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Exploring diversity of Nitrate reducing thermophiles in Nevada hot springs

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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

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Introduction

High rates of denitrification have been measured in Nevada geothermal springs, but little is known about the thermophiles that contribute to this activity (1). Identification 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. We hypothesized that heterotrophic bacteria in the genus Thermus are the most important denitrifiers in the springs. Moreover, other microorganisms including chemolithotrophs may also be important. To test these hypotheses, several different strategies were used to try to enrich and isolate nitrate-reducing microorganisms. Subsequently, microorganisms were identified and their nitrate reduction activities were characterized by growing the most promising microorganisms in order to obtain information on how they behave in situ and how their activities may affect nitrogen cycling in the hot springs.

Introduction of isolates and qualitative analysis of nitrate reduction

Heterotrophic microorganisms were enriched from sediment, water, and sediments amended with different electron donors and incubated at 70°C. Pure cultures were isolated from positive enrichments. The 16S rDNA genes for PCR were amplified by DNA from pure cultures. The 16S rDNA genes were sequenced using Sanger method. Isolates were identified using EzTaxon, National Repository Project, and RDP.

- Three different species of Thermus were found.
- One species of Anaerobacillus was found.
- One species of Geobacter was found.

Quantitative analysis of denitrification during growth

Thermus thermophilus and Thermus dothrii were both grown anaerobically at 70°C in G-DO medium (8), which contains 9 mM nitrate. Nitrite, nitrous oxide and nitrogen gas concentrations were measured periodically throughout incubation. Nitrite concentrations were measured calorimetrically using the colorimetric method with nitrate reductase from Rhizobium melilot. Nitrous oxide and nitrogen gas were measured using gas chromatography (GC-ECO and GC-ECO, respectively). Total gas concentrations were determined using Henry’s Law.

Aims and Methods

- Isolate isolates by using 16S rDNA gene PCR and sequencing.
- Determine the chemotaxis of products of nitrate reduction using activity characteristics of each species.
- Attempt to culture chemolithotrophic and denitrifying thermophiles.

Results

- Identification of isolates and qualitative analysis of nitrate reduction
- Heterotrophic microorganisms were enriched from sediment, water, and sediments amended with different electron donors and incubated at 70°C. Pure cultures were isolated from positive enrichments. The 16S rDNA genes were sequenced using Sanger method. Isolates were identified using EzTaxon, National Repository Project, and RDP.
- Three different species of Thermus were found.
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Table 1. Identification of different bacterial species found in the hot springs. All isolates were cultured aerobically, then selected cultures were grown anaerobically, and tested for nitrate and nitrite reduction activities.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Isolates</th>
<th>NO₃⁻</th>
<th>NO₂⁻</th>
<th>NO</th>
<th>N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermus thermophilus</td>
<td>37</td>
<td>31</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Thermus dothrii</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Anaerobacillus sp.</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

- Nitrite concentrations were measured calorimetrically using the colorimetric method with nitrate reductase from Rhizobium melilot. Nitrous oxide and nitrogen gas were measured using gas chromatography (GC-ECO and GC-ECO, respectively). Total gas concentrations were determined using Henry’s Law.

Discussion

Very little is known about nitrate reduction in hot springs, despite the fact that nitrate (NO₃⁻) is an extremely favorable electron acceptor for anaerobic respiration (2). To date, a few isolated species of thermophilic nitrate reducers from Great Basin hot springs, though their nitrate reduction activities, and assessed the relative contributions of heterotrophic and chemolithotrophic to denitrification in all. Nitrate reducing thermophiles isolated from three species, Thermus Thermophilus, Thermus dothrii, and Anaerobacillus sp. These two genera are known for their ability to reduce nitrate. Four species of Thermus dothrii are nitrite oxidizers: T. thermophilus, T. anaplan, T. scottii, and T. acidophilus. T. thermophilus is capable of complete denitrification to nitrogen gas (3). The biochemical, genetic, and evolution of the nitrate reduction pathway is well-characterized in contrast, although the contribution of denitrification by Thermus Thermophilus strain strain 509, nitrate reduction activities are poorly described (4).

Nitrate is the major product in 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 oxide, consistent with high nitrosating factors measured at 100°C. An electron donor stimulation experiment suggested that denitrification is mainly limited by thermophiles, not chemolithotrophs, which is similar to aquatic and soil–ecosystems.

Future directions

- Continue to enrich collection of nitrate reducing thermophiles from hot springs.
- Continue to cultivate chemolithotrophic nitrate-reducing thermophiles.
- Use nitrogen isotopes experiments by adding cell growth media.
- Perform denitrification kinetics experiments with Anaerobacillus sp. to determine the stoichiometry of nitrogen products from denitrification.
- Quantify heterotrophic and chemolithotrophic nitrate reduction in sediments using quasi-isotopic FISH for nitrate reduction rates.

References


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