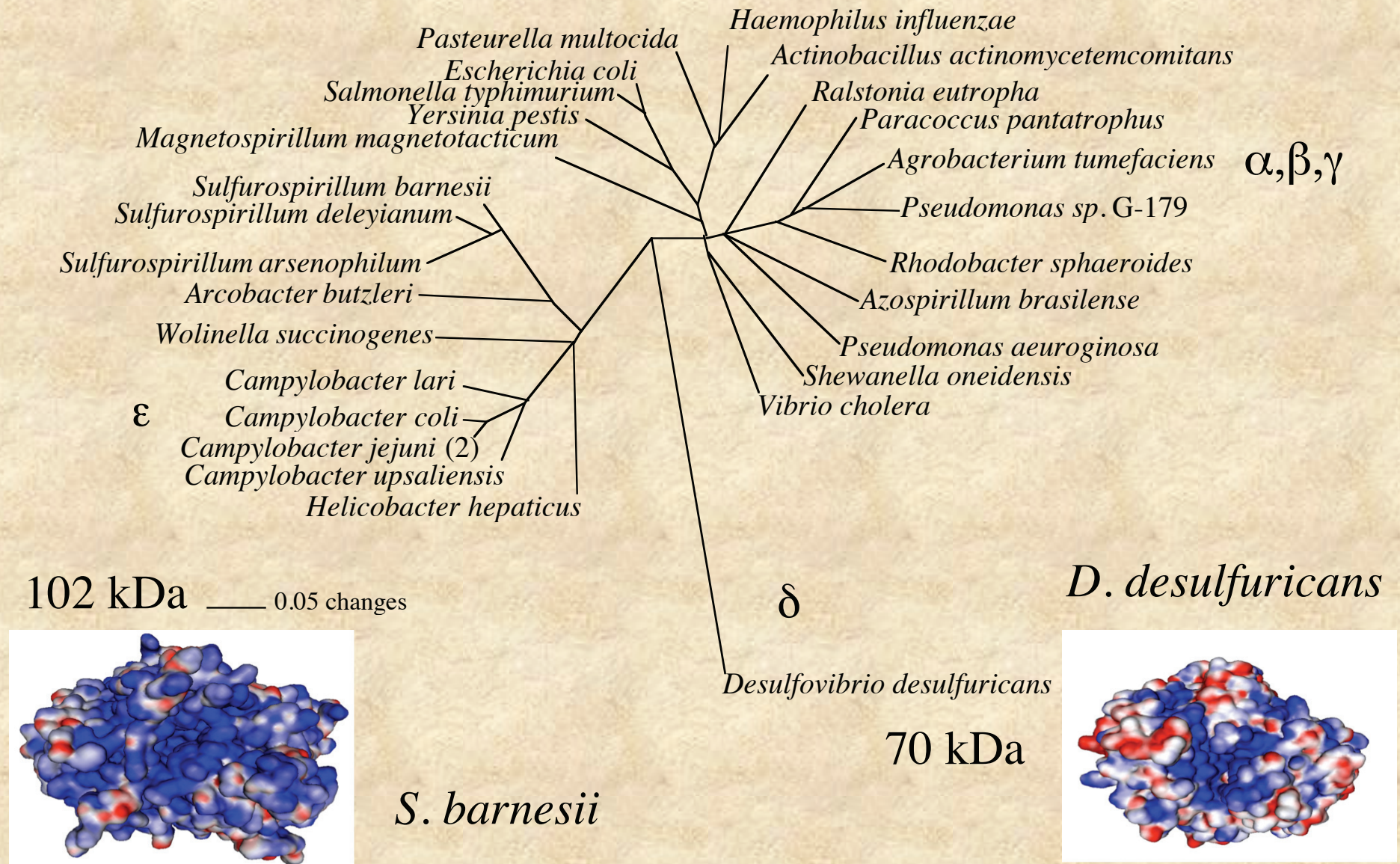
All three organisms reduce nitrate to ammonia in a two step process involving nitrate reductase and nitrite reductase.

G. metallireducens possesses a unique Nar-type nitrate reductase (NarCGHJI).

S. barnesii and *D. desulfuricans* have a periplasmic nitrate reductase (NapAGHBFLD, NapCMADGH).

All three have a pentaheme cytochrome *c* nitrite reductase (Nrf). Preliminary studies have shown Nrf from *G. metallireducens* and *S. barnesii* can be oxidized by chromate. Our hypothesis is that Nrf can function as a chromate reductase and is the link between nitrate and chromium reduction.

Not all nitrate reductases are alike: Size (MW) and surface charge of NapA of *D. desulfuricans* and *S. barnesii* are significantly different



