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GENES AND GENOMES

A Physiological and Genomic Comparison of *Nitrosomonas* Cluster 6a and 7 Ammonia-Oxidizing Bacteria

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Abstract

Ammonia-oxidizing bacteria (AOB) within the genus *Nitrosomonas* perform the first step in nitrification, ammonia oxidation, and are found in diverse aquatic and terrestrial environments. *Nitrosomonas* AOB were grouped into six defined clusters, which correlate with physiological characteristics that contribute to adaptations to a variety of abiotic environmental factors. A fundamental physiological trait differentiating *Nitrosomonas* AOB is the adaptation to either low (cluster 6a) or high (cluster 7) ammonium concentrations. Here, we present physiological growth studies and genome analysis of *Nitrosomonas* cluster 6a and 7 AOB. Cluster 6a AOB displayed maximum growth rates at ≤ 1 mM ammonium, while cluster 7 AOB had maximum growth rates at ≥ 5 mM ammonium. In addition, cluster 7 AOB were more tolerant of high initial ammonium and nitrite concentrations than cluster 6a AOB. Cluster 6a AOB were completely inhibited by an initial nitrite concentration of 5 mM. Genomic comparisons were used to link genomic traits to observed physiological adaptations. Cluster 7 AOB encode a suite of genes related to nitrogen oxide detoxification and multiple terminal oxidases, which are absent in cluster 6a AOB. Cluster 6a AOB possess two distinct forms of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and select species encode genes for hydrogen or urea utilization. Several, but not all, cluster 6a AOB can utilize urea as a source of ammonium. Hence, although *Nitrosomonas* cluster 6a and 7 AOB have the capacity to fulfill the same functional role in microbial communities, i.e., ammonia oxidation, differentiating species-specific and cluster-conserved adaptations is crucial in understanding how AOB community succession can affect overall ecosystem function.

Keywords Ammonia-oxidizing bacteria · *Nitrosomonas* · Nitrification · Niche differentiation · Ammonium availability

Introduction

Nitrification, the oxidation of ammonia to nitrate, plays a crucial role in cycling nitrogenous compounds within both natural and engineered ecosystems. Nitrification is essential for the removal of inorganic nitrogen compounds from wastewater [1]; however, it also contributes to fertilizer loss [2], the eutrophication of aquatic environments

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[3–5], and the release of greenhouse gases such as nitrous and nitric oxide [6, 7].

Aerobic nitrification is performed either as a two-organism process by ammonia-oxidizing bacteria (AOB) [8] or archaea (AOA) [9] and nitrite-oxidizing bacteria (NOB) [10], or as a single organism process by COMplete AMMonia OXidizers (comammox) [11, 12]. All canonical AOB belong to two monophyletic orders within the *Gammaproteobacteria*, the *Betaproteobacteriales* and the *Nitrosococcales*. Recently, changes to microbial taxonomy based on whole genome phylogeny have proposed that the class previously known as the *Betaproteobacteria* be reclassified as a new order, the *Betaproteobacteriales*, within the class *Gammaproteobacteria* [13]. AOB within the *Betaproteobacteriales* are divided into *Nitrosomonas* and *Nitrosospira* AOB, which are abundant in a wide range of environmental and engineered ecosystems [14, 15]. *Nitrosomonas* AOB are further subdivided into six clusters based on either 16S rRNA or *amoA* gene phylogeny, which correlate with previously observed physiological adaptations [9, 16]. Cluster 6b (*Nitrosomonas marina* lineage), cluster 9 (*Nitrosomonas* sp. Nm143 lineage), and the *Nitrosomonas cyrotolerans* lineage AOB are all commonly detected in marine or other saline environments [15], whereas cluster 8 (*Nitrosomonas communis* lineage) AOB have mainly been isolated from eutrophic soil and aquatic environments [8]. To date, there are no cultured cluster 5 (environmental lineage) AOB isolates, but they have been detected in a wide variety of environmental habitats [15–17]. This study is focused on differentiating between cluster 6a (*Nitrosomonas oligotropha* lineage) and cluster 7 (*Nitrosomonas europaea/mobilis* lineage) AOB. Cluster 6a AOB are characterized by a high substrate affinity and their sensitivity to high ammonium and nitrite concentrations. In contrast, cluster 7 AOB are characterized by a low substrate affinity and a tolerance for high ammonium and nitrite concentrations [8, 15, 18].

