Quantification of Prokaryotic Gene Expression in Shallow Marine Subsurface Sediments of Aarhus Bay, Denmark

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Outline

- Background information
- Experimental methods
- Results
- Discussion/Conclusions
Objectives

- To link the dynamics of prokaryotic gene expression and geochemical rate determinations for use in description of metabolically-active sulfate-reducing prokaryotes (SRP)
- Place SRP into context of abundance and metabolic activity of Bacteria and Archaea through community level 16S rRNA quantitation
- Identify the metabolically-active SRP through taxonomic description of mRNAs

✓ Humphrys, 2009
Ecology of Sulfate-reducing Prokaryotes (SRP)

- Broad phylogenetic groups
- 7 lineages: 5 Bacteria, 2 Archaea
- 23 genera within the Deltaproteobacteria

- Broad metabolic diversity
  - Heterotrophs, autotrophs, syntrophs

- Radiotracer assay and good functional gene target for SRP

- dsr analysis has shown that SRP diversity much larger than previously thought

- Few studies have quantified in situ gene expression in marine sediments

Muyzer and Stams, 2008
Expression of genes for SRP

- Expression of respiratory genes correlated with rates of metabolism in pure cultures and in the field
- However, respiratory gene expression shown respond to other parameters besides rates
- Expression of genes linked to central carbon metabolism may provide a better proxy for rates

✓ Neretin et al., 2003; Chin et al., 2004; 2008; Holmes et al., 2005; Villanueva et al., 2008
Functional gene targets

- Desired target = phylogenetically informative gene that is highly conserved and unique to a distinct group and for which expression patterns are correlated to metabolic rates

- **SRP** - Dissimilatory bisulfite reductase gene (*dsrAB*)
  - highly conserved amongst the SRP

- **FeRP** - Citrate synthase (*gltA*)
  - highly conserved amongst Desulfuromonadales (Geobacteraceae + Desulfuromonadaceae)
  - present in all members examined, more closely related to that of eukaryotes than other prokaryotes
  - key gene in the incorporation of acetate into the TCA cycle
  - Desulfuromonadales often predominate in Fe(III)-reducing zones
Sulfate reduction in the marine subsurface

- Aarhus Bay, Denmark
  - Wealth of background data (Jørgensen, Ingvorsen, Finster, Ramsing, and many others)
  - Well-defined sulfate-to-methane-transition
- Research cruise on 27 March, 2007, to site M1
Aarhus Bay Sampling - 27 March 2007
Methods

Biogeochemistry

Profiling of porewater and solid-phase geochemistry to 5 m below surface

$^{35}$S radiotracer determination sulfate reduction rates

Molecular Microbiology

Quantitation of dsrAB gene transcript levels

Quantitation of 16S rRNA for Archaea and Bacteria

16S rRNA and dsrAB gene sequence analysis
Experimental Approach - Molecular Analysis

Extraction and purification of mRNA and total RNA from sediment

cDNA synthesis with gene specific primer → Amplification and quantitation via qPCR → Statistical analysis → Clone library construction & comparative sequence analysis

Amplification of cDNA (method validation and quality control)

Adapted from K. Chin
Results

BIOGEOCHEMISTRY

GENE EXPRESSION

SEQUENCE ANALYSIS
Sediment Depth (cm)

- Sulfate-to-methane-transition (SMTZ) zone at 1.6 to 2.2 m depth
- Sulfate persists to > 5 m depth in the sediment column
Sulfate Reduction Rates

- Vary with depth by 6 orders of magnitude
- Measured to > 5 m depth
16S rRNA QUANTITATION

- rRNA ~ 3 orders of magnitude > for Bacteria at surface; equivalent or < at depth
- Bacterial rRNA parallels with SRR and dsr expression

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**16S Ribosomal RNAs (RNA)**

- Bacteria
- Archaea

<table>
<thead>
<tr>
<th>Sediment Depth (cm)</th>
<th>Copies ug(^{-1})</th>
<th>Copies g(^{-1})</th>
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<td>10^2</td>
<td></td>
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<td>10^4</td>
<td></td>
<td></td>
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<tr>
<td>10^6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^8</td>
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<tr>
<td>10^10</td>
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<table>
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<th>Copies ug(^{-1})</th>
<th>Copies g(^{-1})</th>
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<td>10^5</td>
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<td>10^7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^9</td>
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</table>
GENE EXPRESSION - *dsrAB*

**mRNA Copy Number**

**DNA Copy Number**

- Copies ug\(^{-1}\)
- Copies g\(^{-1}\)
Six libraries were constructed using universal PCR primers 27F and 1492R.