Nitrate Enhanced Chromium Reduction

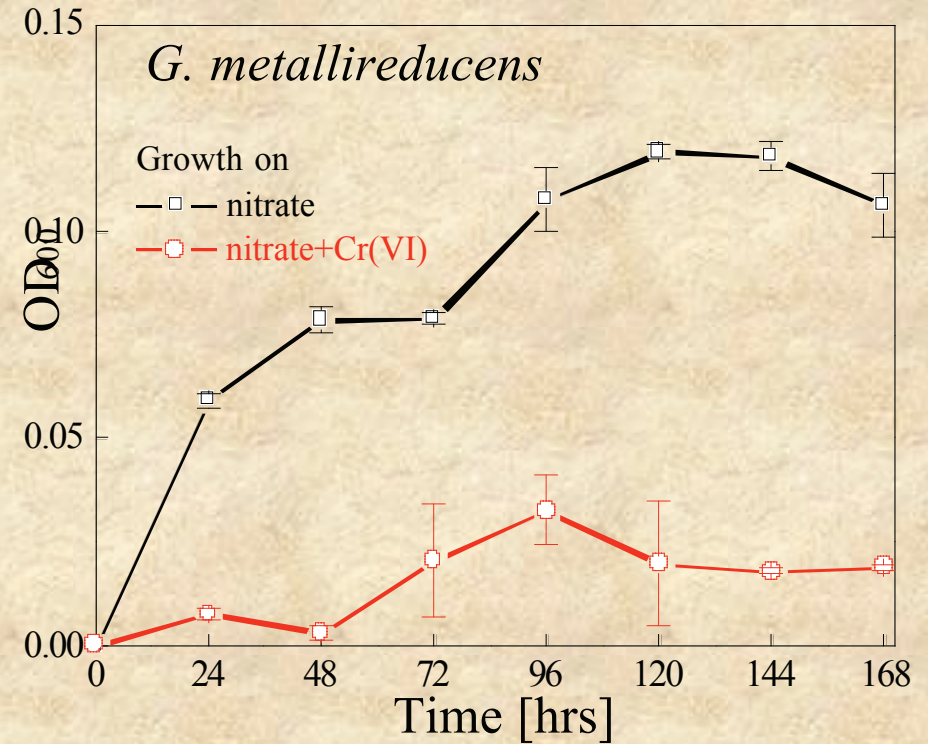
Specific aim 1. To investigate the affects of chromate on nitrate respiration in *G. metallireducens*, *S. barnesii*, and *D. desulfuricans*. Preliminary experiments demonstrated that chromate affects growth with nitrate differently in each of the three organisms. The effect of Cr(VI) concentration on the kinetics of both growth and reduction of nitrate, nitrite, and Cr(VI) in these three organisms is to be determined.

Specific aim 2. To develop a profile of bacterial enzymes involved in nitrate transformation (e.g., oxidoreductases) using a proteomic approach. The proteome of the three species grown under different growth conditions will be compared to identify proteins involved in nitrate and chromate metabolism. It is possible that several proteins are upregulated/downregulated under these conditions.

Specific aim 3. To investigate the function of periplasmic nitrite reductase (Nrf) as a chromate reductase. Nrf from *G. metallireducens*, *S. barnesii*, and *D. desulfuricans* will be purified and the kinetics (K_m , V_{max} , K_{cat}) of chromate reduction and other biochemical characteristics determined.

Specific aim 4. To develop a strategy to maximize microbial chromium reduction in the presence of nitrate. The results from specific aims 1-3 will provide insight into possible amendments and manipulations for enhanced in situ remediation. This will be accomplished using both pure culture and natural populations (sediment slurries).