Whole genome sequencing has made it possible to link observed physiological adaptations with conserved genomic traits, an approach utilized to investigate physiological adaptations in cold adapted *Saccharomyces cerevisiae* [19], high light adapted *Prochlorococcus* ecotypes [20], and AOA adapted to alkaline environments [21]. However, the small number of available AOB genome sequences had previously limited genomic comparisons within and between clusters of *Nitrosomonas* AOB [22]. Comparative genomics between AOB species within clusters 6a and 7 will shed light on the physiological adaptations of these AOB to different environmental conditions such as ammonium availability or nitrite sensitivity and allow for better modeling of the functional potential of AOB species.

Here, we present the analysis of draft genome sequences of two cluster 6a (*Nitrosomonas oligotropha* Nm45 and *Nitrosomonas* sp. JL21) and two cluster 7 (*Nitrosomonas* sp. GH22 and *Nitrosomonas* sp. HPC101) AOB. Comparative genomics and ecophysiological studies with these and previously characterized AOB such as *Nitrosomonas* sp. Is79, *Nitrosomonas* sp. AL212, *Nitrosomonas eutropha*, and *N. europaea* were utilized to elucidate conserved and differentiating traits between cluster 6a and 7 AOB, as both are often observed co-occurring in a wide range of aquatic and terrestrial environments.

Material and Methods

Cultures and AOB Growth Medium

The AOB *Nitrosomonas* sp. Is79 [23], *Nitrosomonas* sp. AL212 [24], *N. oligotropha* Nm45 [25], *Nitrosomonas* sp. JL21 [26], *Nitrosomonas* sp. HPC101 [24], *Nitrosomonas* sp. GH22 [26], and *N. eutropha* [25] were utilized. AOB cultivation and growth experiments were performed in a mineral salt medium containing 10 mM NaCl, 1 mM KCl, 1 mM $CaCl_2*2H_2O$, 0.2 mM $MgSO_4*7H_2O$, and 1 ml l^{-1} trace element solution [27, 28]. Medium was supplemented with $(NH_4)_2SO_4$ as required and HEPES buffer was added in a fourfold molar ratio to the initial ammonium concentration. The pH was adjusted to 7.8 with NaOH before autoclaving. Sterile KH_2PO_4 was added after autoclaving to a final concentration of 0.4 mM [28].

Ecophysiological Experiments

Growth rates based on ammonia oxidation were determined from AOB cultures grown in the dark, at 27 °C, across a range of initial ammonium (0.1–10 mM) and nitrite (0.1–5 mM) concentrations. The ability of AOB to utilize urea as an ammonium source was determined by the concentration of nitrite produced when AOB were grown in medium containing ammonium (0.5 mM) and sterile-filtered (0.2 μm) urea (0.25 mM). Samples from all experiments were taken at regular intervals, centrifuged (20 min, 28,000×*g*, 4 °C), and cell-free supernatants were stored at − 20 °C. Colorimetric assays were used to determine ammonium and nitrite concentrations [29, 30]. Growth rates based on ammonia oxidation were determined by calculating the slope of the log-transformed nitrite concentrations against time assuming a correlation with growth [28, 31].

Genomic DNA Isolation

Cellular biomass from *N. oligotropha* Nm45, *Nitrosomonas* sp. JL21, *Nitrosomonas* sp. HPC101, and

Nitrosomonas sp. GH22 was collected by centrifugation (20 min, 22,000×*g*, 4 °C). High molecular weight DNA was isolated using the Joint Genome Institute bacterial genomic DNA isolation cetyltrimethylammonium bromide (CTAB) protocol (http://my.jgi.doe.gov/general/protocols/JGI-Bacterial-DNA isolation-CTAB-Protocol-2012.pdf) and was stored at − 80 °C.

