Title: **Nitrate Enhanced Microbial Cr(VI) Reduction**
Project ID: **0013016**
Prog Mgr: **Robert T. Anderson Division: SC-23.4**
PI: **John F. Stolz**
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A major challenge for the bioremediation of radionuclides (i.e., uranium, technetium) and metals (i.e., Cr, Hg) is the co-occurrence of nitrate as it can inhibit metal transformation. Denitrification (reduction of nitrate to dinitrogen gas) is considered the most important ecological process. For many metal and metalloid reducing bacteria, however, ammonia is the end product through respiratory nitrate reduction (RNRA). The focus of this work was to determine how RNRA impacts Cr(VI) transformation. The goal was to elucidate the specific mechanism(s) that limits Cr(VI) reduction in the presence of nitrate and to use this information to develop strategies that enhance Cr(VI) reduction (and thus detoxification).

Our central hypothesis was that nitrate impacts the biotransformation of metals and metalloids in three ways 1) as a competitive alternative electron acceptor (inhibiting transformation), 2) as a co-metabolite (i.e., concomitant reduction, stimulating transformation), and 3) as an inducer of specific proteins and pathways involved in oxidation/reduction reactions (stimulating transformation). We identified three model organisms, *Geobacter metallireducens* (mechanism 1), *Sulfurospirillum barnesii,* (mechanism 2), and *Desulfovibrio desulfuricans* (mechanism 3). Four specific aims were proposed.

Specific aim 1. To investigate the affects of chromate on nitrate respiration in *G. metallireducens, S. barnesii,* and *D. desulfuricans*. Our preliminary experiments demonstrated that chromate affects growth with nitrate differently in each of the three organisms. We will investigate the role of Cr(VI) concentration on the kinetics of both growth and reduction of nitrate, nitrite, and Cr(VI) in these three organisms.

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

Specific aim 3. To investigate the function of periplasmic nitrite reductase (Nrf) as a chromate reductase. We have developed the methods to purify Nrf from *G. metallireducens*, *S. barnesii*, and *D. desulfuricans*. We will determine the kinetics (K_m, V_{max}, K_{cat}) of chromate reduction and other biochemical characteristics of Nrf from the three different organisms using enzyme assays, electrochemistry, and EPR spectrometry.

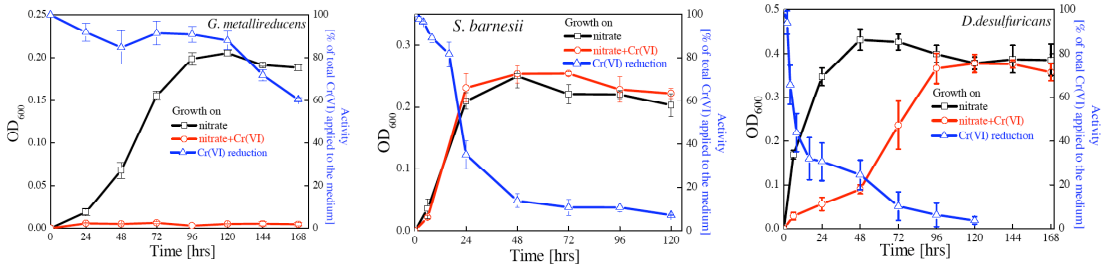
Specific aim 4. To develop a strategy to maximize microbial chromium reduction in the presence of nitrate. The results from specific aims 1-3 will provide insight into possible amendments and manipulations for enhanced in situ remediation. This will be accomplished using both pure culture and natural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

Results

We had originally hypothesized that Cr(VI) reduction by *G. metallireducens* would be inhibited under dissimilatory nitrate reducing conditions as nitrate is more thermodynamically favorable as an electron acceptor than Cr(VI). Our previous investigation of nitrate respiration also indicated a prominent role for cytochromes which could be oxidized by Cr(VI). We found, however, that growth on nitrate is actually inhibited completely by the presence of Cr(VI) (Fig 1A). That led us to investigate the changes in proteome of nitrate grown cells exposed to Cr(VI). These analyses indicated that aldehyde oxidoreductase and an iron-containing alcohol dehydrogenase are up regulated upon Cr(VI) exposure, suggesting the cells are responding to oxidative stress. Furthermore, proteins associated with chemotaxis and motility are upregulated, suggesting electron conducting pili may be involved in Cr(VI) reduction. This is an alternative hypothesis to the current model where Cr(VI) is abioticallly reduction by the Fe(II) produced through dissimilatory iron reduction. Furthermore, cells of *G. metallireducens* grown in liquid culture (e.g., nitrate medium) do not produce pili, so induction of pili expression by exposure to Cr(VI) is a significant change in physiology.