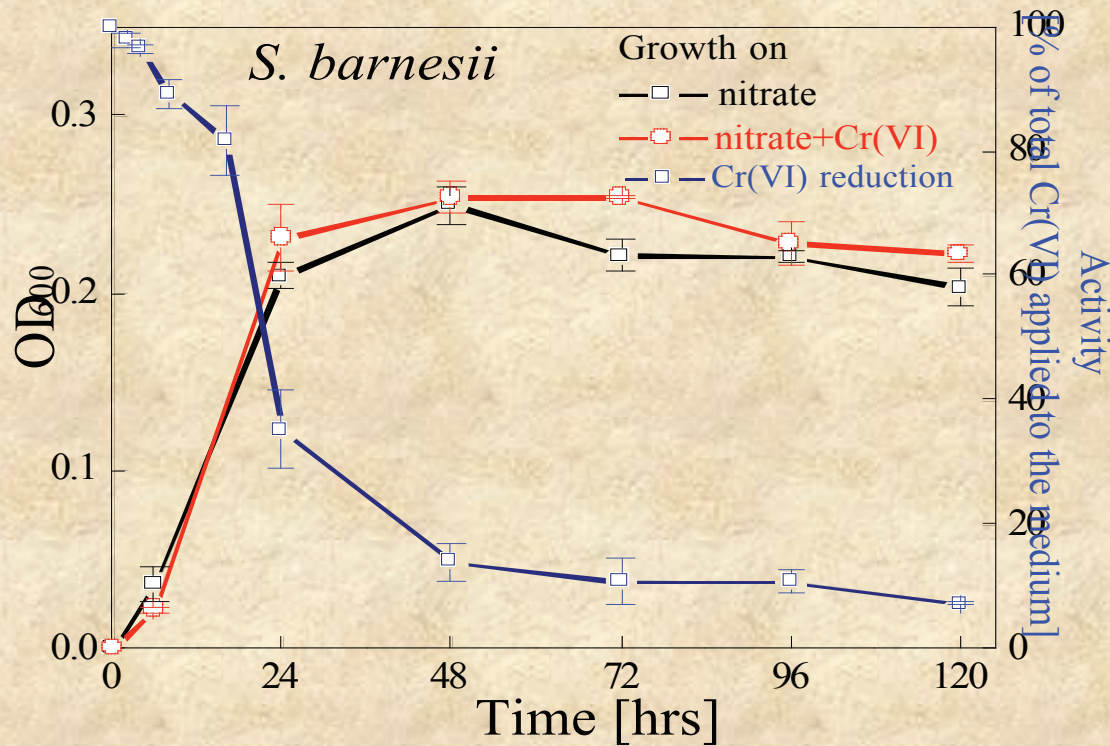
Nitrate Enhanced Chromium Reduction



Cr(VI) inhibits growth on nitrate

Nitrate Enhanced Chromium Reduction

Simultaneous reduction of Cr(VI) and nitrate, no growth inhibition

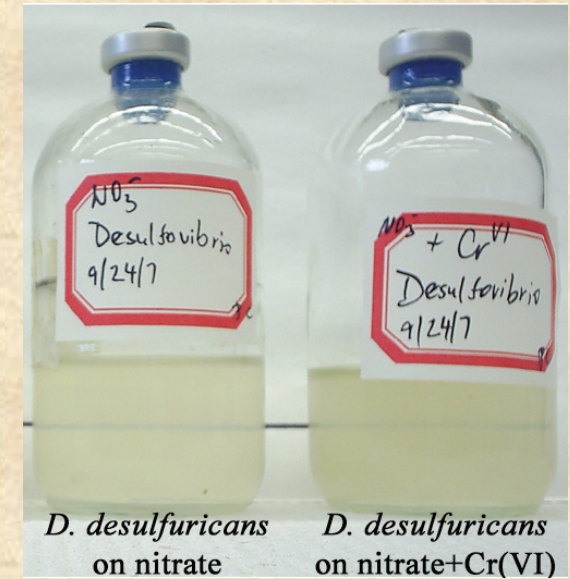
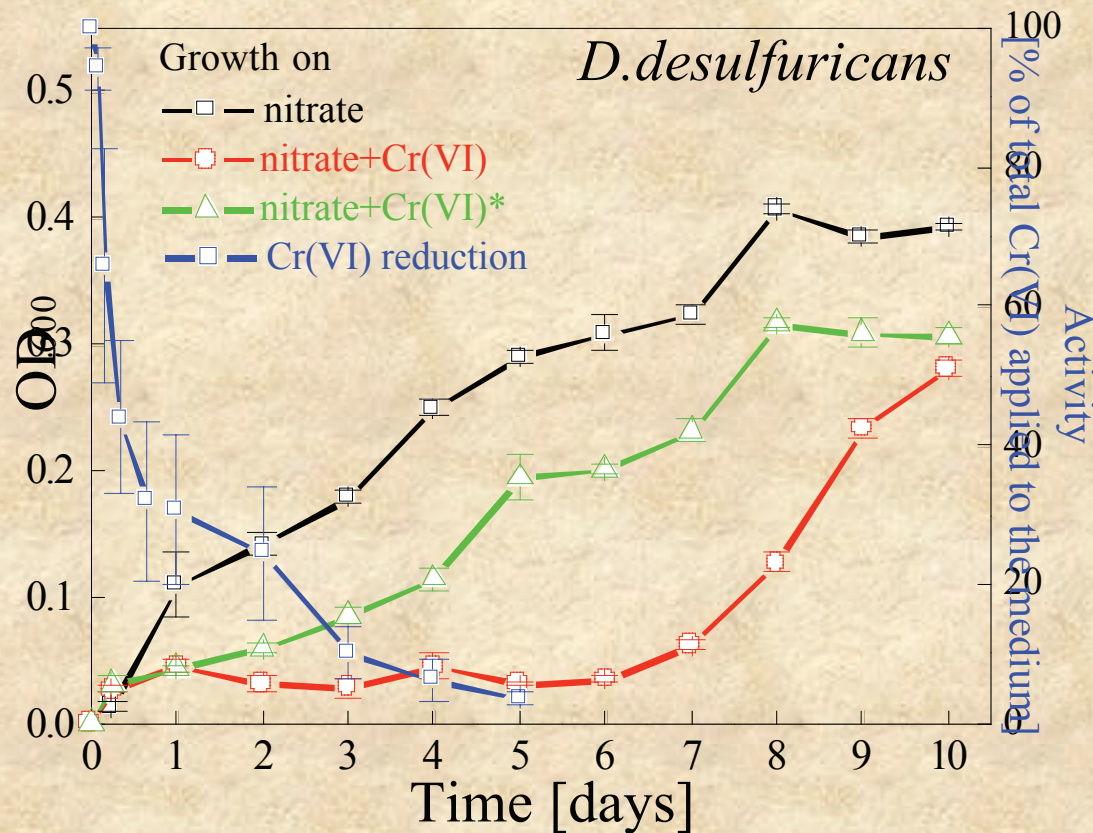


Nitrate Enhanced Chromium Reduction

Cr(VI) reduced first, then growth.

Lag phase can be reduced by reinoculum

Initial “die off” also seen in *D. vulgaris*



Proteomics Investigation:

Cells were harvested during log phase and stationary phase, lysed by French Pressure cell and 2-D gel electrophoresis run on the ETTAN system from GE.

