Dissimilatory Metal Reduction by *Anaeromyxobacter*

Frank E. Löffler

*School of Civil & Environmental Engineering*
*School of Biology*
*Georgia Institute of Technology*
Performers

Dr. Frank Löffler  
Dr. Rob Sanford  
Dr. Qingzhong Wu  
Sara Henry
Performers

Dr. Frank Löffler  
Dr. Rob Sanford

Dr. Qingzhong Wu  
Sara Henry
Isolation of *Anaeromyxobacter*

\[
\begin{align*}
\text{2-Cloro-phenol} & \quad 2H^+ + 2e^- \\
\text{H}^+ + \text{Cl}^- & \quad \text{OH} \\
\end{align*}
\]

\[\Delta G^\circ = -157 \text{ kJ/rx with H}_2\]

**Respiratory Reductive Dechlorination**

*(De)Chlororespiration*

**Anaeromyxobacter - Phylogeny**

Phylogenetic tree based on nearly complete 16S rRNA gene sequences

- **Nannocystis exedens**
- **Polyangium cellulosum**
- **Chondromyces crocatus**
- **Cystobacter fuscus**
- **Myxococcus xanthus**
- **Geobacter metallireducens**
- **Pelobacter carbinolicus**
- **Desulfovibrio desulfuricans**
- **Desulfbacter curvatus**
- **Shewanella putrefaciens**
## Anaeromyxobacter - Properties

### Electron Acceptors
- Ortho-substituted halophenols
- Oxygen
- Nitrate
- Nitrite
- Fumarate
- Soluble and insoluble oxidized metal species

### Electron Donors
- Acetate
- Hydrogen
- Succinate
- Pyruvate
- Formate
- Lactate
**Anaeromyxobacter - Metal Reduction**

Yellow-Brownish

**Fe**\(^{3+}\) → **Fe**\(^{2+}\) reduced insoluble immobile

**U**\(^{6+}\) oxidized soluble mobile

Clear

**Anaeromyxobacter dehalogenans** strain 2CP-C

A. dehalogenans Couples Growth to Uranium (VI) Reduction

Mineral salts medium, acetate, H₂
2% (v/v) inoculum

![Graph showing the relationship between time, uranium (VI), and cells.](image-url)
U(VI) Reduction Requires H₂

Cells grown with:
- Acetate/fumarate (1 mM)
- 2% inoculum
- U(VI), fumarate (1 mM), ± H₂

Acetate/NO₃⁻ (0.5 mM),
- 2% inoculum
- U(VI), NO₃⁻ (0.5 mM), ± H₂
Effect of Fumarate on Uranium (VI) Reduction by *A. dehalogenans*

Resting cell experiment

Cells grown with acetate/fumarate

Washed cell suspensions amended with H₂, U(VI) ± fumarate

![Graph showing the effect of fumarate on uranium (VI) reduction by A. dehalogenans.](image)
Effect of Nitrate on Uranium (VI) Reduction by *A. dehalogenans*

2CP-C grown with acetate/NO$_3^-$ (0.5 mM)
U(VI) and H$_2$ added after NO$_3^-$ had been consumed (day 3)
A. dehalogenans strain 2CP-C Displays Social Motility Flares

A. dehalogenans strain 2CP-C Displays Social Motility Flares

• Gliding Motility
• Chemotactic Response

Anaeromyxobacter dehalogenans strain 2CP-C genome sequenced

JGI
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE
A Glimpse of the *Anaeromyxobacter dehalogenanans* Draft Genome

- 5 MB
- 89 contigs
- 74.8% GC
- 4,313 candidate protein-encoding genes
- single 16S rRNA gene
- 2 RDase genes
- gene clusters for type IV-pilus-based and flagellar motility
- 7 chemotaxis gene clusters
- 18 genes encoding MLPs

93 genes with CXXCH motif
15 genes > 10 CXXCH motifs
1 with 20 CXXCH motifs
1 with 26 CXXCH motifs
1 with 32 CXXCH motifs

Ryan Wagner
Field Research Center (FRC), Oak Ridge, TN

- Area 1
  - TPB16+
  - FW016+
  - FW026+
  - FW106+

- Area 2
  - FW029+

- Area 3
  - FW101*
  - FW102*

- S-3 Ponds Cap
  - FW101*

Stimulated
- FW026+
- FW106+

Not stimulated
- FW029+

* multiport wells
+ wells
Specific Detection of *Anaeromyxobacter dehalogenans* 2CP-C at the FRC

