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Insights into the physiology of ammonia-oxidizing microorganisms

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Nitrification is the aerobic process of the nitrogen cycle that converts ammonia to nitrate and is facilitated by ammonia-oxidizing and nitrite-oxidizing microorganisms. Ammonia-oxidizers are unique chemolithotrophs that evolved specialized networks of electron carriers to generate proton motive force using ammonia as a sole energy source as well as mechanisms to tolerate cytotoxic intermediates of their metabolism. Cultivation and genome sequencing of ammonia-oxidizing bacteria (AOB), archaea (AOA), and comammox bacteria (i.e. COMplete AMMonia OXidizers) have revealed new enzymology, mechanisms to tolerate low pH and hypoxia, and mechanisms for production of the potent greenhouse gas, nitrous oxide. The role of ammonia-oxidizers in natural and engineered environments is of keen interest as they are essential to the nitrogen cycle, wastewater treatment, and flux of greenhouse gases to the atmosphere.

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Introduction

Nitrification is a microbial process that aerobically converts ammonia to nitrate, and joins nitrogen fixation and denitrification as primary functions of the global nitrogen cycle [1]. Nitrification is initiated by the oxidation of ammonia by specialized groups of bacteria 'AOB' and archaea 'AOA.' For nearly 125 years, ammonia-oxidizing microorganisms were thought to produce only nitrite from their metabolism, which was released as the substrate for nitrite-oxidizing bacteria to produce nitrate. In 2015, the first reports of 'comammox,' a bacterium that performs complete ammonia oxidation from ammonia to nitrate, were published [2,3]. This discovery has renewed interest in characterizing and discriminating the enzymology, regulation, and respective niches of AOB, AOA, and comammox in natural and engineered environments. This review primarily focuses on studies published from 2016 to 2018, covering the topics of isolation and cultivation of new ammonia-oxidizers, genomics and metabolic modeling, enzymology, and roles of ammonia-oxidizers in complex ecosystems. Because of the contribution of ammonia-oxidizers to the potent greenhouse gas, nitrous oxide (N_2O), insights into pathways and abiotic processes leading to N_2O are highlighted.

Cultivation of new ammonia-oxidizers

Our understanding of ammonia-oxidizers and the complexities of their physiology has been strongly facilitated by studying axenic cultures. The first isolation of an ammonia-oxidizer, *Nitrosomonas europaea*, was reported in 1890 by Sergei Winogradsky [4]. Since then, several strains of AOB representing two classes of Proteobacteria (Betaproteobacteria and Gammaproteobacteria), AOA in the subphylum Thaumarchaeota, and comammox bacteria in the Nitrospirae phylum have been brought into culture from numerous ecosystems including soils, freshwater, marine systems, estuaries, hot springs, hot water pipe biofilms, aquaria, sediments, wastewater treatment facilities, drinking water systems, and many others [5,6••]. Isolation of ammonia-oxidizers is not a trivial task as they resist losing the tight partnerships formed with microbes that detoxify their metabolic intermediates, protect them from oxidative stress [7], or perform reciprocal feeding functions, such as cyanate degradation to ammonia by associated nitrite-oxidizers [8]. Ammonia-oxidizers often have long generation times and are sensitive to environmental factors like substrate concentration, temperature, light, pH, and oxygen [9]. Ammonia-oxidizers are also highly sensitive to reactive oxygen species. The requirement of pyruvate in cultivation medium of AOA was initially thought to sustain mixotrophic metabolism, but was instead found to detoxify hydrogen peroxide [10•]. Because of the challenges with axenic cultivation, the majority of available genome sequences for AOB, AOA, and comammox bacteria are from metagenomic data or enrichment cultures rather than from isolates. Published reports of recently isolated ammonia-oxidizers into axenic culture are listed in Table 1.

