Genomic and Proteomic Analysis of Deep Underground Microbial Communities

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Collaborations

- Proteomics
  - Ken Reardon

- Genomics
  - Amy Pruden

- Geomicrobiology
  - Alexis Templeton

Identify Novel Functions in the Deep Subsurface

Broader collaborations: *in situ* measurements of who’s there and energy sources (Kieft, Spear, et al.)....
Our hypothesis….

- Constraints in the subsurface…
  - e.g., lack of sunlight, oxygen, and limited nutrients, water, & organic C
- contribute to the evolution of novel forms of metabolism….
  - likely requiring chemolithotrophic means of obtaining energy for growth and maintenance.
Community / Tools Hierarchy

Structure

Function

Expression

Functional genes

mRNA

transcription

translation

protein

Target: 16S rRNA
Who?
Cloning, SSCP, DGGE
How many?
FISH, qPCR
Disadvantages?
Difficult to know who is doing what.

Target: functional genes
Potential to do what?
Cloning, SSCP, DGGE
How much potential?
FISH, qPCR
Disadvantages?
Gene may be present but inactive.

Target: mRNA
What are they doing?
RT-PCR,
Microarrays, differential display
Disadvantages?
Microbial mRNA difficult to work with, techniques still developing.

Target: protein
What is their final product?
2-D gel electrophoresis/mass spectrometry
Disadvantages?
Frontier of knowledge, techniques not developed.
Bio Basics

DNA → messenger RNA (mRNA) → translation → modified protein

post-translational modification


transcription

-DNA

post-translational modification

-DNA

modified protein

-mRNA (mRNA)
Bio Basics

- A-C-C-G-G-C-T-T-A-
- T-G-G-C-C-G-A-A-T-
- U-G-G-C-C-G-A-A-U-

transcription

translation

post-translational modification

modified protein

-trp-pro-asn-

protein

messenger RNA (mRNA)

genomics

DNA
Bio Basics

-DNA-  messenger RNA (mRNA)  protein

transcription  translation  post-translational modification

-DNA-  messenger RNA (mRNA)  protein

modified protein  transcriptomics
Bio Basics

proteomics

post-translational modification

-trp-pro-asn-

translation

-U-G-G-C-C-G-A-A-U-

transcription

-A-C-C-G-G-C-T-T-A-

-T-G-G-C-C-G-A-A-T-

DNA

modified protein

protein

messenger RNA (mRNA)
Metagenomics vs. metaproteomics

- **Metagenomics**
  - Direct cloning, sequencing, assembly and annotation of DNA from microbial communities
  - Provide valuable information on potential ecological function based on sequence
  - **BUT:** accurately predicting function almost impossible without information on what proteins are synthesized under specific conditions

- **Metaproteomics**
  - Provide a direct measurement of functional gene expression in terms of the presence, relative abundance and modification state of proteins
  - Can identify and link proteins to their *in situ* function in the environment
Proteomics: Proof-of-Principle

- Use a model system – cadmium shock – to understand:
  - Can significant proteome shifts be detected?
  - What does proteomics reveal about the meta-organism response to cadmium?
  - Is proteome analysis more sensitive than measurement of population changes?
Proteomics methodology

- Isolation and preparation
  - Sample specific
  - Lysis, purification, solubilization
  - Cytoplasmic or membrane proteins

- Separation
  - 2D electrophoresis
  - Liquid chromatography

- Quantification

- Identification
  - Mass spectrometry
Methods

- Growth on liquid rich medium
- After 20 h of growth, 10 mg/L Cd\(^{2+}\) added
- Harvesting after 15 minutes, 1, 2 or 3 hours
- Protein extraction and purification
- 2D-PAGE
- MALDI-TOF/TOF
- Mascot searches against NCBInr
Mixed culture proteome - Cd exposure
Protein expression analysis

Protein expression over time

Ratios of expression Cd/Ct

Time points

Controls vs. Cadmium over different time points (15 min, 1 h, 2 h, 3 h)
Summary of differentially expressed proteins*

<table>
<thead>
<tr>
<th>Time point</th>
<th>Down-regulated</th>
<th>Up-regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>2%</td>
<td>17%</td>
</tr>
<tr>
<td>1 h</td>
<td>6%</td>
<td>10%</td>
</tr>
<tr>
<td>2 h</td>
<td>1%</td>
<td>8%</td>
</tr>
<tr>
<td>3 h</td>
<td>6%</td>
<td>7%</td>
</tr>
</tbody>
</table>

* 3x change
Identified proteins - 100 IDs

- Metabolism, 42%
- Defense, 14%
- Replication / transcription, 8%
- Transport, 6%
- Cell structure, 6%
- Energy, 10%
- Protein synthesis and storage, 14%
More on MS findings