We have demonstrated that *D. desulfuricans* reduces the Cr(VI) in the medium prior to respiratory growth on nitrate (Fig 1B). Proteomics analysis has revealed significant changes in the proteomes of cells grown on nitrate alone versus cells grown on nitrate in the presence of Cr(VI). When the cells are initially exposed to Cr(VI) a suite of proteins, including nitrate and nitrite reductase, are down regulated. These proteins return to control levels (e.g., nitrate alone) after the complete reduction of Cr(VI). We also have found a redox active secondary metabolite (possibly a ketoquinone) secreted into the medium that rapidly reduces Cr(VI). Experiments indicate that the activity is associated with the filtrate and not the cells, and that the compound is heat and air stable. Activity assays also showed clear inhibition of nitrite reduction by Cr(VI). Thus we conclude that *D. desulfuricans* responds to Cr(VI) exposure by down-regulation of the respiratory pathway and release of an extracellular metabolite that reduces the Cr(VI).

In *S. barnesii*, we have demonstrated that this organism can simultaneously reduces Cr(VI) while respiring nitrate (Fig 1C). Activity assays revealed that Cr(VI) is reduced by Rar, a protein that is over-expressed under these growth conditions (Chovanec et al., 2009). *S. barnesii* also produces an extracellular metabolite that can reduce Cr(VI), however, Cr(VI) reduction rates are as great in the whole cell fractions indicating that Rar can function as a Cr(VI) reductase.



A B C

Figure 1. Growth on nitrate in the presence of Cr(VI) as compared with nitrate alone. A) *G. metallireducens,* B) *S. barnesii*, C) *D. desulfuricans* 27774

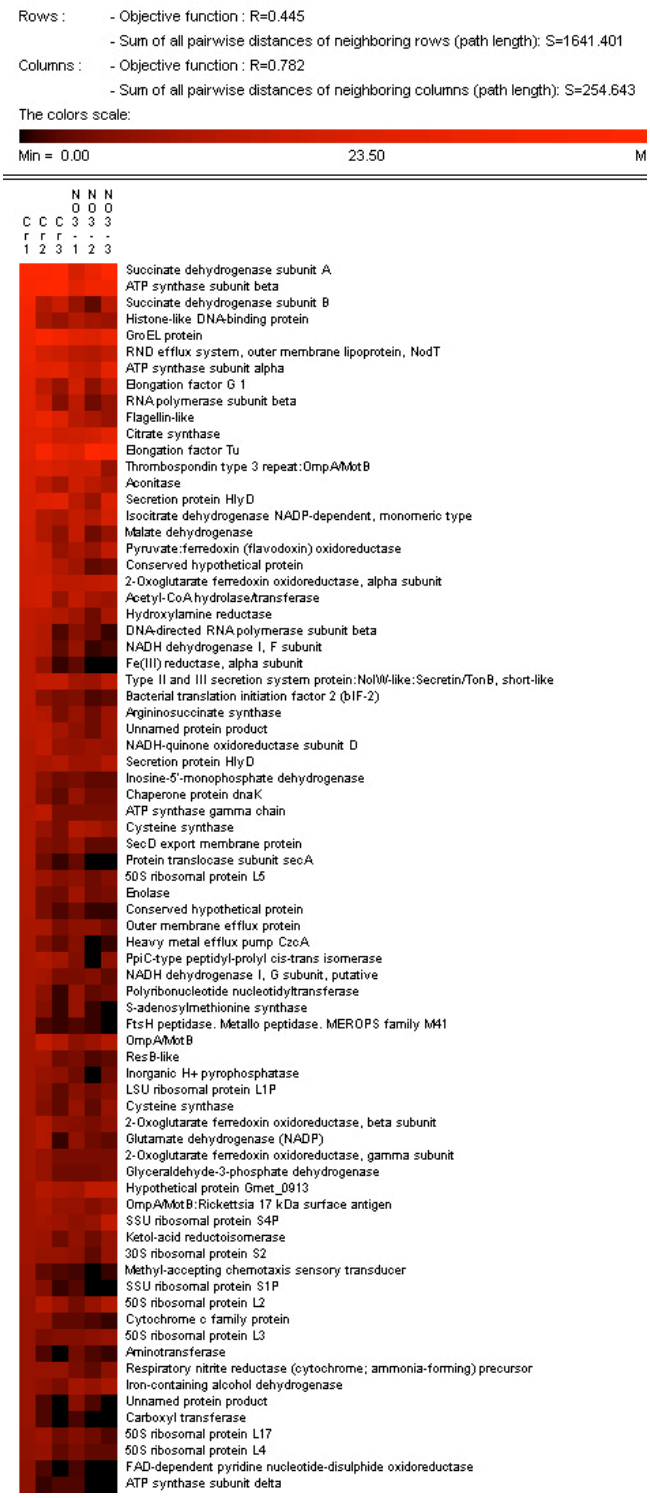


Figure 2 Heat map visualization (Permut Matrix program, Caraux and Pinloche, 2005) of relative differences in protein abundance between the nitrate grown (NO_3^-) and chromate exposed cells (Cr) samples of *G. metallireducens* obtained by LC-MS/MS. Three biological replicates for each condition are visualized in a single column (75 out of 700 proteins shown).