Next-Generation Sequencing

Whole genome sequencing libraries were prepped with the Nextera XT library prep kit as per manufacturer's instructions and sequenced on an Illumina 1.9 HiSeq (Illumina, San Diego, CA, USA). Libraries of *N. oligotropha* Nm45, *Nitrosomonas* sp. JL21, *Nitrosomonas* sp. HPC101, and *Nitrosomonas* sp. GH22 generated paired end reads of 17,153,696/14,546,069/8,383,203 and 9,187,862 base pairs respectively.

Genome Assembly and Annotation

De novo assembly of *N. oligotropha* Nm45, *Nitrosomonas* sp. JL21, *Nitrosomonas* sp. HPC101, and *Nitrosomonas* sp. GH22 was performed using Genomics Workbench 7.5 with the Bacterial Genome Finishing Module (CLC bio, Aarhus, Denmark), with standard default settings. The assembly resulted in 70, 87, 32, and 80 contigs with a coverage of 1287, 933, 1682, and 2060 for *N. oligotropha* Nm45, *Nitrosomonas* sp. JL21, *Nitrosomonas* sp. HPC101, and *Nitrosomonas* sp. GH22, respectively.

The genomes were annotated with Prokka version 1.10 [32]. Annotations were called first from a library comprised of *N. europaea*, *N. eutropha*, *Nitrosomonas* sp. Is79, and *Nitrosomonas* sp. AL212 genome sequences before being called from the general Prokka database. Genome comparisons were performed using CLC Genomics Workbench 7.5, the integrated microbial genomes (IMG) database [33] and comparative analysis system, and Genoscope [34].

Detection of Genes Absent in Draft Genomes by PCR and Sequence Analysis

PCR and subsequent amplicon sequencing was used to determine the presence of several genes that were not detected in the assembled draft genomes. Primers were designed with the NCBI-Primer-BLAST tool (Table S2). PCR products were cleaned using the EZ-10 Spin Column PCR Products Purification Kit (Biobasic, Markham, Ontario, Canada) and sequenced using a BigDye Terminator (version 3.1) cycle sequencing kit on an Applied Biosystems 3730xl DNA analyzer (Life Technology Corporation, Carlsbad, CA, USA). All sequences were edited with the 4Peaks program (A. Griekspoor and T. Groothuis, The Netherlands Cancer Institute).

Phylogenomic Analysis

A maximum likelihood phylogenomic tree was created using the 34 concatenated and aligned universal marker genes identified with CheckM [35]. The tree was constructed using the software package MEGA [36].

Deposited Nucleic Acid Sequence Accession Numbers

The four AOB sequenced in this study are part of BioProject 284623 and are listed as BioSamples SAMN03731130–SAMN03731132 and SAMN0304077. Genome sequence information for *N. oligotropha* Nm45, *Nitrosomonas* sp. GH22, *Nitrosomonas* sp. JL21, and *Nitrosomonas* sp. HPC101 is accessible from NCBI under the accession numbers: QRBZ00000000, QRCA00000000-QRCC00000000 respectively. Individual gene sequences that were generated have also been deposited to Genbank and have the following accession numbers: MK224460-MK224464, MK688461, and MK715451.