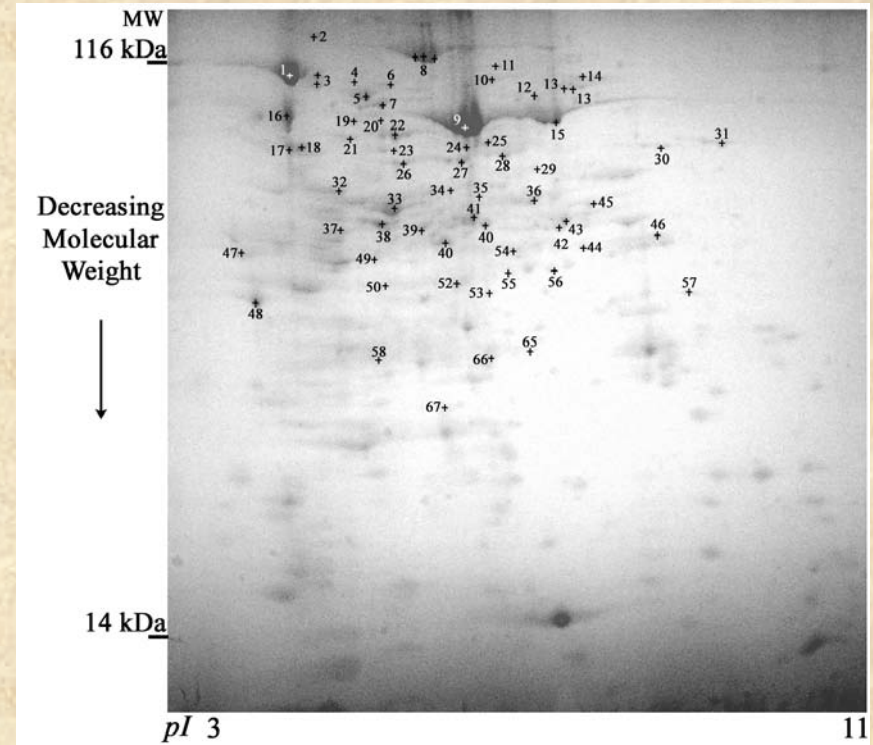
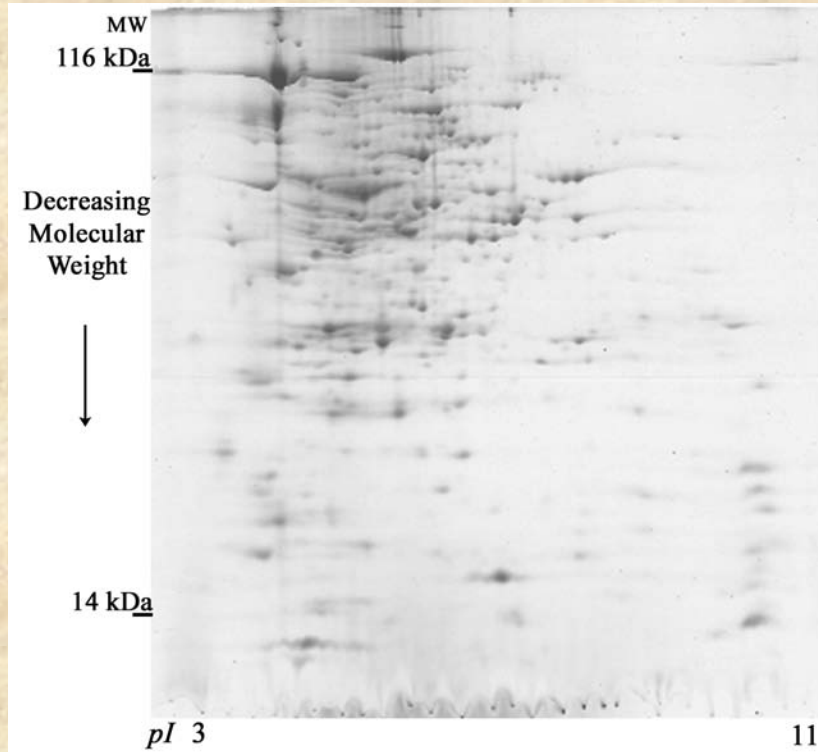
The gels were stained with colloidal coomassie blue or silver. Protein spots of interested were removed from the gels, subject to trypsin digest, and cleaned up using “zip-tips”.

MALDI-TOF was run on an ABI Voyager DE-STR, and the data analyzed with MASCOT (Matrix Science) using a mass tolerance of +/- 0.1

LC-MS-MS was run on a ProteomeX (LC) ThermoFinnigan LCQ Deca XP (MS/MS)

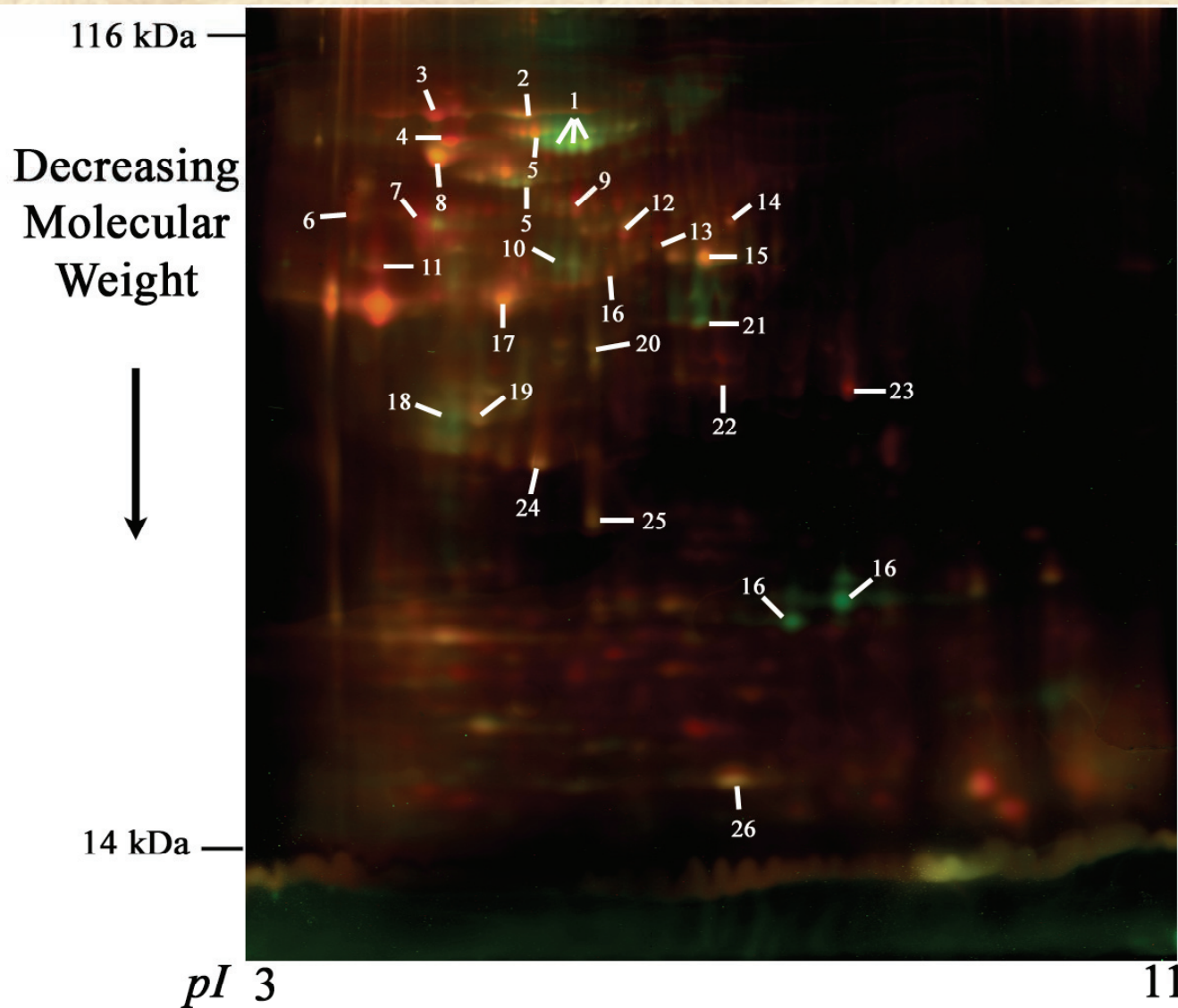
Genome Enabled Proteomics:

Alkaliphilus oremlandii strain OhILAs



Over 200 proteins identified using MALDI-TOF MS

Genome Enabled Proteomics: *Alkalilimnicola ehrlichii*



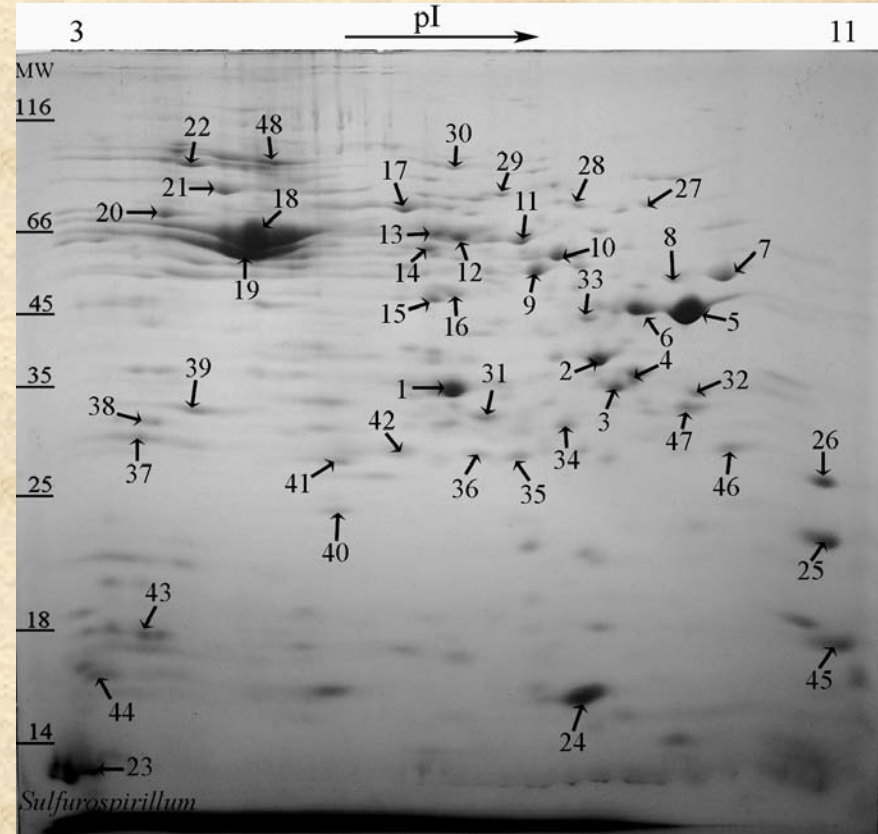
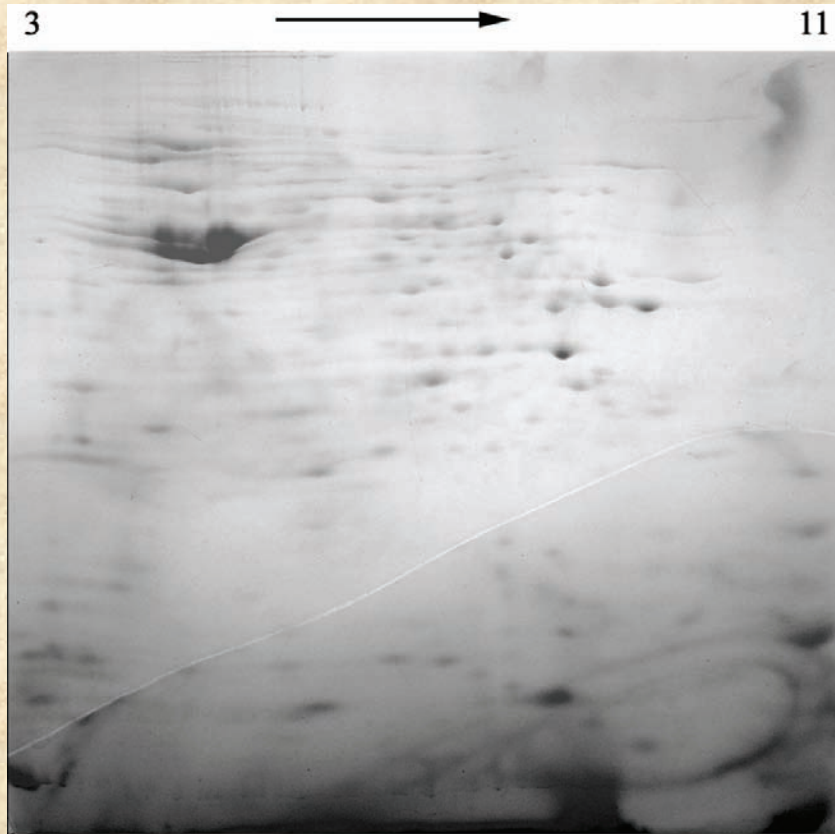
Green - anaerobic

- 1. Nitrous oxide reductase
- 10. Nitrate transporter
- 16. Fructose bisphosphate aldolase
- 18. Triosphosphate isomerase
- 21. Phosphoribulose kinase

