Aug 6th, 9:30 AM - 12:00 PM

Exploring diversity of Nitrate reducing thermophiles in Nevada hot springs

Jenny Lam
University of Nevada, Las Vegas

Jeremy A. Dodsworth
University of Nevada, Las Vegas

Brian P. Hedlund
University of Nevada, Las Vegas

Repository Citation
https://digitalscholarship.unlv.edu/cs_urop/2009/aug6/6

This Event is brought to you for free and open access by the Undergraduate Research at Digital Scholarship@UNLV. It has been accepted for inclusion in Undergraduate Research Opportunities Program (UROP) by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.
High rates of denitrification have been measured in Nevada geothermal hot springs, but little is known about the thermophiles that contribute to this activity. We hypothesize that heterotrophic bacteria in the genus *Thermus* are the most important denitrifiers in the springs. Alternatively, other microorganisms including chemolithotrophs may also be important. To test these hypotheses, several different strategies will be used to try to enrich and isolate nitrate-reducing microorganisms. Isolates will be identified by 16S rRNA gene PCR and sequencing. Subsequently, representative isolates will be chosen for nitrate reductase gene (*narG*) sequencing and for studies on the kinetics of nitrate reduction at high temperature. These data will provide information on how these microorganisms may behave *in situ* and how their activities may affect nitrogen cycling in the hot springs.
Exploring Diversity of Nitrate Reducing Thermophiles in Nevada Hot Springs

Jenny Lam, Jeremy A. Dodsworth and Brian P. Hedlund
School of Life Sciences, University of Nevada, Las Vegas

Introduction

High rates of denitrification have been measured in Nevada geothermal springs, but little is known about the thermophiles that contribute to this activity. Denitrification is a form of anaerobic respiration in which nitrate (NO₃⁻) is converted to nitrogen gas (N₂) in a multi-step pathway, avoiding various intermediates (Fig. 1). It is necessary to cultivate and characterize nitrate reducing microorganisms in order to determine which thermophiles contribute to denitrification. The hypothesis that thermophiles bacteria in the genus Thermus are the most important denitrifiers in this springs. Moreover, other microorganisms including chemolithotrophs may also be important. To test these hypotheses, several isolation strategies were used to enrich and isolate nitrate-reducing microorganisms. Subsequently, microorganisms were identified and their nitrate reduction activities were characterized by determining their ability to reduce nitrogen compounds in the hot springs.

\[
\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2 \text{O} \rightarrow \text{N}_2
\]

Figure 1. The process of denitrification with all the nitrogen intermediates.

Aims and Methods

1. Expand collection of heterotrophic, nitrate reducing thermophiles from Great Boiling Spring (GBS) and Sandy’s Spring West (SSW).
2. Isolate isolates by using 16S ribosomal RNA gene PCR and sequencing.
3. Determine the chemolithotrophs of products of nitrate reduction using representatives of each species.
4. Attempt to cultivate chemolithotrophs and denitrifying thermophiles.

Results

Identification of isolates and qualitative analysis of nitrate reduction.

Figure 2. Geothermal spring and bacteria that inhabit it. (a) Great Boiling Spring (GBS). (b) Thermus thermophilus strain GBS-1-6-2. (c) Thermus thermophilus strain GBS-1-6-2. (d) denitrifying bacteria isolates/photographs show GBS-1-6-2.

Table 1. Identification of different bacterial species found in the hot springs. All isolates were isolated vertically, then selected cultures were grown anaerobically, and tested for nitrate and nitrite reducing activities.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>GBS</th>
<th>Acidum</th>
<th>NO</th>
<th>NO₂</th>
<th>NO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermus thermophilus</td>
<td>37</td>
<td>9</td>
<td>20</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Thermus属</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Thermus aerophilus</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Thermus属</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
| One species of Geobacillus was found.

Figure 3. Denitrification experiments were representative denitrifiers to track different nitrate reduction products at different times of growth. Error bars represent SD determined with representative samples.

Quantitative analysis of denitrification during growth.

