Biomolecular Mechanisms Controlling Metal and Radionuclide Transformations in *Anaeromyxobacter dehalogenans*

Alex S. Beliaev (PI), Frank E. Löffler, Robert A. Sanford, Jim K. Fredrickson
Collaborating Team

Georgia Tech:
- Frank Löffler
- Qingzhong Wu
- Sara Thomas
- Ryan Wagner

Univ. Illinois, U-C:
- Robert Sanford

Pacific Northwest National Laboratory
Operated by Battelle for the U.S. Department of Energy

- Alex Beliaev
- Matt Marshall
- Yang Zhang
- Andy Plymale

- Jim Fredrickson
- David Culley
- David Kennedy
- Alice Dohnalkova
**Anaeromyxobacter dehalogenans 2CP-C**

- Gram-negative, facultatively anaerobic organism
- Isolated from anaerobic enrichments (tropical soil) with monochlorophenol
- Capable of reductive dechlorination (dechlororespiration) coupled to growth

Phylogeny of *Anaeromyxobacter*

Phylogenetic tree based on nearly complete 16S rRNA gene sequences
Electron Acceptors

- Oxygen
- Ortho-substituted halophenols
- Nitrate
- Nitrite
- Fumarate

- Soluble and insoluble oxidized metal species

Electron Donors

- Acetate
- Hydrogen
- Succinate
- Pyruvate
- Formate
- Lactate

Metabolic properties of A. dehalogenans 2CP-C
Metal reduction $A. \ dehalogenans\ 2CP-C$

$\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$

$\text{U}^{6+} \rightarrow \text{U}^{4+}$


$\text{Anaeromyxobacter dehalogenans strain 2CP-C}$
**A. dehalogenans 2CP-C couples U(VI) reduction to growth**

Mineral salts medium, acetate, $H_2$
2% (v/v) inoculum

![Graph](image.png)

- **U(VI)**: Red line with square markers.
- **Cells**: Blue dashed line with triangle markers.
- **U(VI), no cells**: Red dashed line with square markers.
- **Cells, no U(VI)**: Dark blue dashed line with triangle markers.

**Axes:**
- **Y-axis**: Uranium (VI) [µM]
- **X-axis**: Time (days)
- **Y-axis 2**: Cells per ml ($x10^6$)

Legend:
- **U(VI)**
- **Cells**
- **U(VI), no cells**
- **Cells, no U(VI)**
Effect of nitrate on U(VI) reduction by *A. dehalogenans* 2CP-C

2CP-C grown with acetate/NO$_3^-$ (0.5 mM)
U(VI) and H$_2$ added after NO$_3^-$ had been consumed (day 3)
Field Research Center (FRC) - Oak Ridge, TN

* multiport wells
+ wells
Project objectives

- Identify genes involved in metal/radionuclide reduction in *A. dehalogenans* strain 2CP-C regulated under different redox conditions
- Compare the pathways of metal/radionuclide reduction in *A. dehalogenans* 2CP-C to those found in other DMRB
- Identify the key environmental factors specific to subsurface environments that affect the expression of *A. dehalogenans* genes involved in metal/radionuclide reduction
• Carry out resting cell experiments using non-growth conditions to study the kinetics of U(VI) and Tc(VII) reduction in *A. dehalogenans* strains 2CP-C and 2CP-1

• EM imaging of U(VI)-reducing cells (strains 2CP-C and 2CP-1) to study the localization of U(IV) material

• Compare the data with previous results generated for other metal-reducing organisms (*Shewanella*)

• Identify target genes putatively involved in metal and radionuclide reduction in *Anaeromyxobacter*
Tc (VII) reduction by *A. dehalogenans*

Resting cell assays w/ H₂, 2x10⁸ cells

**Aqueous Tc(VII), µM**

- MR-1
- 2CP-C
- 2CP-1

**Time, h**

0 5 10 15 20 25
U(VI) reduction by *A. dehalogenans*

Resting cell assays w/ H₂, 2x10⁸ cells
U(VI) reduction by A. dehalogenans (acetate, 30°C)

Resting cell assays w/ H₂, 2x10⁸ cells
Reduced U(IV) nanoparticle localization in *A. dehalogenans* (*H₂*, 30°C)