We have demonstrated that although *G. metallireducens* can reduce Cr(VI), growth on nitrate is inhibited and exposure to Cr(VI) induces changes in its proteome (Fig 2). We have purified and characterized a multi-heme cytochrome C (nine heme binding motifs CXXCH, calculated molecular mass of 51.5 kDA, pI of 7.05.) with nitrite and Cr(VI) reductase activity, that was annotated as hypothetical (Gmet_0913, GI:78193388). In our investigation of *D. desulfuricans* strain 27774 we found changes in the proteome during the prolonged lag phase with concomitant Cr(VI) reduction as well as brief exposure to Cr(VI). We isolated desulfoviridin (62, 51, and 35 kDa subunits) and found that it had nitrite reductase activity that was inhibited by Cr(VI) which preferentially oxidized the siroheme. For *S. barnesii* we initiated an in depth study of the biochemistry of nitrate respiration in this organism, purifying the nitrate (Nap) and nitrite (Nrf) reductases, as well as a metalloid reductase (Rar).

Geobacter metallireducens

Cells were grown anaerobically with 20 mM sodium nitrate (control sample) and then exposed to 100 uM chromate (treated sample). Controls and treated cultures were harvested after 72 h. Equal volume of 2,2,2 trifluoroethanol (TFE) was added to 20µg of cell lysates. Samples were reduced, alkylated, completely dried in a Speed Vac and resuspended in 50µL of 100 mM ammonium bicarbonate buffer. Samples were acetone precipitated, trypsin digested and reconstituted in 20µL of 0.1% formic acid for LC-

MS/MS analysis. Peptides (3ug/injection) were analyzed on Agilent's 6530 HPLC-Chip q-TOF mass spectrometer using a 50 min gradient. The CID data was analyzed and semi-quantified with Spectrum Mill as well as Mascot bioinformatics tool.

A 3 uL of peptides (6 ug of digested sample) were injected onto an LC/MS system consisting of an 1200 Series liquid chromatograph, HPLC-Chip Cube MS interface, and 6530 QTOF mass spectrometer (Agilent Technologies), and an HPLC-Chip (Agilent Technologies) with a 160-nl enrichment column and a 150-mm x 75-mm analytical column packed with Zorbax 300SB-C18 5-mm particles. Peptides were loaded onto the enrichment column with 97% solvent A (water with 0.1% formic acid). They were then eluted with a gradient from 3% B (acetonitrile with 0.1% formic acid) to 40% B in 60 min, 50% in 70 min, followed by a steep gradient to 90% B in 5 min at a flow rate of 0.4mL/min. The total runtime, including column reconditioning, was 80 min. The column effluent was directly coupled to a 6530 QTOF (Agilent) mass spectrometer via a HPLC-Chip Cube nanospray source operated at capillary voltage 1850 V with a capillary current of 1.9 uA in 2GHz. The MS data was acquired in the positive ionization mode using Mass-Hunter Workstation. Fragmentor voltage, skimmer voltage and octopole RF were set to 180 V, 65 V and 750 V, respectively. Auto MS/MS was performed with a total cycle time of 1.97 s. In each cycle MS spectra was acquired at 5Hz (5 spectra/sec) (m/z 250-1500) and the five most-abundant ions (with charge states +2, +3, and > +3) exceeding 2000 counts were selected for MS/MS at 3Hz (3 spectra/sec) (m/z 50-3200). Medium isolation (4 m/z) window was used for precursor isolation. Collision energy with slope of 3V/(100Da) and Offset 2Vwas used for fragmentation. Reference mass correction was activated using reference mass of 922.009798. Precursors were set in an exclusion list for 0.25 min after one MS/MS spectra.

CID data was searched against the custom *G. metallireducens* database. Peak lists were created with the Spectrum Mill Data Extractor program with the following attributions: scans with the same precursor ±0.05 m/z were merged within a time frame of ±10 s. Precursor ions needed to have a minimum signal to noise value of 25. Charges up to a maximum of 7 were assigned to the precursor ion, and the 12C peak was determined by the Data Extractor. The custom database was searched for tryptic peptides with a mass tolerance of ±10ppm for the precursor ions and a tolerance of ± 50ppm for the fragment ions. Two missed cleavages were allowed. A Spectrum Mill autovalidation was performed first in the protein details followed by peptide mode using a.) in protein details mode; protein score >15, peptide score (scored percent intensity [SPI]) charge +1 (>6, >60%), peptide charge +2 (>5, >50%), peptide charge +3 (>6, >60%), peptide charge +4 (>7, >60%), peptide charge +5 (>11, >50%), peptide charge +2 (>4, >80%), forward minus reversed score threshold >1, and rank 1 minus rank 2 score threshold >1 ; b.) in peptide mode: SPI charge +1 (>13, >70%), peptide charge +2 (>13, >70%), peptide charge +3 (>13, >70%), peptide charge +4 (>13, >70%), peptide charge +5 (>15, >70%), forward minus reversed score threshold >2, and rank 1 minus rank 2 score threshold >2. All protein hits found in a distinct database search by Spectrum Mill are non-redundant.