Results and Discussion

Ammonia Oxidation-Dependent Growth Rates

The four AOB sequenced in this study were grouped into *Nitrosomonas* cluster 6a or cluster 7 by their phylogenomic position (Fig. 1). Cluster 6a AOB (*Nitrosomonas* sp. Is79, *N. oligotropha* Nm45, *Nitrosomonas* sp. JL21, and *Nitrosomonas* sp. AL212) displayed maximal growth rates with initial ammonium concentrations of ≤ 1 mM, where cluster 7 AOB (*N. eutropha*, *Nitrosomonas* sp. GH22, and *Nitrosomonas* sp. HPC101) were tolerant of much higher initial ammonium concentrations and exhibited maximal growth rates at ammonium concentrations ≥ 5 mM (Fig. 2a). This agrees with previous observations that cluster 6a and 7 AOB are adapted to environments with low and high ammonium concentrations respectively [8, 15, 18, 37]. The adaptation of cluster 6a and 7 AOB to low and high ammonium environments may be explained by their respective ammonia oxidation kinetics. Cluster 6a AOB have an apparent half saturation constant ($K_{m(app)}$) of ~ 0.24–3.6 μM NH_3 whereas cluster 7 AOB have a $K_{m(app)}$ of ~ 12.5–160 μM NH_3 [26, 27, 38–48]. Cluster 7 AOB were also much more tolerant to high initial nitrite concentrations (5 mM), whereas growth of all four cluster 6a AOB was fully inhibited at initial nitrite concentrations of 5 mM (Fig. 2b).

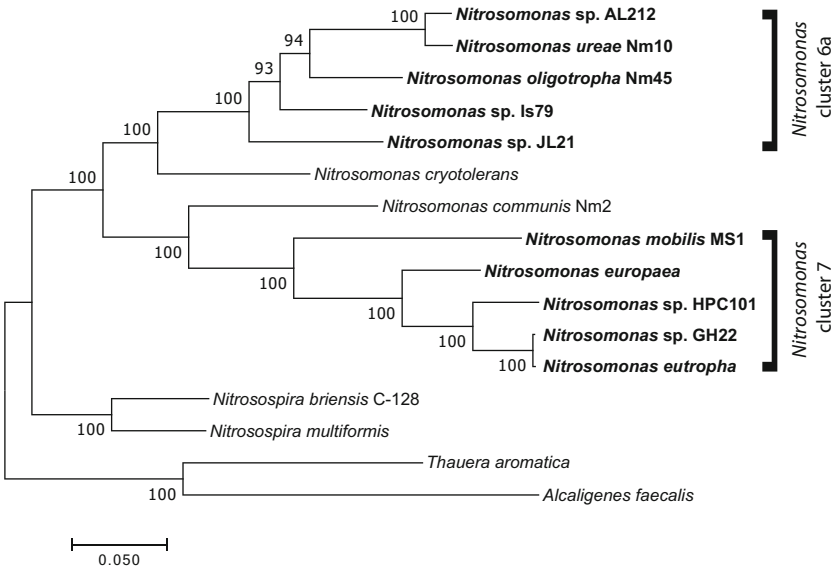

Fig. 1 The phylogenomic relationship of *N. oligotropha* Nm45, *Nitrosomonas* sp. JL21, *Nitrosomonas* sp. HPC101, and *Nitrosomonas* sp. GH22 relative to other sequenced AOB. A phylogenomic tree was obtained from the alignment of 34 concatenated universal marker genes identified by CheckM [35]. Numbers adjacent to the branches are support values from 100 maximum likelihood bootstrap replicates. Genome (draft) sequences analyzed and compared in this study are presented in bold

Genome Assembly and Genes Involved in Ammonia Oxidation

The general genome information and strain origin of the two cluster 6a (*N. oligotropha* Nm45 and *Nitrosomonas* sp. JL21) and two cluster 7 (*Nitrosomonas* sp. GH22 and *Nitrosomonas* sp. HPC101) AOB sequenced in this study are reported (Table 1). Each of the four draft genomes encodes all the core genes expected for ammonia oxidation in AOB. Although not resolved in the four draft genomes presented here, it is presumed that each of these AOB encodes two or more nearly identical copies of both the ammonia monooxygenase