Libraries at 20, 40, 61, 170, 340, and 465 cm depth below seafloor.
**16S rRNA Phylogeny**

<table>
<thead>
<tr>
<th>phylum</th>
<th>%</th>
<th>Library</th>
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<tbody>
<tr>
<td>Spirochaetes</td>
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<tr>
<td>Chloroflexi</td>
<td>3.7</td>
<td></td>
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<tr>
<td>Bacteroidetes</td>
<td>2.0</td>
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<tr>
<td>Proteobacteria</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>Firmicutes</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>unclassified_Bacteria</td>
<td>78.3</td>
<td></td>
</tr>
</tbody>
</table>

\[ n = 244 \]

**Proteobacteria = 34**

85 % of total **Proteobacteria**

11.8 % of total library

Taxa of interest represent a small fraction of clone libraries
Six libraries also constructed for *dsrB* from primers 2060F and 4R

Sequences were translated in-frame for protein comparison

Majority of clones affiliated with other uncultured SRP
Sulfate Reduction Rates vs. Gene Expression

- Obtained by normalizing sulfate reduction rates to \( dsrAB \) mRNA expression values.
- Comparison of time-integrated and instantaneous rate data is not absolute as mRNA residence time is not addressed.
**Sulfate Reduction Rates vs. Gene Expression**

![Graph](image.png)

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Mean [SO$_4^{2-}$] (mM)</th>
<th>Mean SRR (nmol SO$_4^{2-}$ cm$^3$ d$^{-1}$)</th>
<th>dsrAB Transcripts (copies g sediment$^{-1}$) (w/w)</th>
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<tbody>
<tr>
<td>20</td>
<td>19.2</td>
<td>$6.55 \times 10^3$</td>
<td>$2.72 \times 10^6$</td>
</tr>
<tr>
<td>40</td>
<td>16.9</td>
<td>$3.49 \times 10^2$</td>
<td>$2.72 \times 10^5$</td>
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<tr>
<td>61</td>
<td>14.8</td>
<td>$1.27 \times 10^2$</td>
<td>$5.71 \times 10^4$</td>
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<tr>
<td>170</td>
<td>0.58</td>
<td>$5.18 \times 10^0$</td>
<td>$7.42 \times 10^4$</td>
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<tr>
<td>340</td>
<td>0.34</td>
<td>$9.79 \times 10^{-1}$</td>
<td>$9.01 \times 10^3$</td>
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<tr>
<td>465</td>
<td>0.18</td>
<td>$4.24 \times 10^{-1}$</td>
<td>$2.25 \times 10^3$</td>
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</table>

Mean [SO$_4^{2-}$] (mM)
Mean SRR (nmol SO$_4^{2-}$ cm$^3$ d$^{-1}$)
dsrAB Transcripts (copies g sediment$^{-1}$) (w/w)
Conclusions

- Expression profiles of dsrA were directly correlated with sulfate reduction rates and Bacterial rRNA content.
- mRNA analysis provides a valuable molecular proxy for interrogation of in situ sulfate-reducing communities in marine sediments.
- mRNA expression confirmed that sulfate reduction cellular machinery remains active at low sulfate, below the SMTZ.
- May be explained by fermentation, syntrophy, or growth on alternative electron acceptors.
- Transcript-specific SRR indicates shift in regulation of expression at SMTZ.
Conclusions

- 16S rRNA phylogeny detects similar SRP lineages, but likely underrepresents SRP community relative to dsr
- SRP sequence analysis provides a direct link between gene expression and taxonomy
- SRP communities show some phylogenetic clustering when compared to sediment depth
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