**Whole mounts:**
U(IV) material is present as a heavy decoration of extracellular polymers. Cells are covered with a very fine coat. Material shows an excellent match with uraninite (very similar to *Shewanella* experiments). A typical particle size is in a 5 nm range, but also with d-lines extending 10+ nm. An interesting distribution of U was on many cells – uraninite forming nodular patterns.
Reduced U(IV) nanoparticle localization in *A. dehalogenans* (*H₂*, 30°C)

*Thin sections:* Reduced U(IV) is bound to dispersed (amorphous) extracellular material which forms dendric-like structures. The U nanoparticles are > 5 nm in diameter. Absolutely no periplasmic deposition is found.
Thin sections:
U material is also bound to organized sheet-like EPS structures which are abundant throughout the sample and appear to have the same familiar morphology as known from *Shewanella* species. A thin (lipid?) core layer that is clear of UO₂, but surrounded by U nanoparticles from both sides is visible (D,E). Frequently, cells are attached to these structures (C), as if sheets are spinning out of the cell membranes, incorporating them into their network.
Are the molecular mechanisms of U(VI) reduction in *Anaeromyxobacter* similar to those in other DMRB?

In *S. oneidensis* MR-1, UO$_2$ nanoparticles accumulate in multiple locations: extracellular to the cells, periplasmic, and at high densities with an exopolymeric substance (EPS).

Immune-electron microscopy (EM) reveals that OM-associated, c-type decaheme cytochromes of (MtrC and OmcA) in MR-1 were primarily localized with the extracellular UO$_2$-EPS matrix.
Are c-type cytochromes involved in radionuclide/metal reduction in *Anaeromyxobacter* species?

A glimpse of the *A. dehalogenans* 2CP-C draft genome

- 93 genes with CXXCH motif
- 15 genes > 10 CXXCH motifs
- 1 with 20 CXXCH motifs
- 1 with 26 CXXCH motifs
- 1 with 33 CXXCH motifs
Organization of a 40-kb c-type cytochrome gene cluster in *A. dehalogenans* 2CP-1
Organization of a 40-kb c-type cytochrome gene cluster in *A. dehalogenans* 2CP-1

**Putative c-type cytochromes**

**Tetratricopeptide repeat proteins**
(protein-protein interactions and assembly of multiprotein complexes, type III secretion chaperons)

**Organization of a 40-kb c-type cytochrome gene cluster in A. dehalogenans 2CP-1**
Organization of a 40-kb c-type cytochrome gene cluster in *A. dehalogenans 2CP-1*

LPS/EPS biosynthesis cluster

VCBS domain protein (putative role in adhesion)

Putative lipoproteins

Putative c-type cytochromes

Tetratricopeptide repeat proteins (protein-protein interactions and assembly of multiprotein complexes, type III secretion chaperons)
Development of a genetic system for *A. dehalogenans* 2CP-C

Plasmids conjugated into *A. dehalogenans* 2CP-C
Development of genetic system for *A. dehalogenans* 2CP-C

*A. dehalogenans* 2CP-C with the RSF1010 BHR plasmid p519ngfp (expression of GFP using constitutive *lac* promoter)
Next steps

• Targeted mutagenesis to identify the function of the high-molecular c-type cytochromes found in 2CP-C

• Identification of genes essential for metal & radionuclide reduction through random mutagenesis

• Generation of whole-genome microarrays to study gene expression under different respiratory conditions

• Comparative genomic studies of different *Anaeromyxobacter* strains (2CP-C, 2CP-1, strain K, FW-109)
Related Poster Presentations

- Q. Wu et al., “Uranium (VI) reduction by Anaeromyxobacter dehalogenans”, PI - F.E. Löffler


Relevant characteristics of *A. dehalogenans*

- High rates of ferric iron reduction (constitutive)
- Reduce (immobilize) U(VI)
- Rapidly reduce chlorophenols to phenol
- Metabolic versatility (e⁻ acceptor and e⁻ donor)
- Genome analysis suggests abundance of c-type cytochromes
- *Anaeromyxobacter* 16S rRNA gene sequences retrieved from high NO₃⁻, low pH, radionuclide-contaminated FRC site
Domain alignment of Adeh_3077

