CT23 - Archaea: important players in diverse microbial ecosystems

Archaeal dominated ammonia-oxidizing communities in Icelandic grassland soils are moderately affected by long-term N fertilization and geothermal heating
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The contribution of ammonia-oxidizing bacteria and archaea (AOB and AOA, respectively) to the net oxidation of ammonia varies greatly between terrestrial environments. To better understand, predict and possibly manage terrestrial nitrogen turnover, we need to develop conceptual and mechanistic models of ammonia oxidation as a function of environmental conditions, including the ecophysiology of the associated organisms. We examined the discrete and combined effects of mineral nitrogen deposition and geothermal heating on ammonia-oxidizing communities by sampling soils from a long-term fertilisation site along a temperature gradient in Icelandic grasslands. Microarray, clone library, and quantitative PCR analyses of the ammonia monoxygenase subunit A (amoA) gene accompanied by physico-chemical measurements of the soil properties were conducted. In contrast to most other terrestrial environments, the ammonia-oxidizing communities studied here consisted almost exclusively of archaea. Their bacterial counterpart proved to be undetectable, hence suggesting minor relevance of AOB for ammonia oxidation in these soils. Our results also show that fertilization and local warming by geothermal activity has a limited effect on both soil chemistry and detectable ammonia-oxidizing communities: only a subset of the detected AOA phylotypes was present in higher temperature soils. In the fertilized soils, the AOA abundance was consistently higher than in the control soils. Differences in distribution and structure of AOA communities were best explained by pH and clay contents irrespective of soil temperature or fertilizer treatment in these grassland soils. These findings indicate that pH and clay contents may be variables with a more general potential for niche-selection of archaeal ammonia oxidizers, while temperature and N availability may constitute intra-AOA niches in soil.

The metabolism of polar archaea: is urea playing a role?
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An annual recurrent growth of archaea in winter surface waters has been detected in polar systems. However, the sources of carbon and energy sustaining their growth remain elusive. We collected data on abundance and metabolic activity of Thaumarchaeota in Arctic and Antarctic waters by in situ single-cell analyses. The abundance of archaea grew one order of magnitude throughout the winter in Arctic waters. Yet, paradoxically, analyses by MicroAutoRadiography combined with Fluorescence In Situ Hybridization (MAR-FISH) revealed an unexpectedly low metabolic activity of Thaumarchaeota for both polar systems. Less than 5% of all thaumarchaeal cells took up leucine or bicarbonate, inconsistent with currently recognized heterotrophic and autotrophic archaeal lifestyles. To better understand how archaea obtain energy and carbon for growth, we analyzed a metagenome collected during the Arctic winter, when the Thaumarchaeota population was at its maximum of abundance (18% of cell counts). The metagenomic analysis revealed that archaeal amoA genes were abundant, indicating that polar archaea have the potential for ammonia oxidation. Furthermore, the presence of archaeal genes involved in urea transport
and degradation suggests that Arctic archaea may use urea as an alternative source of ammonia, and thus energy for growth. Genes encoding ureases were also detected in Antarctic waters, confirming that urea degradation pathways are widespread among polar Thaumarchaeota, and hinting at the potential importance of urea to sustain their growth.

