Bacterial Diversity of an Abandoned Mine Land Soil in Southeast Kansas

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Bacterial Diversity of an Abandoned Mine Land Soil in Southeast Kansas

Rachel Bechtold
PSU 2017 Research Colloquium
Background

• Acid mine drainage (AMD) occurs from pyrite exposure to water and oxygen

• AMD infiltrates local soil and streams

• Bacterial diversity can indicate ecosystem health

• An ideal site for AMD study is Monahan Outdoor Education Center
Bioremediation

- **Sustainability** is economical, healthy, long-term planning
- Biotic techniques can be combined with abiotic, enhancing change
- Many environmental elements are interrelated, e.g. iron and pH

![PH and Iron (Log10)](chart)
Why bacteria?

• Environmental conditions **change over time**
  • e.g. pH changes with weather fluctuations

• Additional data, such as a record of bacterial diversity, can make the study more **valid**

• Microbes **are definite indicators** of environmental change

• This **study is novel** in that only physicochemical characteristics have been measured previously

Sulfate-reducing bacteria (SRB) and Bioremediation

Chemistry of AMD
General equations for this process are:
• $2\text{FeS}_2 + 7\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{Fe}^{2+} + 4\text{SO}_4^{2-} + 4\text{H}^+$
• $4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ \rightarrow 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$
• $4\text{Fe}^{3+} + 12\text{H}_2\text{O} \rightarrow 4\text{Fe(OH)}_3 + 12\text{H}^+$
• $\text{FeS}_2 + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} \rightarrow 15\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+$

The net effect of these reactions is to release $\text{H}^+$, which lowers the pH, produces sulphate ions.

- $\text{H}^+$ ions are released, ferrous iron becomes ferric iron
- At pH levels of 3.5, ferrous hydroxide (yellowboy) forms and $\text{H}^+$ ions increase
- SRB could reverse the process of pyrite oxidation

Hypothesis: Differences exist in the bacterial diversity of remediated vs. unremediated sites

Goals of the present study:

(i) To assess soil bacterial diversity and to evaluate physicochemical characteristics of soil
(ii) To isolate acidophiles

The findings of this study would also help in understanding the timeline of *ecosystem recovery paralleled with wetland development and/or bioremediation*. 
Methods

1. Sample collection
2. Physicochemical analysis
3. Bacterial concentration and identification by biochemical and molecular techniques
4. Isolation of acidophilic bacteria
1. Soil sampling in fall 2015 and summer 2016

Monahan Outdoor Education Center
2. Physicochemical analysis

Soil samples digested and analyzed using an ICP OES (inductively-coupled plasma optical emission spectroscopy). [K-State Geology]

<table>
<thead>
<tr>
<th>Location/Sample ID</th>
<th>Texture</th>
<th>pH</th>
<th>As (mg/kg)</th>
<th>Mn (mg/kg)</th>
<th>Fe (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mine Soil (A)</td>
<td>Silt</td>
<td>6.6±0.0</td>
<td>10.11</td>
<td>614.69</td>
<td>28.72</td>
</tr>
<tr>
<td>Top Mound (B)</td>
<td>Sand/Clay</td>
<td>6.6±0.2</td>
<td>13.82</td>
<td>877.58</td>
<td>35.50</td>
</tr>
<tr>
<td>Lake S. (C)</td>
<td>Clay</td>
<td>6.6±0.0</td>
<td>15.52</td>
<td>932.66</td>
<td>38.93</td>
</tr>
<tr>
<td>Ditch (D)</td>
<td>Gravel</td>
<td>2.6±0.2</td>
<td>10.39</td>
<td>433.40</td>
<td>169.59</td>
</tr>
<tr>
<td>Lake N. (E)</td>
<td>Clay/Silt</td>
<td>6.6±0.0</td>
<td>8.52</td>
<td>997.96</td>
<td>34.13</td>
</tr>
</tbody>
</table>
3. Bacterial concentration
Calculated CFU/g based on dilution plating

Plotting season to site
3. Identification of bacterial isolates
Selective-Differential Media and Biochemical Testing

- Nitrate reduction
- Citrate utilization
- Cysteine desulfurization
- Phenylalanine deamination
- Indole production
- Gelatin & starch hydrolysis
- Catalase
- Oxidase
Results of carbohydrate fermentation and biochemical tests

Metabolically, bacterial isolates were highly diverse
3. **Identification of bacterial isolates**

*Using PCR amplification of 16S rRNA gene and sequencing*

- **PCR**
- **Gel electrophoresis**
- **Purification and quantitation**

1.5 kb
Total bacterial diversity based on sequence analysis of 58 isolates

[K State sequencing facility]
4. Screening for acidophilic bacteria

A total of 17 acidophilic bacterial isolates identified

Citric acid buffer

<table>
<thead>
<tr>
<th>pH3 (N=5)*</th>
<th>pH4 (N=8)</th>
<th>pH5 (N=7)</th>
<th>pH6 (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pantoea agglomerans</strong></td>
<td><strong>Bacillus simplex</strong></td>
<td><strong>Paenibacillus alvei</strong></td>
<td><strong>Bacillus megaterium</strong></td>
</tr>
<tr>
<td><strong>Pseudomonas syringae</strong></td>
<td><strong>Frigoribacterium endophyticum</strong></td>
<td><strong>Curtobacterium flaccumfaciens</strong></td>
<td><strong>Microbacterium oleivorans</strong></td>
</tr>
<tr>
<td><strong>Bacillus subterraneus</strong></td>
<td><strong>Jeotgalibacillus campisalis</strong></td>
<td><strong>Pantoea agglomerans</strong></td>
<td><strong>Bacillus thuringiensis</strong></td>
</tr>
<tr>
<td><strong>Bacillus pumilus</strong></td>
<td><strong>Bacillus pumilus</strong></td>
<td><strong>Bacillus pumilus</strong></td>
<td><strong>Bacillus pumilus</strong></td>
</tr>
<tr>
<td><strong>Terrabacillus saccharophilus</strong></td>
<td><strong>Frigobacterium endophyticum</strong></td>
<td><strong>Bacillus pumilus</strong></td>
<td><strong>Bacillus pumilus</strong></td>
</tr>
</tbody>
</table>
Future study

➢ Physical remediation can be combined with biotic remediation, bench scale trials first

➢ Diverse metabolic profiling of the isolates can be improved upon with metagenomic data
A baseline measurement of bacterial diversity and soil chemistry of AMD site is novel in its kind in southeast KS. Acidophilic bacterial strains from this study could be used along with anoxic limestone drains or wetlands to expedite the process of bioremediation and restoration of natural habitat for plants and animals.
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