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

Exploring diversity of Nitrate reducing thermophiles in Nevada hot springs

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Repository Citation

Lam, Jenny; Dodsworth, Jeremy A.; and Hedlund, Brian P., "Exploring diversity of Nitrate reducing thermophiles in Nevada hot springs" (2009). *Undergraduate Research Opportunities Program (UROP)*. 6.  
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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 Nitratreducing Thermophiles in Nevada Hot Springs

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Introduction
High rates of denitrification have been observed in Nevada geothermal springs, but little is known about the thermophiles that contribute to this activity. Identification is a key step in anaerobic respiration in which nitrate (NO₃⁻) is converted to nitrogen gas (N₂) via a multi-step pathway, involving various intermediates (Fig. 1). It is necessary to cultivate and characterize these nitrate reducing microorganisms in order to determine which thermophiles contribute to denitrification. We hypothesized that thermophilic bacteria in the genus Thermus are the most important denitrifiers in these springs. Microscopically, other microorganisms including chemolithotrophs may also be present. To test these hypotheses, several different strategies were used to enrich and isolate nitrate-reducing microorganisms. Subsequently, microorganisms were identified and their nitrate reduction activities were characterized by following the conversion of nitrate to nitrogen gas.

Results
Identification of isolates and qualitative analysis of nitrate reduction
Retro-enzymes were enriched from sediments, water, and sediments and isolated at 70°C. Pure cultures were isolated from positive enrichments, and DNA was PCR amplified using a primer on a culture. 16S rRNA genes were sequenced using Sanger method. Bacterial were identified using EzTaxon, National Institute Project, and CBG.
- Three different strains of Thermus were found.
- One species of Acidobacteria was found.
- One species of Geobacillus was found.

Quantitative analysis of denitrification during growth
Thermus thermophilus and Thermus aquaticus were both grown anaerobically at 70°C with glucose medium D, which contains 10 mM nitrate, nitrite, nitric oxide and nitrogen gas. Cultures were monitored for 96 hours. Nitrate concentrations were measured periodically using the colorimetric method with the kits from Megazyme. Nitrite, nitric oxide and nitrogen gas were measured using chemoluminescence (CLC-8000 and CLC-1000, respectively). Total gas concentrations were determined using Henry's Law.

Aims and Methods
- Expand collection of thermophilic nitrate reducing thermophiles
- Form Great Boiling Spring (GBS) and Sandy’s Spring West (SSW)
- Identify isolates by using 16S rRNA gene PCR and sequencing
- Determine the chemolithotrophy products of nitrate reduction using representatives of each species.
- Attempt to culture chemolithothrophic and denitrifying thermophiles.

Isolates, isolation strategy, and electron donors
Isolates were obtained from different locations in the hot springs by direct plating or by plating after enrichment with different organic compounds.

Discussion
Very little is known about the community of nitrate reducing thermophiles. However, the fact that nitrate (NO₃⁻) is a strong oxidizer of terminal electron acceptor or anaerobic respiration (3). In this study, we isolated a large collection of thermophilic acetate reductors from Great Boiling Spring, studied their nitrate reducing activities, and assessed the relative contributions of thermophiles and chemolithotrophs to denitrification in situ.

Nitratreducing Thermophiles identified by Three species, Thermus thermophilus, Thermus aquaticus, and Acidobacter were isolated. These three genera are known for their ability to reduce nitrate. Four species of Thermus were isolated from GBS. T. thermophilus, T. aquaticus, T. scotoductus, and T. acidovorans. T. thermophilus is capable of complete denitrification to nitrogen gas (4). The biodegradation of nitrate and evolution of the nitratereduction pathway is well characterized in contrast, although there is no reference to denitrification of Thermus strains, nitrate reduction activities are poorly described (4).

Nitrate is the major product for the three Thermus strains tested, suggesting a role in conversion of nitrate to nitrite in situ. In addition, T. aquaticus produces large amounts of nitrous oxide, consistent with the high activity of nitrous oxide reductase measured in GBS. An electron donor stimulation experiment suggested that denitrification is mainly coupled to hydrogen, not chemolithotrophy, which is similar to aquatic and soil-ecosystems.

Future directions
- Continue to expand collection of isolated thermophilic nitrate reducing Thermus
- Continue to cultivate chemolithotrophic and denitrifying Thermus
- Use nanofluidic techniques for extracellular gene expression
- Perform chemotrophic isolation experiments with Acidobacter to determine the stability of nitrogen products from denitrification
- Quantify heterotrophic and chemolithotrophic nitrate reduction in sediments by using isotope tracer for nitrate reduction rates

References

Acknowledgements
Thank you to all who contributed to this project. We appreciate all the help and support from the Nevada Bureau of Mines and Geology. This work was supported by NSF grant number ECS-01109730 and NRM grant number P20 RR01564 from the NIBRS program of the National Center of Research Resources.