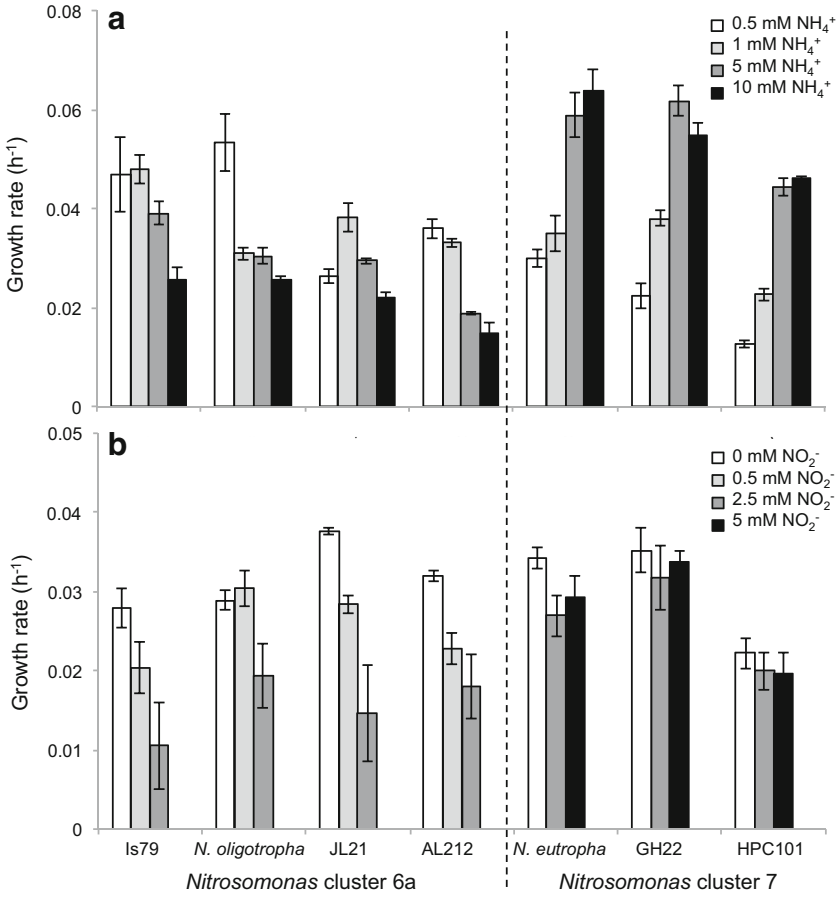

Fig. 2 Influence of initial **a** ammonium and **b** nitrite concentrations on the ammonia oxidation-dependent growth rates of *Nitrosomonas* cluster 6a and 7 AOB (mean ± SD; $n = 3$). Growth rates calculated with various initial nitrite concentrations were all determined in medium with an initial ammonium concentration of 1 mM. No growth was observed by any cluster 6a AOB in medium with an initial nitrite concentration of 5 mM

Table 1 Sample origin and general genomic features of two *Nitrosomonas* cluster 6a and 7 AOB draft genome sequences

	Nitrosomonas cluster 6a		*Nitrosomonas* cluster 7	
	N. oligotropha	JL21	GH22	HPC101
Reference	[25]	[26]	[26]	[24]
Origin	Hamburg, Germany	Tsukuba, Japan	Tsukuba, Japan	Matsudo, Japan
Environmental habitat	Soil	Laboratory activated sludge	Laboratory activated sludge	Laboratory activated sludge
Genome size (Mb)	3.12	3.17	2.54	2.52
Contigs (no.)	70	87	80	32
G+C content (%)	49.2	47.8	48.1	49.3
Number of CDSs	2908	2922	2405	2344
Number of tRNA genes	38	38	39	41
Estimated completeness (%)[1]	99.73	99.84	99.53	99.53
Estimated contamination (%)[1]	0.15	0	0.12	0.59
Average nucleotide identity (ANI)[2]	*Nitrosomonas* sp. Is79	*Nitrosomonas* sp. Is79	*N. eutropha*	*N. eutropha*
	77.5	74.1	99.03	81.9

[1] Completeness and contamination estimates were generated based on CheckM predictions

[2] The most similar AOB, based on ANI, is provided for each draft genome. ANI values were calculated with the EZgenome program [49]

(*amoCABED*) and hydroxylamine dehydrogenase (*haoA-haoB-*cytochromec_{554}-cytochromec_{m552}) gene clusters based on previously sequenced *Nitrosomonas* AOB genomes (Table 2, Supplemental Table 1) [23, 50–56].

