Mechanisms of U(VI) Reduction and Sediment Growth in *Desulfovibrio*

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Goals

• Identify mechanisms of survival of sediment bacteria in U(VI) contaminated sediments.
  – This was further refined to
  1. Identification of mechanisms of dealing with U(VI)
  2. Identification of mechanisms of survival in Anoxic sediments.
Desulfovibrio G20

- Isolated from an oil producing reservoir.
- 4.1 Mb, 3598 candidate genes
- Doubling time is ~4.6 hours
Mechanisms of U(VI) reduction

PERIPLASM

Fe H₂ase; -410 mV

2H⁺ + 2e⁻

2H⁺ + 2e⁻

Hmc; ~180 mV

APS, SO₃ reductase, etc

SO₄²⁻ -220 mV

S²⁻

NiFe H₂ase; -410 mV

2Fe(III)/U(VI) (+770 mV/+334 mV)

2Fe(II)/U(IV)

H₂

2H⁺

2H⁺

2e⁻

2e⁻

Cyt c₃; ~-300 mV

Lactate → Pyruvate → Acetate

2 e⁻

2H⁺ + 2 e⁻

2H⁺ + 2 e⁻

2H⁺ + 2 e⁻

2H⁺ + 2 e⁻

2H⁺ + 2 e⁻

2H⁺ + 2 e⁻
Identification of Genes involved in U(VI) Response

Inoculate STM mutants to 96-well plate

Grown with 2 mM U(VI) → Grown without 2 mM U(VI)

Check growth after two days → Pick the mutants that cannot grow with 2 mM U(VI)

Recheck the growth with 2 mM U(VI) in the serum tubes
10 mutants
Potential Functions of mutated genes in U(VI) Sensitive Mutants

- DNA repair
- rRNA methylation
- Protein Renaturation
Washed Cell U(VI) reduction test by 24 mutants

Growth Experiment with B11E9

![Graph showing U(VI) concentration over time for different mutants and B11E9.](image)
The Region surrounding the mutation in B11E9

D. vulgaris
Mechanism of As(VI) Reduction

QuickTime™ and a None decompressor are needed to see this picture.
Loss of As(V) Tolerance.

- B11E9 also lost As(V) resistance when grown in lactate sulfate media with 20mM As(V)
Growth and U(VI) reduction of G20 with Cd
Mechanism of U(VI) Reduction

QuickTime™ and a
None decompressor
are needed to see this picture.
U(VI) Reduction by *E. coli* transformant
Signature Tagged Mutagenesis

- First developed to identify virulence genes in pathogens.
- Use the technique to identify functions involved in sediment growth. Different from functions needed for lab growth.
Overview of signature tagged mutagenesis (STM)

1. Tagged transposon moved into bacteria
2. Mutants assembled in microtiter dishes
3. Pooled liquid cultures
4. Sediment incubations
5. Recover viable cells and amplify tag region
6. PCR generation of tagged probes and hybridization to complimentary tag target
7. Replicate tags onto solid surface (membrane)
Growth of potential non-survival mutants of *Desulfovibrio* in sediment
Screened ~5000 mutants for each bacterium by STM and identified 100 mutants in G20 and 46 mutants in MR-1

Recurring Themes

DNA repair
Transcriptional regulators
Phage-related proteins
Transport proteins (multidrug efflux)
Conserved and hypothetical proteins
DNA repair

<table>
<thead>
<tr>
<th>In situ CI</th>
<th>In vitro CI</th>
<th>Gene</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.11±0.002</td>
<td>0.571</td>
<td>recG</td>
<td>ATP-dependent DNA helicase RecG</td>
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<tr>
<td>0.39±0.05</td>
<td>ND</td>
<td></td>
<td>helicase (SO0368)</td>
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<tr>
<td>0.4±0.42</td>
<td>ND</td>
<td></td>
<td>conserved hypothetical (SO1652)</td>
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<tr>
<td>0.85±0.38</td>
<td>ND</td>
<td></td>
<td>helicase (SO2744)</td>
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</table>

- Sediment conditions may be mutagenic
- These genes may correct DNA defects resulting from sediment mutagens (e.g., organic acid fermentation products)
Response to mutagens

- One mutant B12(pF11) belongs to **UmuC** family.
- It has 53.83% similarity to *D. vulgaris* umuC protein.
Protection from a Severe Mutagenic Event

The SOS response

### STM mutants in MR-1 with potential role in drug efflux

<table>
<thead>
<tr>
<th>In situ CI</th>
<th>In vitro CI</th>
<th>Gene</th>
<th>Product</th>
<th>COG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.28±0.06</td>
<td>1.3±0.26</td>
<td>mexF</td>
<td>RND multidrug efflux transporter MexF</td>
<td>Cation/Multidrug efflux pump</td>
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<tr>
<td>0.59±0.07</td>
<td>1.3±0.54</td>
<td>HlyD family secretion protein</td>
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<td>Multidrug resistance efflux pump</td>
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<td>0.43±0.14</td>
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<td>toxin secretion, membrane fusion protein</td>
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<tr>
<td>0.3</td>
<td>1.3±0.78</td>
<td>transcriptional regulator, TetR family</td>
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<td>Transcriptional regulator</td>
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</table>
mexF deletion cannot grow in the presence of 5 µg/ml chloramphenicol
Tripartite efflux pump system

- Outer membrane
- Periplasmic space
- Inner membrane
- Outer membrane protein
- Membrane fusion protein (MexE)
- RND Exporter (MexF)

Antibiotic

RND = resistance-nodulation-division

Adapted from Aeschlimann, 2004
<table>
<thead>
<tr>
<th>organism</th>
<th>size (aa)</th>
<th>identity</th>
<th>similarity</th>
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<tbody>
<tr>
<td>B. japonicum USDA 110</td>
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<td>600/1035 (57%)</td>
<td>745/1035 (71%)</td>
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<tr>
<td>Magnetococcus MC-1</td>
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<td>M. magnetotacticum MS-1</td>
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<td>576/1038 (55%)</td>
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</tbody>
</table>

### α-proteobacteria

- Bacteroides thetaiotaomicron VPI-5482
- Dechloromonas aromatica RCB
- Geobacter metallireducens GS-15
- Wolinella succinogenes

### β-proteobacteria

- Acinetobacter ADP1
- Desulfovibrio strain G20
- D. vulgaris
- Geobacter metallireducens GS-15
- Wolinella succinogenes

### γ-proteobacteria

- Alcaligenes faecalis
- Anabaena variabilis
- C. watsonii WH8501
- G. violaceus PCC 7421

### δ/ε-proteobacteria

- Acinetobacter ADP1
- Desulfovibrio strain G20
- D. vulgaris
- Geobacter metallireducens GS-15
- Wolinella succinogenes

### Other

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- α-proteobacteria
- β-proteobacteria
- γ-proteobacteria
- δ/ε-proteobacteria

### Other

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- Dechloromonas aromatica RCB
- Geobacter metallireducens GS-15
- Wolinella succinogenes
What have we learned from STM

• Identified a number of genes and cell functions that are required for life in the real world but not needed for growth in culture.

• Demonstrated for the first time the importance of specific genes as cells are growing in natural environments.

• Raised many questions regarding function of specific genes. Regulatory genes. Protection from toxins. DNA repair, etc.
Acknowledgments

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• Qingwei Luo, Jennifer Groh, Xiangkai Li and Nydia Castaneda.
• EMSL