Red - aerobic

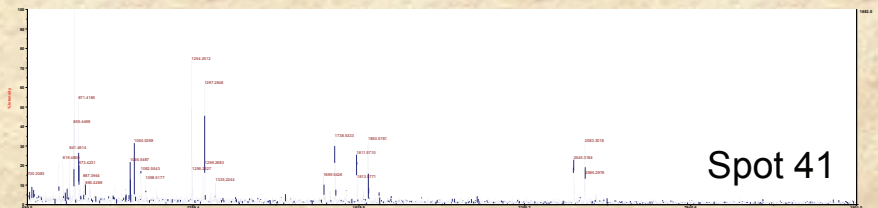
- 4. TonB dependent Cu receptor
- 23. Oxoglutarate dehydrogenase
- 24. Superoxide dismutase

S. barnesii proteome: Nitrate vs Nitrate + Cr(VI)



Spot 18

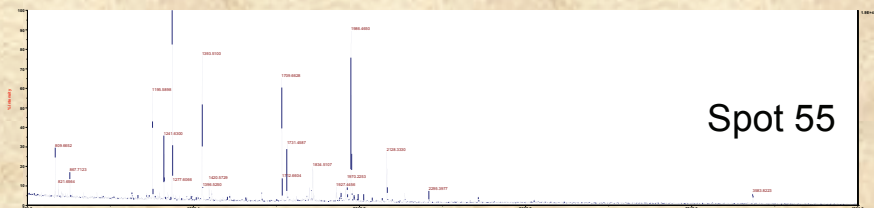
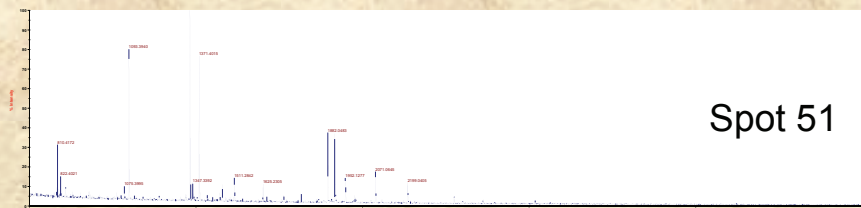
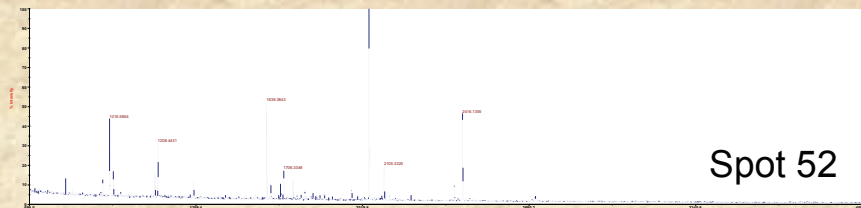
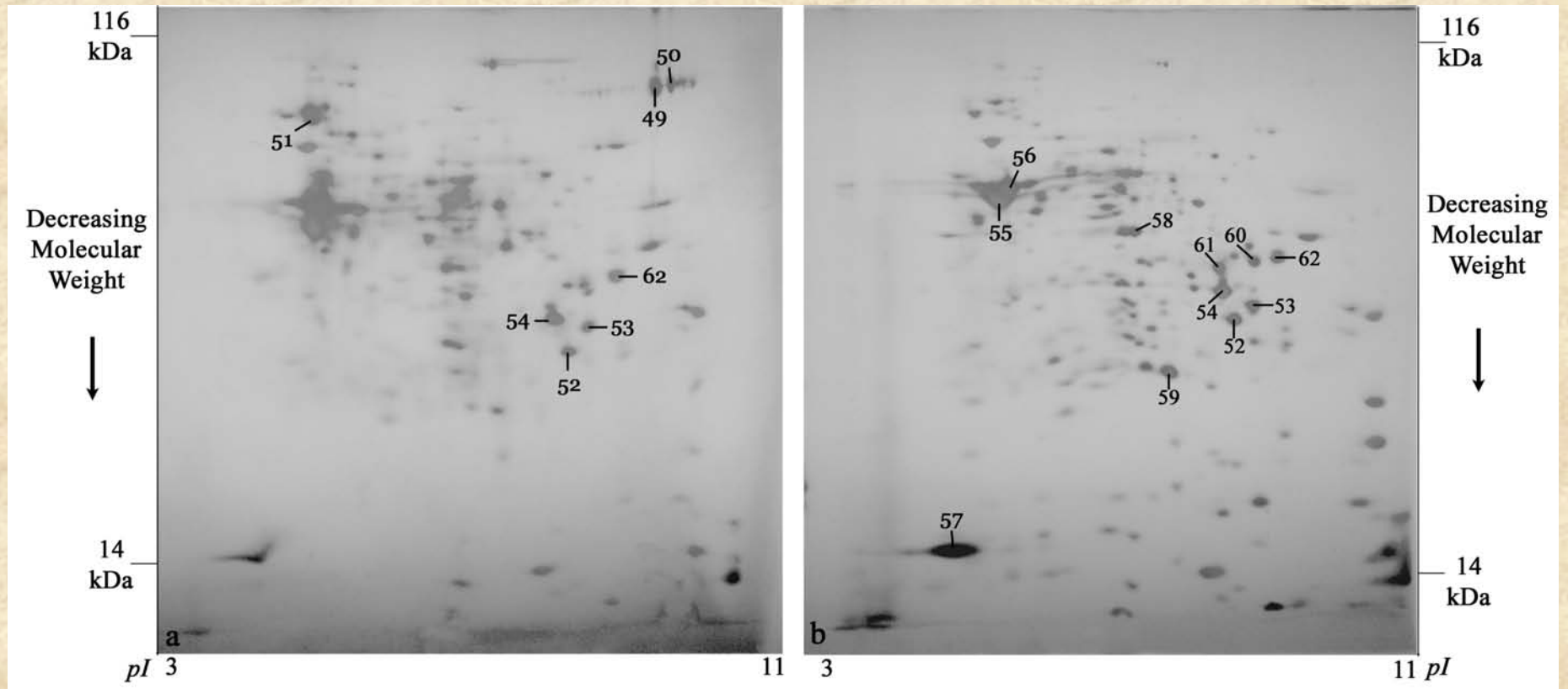
Metalloid reductase RarA *S. barnesii*



