Abstract

Nitrification is the process by which ammonia is oxidized to nitrate, and is an important biogeochemical reaction in the global nitrogen cycle. This process is catalyzed by ammonia monooxygenase (AMO), which is encoded by the *amoA* gene. Both bacteria (ammonia-oxidizing bacteria, or AOB) and archaea (ammonia-oxidizing archaea, or AOA) are capable of carrying out ammonia oxidation. This project focuses on the phylogenetic analysis of novel amoA genes from microorganisms found in caves. Through this project, amoA genes from two new metagenomes from Lehman Caves in Nevada and Lechuguilla Cave in New Mexico are being analyzed. Other *amoA* sequences used for phylogenetic comparison are being compiled from the scientific literature, including amoA studies that go back more than 23 years, as well as from metagenomes from other cave systems. At least one bacterial and one archaeal amoA have been identified so far, and preliminary BLAST analysis showed that these likely originated from close relatives of known ammonia oxidizers, including *Nitrosomonas* spp., and an unknown archaea. Detailed phylogenetic analysis shows that the amoA from the ammonia-oxidizing bacterium is most closely related to *Nitrosomonas* spp. and to amoA recovered from other caves, as well as several pmoA sequences, indicating potential use of trace methane. We are now performing additional phylogenetic analyses to further classify these new *amoA* sequences and explore the evolution of ammonia-oxidizing microorganisms in caves, as well as to further evaluate the potential use of trace methane as an energy resource in desert caves. The work from this project will be used in future research aimed at uncovering new ammonia-oxidizing cave microorganisms and exploring their role in the subterranean nitrogen cycle.

Introduction and Research Questions

Introduction

Nitrification (an important process in the global nitrogen cycle) is catalyzed by ammonia monooxygenase, and encoded on the *amoA* gene. Both bacteria and archaea are carriers of the gene and are capable of dissimilatory ammonia oxidation. Through this project, phylogenetic analysis of novel *amoA* genes from two new metagenomes from Lehman Caves in Nevada and Lechuguilla Cave in New Mexico are being analyzed. **Research Questions**

- What ammonia oxidizing organisms are present in desert cave systems?
- How can we use different phylogenetic analyses to best resolve their evolutionary relationships?



C: Lehman Cave sample collection site.

Phylogenetic analysis of ammonia monooxygenase (amoA) genes from desert caves Kenyan Phlieger¹, Katelyn Green², Daniel Jones^{1,3} Departments of ¹Earth and Environmental Science, ²Biology, New Mexico Institute of Mining & Technology, Socorro, NM 87801 ²National Cave and Karst Research Institute, Carlsbad, NM, 88220

D: Location of Lehman Cave in GBNP.



Figure 2. Bacterial Tree built in geneious prime using Maximum Likelihood, Neighbor Joining, Jukes-Cantor method. Bootstrapping values below 50 have been omitted.

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Results

Both a bacterial and an archaeal *amoA* gene have been identified. Preliminary BLAST analysis showed that these likely originated from close relatives of known ammonia oxidizers, and an unknown archaea. Detailed phylogenetic analysis shows that the *amoA* from the ammonia-oxidizing bacterium is most closely related to Nitrosomonas spp. and to amoA recovered from other caves, as well as the unexpected discovery of several *pmoA* sequences, indicating



Figure 3. Archeal Tree built in geneious prime using Maximum Likelihood, Neighbor Joining, Jukes-Cantor method

Future Directions

Results from this project will guide future research aimed at uncovering new ammonia-oxidizing cave microorganisms and exploring their role in the subterranean nitrogen cycle. Future research on trace

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