The method of Choi et al. was used in quantitating the expressed cellular proteins by determining the number of peptides. The cells grown on nitrate and exposed to chromate were grown in three separate experiments, and in one case, samples were injected three times for protein identification. For each experiments mean, standard deviation were calculated. Relative abundance was determined from these average values.

Under these growth conditions, LamB porin family protein, succinate dehydrogenase subunit A, succinate dehydrogenase subunit B, ATP synthase subunit beta, GroEL protein, RNA polymerase subunit beta, pyruvate:ferredoxin (flavodoxin) oxidoreductase, ATP synthase subunit alpha, Fe(III) reductase, alpha subunit, malate dehydrogenase, flagellin-like protein, citrate synthase and OmpA/MotB are among the most abundant. The most abundant LamB porin family protein was significantly over-expressed after 12 hrs chromate exposure. It is interesting to note that pyruvate ferredoxin oxidoreductase is found to be high in abundance, a feature we recently reported in another bacterium, *Alkaliphillus oremlandii* grown on lactate. 363 and 442 proteins were identified in cells grown on nitrate only, and cells grown on nitrate and exposed to chromate, respectively. In chromate exposed cells, many proteins went higher in abundance that those found to be down-regulated. Of particular interest is the higher abundance of outer membrane efflux protein (Gmet_1549) and heavy metal efflux pump CzcA (Gmet_1547) which indicates that certain bacterial (heavy metal) resistance mechanisms are involved. It is interesting to note that there are highly expressed two subunits of succinate dehydrogenase, A and B, respectively. Subunit B showed a significant increase in abundance in the sample of the cells exposed to chromate.

In an attempt to purify the Nrf homolog from cells of *G. metallireducens* grown on nitrate, a

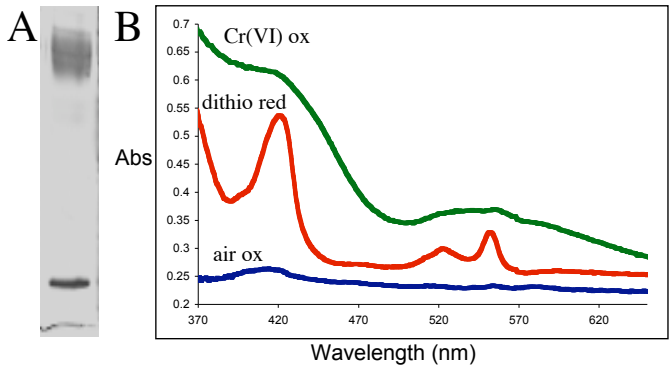


Figure 3. Multiheme c-cytochrome of *G. metallireducens*. A) SDS-PAGE of band G-4 from the Preparative native gel (Figure 5A). B) Electronic spectra of the as purified oxidized protein (air ox), dithionite reduced protein (dithio red), and reoxidized with chromate (Cr(VI) ox).

multiheme c-cytochrome was identified. A distinct prominent pink band that separated in a preparative gel was found to contain to proteins of relative molecular masses of 39 kDa and 9 kDa respectively (Fig. 3). The larger protein was identified by MALDI-TOF MS as hypothetical protein Gmet_0913 (GI:78193388). Our analysis indicates it is a multi-heme cytochrome c, with nine heme binding motifs (CXXCH), a calculated molecular mass of 51.5 kDA, and a pI of 7.05. It has homologs in other *Geobacter* species include *G. lovleyi*, *G. bemidjiensis*, and *G. uraniireducens*. The dithionite reduced spectrum has absorbance maxima at 408, 522, and 551 nm. The dithionite reduced cytochrome could be oxidized with nitrite or Cr(VI) (Fig. 6B).

Desulfovibrio desulfuricans

In order to investigate Cr(VI) induced changes in the proteome, proteins were profiled by 'shot-gun' proteomic approach with LC-MS/MS. Cell lysates from *D. desulfuricans* expressed under three different conditions: cells grown on nitrate only, cells grown on nitrate in the presence of chromate, and nitrate grown cells exposed to chromate for 24 hr, revealing that many of the over 400 proteins identified were down-regulated (Fig 4). These proteins return to control levels (e.g., nitrate alone) after the complete reduction of Cr(VI). We also have found a redox active secondary metabolite (i.e., ketoquinone) secreted into the medium that rapidly reduces Cr(VI). We conducted a series of experiments with live and heat killed cells that indicate the activity is associated with the filtrate and not the cells, and that the compound is heat and air stable (Table 1).

