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Denitrification in Great Basin hot springs

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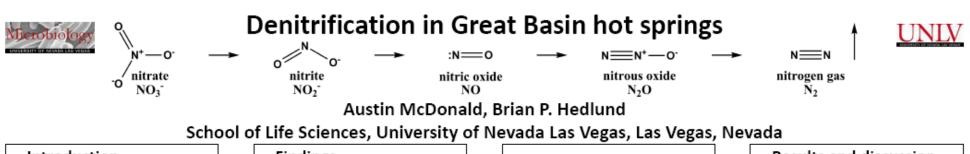
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Austin McDonald Mentor - Brian Hedlund

Hydrogen has been proposed to fuel primary production in the *Aquificae*dominated hot springs of Yellowstone National Park (Spear, et al. 2005), a finding the authors generalized to all hot springs. However, clone libraries derived from Great Basin springs contain few 16S ribosomal RNA (rRNA) gene sequences from *Aquificae* and many from unknown microorganisms. In the same springs, alternative electron donors rival the reducing power of hydrogen. This project will try to cultivate the uncharacterized microbes of two Great Basin springs and determine which electron donors they can use.

Nitrogen is key to life. In its reduced form, ammonia, it is a primary constituent of nucleic acids and proteins. In its oxidized form, nitrate, it frequently substitutes for oxygen in anoxic conditions as microbes' preferred electron acceptor for In this capacity, it drives energy capture-typically, though not respiration. always, in the process of denitrification [8]. Understanding the supply, demand, and interconversion of nitrogen through an ecosystem is essential to understanding the life within it. Although denitrification has been predicted to occur within hot springs on thermodynamic grounds, and some thermophilic isolates reduce nitrate, denitrification has never been examined in a hot spring. The hot springs of the Great Basin are under studied reservoirs of novel metabolisms and microbes, and are well worth in-depth exploration. Our project adapts techniques regularly used in marine and soil microbiology [6,7,9] to higher temperatures to test our hypothesis: that some thermophiles with in the hot springs respirenitrate, in the process of denitrification, for a significant amount of energy capture.



Introduction

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NH3 ammonia (groundwater)	ammonia exidation)	(which microbes?) N=N (which microbes?) N=N nitrogen gae (atmosphere
		nitrate	

Aims and methods

- Test the denitrification hypothesis at two representative springs: GBS and SSW.
 Measure mitrogen species in situ with colorimetric
 - a) Measure mitrogen species in situ with colorimetric assays [6] to determine whether the springs can support denitrification
 b) Measure denitrification activity with the acetylene
- b) Measure demonstration activity with the acetylene (C₁H₂) inhibition method described by Moster [7]
 2) Link observed denitrification activity to specific
- Link observed demunication activity to specific microbes.
- Through culturing, determine whether isolates can reduce mirsts and, if so, identify mirsts reduction produces using colorimetric assays [6] and gas chromatography.
- b) Determine whether isolates possess nurG genes, which succede a catalytic subunit of nitrate reductase found in all known denitrifiers [9].

Findings

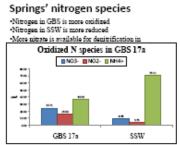


Figure 2. Comparison of nitrogen species in the springs, NO₁ wavelet bill, wavelet bill, wavelet bill, which destification in the hot springs. Spring water was tested on-site with a coloritettic water analysis it (LAMotte 42001), NO₁ undergoses indicted description in the presence of unifinitaritie and a subbill coupling magnet to form a red day [6]; NO₂ (in reduced to NO₂ with coupling and in the same mannet. NH₂ "masks with Resultr's reagent to form a yellow molecule.

Springs' denitrification activities

GBS denitrification is high
SSW denitrification is on par with marine rediment [4]
Denitrification appears to be limited by NO₄.

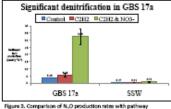
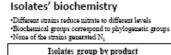


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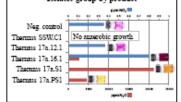


Figure 4. A comparison of the nitrate reduction products of different thermal totales. Specificated and form each totales was assayed, as was unbockleder medium. As in the springs, NO, is determined colorismitically, and N₂D y CC-CD. Interface Durham tubes were included for the detection of N₂, but none was measured. The yellow color developed from totales S1 and PS1 during disactization may be due to lack of NO₂ : It also might indicate unintended methors with an artise.

Isolates' narG genes

-Groupings correlate with observed activity ward; and 165 rRNA relationships are similar -Possible to identify desirilitest by comparison of a marG survey [9] with 165 rRNA surveys?

- 4	Annual a March Program	
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		ur holetes' 165 rRNA and nor cotstrap value, nord genes w

Acknowledgements

generated by the PhyML maximum likelihood algorithm [5].

Thank yea to Kyle particularly, for isolating those gays. Thank you to the Lab Jammy, Robin, Mitch, Rachal, Tanty, Cailin, Coly, and even Day, you are the most helpful and intelligent people I know. Thank you Kann, for betimeling a nat, and Colin Purington, for your advise and ample posters. Finally, thenk you to my family for continuing support. NSF geneta 4047416 and MCD-0346885 supported this project.

Results and discussion

Denitrification was significant in GBS when measured (Fig. 2), well above the levels found in marine sodiment [3]. In SSW, the rate of denitrification (Fig. 2), while much less than in .(GBS was still comparable to that of marine sodiment [3].

The two springs feed from the same source [11], but are different in mirrogen oxidation states (Fig. 1). This may drive differences in desiribition activity (Fig. 2) by limiting mirrate diffusion to sedimant microbes. We hypothesise that microbial manumois oxidation in GBS produces the mirrate meeded for demitrification , but that in SSW, other metabolisms such as sulfide excidation outcompete emmouin oxidation.

The examination of Thermus isolates in the lab is only a preliminary step in matching in sim destinification activity to specific microbes. However, the tigorous reduction of mirrate by GBS isolates (Fig. 3) suggests that Thermus species play a role in denimification in the springs: further, the correlation between nurG and 165 rRNA phylogenetic trees (Fig. 4) may allow the prediction of the identity of denitrifiers within the springs.

Having established denitrification within the springs, we may begin mapping activity to microbes; see the future directions section below.

Future directions

- Place a lower bound on denitrifier density with "most probable number" scrild dilution experiments [2] - Survey $\kappa_B G$ genes with highly degenerate primers to determine the diversity of possible mirror reducers in the

springs • Survey expressed *norG* genes using reverse-transcriptase based amplification of mRNA

 Quantify the amount of specific marG DNA and RNA alleles with real-time PCR.

Investigate animonia oxidation using similar techniques
Continue isolation attempts with more sophisticated

methods, including use of a continuous culture bioreactor • Employ one of the hardier *Thermus* isolates as the nitrate detector in a high-temperature biosensor

Extend model to predict denitrification activities of other

springs

For more information

Phase contact me at moderne?#@gawhenenede.adu. More information on this and related projects can be obtained at http://foculycarite.edu/hedland.

Also, take a card!

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