Pinning down a metabolism for widespread, abundant, uncultured archaea through single cell genomic analysis
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Archaea of the Miscellaneous Crenarchaeotal Group (MCG) and Marine Benthic Group D (MBG-D) have only ever been identified by 16S rRNA sequences and are evolutionarily distant from any cultured organisms. Their 16S rRNA sequences have been found in a wide variety of anoxic sediments, but no metabolism has been directly linked to them. The MCG are environmentally widespread, phylogenetically diverse and tend to dominate lower activity sediments (Kubo et al., in press). We found that MCG and MBG-D comprise the bulk of the archaeal population in Aarhus Bay sediments, using qPCR as well as 454 FLX sequencing of archaeal 16S rRNA genes. In order to link phylogenetic identity to metabolic capacity of both MCG and MBG-D, we isolated a single cell of each from Aarhus Bay sediments, and sequenced their genomes using a hybrid approach of 454 FLX and Illumina methods. Out of the 56 single copy genes conserved in all currently available archaeal archaea, we found 14 and 29, MCG and MBG-D, respectively. This shows that these genomes are far from complete, but it allows a more detailed analysis of the phylogeny than that based on the 16S rRNA gene alone. A concatenation of these conserved genes provide support for an evolutionary grouping of the MCG deep within the mesophilic Crenarchaeota (newly named as the Phylum Thaumarchaeota) and the MBG-D within the Order Thermoplasmatales in the Phylum Euryarchaeota. We found the genomes of MCG and MBG-D to contain close homologues of extracellular cysteine proteases that are specialized for breaking down native proteins in highly reducing environments such as marine sediments. One such protease, C25 gingipain, has never before been detected in archaea. MCG and MBG-D also contain genes for membrane-associated or extracellular peptidases capable of breaking oligopeptides into di- and tri-peptides small enough to transport into the cell. This is coupled to the presence of transporters for small peptides, as well as nearly complete pathways for amino acid fermentation to pyruvate and a tricarboxylic acid cycle. Therefore, protein fermentation could provide a complete metabolism for MCG and MBG-D, including both carbon and energy sources, yet other metabolisms may be discovered as the genome is completed. The physiologies of MCG and MBG-D most likely diverge from those of their aerobic autotrophic relatives, and archaea may directly contribute to the breakdown of complex organic matter in anoxic marine sediments.
Phylogenetic and functional attributes of a novel deeply-rooted archaea from high-temperature acidic iron-oxide mats
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Geothermal systems in Yellowstone National Park (YNP) provide an outstanding opportunity to understand the origin and evolution of metabolic processes necessary for life in extreme environments including low pH, high temperature, low oxygen and elevated concentrations of reduced iron. Previous phylogenetic studies of acidic ferric iron mats from YNP have revealed considerable diversity of uncultivated and undescribed archaea. The goal of this study was to obtain replicated de novo genome assemblies for a dominant archaeal population inhabiting acidic iron-oxide mats in YNP. We present evidence that these replicate genome assemblies represent a deeply-rooted, phylum-level lineage in the domain Archaea (referred to as ‘novel archaea group 1 (NAG1)’). This finding has significant implications for understanding the phylogeny, ecology and evolution of the archaea, as well as the evolution of key metabolic pathways in higher eukaryotes.

The NAG1 populations falls within a suggested sixth phylum in the domain Archaea along with the Euryarchaeota, Crenarchaeota, Korarchaeota, Nanoarchaeota and Thaumarchaeota. Additionally, NAG1 is one of the most deeply-rooted of these lineages with a number of unique bacterial-like pathways including a form I CO dehydrogenase complex (hypothesized for energy conservation), and those necessary for cofactor biosynthesis and transport of metabolites. The analysis of these pathways has implications for understanding ancient archaeal metabolisms and their possible role in the divergence of the three domains of life. The highly-constrained realized niche of these organisms suggests metabolic capabilities important for survival in extant Fe mats, as well as in critical periods during Earth’s evolutionary history (the ‘Great Oxidation Event’).

De novo assemblies of NAG1 populations contain genes involved in the metabolism of oxygen including a TypeA heme copper oxidase, abd-type terminal oxidase and a putative oxygen sensing protoglobin. Data presented in this study supports the hypothesis that the NAG1 lineage was among one of the first groups to utilize TypeA HCOs for oxygen respiration, and that this was occurring in high-temperature, acidic ferrous iron systems possibly before the proposed rise in oxygen to present atmospheric levels. Furthermore, the NAG1 HCO may represent an evolutionary link between the TypeB HCOs found primarily in iron and sulfur geothermal systems and TypeA HCOs found in aerobic bacteria and higher Eukaryotes.

Organisms within the NAG1 lineage are important in many of the acidic Fe-rich mats studied in YNP (inferred from 16S rRNA gene distribution) and exhibit unique biochemical signatures (inferred from genome sequence) that, if preserved in the fossil record, would provide additional tools for understanding the importance of this habitat type in the sedimentary record. The discovery and description of NAG1 is critical to our understanding of microbial community structure and function in extant thermophilic iron mats of YNP. Further characterization of this newly described lineage will provide insight regarding the evolution of archaea and the possible importance of these organisms in early Earth environments, which may have modern-day analogues active in YNP today.
The ongoing mystery of anaerobic methanotrophy: ecological physiology of anaerobic methanotrophy and the implications for carbon and sulfur cycling
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Anaerobic methanotrophy has been the subject of much research over the past 20 years. In that time, we have observed that anaerobic methanotrophy in marine systems is mediated by a consortia of archaea and bacteria, as evidenced by the physical association of methanogen-like archaea and sulfate-reducing bacteria. Despite many efforts, the mechanisms underlying this relationship remain unknown. It has, however, been shown that the archaea possess key genes and express key enzymes involved in canonical methanogenesis, which they putatively use in methanotrophy. Methanotrophic archaea also fix both inorganic carbon and nitrogen. Laboratory studies reveal that anaerobic methanotrophs can and do partner with a greater diversity of bacteria than previously recognized, coupling methane oxidation to other oxidants. In seeming contrast to the growing body of data on anaerobic methanotrophy, ecological and theoretical studies suggest that anaerobic methanotrophs may be methanogenic as well.