	NITRATE			CHROMATE			EXPOSURE			
AVERAGE	SD	%AA	AVERAGE	SD	%AA	AVERAGE	SD	%AA	Accession	Protein Name
82.25	9.87	64	61.92	23.62	66	55.08	7.74	62	219869690	Adenylylsulfate reductase, alpha subunit
50.83	4.67	71	37.33	5.80	71	31.50	3.53	67	219869115	Chaperonin GroEL
48.17	5.18	53	35.58	11.16	52	30.17	4.17	44	219867878	Pyruvate flavodoxin/ferredoxin oxidoreductase domain protein
45.42	4.50	74	15.75	6.92	56	32.75	9.08	59	219867758	NAD(P)(+) transhydrogenase (AB-specific)
42.58	5.33	77	32.67	11.51	79	25.50	4.68	82	219868540	Inorganic diphosphatase
39.25	5.03	71	34.92	11.37	72	32.83	3.49	71	219868031	Sulfate adenylyltransferase
31.75	3.41	81	30.25	5.12	79	26.75	2.14	78	219869192	Translation elongation factor Tu
29.08	3.34	67	26.17	4.22	67	23.75	3.65	60	219869855	Sulfite reductase, dissimilatory-type alpha subunit
28.67	1.37	61	17.75	3.96	53	20.50	2.32	60	219869101	Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP(+))
27.67	6.27	63	15.58	4.27	55	9.08	3.80	56	219869537	OmpA/MotB domain protein
27.25	17.63	36	22.42	17.52	40	1.50	2.39	9	219868157	Conserved hypothetical protein
23.67	2.15	61	21.75	4.37	46	21.42	2.43	48	219868492	Sulfite reductase, dissimilatory-type beta subunit
23.58	2.15	66	20.42	5.18	64	17.92	2.31	57	219869749	ATP synthase F1, beta subunit
23.42	4.64	54	12.75	5.07	48	13.58	4.52	54	219867672	Histone family protein DNA-binding protein
22.50	2.68	43	10.50	5.13	34	14.42	3.23	42	219867666	Nitrite reductase (cytochrome; ammonia-forming)
21.75	3.31	68	15.92	4.76	70	18.75	2.53	70	219869504	Iron-containing alcohol dehydrogenase
18.33	3.42	54	6.00	3.72	29	9.42	2.84	40	219869098	Succinate dehydrogenase or fumarate reductase, flavoprotein subunit
17.17	1.53	42	13.17	4.67	45	11.50	3.40	35	219869630	Translation elongation factor G
16.92	2.02	71	4.17	3.10	32	6.67	1.50	39	219868359	Glycine cleavage system T protein
16.25	2.22	59	8.00	2.04	35	10.42	2.07	46	219868362	Glycine dehydrogenase (decarboxylating)
15.50	2.91	61	8.75	4.49	47	11.17	2.62	48	219869391	Hybrid cluster protein
15.00	1.35	42	7.50	3.78	32	6.17	3.97	30	219867877	Phosphate acetyltransferase
14.75	2.83	40	13.75	4.97	40	9.42	1.62	30	219869747	ATP synthase F1, alpha subunit

Figure 4 Relative differences in protein abundance between nitrate grown (NO_3^-), nitrate with Cr(VI), and chromate exposed cells (Cr(VI)) of *D. desulfuricans* obtained by LC-MS/MS (23 of 400 shown).

Table 1. Cr(VI) reduction in cells and filtrate of *D.desulfuricans* as measured by the diphenylcarbazide method (A540nm)

Time	Heat Killed Cells	Filtred Spent Media Autoclaved	Cells autoclaved	Filtered Spent Media
0	0.0907±0.019	0.098±0.008	0.087±0.013	0.067±0.013
2hrs	0.005±0.006	0.004± 0.002	0.039±0.018	0.013±0.008
6hrs	0	0	0.022±0.01	0.003±0.001
12hrs	0	0	0.016±0.008	0.003±0.001
24hrs	0	0	0.005±0.003	0
48hrs	0	0	0	0

In an attempt to purify the Nrf homolog from cells of *D.desulfuricans* grown on nitrate, desulfoviridin was identified. A distinct prominent green band that separated in a preparative gel found to be comprised of desulfoviridin. SDS/PAGE of fraction D-5 (Fig. 5) shows the presence of the α (62 kDa), β (51 kDa) and γ (35 kDa) subunits. The identity of the 62 kDa band was confirmed by MALDI-TOF MS. *Desulfovibrio vulgaris* dSiR has been reported to reduce nitrite and hydroxylamine. Activity assays using this fraction (D-5) indicated that it had nitrite reductase activity and that this activity was inhibited by Cr(VI) as it preferentially oxidized the siroheme.

