Can Hydroxylamine or Phenylacetylene Differentiate Ammonia Oxidizing Activity of Bacteria & Archaea?

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1. INTRODUCTION

Microbial oxidation of ammonia is a key process in global cycling of nitrogen. It determines the balance between oxidized and reduced forms of nitrogen in the environment. Hence knowledge of the key players in nitrification and measurement and prediction of their activity is essential. The ability to oxidize ammonia was typically thought to be an obligatory aerobic, chemoautotrophic process restricted to just a few groups within the Proteobacteria of the domain bacteria (Fig 1). However recently organisms such as *Nitrosopumilus maritimus* belonging to crenarchaeota group of the domain Archaea have been isolated and were shown to oxidize ammonia autotrophically (Fig 1)[1].

By using an inhibitor that targets only one of the ammonia oxidizing communities, the amount of oxidation performed by each can be determined. Two potential inhibitors were analyzed, hydroxylamine and phenylacetylene. Hydroxylamine is an intermediate in the oxidation of ammonia to nitrite by ammonia oxidizing bacteria (AOB) (Fig 2). This process is carried out by ammonia monooxygenase enzyme encoded by the *amoABC* genes and by the hydroxylamine oxidoreductase enzyme encoded by the *hao* gene (Fig 2). Although homologs for all three *amo* genes were identified in ammonia oxidizing archaea (AOA), *hao* gene homolog has not been identified so far (Fig 2). Phenylacetylene is a known inhibitor of AOB by inhibiting *amo*[2].

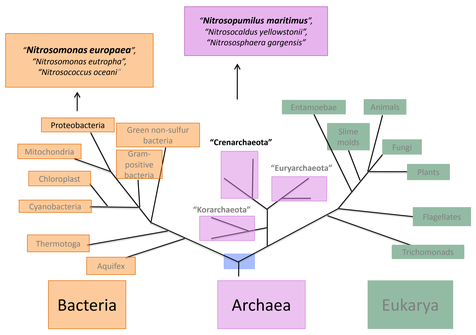

Fig 1. Universal phylogenetic tree as determined from comparative ribosomal RNA sequencing.

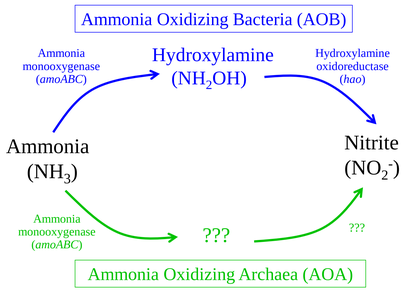

Fig 2. Catabolism of ammonia and genes so far known to be linked to this process in ammonia oxidizing bacteria (AOB) & ammonia oxidizing archaea (AOA)

2. UPTAKE OF HYDROXYLAMINE BY *Nitrosomonas europaea* (an AOB) & *Nitrosopumilus maritimus* (an AOA)

In this project, we have studied uptake of hydroxylamine (NH_2OH) by pure cultures of both *Nitrosomonas europaea* & *Nitrosopumilus maritimus*. The results show that *N. europaea* and *N. maritimus* can oxidize a portion of the added hydroxylamine (Fig 3 and 4). Hydroxylamine did not completely inhibit *N. europaea* or *N. maritimus'* capability to oxidize ammonia (Fig 3 and 4). *N. maritimus*, however, is more sensitive to higher concentrations of hydroxylamine (Fig 4). Because both cultures can oxidize hydroxylamine, it is not a good inhibitor for distinguishing ammonia oxidizing communities in the environment.

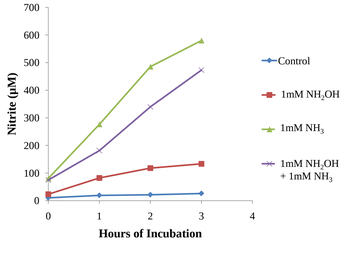

Fig 3. Growth curves of *N. europaea*, in presence of varying concentrations of NH_3 and NH_2OH

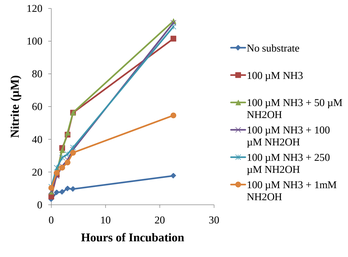

Fig 4. Growth curves of *N. maritimus*, in presence of varying concentrations of NH_3 and NH_2OH

3. UPTAKE OF HYDROXYLAMINE BY ENVIRONMENTAL SAMPLES

We have also studied the uptake of hydroxylamine by ammonia oxidizing communities found in soil collected from Hislop Farms. The environmental samples were capable of producing nitrite in the presence of hydroxylamine (Fig 5 and 6). In addition, hydroxylamine enhanced the nitrification potential in the soils (Fig 5 and 6). The oxidation of hydroxylamine can probably be attributed to AOB based on Figures 3 & 4 and on PCR results using bacteria and archaea amoA primers (Fig 7).

