Biogeochemical Controls Over Organohalide-Respiring Chloroflexi

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Contaminated Sites in the U.S.

- 1,322 Superfund sites
- 3,747 RCRA sites
- >450,000 Brownfields

166 MILLION PEOPLE Live Within 3 MILES of a Superfund or a RCRA Corrective Action Site, Equal to 53% OF THE U.S. POPULATION Including 55% OF ALL CHILDREN UNDER AGE 5

... and in Europe

Majority of sites impacted with chlorinated compounds

- Remediated: 58
- Contaminated: 127
- Potentially contaminated: 1170
Reductive Dechlorination: A Process that Leads to Contaminant Detoxification In Situ

![Chemical Reaction Diagram]

Organohalide Respiration

Populations Involved in Reductive Dechlorination of CEs

Geobacter lovleyi, Dehalobacter, Sulfurospirillum, Desulfuromonas, Desulfitobacterium

Dehalococcoides mccartyi

Sung et al. 2006
AEM, 72:2775

Löffler et al. 2013
IJSEM, 63:625
Populations Involved in Reductive Dechlorination of Chlorinated Ethenes

1,173 GW samples, 111 sites
849 samples: Dhc & Dhgm
65%: Dhgm outnumber Dhc

Yang et al. ISME J. 2017. 11:2767-2780
Populations Involved in Reductive Dechlorination of Chlorinated Ethenes

1,173 GW samples, 111 sites
849 samples: Dhc & Dhgm
65%: Dhgm outnumber Dhc

Dehalococcoides (Dhc)
Dehalogenimonas (Dhgm)
Structural Basis of Organohalide Respiration

Dimer of PceA
*Sulfurospirillum multivorans*

2 FeS Clusters

Corrinoid Cofactor

Corrin ring

Dimethylbenzimidazole (DMB)

Upper ligand

Adenosylcobalamin

Methylcobalamin

Cyanocobalamin

Vitamin B₁₂
Lower Bases

- Benzimidazole (Bza) type
- Nucleobase type
- Phenol type
- Naphthimidazole

De novo Biosynthesis of Corrinoids

**Bacteria**
(n = 56,902)
- Complete
- Incomplete or missing

**Archaea**
(n = 1,362)
- Complete
- Incomplete or missing

- Geobacter lovleyi → Corrinoid prototroph
- Dehalococcoides mccartyi → Corrinoid auxotroph
**Dhc & Dhgm: Strict Requirement for Corrinoid**

- **No B$_{12}$**
  - No dechlorination

- **Limited B$_{12}$** [1 µg/L]
  - VC stall

- **Sufficient B$_{12}$** [25 µg/L]
  - Complete Dechlorination (Detoxification)
Specific Aims

• **Aim 1:** Explore the specific cobamide requirements of organohalide-respiring *Dhc* relevant for detoxification of chlorinated ethenes

• **Aim 2:** Demonstrate that geochemical conditions affect the specific cobamide pool, and hence *Dhc* activity

• **Aim 3:** Identify community and *Dhc* biomarkers that indicate when cobamide and/or lower base bioavailability limit *Dhc* reductive dechlorination activity
**Simplified Model of Organohalide Respiration**

Dehalococcoides mccartyi strain BAV1

- Corrinoid auxotroph

Geobacter lovleyi
- Corrinoid prototroph

\[
R\text{-Cl} + H^+ \rightarrow R\text{-H} + Cl^-
\]

\[
H_2 \rightarrow 2 H^+ \rightarrow 2 e^- \rightarrow ATP
\]
Who Supplies Corrinoid to *Dhc* and *Dhgm*?