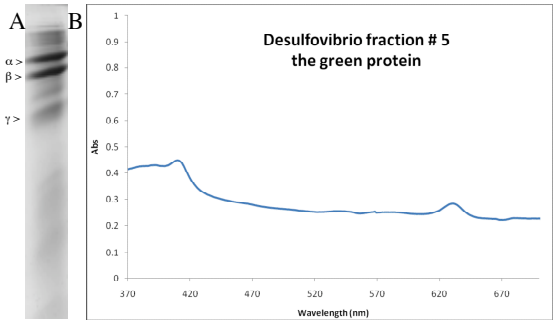


Figure 5. Desulfoviridin from *D. desulfuricans*. A) SDS-PAGE showing three subunits, B) Electronic spectrum showing the absorbance maxima at 408 and 630 nm.

Sulfurospirillum barnesii

For the purification of Nrf and Nap, CHAPS solubilized membrane fractions were first treated with ammonium sulfate (30% saturation). Proteins remaining in the supernatant after centrifugation were loaded onto a DEAE anion exchange column and eluted with a NaCl gradient of 300 mM NaCl + 300 mM Tris-HCl. A large percentage of Nrf was collected in the wash eluent but was also found in subsequent fractions. Nap eluted at higher ionic strength (Fig. 6). Rar was found in many fractions (Fig. 6). Rar could be solubilized with the detergent n-octyl-β-D-glucopyranoside (OBGP) and purified with subsequent ammonium sulfate precipitation as it remains soluble in 75% saturation.

In gel activity assays indicate that Rar was shown to couple methyl viologen oxidation to nitrite reduction (Fig. 7). In our investigation of Cr(VI) reduction in *S. barnesii*, we found that a protein we previously identified as redox active (redox active reductase or Rar), was capable of coupling the oxidation of methyl viologen to Cr(VI) reduction. This was confirmed using native PAGE and MALDI-TOF (Fig. 6). Rar was purified to homogeneity using the following scheme. Cells of *S. barnesii* were grown on 20 mM nitrate and 15 mM lactate medium in an anaerobic fermentor (14 L). Cells (9-13g) were harvested in the log phase of growth (~12 hrs) and lysed by French Pressure Cell. Protease inhibitor cocktail and DNAse were added to cold lysate immediately after. The membrane fraction was obtained by ultracentrifugation (50K x g). The membrane pellet was suspended in 50 mM Tris- Cl buffer pH 7.7,

Rar was solubilized from the membrane suspension with n-octyl-b-D-glucopyranoside (OBGP) and enriched by subsequent ammonium sulfate precipitation (it remains soluble in 75% saturation). The resulting supernatant was dialyzed to lower the salt concentration. The desalted fraction was loaded onto a DEAE anion exchange column and eluted with a NaCl gradient of

300 mM NaCl + 300 mM Tris-HCl. Rar was found in many fractions. In gel activity assays indicated that Rar can couple methyl viologen oxidation to nitrite reduction (Fig. 7) as well as other metal and metalloid oxyanions.

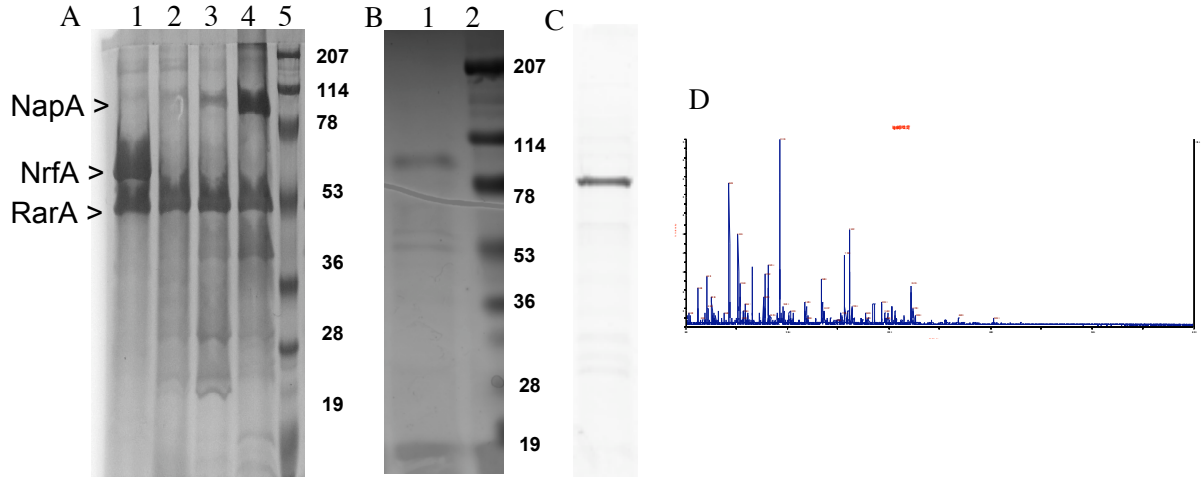


Figure 6 Nap, Nrf, and Rar from *S. barnesii*. A) SDS-PAGE of fractions collected from the first DEAE ion exchange column. Lane 1, fraction eluted with 100 mM NaCl containing NrfA and RarA, lane 2, fraction eluted with 125 mM NaCl with only RarA, lane 3, fraction eluted with 150 mM NaCl will all three, lane 4, a fraction eluted with 200 mM NaCl containing NapA, RarA, and NrfA. Lane 5, molecular weight standards. B) Purified Nap after a second DEAE ion exchange column (lane 1), C) Purified NrfA (62 kDa) eluted with low salt off a DEAE ion exchange column.. D) MALDI-TOF MS of NapA from B).