Average nucleotide identities (ANI) were calculated to compare the newly sequenced AOB to previously sequenced strains. Based on ANI values (Table 1), each of the sequenced AOBs is individual species (> 95% cutoff), with the exception of *Nitrosomonas* sp. GH22, which is a strain of *N. eutropha* (99.03% ANI). However, *Nitrosomonas* sp. GH22 does not encode the ~ 117 kbp (126 genes) genomic island previously identified in the genome of *N. eutropha*. The genomic island contains several putative genes for heavy metal resistance and is flanked on either side by tRNA-Gly and tRNA-Cys genes respectively [50]. While the genome of *Nitrosomonas* sp. GH22 is syntenous with the genome of *N. eutropha* on both sides of the genomic island, the tRNA-Gly and tRNA-Cys genes are adjacent in the genome of *Nitrosomonas* sp. GH22.

Nitrosocyanin

The red copper protein, nitrosocyanin, has so far only been detected in AOB. This conserved presence in AOB has led to the hypothesis that nitrosocyanin may be essential for ammonia oxidation or energy generation in AOB [57–59]. In support of this hypothesis, nitrosocyanin has been identified as one of the most abundant proteins in several AOB [60]. It has also been suggested that nitrosocyanin may function as a nitric oxide oxidase and therefore be a third enzyme involved in the core ammonia oxidation pathway proposed by Lancaster et al. [61]. In this case, nitrosocyanin would convert nitric oxide produced by HAO to nitrite [62]. However, the absence of nitrosocyanin in the closed genome of *Nitrosomonas* sp. Is79 [23] seems to refute the hypothesis that nitrosocyanin is essential for all AOB. However, this does not exclude the possibility that nitrosocyanin functions as a nitric oxide oxidase or is involved in energy generation in AOB when present. Despite nitrosocyanin not being conserved in all AOB, the presence of functionally redundant enzymes cannot be ruled out as long as the exact function of nitrosocyanin remains unknown.

Nitric Oxide Metabolism

The process of ammonia oxidation in AOB produces the reactive nitrogen species, hydroxylamine (NH_2OH), nitrite, nitric oxide, and nitrous oxide [6, 7, 62, 63], which contribute to nitrosative stress [64]. All AOB investigated here encode the copper-containing nitrite reductase (*nirK*), cytochrome *c*'-beta, and cytochrome P460 (Table 2, Supplemental Table 1), which are involved in nitrogen oxide transformations [65]. The cluster 7 AOB encode the heme copper nitric oxide reductase (sNOR) [66] and the nitrosative stress master tran-

Table 2 Select genomic inventory of sequenced *Nitrosomonas* cluster 6a and 7 AOB. The presence (+) or absence (−) of genes in each complete or draft genome is indicated. The ability of each AOB to utilize urea as an ammonium source is also provided. Locus tags as well as more detailed information on gene duplications can be found in the supplementary material (Supplemental Table 1)