Spot 41

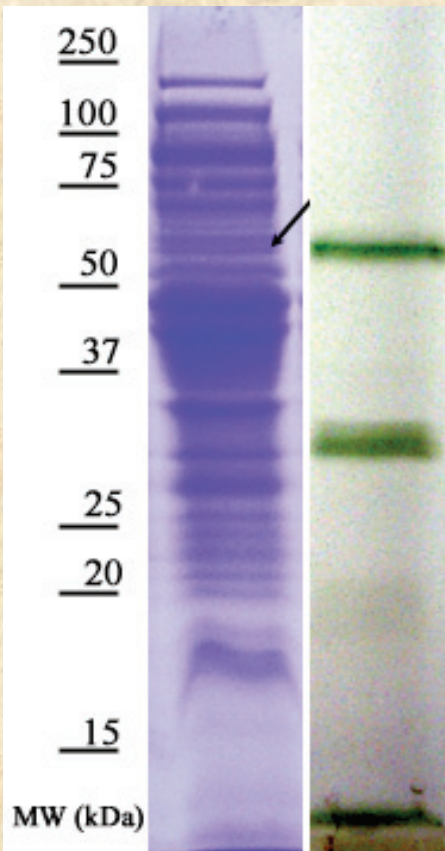
Ribosome recycling protein *H. hepaticus*

D. desulfuricans proteome: Nitrate vs Nitrate + Cr(VI)



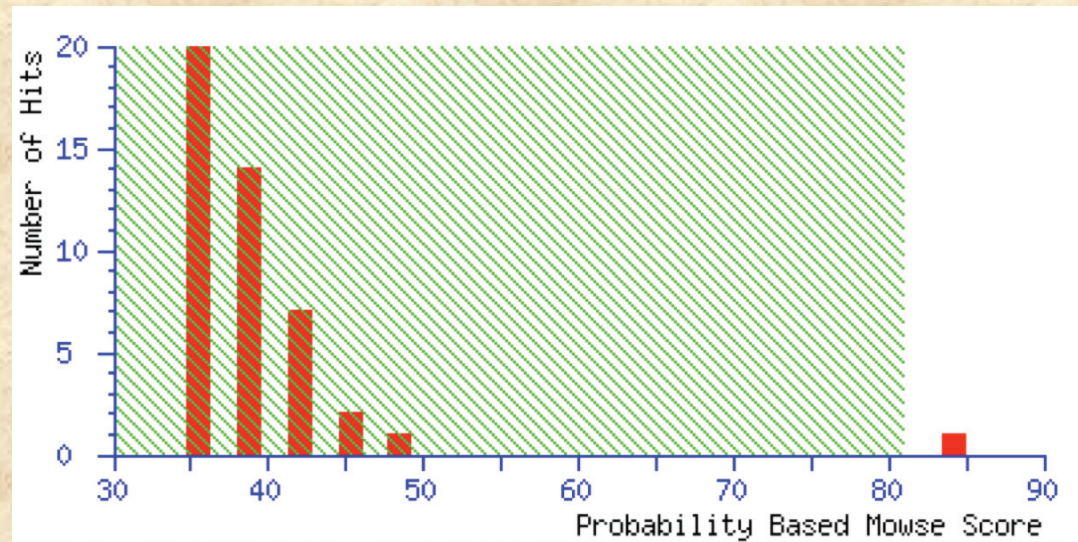
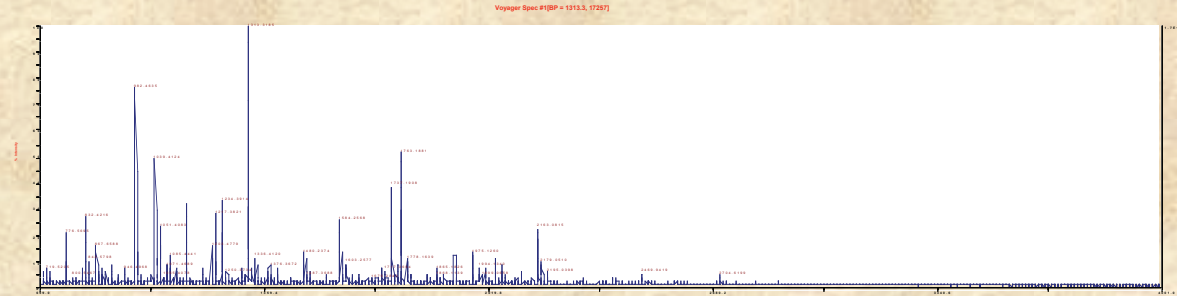
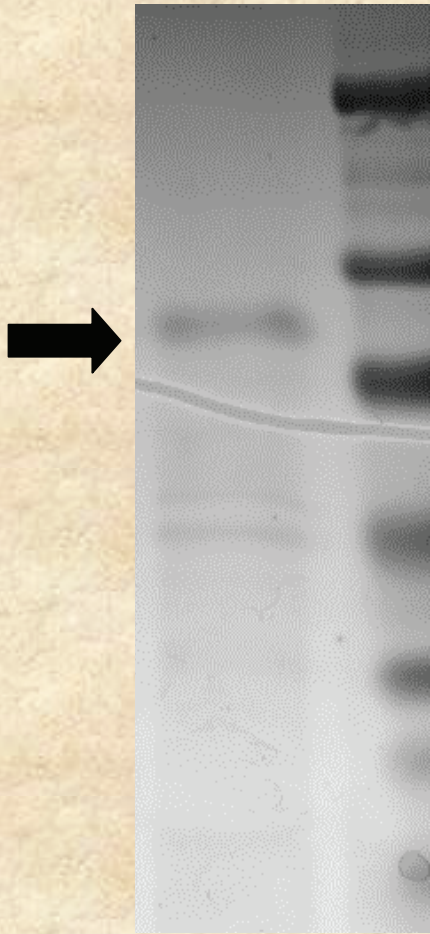
S. barnesii periplasmic nitrite reductase (Nrf)

Protein matches from LC-MS-MS analysis.