Nested PCR with *Anaeromyxobacter*-specific primers

<table>
<thead>
<tr>
<th>Location</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. FBR</td>
<td></td>
</tr>
<tr>
<td>2. FW016</td>
<td>1</td>
</tr>
<tr>
<td>3. FW026</td>
<td>3</td>
</tr>
<tr>
<td>4. FW029*</td>
<td>1</td>
</tr>
<tr>
<td>5. FW101-2</td>
<td>3</td>
</tr>
<tr>
<td>6. FW102-3</td>
<td>3</td>
</tr>
<tr>
<td>7. FW106</td>
<td>3</td>
</tr>
<tr>
<td>8. TPB16</td>
<td>2</td>
</tr>
<tr>
<td>9. Strain 2CP-C</td>
<td></td>
</tr>
</tbody>
</table>

* detected by RTm PCR (1.1 x 10^4 cells/ml)
Geobacter sp.
Strain SZ
**Geobacter Strain SZ - Phylogeny**

Phylogenetic tree based on nearly complete 16S rRNA gene sequences

**Youlboong Sung**

---

*Geobacter sp. strain SZ (AY914177)*

- **KB-1 1 (AY780563)**
  - **TDC-418 (AF447133)**
  - **Trichlorobacter thiogenes (AF223382)**
  - **TANB142 (AY667270)**

---

**FTLM68 (AF529127)**

- **TSAC03 (AB186845)**
  - **ZZ12C10 (AY214184)**

---

**Geobacter humireducens (AY187306)**

- **Geobacter bremiensis (U96917)**
  - **Geobacter thiogenes (AF223382)**

---

**FTLpost101 (AF529129)**

- **Geobacter chapellei (U41561)**
  - **Pelobacter propionicus (X70954)**

---

**Geobacter sulfurreducens (U13928)**

- **Geobacter hydrogenophilus (U46860)**
  - **Geobacter grbiciae (AF335183)**
  - **Geobacter metallireducens (L07834)**

---

**KB-1 2 (AY780557)**

- **Geobacter pelophilus (U96918)**
- **Geobacter hephaestus (AY737507)**

---

**Desulfuromonas michiganensis (AY221993)**

---

* env. clone seq.

★ clone from dechlorinating culture/contaminated site
Geobacter sp. Strain SZ

- Chloroethenes [µmol/bottle]
  - PCE
  - cis-DCE
  - TCE

- Time (d)
  - 0 2 4 6 8

- Fe (II) / Fe (III) [mM]
  - Fe (III)
  - Fe (II)

- U(VI) [µM]
  - cis-DCE
  - Killed Control

- Time (d)
  - 0 5 10 15
Specific Detection of *Geobacter* sp. Strain SZ at the FRC

<table>
<thead>
<tr>
<th>Location</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. FBR</td>
<td></td>
</tr>
<tr>
<td>2. FW016</td>
<td>1</td>
</tr>
<tr>
<td>3. FW026</td>
<td>3</td>
</tr>
<tr>
<td>4. FW029</td>
<td>1</td>
</tr>
<tr>
<td>5. FW101-2</td>
<td>3</td>
</tr>
<tr>
<td>6. FW102-3</td>
<td>3</td>
</tr>
<tr>
<td>7. FW106</td>
<td>3</td>
</tr>
<tr>
<td>8. TPB16</td>
<td>2</td>
</tr>
<tr>
<td>9. Strain SZ</td>
<td></td>
</tr>
</tbody>
</table>
Field Research Center (FRC), Oak Ridge, TN

0 200 400 feet

North

Stimulated

Not stimulated

Area 1

Area 2

Area 3

S-3 Ponds Cap

Geo FW102*

Geo FW026+

Geo FW106+

Geo FW029+

Geo FW016+

Geo FW101*

TPB16+

* multiport wells
+ wells
Take Home Messages

- Members of the “dechlorinating Geobacter” group detected at the FRC
- *Anaeromyxobacter dehalogenans* strain 2CP-C grows on the expense of U(VI) reduction
- Facultative, versatile metabolism
- Requires H₂ for U(VI) reduction
- Specific qualitative and quantitative tools available
- *Anaeromyxobacter* detected at the FRC
Take Home Messages

Anaeromyxobacter - another model organism for studying electron transfer to toxic metals and rads
The Team

Frank Löffler
Qingzhong Wu
Sara Henry
Youlboong Sung
Ryan Wagner
John Kirby
Martial Taillefert
Tom DiChristina
Robert Sanford
Relevant Characteristics of Anaeromyxobacter Species

- Rapidly reduce chlorophenols to phenol
- High rate ferric iron reduction (constitutive)
- Reduce (immobilize) U(VI)
- Metabolic versatility (e^- acceptor and e^- donor)
- Pilus-based motility
- Genome analysis suggests chemotaxis and flagellar motility
- Anaeromyxobacter 16S rRNA gene sequences retrieved from high NO_3^-, low pH, radionuclide-contaminated FRC site