	Nitrosomonas cluster 6a					*Nitrosomonas* cluster 7				
	N. oligotropha This study	Is79 [23]	JL21 This study	AL212 [53]	*N. urea* [54]	*N. eutropha* [50]	GH22 This study	HPC101 This study	*N. europaea* [52]	*N. mobilis* [51]
Energy generation										
Ammonia monooxygenase (*amoC*/*A*/*B*/*E*/*D*)	+[1]	+	+*	+	+	+	+*	+	+	+
Hydroxylamine dehydrogenase (*haoA*/*haoB*/*c554*/c_m*552*)	+	+	+	+	+	+	+*	+*	+	+
Ammonia transporter	+	+	+	+	+	−	−	−	+	+
Nitrosocyanin	+*	−	+	+	+	+	+	+	+	+
Terminal oxidases										
Cytochrome *c* oxidase aa_3	+	+	+	+	+	+	+	+	+	+
Quinol oxidase bo_3	−	−	−	−	−	+	+	−	−	−
Cytochrome c oxidase cbb_3	−	−	−	−	−	+	+	−	−	−
Urea utilization										
Ability to utilize urea	Yes	No	Yes	Yes	Yes	No	No	No	No	No
Urease	+	−	+	+	+	−	−	−	−	−
Urea transporter	+	−	+	+	+	−	−	+	+	+
Urea carboxylase	+	+	+	+	+	+	+	+	+	+
Associated protein 1	+	+	+	+	+	+	+	+	+	+
Associated protein 2	+	+	+	+	+	+	+	+	+	+
Urea allophanate hydrolase	−	+	+	+	+	−	−	−	−	−
Hydrogen utilization										
Hydrogenase	+	+	+	−	−	−	−	−	−	−
Nitrogen oxide metabolism										
Nitrite reductase (*nirK*)	+	+	+	+	+	+	+	+	+	+
Nitrite reductase cluster genes (*ncgC*/*B*/*A*/*nsrR*)	−	−	−	−	−	+	+	+	+	−
Cytochrome *c* oxidase (NO reductase sNOR)	−	−	−	−	−	+	+	+	+	+
cnorCBQD	−	−	+	+	−	+	+	−	+	+
Cytochrome c' beta	+	+	+	+	+	+	+	+	+	+
Cytochrome P460	+	+	+	+	+	+	+	+	+	+
Carbon fixation										
RuBisCO Form IA (green like)	+	+	+	+	+	+	+	+	+	+
RuBisCO Form IC (red like)	+	+	+	+	+	−	−	−	−	−
Carboxysome gene inventory	−	−	−	−	−	+	−	+	−	−

*Gene or one gene from a cluster is not present in the draft genome sequence, but its presence was confirmed by PCR and sequencing

[1] *amoE* and *amoD* are not present in the draft genome sequence, but its presence was confirmed by PCR and sequencing

scriptional regulator *nsrR* (Table 2, Supplemental Table 1). In addition, all cluster 7 AOB, except *Nitrosomonas mobilis* Ms1, encode the putative nitric oxide-scavenging cluster *ncgABC* [67] (Table 2, Supplemental Table 1). NirK has previously been shown to confer tolerance to high nitrite concentrations [68] and act as an electron shuttle for efficient ammonia oxidation [6, 69]. This may explain why *nirK* but not the entire *nirK* gene cluster (*nirK-ncgCBA-nsrR*) is conserved across all sequenced AOB to date, with the exception of *Nitrosomonas communis* Nm2 [55]. In addition, the presence of the nitric oxide reductase genes (*norCBQD*) is not present in all cluster 6a AOB genomes (Table 2, Supplemental Table 1). Together, the lack of a nitrosative stress master regulator and a reduced nitric oxide-scavenging genetic inventory likely contributes to the observed nitrite sensitivity of cluster 6a AOB. None of the cluster 7 AOB tested displayed a

decreased growth rate in the presence of 5 mM nitrite, whereas the growth rate of all cluster 6a AOB tested was totally inhibited by 5 mM nitrite (Fig. 2b).

Hydrogenase

Aside from ammonia oxidation, several AOB encode for alternative metabolisms such as hydrogen utilization (Table 2, Supplemental Table 1). Several cluster 6a AOB encode putative [NiFe] hydrogenase enzyme complexes, resembling the hydrogenase present in *Nitrosospira multiformis* [70]. However, the hydrogenase genes in *N. multiformis* are encoded as a single gene cluster, whereas they are encoded in multiple gene clusters in cluster 6a AOB (Table 2, Supplemental Table 1). If functional, the oxidation of hydrogen under hypoxic conditions may be directly linked to NAD(P)H production as it is in *Ralstonia eutrophus* [71]. No hydrogenase genes were identified in cluster 7 AOB (Table 2, Supplemental Table 1), although *N. eutropha* and *N. europaea* have previously been reported to utilize hydrogen under anoxic conditions [72]. Physiological experiments are needed to confirm the hydrogen utilization in cluster 6a AOB hypothesized here.