Reference Scan	Peptide	P (pro) P(pep)	Score	MW
	Putative cytochrome c nitrite reductase [Sulfurospirillum barnesii]	4.19E-05	20.20	20144.6
642	R.ALVAAGLKPFEESTHQEK.R	4.19E-05	3.95	980.5
742	K.TAMVVVTPWANGIGK.E	2.93E-04	3.33	596.2
	A Chain A, Cytochrome C Nitrite Reductase From Wolinella Succinogenes	7.80E-07	18.20	55252.2
744	R.IIEQDGELEYFTGK.W	7.80E-07	4.04	2019.5
1675	K.TAHSQGIEGKAMSEEWAR.Y	1.00E+00	0.85	88.6
	Cytochrome c nitrite reductase [Sulfurospirillum deleyianum]	3.93E-08	22.18	57873.5
744	R.LIEEDGELEYFTGK.W	3.93E-08	3.59	1769.0
1199	K.LIRHGQFKADMAIAAHGNYFHAPETLR.L	9.76E-01	0.64	113.5
1652	K.ILAKHGVMDYIAPDFDTK.D	4.17E-01	1.08	264.0

S. barnesii periplasmic nitrate reductase (Nap)



gi|169104652 Mass: 105393 Score: **84** Expect: 0.024 Queries
matched: 31

periplasmic nitrate reductase [Sulfurospirillum barnesii]

Nominal mass (M_r): **105393**; Calculated pI value: **8.77**

Fixed modifications: Carbamidomethyl (C)

Variable modifications: Oxidation (M)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Number of mass values searched: **134**

Number of mass values matched: **31**

Sequence Coverage: **34%**

Matched peptides shown in **Bold Red**

1	MSLSRRDFLK	TTAAASAAAA	VGIGVPAELK	AAGEQAEANW	KWDKAVCRFC
51	GTGCGIMVAT	KEGKIVAVK G	DPAAPVNRGL	NCIKGYFNAK	IMYGADRLKQ
101	PLLRMNDKGE	FDKK GQFKPV	SWKRAFDEME	KHIKAALKVG	GPEAIGVFGS
151	GQYTIQEGYA	AAKMMKAGFR	ANGIDPNARH	CMASAVAGFM	QTFGIDEPAG
201	CYDDIEITDT	IITWGANMAE	MHPILWSRVS	DRKLTSPDRV	KIVNLSTYTH
251	RCSDLADLEI	IFSPSTD LAI	WNYIARE IVY	NHPEAIDWDF	VKKNTIFTTG
301	FANIGYGMRT	EAEAKKLGYS	AKELEVIKKE	DAKWISEKEA	PGLAHLGVKA
351	GDVMKMDK AD	AAGAHWEISF	EDFKKALEPY	TLEYVAKISK	GNPDEKLEDF
401	KVK LQELANL	YIEKNRKVVS	FWTMGENQHQ	RGTWVNEQSY	MVHFLLGK QA
451	KPGDGAFSLT	GQPSACGTAR	EVGTFTHRLP	XDMDVSIPKH	REVSEKIWKL
501	PAGTLNPMGY	QHIMNXHRQI	ESGKIK FAWV	NVCNPYQDTA	NANHWIKAAR
551	ELDNFIVCSD	AYPGISAKVS	DLILPSAMIY	EKWGSYGNAE	RRTQHWRQQV
601	LPVGDAMSDT	WQWVELSKRF	TIK DVWGEQP	IKGGKLPNVI	EAAKAMGYKE
651	TDTMYDVLFA	TPFAKQFKAD	DAIGK GFDNS	EVFGDARKVM	GSDGK EWTTY
701	GFFIQKSIWE	EYRQFGLGHG	HDLADFDTYH	KVRGLKWPVV	DGK ETQWR FN
751	AKYDPYAAKA	GNGDFAFYGD	FAKALKKGNL	VKPTTEETYS	LKNKAKIFFR
801	PYMDPCEMPD	REYDTWLCTG	RVLEHWHSGT	MTMR VPELYR	AVPEALCYMH
851	PEDAKAKGFK	QGDMIWLESR	RGSCARVET	RGRNRTPRGL	VFVPWFDEKV
901	MINKVCLDAT	CPISKQTDYK	KCAVKLYKA		

PROGRESS TO DATE

Specific Aim 1

1. *G. metallireducens* is inhibited by Cr(VI) when using nitrate as the terminal electron acceptor.
2. *D. desulfuricans* reduces the Cr(VI) before growing on nitrate.
3. *S. barnesii* could simultaneously reduce Cr(VI) and nitrate.

Specific Aim 2

1. Proteomics analysis revealed differences in the proteomes of both *S. barnesii* and *D. desulfuricans* when exposed to Cr(VI).
2. Protein identification by MALDI-TOF, however, was limited by the lack of genome sequence data.

Specific Aim 3

1. Progress has been made on the purification proteins involved in nitrate respiration in *S. barnesii*

FUTURE DIRECTIONS (Year 2)

Initiate proteomic analyses of nitrate grown *G. metallireducens* in the presence and absence of chromate

Attempt to identify key proteins from *S. barnesii* and *D. desulfuricans* using LC-MS/MS

Characterize purified Nrf from *S. barnesii*

Purify and characterize Nrf from *D. desulfuricans* strain 27774.

Await release of annotated genome of *D. desulfuricans* strain 27774

Propose sequencing the genome of *S. barnesii*.



Basu/Stolz lab

Peter Chovanec

Courtney Sparacino

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