Similar to *D. desulfuricans*, we also have found a redox active secondary metabolite (i.e., ketoquinone) secreted into the medium that rapidly reduces Cr(VI). However, in experiments

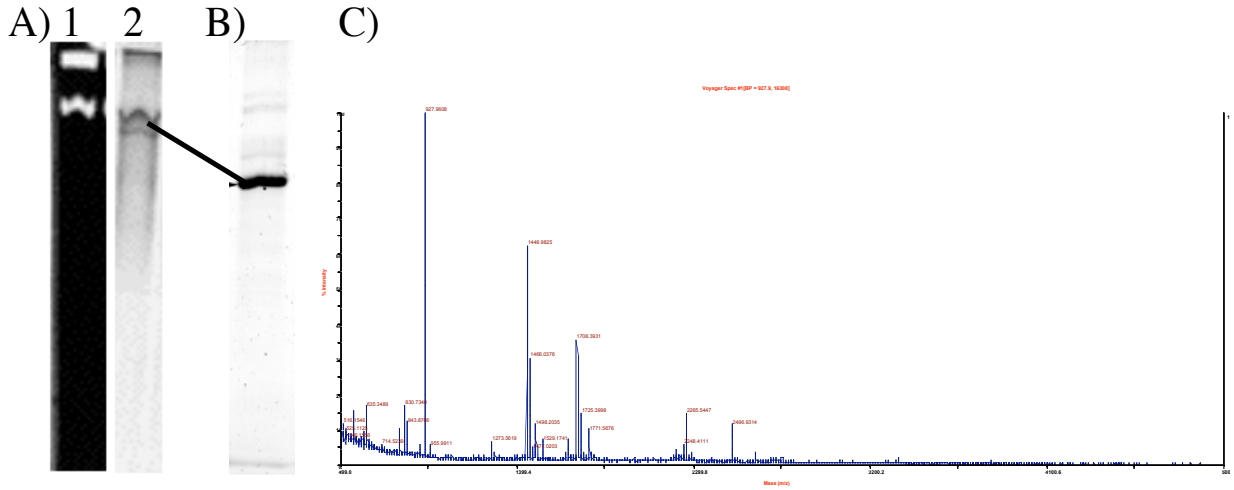


Figure 7. Reductase activity and identification of the non-specific metalloid reductase Rar from *S. barnesii*. A) native gel of OBGP (1%) solubilized membrane proteins developed with nitrite showing two bands of activity (lane 1). Lane 2 is the same gel stained with Coomassie blue. The upper active (clear) band is a fraction containing Nrf, the lower active (clear) band is Rar, B) SDS-PAGE of lower active band excised from A (shown by line), C) MALDI-TOF MS identification the protein excised from gel in B as Rar.

with live and heat killed cells Cr(VI) reductase activity was found both in the filtrate and cell fractions (Table 2). This indicates to us that there are at least two different mechanisms for chromate reduction in *S. barnesii*, one involving an extracellular redox active compound (the ketoquinone-like compound) and one involving the membrane associated Rar. Preliminary analysis of fractions obtained from spent media revealed the presence of several pigmented and fluorescent compounds with different molecular mass (Fig. 8). Which of these possess chromate reductase activity is to be determined.

Table 2. Cr(VI) reduction in cells and filtrate of *S.barnesii* as measured by the diphenylcarbazide method (A540nm)

Time	Heat Killed Cells	Filtred Spent Media Autoclaved	Cells autoclaved	Filtered Spent Media
0	0.075±0.009	0.082±0.048	0.073±0.047	0.138±0.025
2hrs	0.043±0.006	0.039±0.023	0.046±0.030	0.085±0.021
6hrs	0.041±0.010	0.031±0.026	0.027±0.022	0.089±0.025
12hrs	0.024±0.006	0.021±0.026	0.021±0.018	0.086±0.027
24hrs	0.006±0.011	0.010±0.014	0.012±0.017	0.058±0.029
48hrs	0	0.004±0.007	0.009±0.014	0.038±0.019