**Corrinoid**

*Geobacter lovleyi*

**Corrinoid**

*Dehalococcoides mccartyi*
Co-Culture Experiments: Corrinoid Producer / *Dhc mccartyi*

- Cobamide producing microbe: *Geobacter* spp.
- *Dhc mccartyi* auxotroph
- Corrinoid dechlorination activity?
Summary of Co-Culture Experiments

<table>
<thead>
<tr>
<th>Co-Cultures</th>
<th>Dhc Strains</th>
<th>Dhc Growth</th>
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<td></td>
<td>BAV1, FL2</td>
<td>+</td>
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- Geobacter spp. BAV1, FL2 - Sporomusa sp. BAV1, FL2, GT - Acetobacterium sp. BAV1, FL2 - Clostridium aceticum BAV1, FL2 - Methanosarcina barkeri BAV1, FL2, GT
## Summary of Co-Culture Experiments

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Yan et al. 2013. Phil. Trans. R. Soc. B. 368, 20120320
Co-Culture Experiments

### Co-Cultures

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<td><strong>Geobacter lovleyi</strong></td>
<td>BAV1, FL2</td>
<td>+</td>
</tr>
<tr>
<td><strong>Geobacter sulfurreducens Wildtype</strong></td>
<td>BAV1, FL2</td>
<td>-</td>
</tr>
<tr>
<td><strong>Geobacter sulfurreducens + pNJ052</strong></td>
<td>BAV1, FL2</td>
<td>+</td>
</tr>
</tbody>
</table>

**Notes:**
- ? Dhc dechlorination activity?
- Corrinoid Producer: Geobacter lovleyi
- Dhc Strains: BAV1, FL2
- Dhc Growth: +, -, +
- Acetobacterium sp. BAV1, FL2
- Clostridium aceticum BAV1, FL2
- Methanosarcina barkeri BAV1, FL2, GT

**Diagram:**
- 5-Hydroxy-benzimidazole (5-OHbzA) converted to 5-Methoxy-benzimidazole (5-OMebzA)
- Diagrams showing conversion activity

**References:**
- Yan et al. 2013. Phil. Trans. R. Soc. B. 368, 20120320

**Image:**
- Corrinoid auxotroph
- Dhc mccartyi
- Geobacter spp.
- Strain BAV1 Strain FL2
Key *Dhc* RDases

**Dhc str. 195**

\[ \text{PceA/PteA} \rightarrow \text{TceA} \]

**Dhc str. FL2**

\[ \text{..} \rightarrow \text{TceA} \rightarrow \text{..} \]

**Dhc str. BAV1**

\[ \text{..} \rightarrow \text{BvcA} \rightarrow \text{..} \]

**Dhc str. VS**

\[ \text{VcrA} \]

**Dhc str. GT**

\[ \text{..} \]
Lower Base Affects Dechlorination Activity

Strain BAV1 (BvcA)

Dechlorination rates [mnoles Cl\(^{-}\) L\(^{-1}\) d\(^{-1}\)]

Yan et al. 2016. ISME J. 10:1092–1101
Lower Base Affects Dechlorination Activity

Strain GT (VcrA)

Dechlorination rates [mmoles Cl⁻ L⁻¹ d⁻¹]

Yan et al. 2016. ISME J. 10:1092–1101

Lower base affects dechlorination rates and endpoints
Corrinoid Production Under Different Redox Conditions

**Third Creek Site**
Knoxville, TN
- Metal-manufacturing
- Chlorinated solvents

**Commerce Street Superfund Site**
Williston, Vermont
- Multi-tenant industrial park
- TCE, *cis*-DCE, petroleum hydrocarbons, metals (chromium, cadmium, nickel)

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Corrinoid Extraction and Identification
Corrinoids Produced by the Community Under Different Redox Conditions

<table>
<thead>
<tr>
<th>Corrinoids produced</th>
<th>Glucose fermenting</th>
<th>Methanogenic</th>
<th>Iron reducing</th>
<th>Sulfate reducing</th>
<th>Nitrate reducing</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Corrinoid 1" /></td>
<td><img src="image2" alt="Corrinoid 2" /></td>
<td><img src="image3" alt="Corrinoid 3" /></td>
<td><img src="image4" alt="Corrinoid 4" /></td>
<td><img src="image5" alt="Corrinoid 5" /></td>
<td><img src="image6" alt="Corrinoid 6" /></td>
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<tr>
<td><img src="image7" alt="Corrinoid 7" /></td>
<td><img src="image8" alt="Corrinoid 8" /></td>
<td><img src="image9" alt="Corrinoid 9" /></td>
<td><img src="image10" alt="Corrinoid 10" /></td>
<td><img src="image11" alt="Corrinoid 11" /></td>
<td><img src="image12" alt="Corrinoid 12" /></td>
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</table>
Redox Conditions Affect Corrinoid Type(s) and Quantity