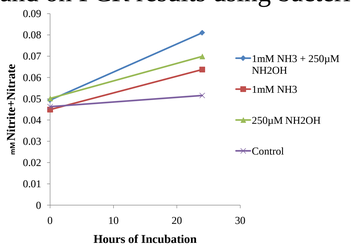

Fig 5. Nitrification potential in Wheat-Fallowed soils of Hislop Farms in presence of NH_3 and NH_2OH

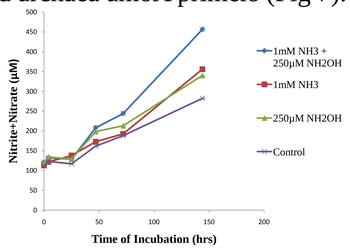

Fig 6. Nitrification potential in Wheat-Wheat soils of Hislop Farms in presence of NH_3 and NH_2OH

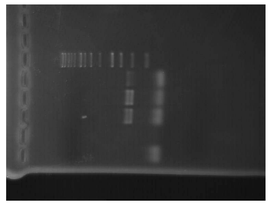

Fig 7. Bacterial amoA DNA was applied while no Archea amoA DNA was found.

2. INHIBITION OF *Nitrosomonas europaea* (an AOB), *Nitrosopumilus maritimus* (an AOA), AND ENVIRONMENTAL SAMPLES WITH PHENYLACETYLENE

Another potential inhibitor that could be used to differentiate ammonia oxidizing communities is phenylacetylene (C_8H_6). Pure cultures of both *Nitrosomonas europaea* & *Nitrosopumilus maritimus* and environmental samples from Hislop Farms received doses of phenylacetylene. *N. europaea* was only capable of oxidizing ammonia in the presence of concentrations of phenylacetylene less than 50µM (Fig 8), while *N. maritimus* was completely inhibited by all concentrations (Fig 9). The environmental sample was also completely inhibited by all concentrations of phenylacetylene (Fig 10). This could be due to the soil containing a large population of AOA or due to lower concentrations of cells in the soil compared to the pure cultures.

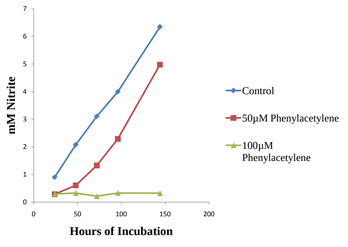

Fig 8. Growth curves of *N. europaea*, in presence of varying concentrations of phenylacetylene

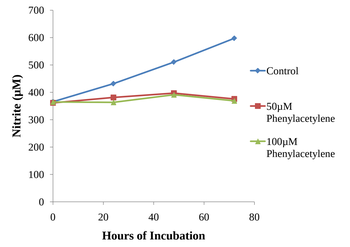

Fig 9. Growth curves of *N. maritimus*, in presence of varying concentrations of phenylacetylene

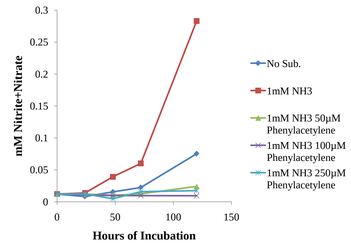

Fig 10. Nitrification potential in Wheat-Wheat soils from Hislop Farms in presence of phenylacetylene

5. CONCLUSION

Hydroxylamine and phenylacetylene were added to pure cultures of *N. europaea* and *N. maritimus* and to environmental samples from Hislop Farms as inhibitors to test for differences in tolerance of AOA and AOB. A difference in tolerance could be used to determine what portion AOA and AOB contribute to the processes of nitrification.

Both *N. europaea* and *N. maritimus* were capable of oxidizing ammonia in the presence of hydroxylamine, but *N. maritimus* was partially inhibited at 1mM. Phenylacetylene completely inhibited ammonia oxidation except for *N. europaea* at 50µM. The two communities showed differences in their tolerances to both inhibitors, but phenylacetylene showed a stronger difference.

Lower concentrations of phenylacetylene and higher concentrations of hydroxylamine need to be tested in the environmental samples to better determine the ammonia oxidizing communities. The ammonia oxidizing communities are being analyzed using molecular techniques to draw more definite conclusions.

REFERENCES

[1]Konneke, M., A. et al., 2005. Nature **437:**543-6;
[2]Lontoh, S., et al., 2008. Environmental Microbiology 2(5): 485-494;