Genomics and metabolic models

It has become commonplace to include a genome sequence with the report of a new microbial isolate

Table 1

Recently isolated ammonia-oxidizing microorganisms

solate	Year	Environment	Genome sequence	Ref.
Gammaproteobacteria (AOB)				
'*Candidatus* Nitrosoglobus terrae'	2017	Acidic soil	Yes	[45]
Nitrosococcus wardiae D1FHS	2016	Eutrophic marine sediment	No	[46]
Betaproteobacteria (AOB)				
Nitrosomonas sp. PY1	2017			
Nitrosomonas sp. NP1		Activated sludge	No	[47]
Nitrosomonas sp. SN1				
Nitrosomonas mobilis Ms1	2016	WWTP granules	Yes	[48]
Thaumarchaeota (AOA)				
'*Candidatus* Nitrosocaldus cavascurensis'	2018	Hot spring	Yes	[26]
'*Candidatus* Nitrosocaldus islandicus'	2018	Hot spring biofilm	Yes	[27]
Nitrosomarinus catalina SPOT01	2017	Temperate Pacific	Yes	[49]
Nitrosopumilus cabalaminigenes HCA1	2017	Tropical marine fish tank	No	[50]
Nitrosopumilus oxyclinae HCE1				
Nitrosopumilus ureiphilus PS0				
'*Candidatus* Nitrosocosmicus exaquare G61'	2017	WWTP	Yes	[51]
'*Candidatus* Nitrosopumilus sp. NF5'	2016	Adriatic Sea	Yes	[52]
'*Candidatus* Nitrosopumilus sp. D3C'				
'*Candidatus* Nitrosocosmicus franklandus'	2016	Neutral pH soil	No	[53]
Nitrospirae (Comammox)				
Nitrospira inopinata	2017	Hot water pipe biofilm	Yes	[6••]

Enrichment cultures, metagenomes, and unpublished strains are not included.

(Table 1). Access to genome sequence information enables characterization and comparison of interesting physiological, regulatory, and evolutionary features of microbes in addition to a platform for building predictive genome-scale metabolic models. Genomic inventory on its own assists with generating hypotheses for interesting physiologies, such as acid tolerance of the AOA isolate, '*Candiatus* Nitrosotalea devanaterra' [11] and related strains [12]. Comparison of four '*Ca*. Nitrosotalea' genome sequences with 23 other AOA genomes revealed 743 shared core proteins [12], which is fewer than the 860 shared core proteins reported in a prior AOA genome comparison [13]. All AOA genomes encode the 3-hydroxyproionate/4-hydroxybutyrate CO_2 fixation pathway, central carbon pathways, and enzymes for ammonia-oxidation. Unique genes in acid-tolerant '*Ca*. Nitrosotalea' genomes include a Na^+/solute symporter, metal transporters, and a chaperone specific for proline-rich proteins [12]. Soil AOA encode unique features such as envelope modifications for biofilm formation, polysaccharide production, and cell–cell adhesion [13]. A prior analysis of the '*Ca*. N. devanaterra' genome alone predicted 51 candidate genes conferring adaptations to low pH including high-affinity substrate transport, membrane impermeability, and a number of cation transporters [11]; however, the more robust comparative analysis found that all but 10 of these genes are in AOA genomes of non-acidophiles [12]. These analyses highlight the need to compare across as many genome sequences as possible to identify unique gene sets that confer specialized functions, but also suggests that gene inventory alone is an inaccurate predictor of specialized pathways and functions.

Genome-scale metabolic network modeling assembles layers of functional information (e.g. genomic, physiological, transcriptomic, proteomic, metabolomic) from a microorganism into a mathematical representation. When iteratively refined with experimental data, the model can accurately predict global metabolic responses of a microbe before confirmation with wet-lab experiments, thus extending functional information far beyond the genome sequence alone. Metabolic network models have been used to predict N_2O formation from nitric oxide (NO)-producing reactions in AOB consortia with nitrite-oxidizing bacteria [14,15] and to describe adaptation of *N. europaea* to anoxic-oxic transitions [16•]. The latter study found that exposure of *N. europaea* to repeated anoxic-oxic cycles resulted in a reduction of N_2O production and that long-term changes in flux of nitrogen oxide metabolites correlated to changes at the protein, rather than transcript, levels [16•]. The implication of this study is that N_2O emissions from AOB are likely overestimated in wastewater treatment operations that regularly utilize anoxic-oxic cycling. With rapidly increasing accessibility to genome sequences, 'omics, and physiological information on ammonia-oxidizing isolates, genome-scale metabolic network modeling is rapidly becoming a primary tool for resolving remaining questions on ammonia-oxidizer metabolism, whole genome regulation, and control of reactive metabolites, like NO and N_2O.

Enzymology of ammonia-oxidation

Ammonia oxidation is a highly specialized metabolism among prokaryotes due to the toxicity of its intermediates and products (i.e. hydroxylamine, NO and nitrite) and the