To address some of these key questions, we have conducted a series of studies at a variety of environments -from hydrocarbon seeps to hydrothermal vents- to better understand the extent to which anaerobic methanotrophic archaea A) are involved in methanogenesis, B) are coupled to other oxidants in situ, and C) participate in thermophilic methanotrophy. In aggregate, these data provide quantitative insight into the rates of methanotrophy and methanogenesis by canonical methanotrophic archaea, as well as their capacity to mediate methanotrophy via alternative electron acceptors.

Responses of the terrestrial ammonia oxidizing archaeon Ca. Nitrososphaera viennensis and the ammonia oxidizing bacterium Nitrosospira multiformis to nitrification inhibitors
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Nitrification inhibitors were used for decades to improve nitrogen fertilizer utilization in farmland. Their modes of action have been studied in few ammonia oxidizing bacteria (AOB) but not in the recently discovered ammonia oxidizing archaea (AOA). The aim of this study was to assess the potency of known nitrification inhibitors against the ammonia oxidation activity of representative archaeal and bacterial ammonia oxidizers present in farmland soils. The effect of selected nitrification inhibitors was assessed on Ca. Nitrososphaera viennensis – the sole AOA isolated from soil – and N. multiformis – a model soil AOB.

Both Ca. N. viennensis and N. multiformis were grown aerobically without shaking in fresh water medium (FWM). Five chemical compounds were investigated separately, including four nitrification inhibitors – allylthiourea, amidinothiourea, dicyandiamide (DCD) and nitrapyrin – and the antibiotic sulfathiazole. Archaeal and bacterial cultures were sampled every 2 days for 10 days, nitrite concentration (NO2-) was measured to determine microbial ammonia-oxidizing activity and half maximal effective concentrations (EC50) were calculated for the different inhibitors.

(1) Allylthiourea, amidinothiourea and DCD clearly inhibited ammonia oxidation in cultures of both N. multiformis and Ca. N. viennensis but the inhibitory effect on the archaeon Ca. N. viennensis was markedly lower. In particular, the EC50 of allylthiourea was 1000 times higher for the AOA culture. Among the nitrification inhibitors selected for this study, DCD was the least potent against Ca. N. viennensis, with an EC50 up to 944.33 μM. (2) The maximal soluble amount of nitrapyrin in the FWM medium reduced nitrite production by only less than 50% in cultures of both strains. (3) Sulfathiazole significantly reduced nitrite production by the bacterium, but had little effect on the archaeon.
The nitrification inhibitors tested showed little effect on the ammonia oxidizing activity of the soil archaeon at concentrations that inhibited the bacterium. Even the copper chelators did not have strong effects on the archaeon, although ammonia oxidation in archaea is supposed to be copper dependent. This different susceptibility might reflect the fundamental differences in the cellular structure of archaea and/or in their ammonia oxidation pathway, which is little explored. According to our results, the currently employed nitrification inhibitors might not affect archaea in soils, but it is also still uncertain under which conditions and to what extent ammonia oxidizing archaea contribute to nitrification in agricultural soils.