Seus et al. 2019. In Preparation
Corrinoid Quantity and Quality Determine *Dhc* Activity

- **No B\textsubscript{12}**
- **Limited B\textsubscript{12} [1 \textmu g/L]**
- **Sufficient B\textsubscript{12} [25 \textmu g/L]**
Impact of $N_2O$ on corrinoid-dependent reductive dechlorination?


N₂O Inhibits Corrinoid-Dependent Reductive Dechlorination

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**Geobacter lovleyi**

- **PCE**
- **TCE**
- **cDCE**

**N₂O [µM]**

- 0
- 10
- 20
- 60

**Fumarate, succinate (mM)**

- 0
- 20
- 100
**Impact of Increased N₂O in Environmental Systems**

<table>
<thead>
<tr>
<th>N₂O (µM)</th>
<th>0.3</th>
<th>12.5</th>
<th>37.4</th>
<th>75</th>
<th>84</th>
<th>143</th>
<th>65.7</th>
</tr>
</thead>
</table>

- $K_{i, N_2O} = 40.8 \pm 3.8$ µM
- $K_{i, N_2O} = 21.2 \pm 3.5$ µM
- $K_{i, N_2O} = 9.6 \pm 0.4$ µM


Adenosylcobalamin Biosynthesis

**Oxygen Pathway**
- Precorrin-3A → Precorrin-3B → Precorrin-4 → Precorrin-5 → Precorrin-6A → Precorrin-6B → Precorrin-8x
- Hydrogenobyrinic acid
- Hydrogenobyrinic acid a,c-diamide

**Late Cobalt Insertion Pathway**
- Cob(I)lrynic acid a,c-diamide
- L-threonine-phosphate

**Early Cobalt Insertion Pathway**
- Cobalt-precorrin-2 → Cobalt-precorrin-3 → Cobalt-precorrin-4 → Cobalt-precorrin-5 → Cobalt-precorrin-6A → Cobalt-precorrin-6B → Cobalt-precorrin-7 → Cobalt-precorrin-8x

**Corrinoid Salvage**
- incomplete corrinoid
- 5,6-dimethylbenzimidazole

**Bacterial Salvage**
- Ado-ribazole-5-phosphate

**Archaeal Salvage**
- Ado-ribazole

**Dehalococcoides genes**
- beta-ribazole-5-phosphate

**Adenosylcobalamin**
- Ado-cobyric acid
- Ado-cobyric acid a,c-diamide
- Ado-GDP-cobinamide
- Ado-GDP-cobinamide-phosphate
- Ado-L-threonine-1-amino-2-propanol-phosphate
High-Throughput qPCR

- Monitor many biomarker genes simultaneously

QuantStudio Open Array Plate
3,072 through-holes
48 subarrays x 64 through-holes/subarray

- Scalable platform (224x12; 112x24; 56x48)
- Cost per reaction reduced from $3.0 to $0.30
- Four plates can be cycled simultaneously, producing up to 12,288 qPCR data points per run

Robot reduces pipetting errors

Kara Murdoch et al. 2019. In Preparation
Take Home Messages

- Corrinoids are essential for most organisms
- Corrinoid-auxotrophic OHRBs (e.g., Dhc, Dhgm) are ideal systems to study corrinoid effects on metabolism
- Corrinoid quantity (flux) affects dechlorination activity
- Corrinoid type (lower base) determines reductive dechlorination rates and end points (i.e., function) in Dhc
- Geochemistry affects corrinoid pool
- Purine is a naturally occurring lower base
- New avenues to manipulate microbial metabolism (function, ecology → biotechnology, medicine)
Peer-reviewed Manuscripts