Thermus thermophilus and Thermus aerophilus were both grown anaerobically at 70°C with 9 mM nitrate, with different nitrate and nitrite nitrate concentrations were measured periodically through incubation. Nitrite concentrations were measured colorimetrically using the denitration method with nitrate from Labfrost. Nitrate and nitrite concentrations were measured using gas chromatography (GC5000 and GC6000, respectively). Total nitrogen concentrations were determined using Henry's Law.

Discussion

Very little is known about denitrification in hot springs, despite the fact that nitrate (NO₃⁻) is an extremely favorable electron acceptor for anaerobic respiration (3). In this study, we isolated a large collection of thermophilic nitrate reducers from Great Boiling Spring, tested their nitrate reduction activities, and assessed the relative contributions of heterotrophic and chemolithotrophic denitrifiers in the hot springs.

Nitrate reducing thermophiles isolated in the hot springs, Thermus thermophilus, Thermus aerophilus, and Thermus属. There are two genera of Thermus that are known for their ability to reduce nitrate. Four species of Thermus were isolated from the hot springs. Thermus thermophilus, T. aerophilus, T. scotodurans, and T. stercorarius. T. thermophilus is capable of complete denitrification to dinitrogen gas (2). The chemolithotrophy, and evolution of the nitrate reduction pathway is well characterized in contrast, although, the relative contribution of denitrification to denitritification strains exists, nitrate reduction activities are poorly described (4).

Nitrite is the major product for the three Thermus strains tested, suggesting a role in conversion of nitrate to nitrite in situ. In addition, T. thermophilus produces large amounts of nitrous oxides, consistent with high nitrate to dinitrogen oxides measured at GBS. In electron donor stimulation experiments, it showed a higher rate of conversion of nitrate to nitrite in situ. In addition, T. thermophilus produced large amounts of nitrous oxides, consistent with high nitrate to dinitrogen oxides measured at GBS. In electron donor stimulation experiments, it showed a higher rate of conversion of nitrate to nitrite in situ.

Future directions

1. Continue to expand collection of nitrate reducing thermophiles from Nevada hot springs.
2. Continue to cultivate chemolithotrophic, nitrate reducing thermophiles.
3. Purify novel nitrate compounds with the aid of nitrate growth.
4. Perform denitrification experiments with Anabaena subalpina to determine the stoichiometry of nitrogen products from denitrification.
5. Determine the chemolithotrophs of products of nitrate reduction using representatives of each species.

Isolate sources, isolation strategy, and electron donors

Table 2. Isolates were obtained from different locations in the hot springs by direct plating or by plating after enrichment with different organic compounds.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>GBS</th>
<th>Acidum</th>
<th>NO</th>
<th>NO₂</th>
<th>NO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermus thermophilus</td>
<td>37</td>
<td>9</td>
<td>20</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Thermus属</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Thermus aerophilus</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Thermus属</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 4. Results of denitrification experiment. Only one strain was tested. Data was collected using Henry's Law.

Chemoautotrophic nitrate reduction

Electron donor stimulation experiments. Electron donor stimulation experiments were performed in order to assess which electron donors were capable of denitrification. Sediment slurries anaerobic spring water were stimulated with 1 and nitrate and several different possible electron donors and the final step in denitritification experiments were performed with 30% of dinitrogen. Slurries were inoculated in the spring and monitored for nitrite oxide production. Nitrite oxide was quantified using GC5000 and total nitrogen concentration was calculated using Henry's Law.

Cultivation experiments

To further determine the possible existence of chemolithotrophic nitrate reducers, sediment was incubated in spring water or Great Boiling Medium D at 70°C containing 1 and nitrate and other possible electron donors, hydrogen, sulfur, and thiosulfate. All cultures were examined microscopically and tested for nitrate and nitrite concentration.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>GBS</th>
<th>Acidum</th>
<th>NO</th>
<th>NO₂</th>
<th>NO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermus thermophilus</td>
<td>37</td>
<td>9</td>
<td>20</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Thermus属</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Thermus aerophilus</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Thermus属</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 5. Cultivation experiment. Data was collected using Henry's Law.

References


Acknowledgements

Thank you to everyone in the Nevada Land Brian, Jeremy, Jose, and Janina. You’ve all been very helpful and made this experience extremely enjoyable.

This work was supported by NSF grant number MCB-051065517206 and NIH grant number R20 RR-05464 as the INBRE program of the National Center of Research Resources.