New insights into the physiology of the ammonia oxidizing archaeon Candidatus Nitrososphaera viennensis
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Nitrification, the two-step process of ammonia and nitrite oxidation, is important for Earth’s nitrogen cycle, for example by providing accessible N-compounds to plants. But nitrifiers are also known to contribute to eutrophication and to produce the greenhouse gas N₂O. The first step of nitrification, the ammonia oxidation, is performed by bacteria and archaea. Ammonia oxidizing archaea of the phylum Thaumarcheota have only recently been described, although they are widespread and abundant in almost all marine and terrestrial environments. As the cultivation of these archaea is very challenging, information about these organisms have been mostly obtained from environmental and molecular studies and from the only pure culture Ca. Nitrosopumilus maritimus, which has been isolated from a marine environment. We have recently isolated Ca. Nitrososphaera viennensis, the first pure culture of this group from a soil environment in Vienna, and obtained a second strain in stable enrichment culture (EN123) from the same environment. Those two cultures enable us to obtain insights into the ecophysiology as well as the carbon and nitrogen metabolism of ammonia oxidizing archaea from soil. Ca. N. viennensis grows up to a maximum cell density of 5*10⁷ cells ml⁻¹ and has a generation time of 45h. Although the strain is able to grow chemolithoautotrophically, high growth rates were only obtained when it was grown in co-culture with bacteria or upon addition of small amounts of some organic substrates, which are intermediates of the central carbohydrate metabolism (for example pyruvate, oxaloacetate, α-ketoglutarate). Nevertheless, only a small amount of organic substrate is incorporated into biomass as shown by a combination of isotope labeling studies with IRMS and NanoSIMS. Ca. N. viennensis tolerates higher concentrations of ammonia, compared to the marine isolate Ca. N. maritimus, is more tolerant against nitrite accumulation and can use urea as an alternative nitrogen source. We have also demonstrated that Ca. N. viennensis produces stable amounts of the greenhouse gas N₂O during growth in a process that is apparently different from that known of bacterial ammonia oxidizers. Our findings extend the knowledge about the metabolic capacities and ecophysiology of ammonia oxidizing archaea and shed light on commonalities and differences among marine and soil isolates.
Ammonia oxidizing archaea and their role in biogeochemical cycling in terrestrial hot spring ecosystems

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Ammonia-oxidizing archaea (AOA) are significant contributors to carbon and nitrogen cycling in mesophilic marine and soil environments. Genomic comparisons between AOA and ammonia-oxidizing bacteria (AOB) have revealed fundamental differences in the physiological of these organisms and their respective pathways for ammonia oxidation and energy metabolism. We recently reported the cultivation of the first thermophilic AOA, *Nitrosocaldus yellowstonii*, from a Yellowstone hot spring. To date, no thermophilic AOB have been described, suggesting that AOA like *N. yellowstonii* may be the only ammonia oxidizers in high temperature environments. To understand the role of AOA to geochemical cycles in hot spring environments, we have examined the physiology and genetics of *N. yellowstonii* in the laboratory and explored its contribution to rates of nitrogen cycling and carbon fixation in *Nitrosocaldus*-dominated hot springs. Like mesophilic AOA, *N. yellowstonii* generates energy from the aerobic oxidation of ammonia to nitrite, and is capable of fixing inorganic carbon using the 3-hydroxypropionate/4-hydroxybutyrate pathway. *N. yellowstonii* can grow on a broad range of ammonia concentrations and can also utilize alternative electron donors such as urea, reflecting the complex geochemistry of its natural environment. Genomic comparisons indicate that, like other AOA, *N. yellowstonii* encodes a putative archaeal ammonia monooxygenase (AMO) but lacks recognizable homologs of bacterial hydroxylamine oxidoreductase (HAO) and of multi-copper oxidases previously predicted to play a role in archaeal ammonia oxidation. Genomic comparisons between AOA reveal that the vast majority of 149 orthologs found exclusively in AOA have no predicted function. Thus, the ammonia oxidation pathway in *N. yellowstonii* and the AOA may involve fundamentally different biochemical steps from what is known in AOB, and may also involve distinct chemical intermediates.

Indeed, we find that *N. yellowstonii* produces significant amounts of nitric oxide (NO) during exponential growth and can be selectively inhibited by NO scavengers, suggesting that NO may be a crucial intermediate in archaeal ammonia oxidation. Molecular surveys and metagenomics indicate that thermophilic AOA related to *N. yellowstonii* are widely distributed in hot springs around the world. *Nitrosocaldus*-like organisms dominate sediments of one such spring, Great Boiling Spring (GBS) in the Great Basin, Nevada. Nearly 10% of sequence reads from the GBS metagenome belong to organisms closely related to *N. yellowstonii*, and resulting contigs show a high degree of conservation of gene content and genomic organization. Preliminary experiment using selective inhibitors of archaeal ammonia oxidation, indicate that thermophilic AOA play an important role in carbon and nitrogen cycling processes in high temperature environments like GBS.