- Proteins with the largest changes (> 4x up-regulated)
  - Outer membrane protein precursor
  - Catalase
  - Translation elongation factors

- Defense proteins (examples)
  - Chaperonin GroEL (HSP60 family)
  - Chaperone Hsp70
  - Peroxiredoxin
  - Superoxide dismutase

- ATP synthase
  - Identification of 5 different subunits from ~ 20 different spots
  - Probably expressed by many different species
Proof-of-Principle: Conclusions

- Proteins can be identified from a community of unsequenced organisms.
- In comparison to traditional 16S rDNA methods, proteomics reveal insights into:
  - functional responses
  - short-term responses
- Short and long term responses can be detected
  - Provides insight into function
HUSEP: Research Plan

- Enrichment cultures
  - manganese oxidizing (current)
  - iron oxidizing (current)
  - sulfate reducing (future?)
  - manganese reducing (future?)
  - others?
Proteomic analysis

- Response to stimuli
  - Increased/decreased e- acceptor
  - Increased/decreased e- donor
  - Shifting e- acceptor/donor

- Identify corresponding proteins
HUSEP: Research Plan

- **Genomic analyses**
  - 16S Profiling- CE-SSCP
  - Design probes
    - FISH (both 16S and newly designed probes)
    - Visualize/quantify microbes of interest
  - Design primers
    - Clone/sequence to discover novel func. genes
    - RT-qPCR (quantify gene expression)
Population characterization – 16S rDNA

<table>
<thead>
<tr>
<th>Peak</th>
<th>Size</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>189.0</td>
<td>Uncultured bacterium</td>
</tr>
<tr>
<td>c</td>
<td>191.1</td>
<td><em>Arthrobacter oxydans</em>, <em>Arthrobacter polychromogenes</em></td>
</tr>
<tr>
<td>d</td>
<td>193.8</td>
<td><em>Acidobacteria</em> sp.</td>
</tr>
<tr>
<td>g</td>
<td>209.5</td>
<td><em>Kaistella koreensis</em></td>
</tr>
<tr>
<td>h</td>
<td>212.9</td>
<td><em>Alcaligenes xylosoxidans</em></td>
</tr>
<tr>
<td>j</td>
<td>226.2</td>
<td>Uncultured bacterium</td>
</tr>
<tr>
<td>l</td>
<td>231.9</td>
<td><em>Burkholderia cepacia</em></td>
</tr>
</tbody>
</table>
Advantageous site characteristics…..

- **Significant microbial diversity**
  - Confirmed by John Spear

- **Thermal and chemical gradients**

- **Diverse geochemistry**
  - $\text{SO}_4^{2-}$, $\text{NO}_3^-$, and $\text{NO}_2^-$ as e- acceptors
  - $\text{NH}_4^+$, Mn, Fe, (H$_2$?) as e- donors
  - Abundant N, but restricted P
  - High inorganic C (autotrophs)
  - Some organic C
  - Trace elements
Expected results

- Growing enrichments under different electron acceptor conditions
  - ID proteins and link them to their functions
- **Functional gene probes for genes corresponding to proteins of potential application**
  - environmental remediation, e.g. AMD
  - autotrophic sulfate reducers
  - Mn as an electron acceptor
- **Link proteins with corresponding microorganisms**
  - FISH & 16S profiling
- **Functional gene primers**
  - Fully sequence novel genes
  - RT-qPCR to quantify gene expression
Feed back and future work

- Communicate with collaborators conducting in situ studies
  - Who’s there in the subsurface versus the enrichment culture?
- Develop protein extraction techniques for subsurface material
- “Life as we don’t know it?”
HUSEP: Technical needs

- **Principle need: access to sites**
  - Explore subsurface heterogeneity
  - Profile with depth, the deeper the better

- **Subsurface labs:**
  - The closer the lab to sample processing, the better
  - Basic micro lab for preservation and sterile on all three campuses and at surface ideal

- **Hazardous materials or equipment:**
  - Acids, solvents, and chemical storage facilities needed

- **Surface facilities and space required:**
  - Samples will be brought up from the sub-surface, thus a surface-level dedicated microbiology lab will be required for sample prep and preservation.
Basic micro lab equipment

- Laminar flow hood
- Anaerobic chamber
- Refrigerated centrifuge
- -80 freezer (Will draw ~10KW power on average)
- Autoclave
- Deionized water
- Ice machine
- Dry ice supply
- Liquid nitrogen supply
- Glassware, spatulas, etc.
- Fume hood
- Solvent, Acid, and Chemical Storage
- Cabinet and lab bench space
Questions??????