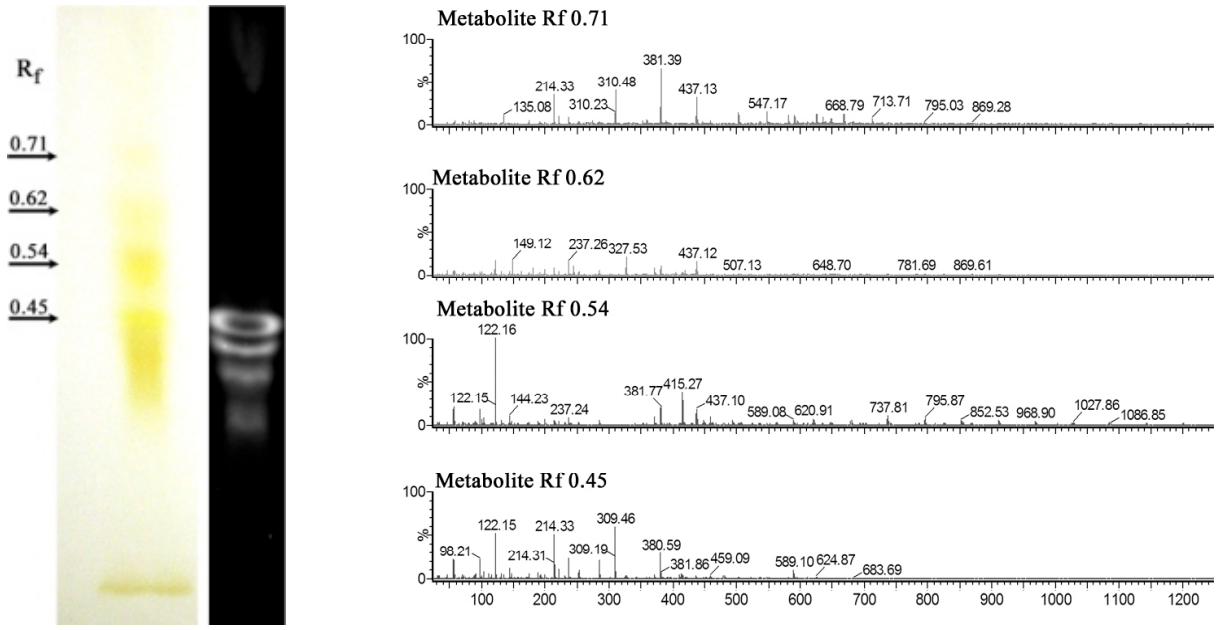


Figure 8. Secreted redox active metabolite from *S. barnesii*. **A)** thin layer chromatography. Lane 1, spots from medium extract of the *S. barnesii*. Lane 2, spots under UV light, **B**, MS of selected metabolites.

Students trained: Courtney Sparacino Watkins (PhD in Chemistry)
Ning Zang (MS – Environmental Science and Management)
Steven Barrows (BS- Biological Sciences)

Post doctoral fellow: Peter Chovanec

Poster Presentations:
Chovanec, P., Basu, P., and Stolz, J.F. 2008 Nitrate enhanced chromate reduction. DOE ERSP PI's Annual Meeting Abstracts

Sparacino, C., Chovanec, P., Stolz, J.F., and Basu, P. 2008. Structure predication and purification of periplasmic nitrate reductase from *Sulfurospirillum barnesii*. American Chemical Society Meeting Abstracts.

Chovanec, P., Sparacino-Watkins, C., Basu, P., and Stolz, J.F. 2009. Chromate reduction under nitrate reducing conditions in *Geobacter metallireducens*, *Sulfurospirillum barnesii*, and *Desulfovibrio desulfuricans*. DOE ERSP PI's annual meeting

Chovanec, P., Sparacino-Watkins, C., Basu, P., and Stolz, J.F. 2009. Chromate reduction under nitrate reducing conditions by three nitrate respiring bacteria. American Society for Microbiology, General Meeting abstracts

Chovanec, P., Sparacino-Watkins, C., Basu, P., and Stolz, J.F. 2010. Proteome changes induced by chromate exposure in *Geobacter metallireducens*. American Society for Microbiology, San Diego CA, general meeting abstracts

Chovanec, P., Sparacino-Watkins, C., Basu, P., and Stolz, J.F. 2010. Proteome changes induced by chromate under nitrate reducing conditions in *Geobacter metallireducens* and *Desulfovibrio desulfuricans*. DOE-ERSP PI's annual meeting, Washington DC, meeting abstracts.

Chovanec, P., Basu, P., and Stolz, J.F. 2011. Proteome changes induced by chromate exposure in *Desulfovibio desulfuricans* strain 27774. American Society for Microbiology, San Diego CA, general meeting abstracts

Publications:
Chovanec, P., Sparacino-Watkins, C., Basu, P., and Stolz, J.F. Chromate reduction under nitrate reducing conditions by three nitrate respiring bacteria. Envir. Sci. Technol. (submitted)

Chovanec, P., Sparacino-Watkins, C., Basu, P., and Stolz, J.F. Proteome changes induced by chromate under nitrate reducing conditions in *Geobacter metallireducens* and *Desulfovibrio desulfuricans* (in preparation)

Chovanec, P., Basu, P., and Stolz, J.F. Proteome changes induced by chromate exposure in *Desulfovibio desulfuricans* strain 27774 (in preparation)