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  ahc25-29: DGYT ATSVNYAT VTH NGALEV YAMA CTSCHDASR ALGAAPPVGVT
  ahc30-34: PEGATLGAP ARDV . . . HY DQKVDTAAG CALCHGDAP PVGAAAPPAG
  ahc20-24: ~~~~ GSCWQSN CTFCGHDP DSVNFAPPAG TRGGATTDR
  ahc13-17: ~~~~ GACGQSN CTSCHGDAR PVGAAAPPAG TRGGATTDR
  ahc6-10: PATILAGTLR TDVAGRRHL NGTVF PSTG CACHGDOPR FRAPLRAPPW
  ahc1-5: ~~~~ TGEAERDAGT ATEERADGAT CTCRCHGRAN TSG . APPD AHG3 ATRI
  ahc35-39: ~~~~ TGYA STTNVYATHV DQRLSVGGLT CTSCHGDAT HTMAAPPFL TGGSETP
  ahc25-29: AVGAHQAYHO QS . AITARPFD DECHLNP T . GLCHIDGT FALVSSLSAS GGS . SPOMD
  ahc30-34: AAGAHAHQHO QS . AITARPFD DEDCILKPT . GLHAPIDGN FAVNWWALAS ASGNVSPTWS
  ahc20-24: AVGAHAHQAHV QG . PLARPLG CAECHVOPA DLEHRVDGDFAVTVTGLAR A . CATAPW
  ahc13-17: AVGAHAHQLH AG . TLRPLP SCPSTVPT DLTVDTGA VELTHQPLR A . CATAPW
  ahc6-10: AVGAHRAHLQ AG . PLGPEFA CTECHYPT GLNIDDP AQITSTGALAR A . CATAPW
  ahc1-5: SVGAHQAHML QS . SHGIALPLG CAEACHVDFP AHPRLAVGDK FRWCTAVAAK ANCTAV.
  ahc35-39: AVGAHAQLHT QG . WSNGS A CTECHYPT DNMUTDGA AQVSDGEL ATGGGVLAPAW
  ahc25-29: . GATCSTY CHGATBA GG TNNWRAMTV VDGTQACACGT CHGAPPFX Y PPGRRAA...
  ahc30-34: . GATCSTY CHGATBA AG CTNVEEWTQ VDGTQACACGT CHGAPPFX Y PPGRRAA...
  ahc20-24: . GATCQTY CHGATBA AG ANYAWEVTY VDGTQACACGT CHGAPPFX Y PPGRRAA...
  ahc13-17: . GATCQTY CHGATBA AG TNQARWRTY VDGTQACACGT CHGAPPFX Y PPGRRAA...
  ahc6-10: RTSACTTY CHGATBA AG ERCRTRWRTY VDGTQACACGT CHGAPPFX Y PPGRRAA...
  ahc1-5: PADATCBBY CHVSLAG . AGAATRTRWR . GSPOEDACGT CHGAPPFX A PPGRQSTQ...
  ahc35-39: GSG . LATAV CHGARYG . GAVITMNGT GSPNEDGACGT CHGAPPFX Y PPGRQSTQ...
  ahc25-29: . . DCOTCH FGAT . GAP AR . . DVHY DQKMDTETAG...
  ahc30-34: . . DCOTCH FGAYT . STVNY . ATSV NY . . ATSV NGALEV YAMA
  ahc20-24: . . DCOTCH FGAT . ATSV NY . . ATSV NGALEV YAMA
  ahc13-17: . . NCOTCH FETV . LAAG . . ELTV . . AHG3 ATRI
  ahc6-10: . . GCOTCH FGTV . NGDNG . . TNYVAAMGHV...
  ahc1-5: . . DCOTCH PATILAGTLR TDVAGRRHL NGTVF PSTG
  ahc35-39: HGAKAP ACH . . CAGYQTQAG YEA VWNKR...
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Reduced U(IV) nanoparticle localization in *A. dehalohegans* (acetate, 30°C)

**Whole mounts:**
The sample with acetate has a large Na signal on top of U/O. Distinctive needle-like large crystals were found throughout the sample. Areas of uraninite with very little Na signal was found also (C). Cells were mostly clean (A), and didn’t show much of exPS or fine material on their surface.