Urease

The ability of AOB to hydrolyze urea is advantageous as it provides substrates for both energy generation (ammonium) and carbon fixation (CO_2). Consistent with previous reports [8, 18, 23], urea utilization is not a conserved physiological trait among cluster 6a AOB (Table 2, Supplemental Table 1). All AOB compared here encode at least one subunit of a urea amidolyase enzyme (UAL); however, the presence of one or both UAL subunits did not correlate with urea utilization in AOB. All AOB able to utilize urea encode the essential structural and accessory proteins for a urease enzyme (*ureABCDEFG*) and a urea transporter (Table 2, Supplemental Table 1). Notably, alternative metabolisms such as urea and hydrogen utilization are not conserved among cluster 6a AOB. This is unexpected, as alternative metabolisms would increase the amount of available substrates and be advantageous in the low substrate environments *Nitrosomonas* cluster 6a AOB are adapted to. Urea and hydrogen utilization appear to be species-specific physiological adaptations and not directly related to an adaptation to low or high ammonium environments.

Terminal Oxidases

All AOB characterized here encode a cytochrome *c* oxidase aa_3 low affinity terminal oxidase. In addition, several cluster 7 AOB also encode for two additional terminal oxidases, the low affinity quinol oxidase bo_3 and the high affinity cytochrome *c* oxidase cbb_3 (Table 2, Supplemental Table 1). Possessing a genetic inventory to cope with fluctuating oxygen concentrations appears to be species-specific adaptation among cluster 7 AOB but not among cluster 6a AOB. Encoding multiple proton pumping terminal oxidases with a range of O_2 affinities would provide cluster 7 AOB a competitive advantage over cluster 6a AOB as well as other microorganisms in aquatic or terrestrial environments that have constantly fluctuating O_2 concentrations. This approach has been previously documented in *Pseudomonas putida*, which can differentially regulate multiple terminal oxidases to adapt to different O_2 concentrations [73].

Carbon Fixation

Cluster 6a and 7 AOB each encode different genetic inventories to cope with fluctuating CO_2 concentrations. All AOB characterized here encode a Form IA green-like (high affinity) (RuBisCO) (Table 2, Supplemental Table 1). In addition, all cluster 6a AOB encode an additional Form IC red-like (low affinity) RuBisCO. Some, but not all, cluster 7 AOB encode genes for carboxysome biosynthesis (Table 2). Although cluster 6a and 7 AOB appear to utilize different strategies for dealing with fluctuating environmental CO_2 concentrations, utilizing multiple terminal oxidases with different affinities or carboxysomes to concentrate CO_2 can each be effective [74, 75].

Conclusions

This study compares the ecophysiological and genomic characteristics of cluster 6a and 7 AOB within the genus *Nitrosomonas*, adapted to low and high ammonium environments, respectively. Several conserved genomic traits were identified highlighting differences between cluster 6a and 7 AOB: urea and hydrogen utilization, and nitric oxide metabolism, as well as strategies to cope with fluctuating O_2 and CO_2 concentrations. For example, cluster 7 AOB encode genes involved in nitrogen oxide detoxification, which are not found in cluster 6a AOB and they are tolerant to higher nitrite concentrations than cluster 6a AOB. The presence of the nitrogen oxide detoxification genes could help the cluster 7 AOB withstand higher nitrite concentrations. Differentiating ecophysiological characteristics conserved among groups of AOB as well as species-specific adaptations will prove crucial in understanding how AOB community succession will affect overall ecosystem function. As oligotrophic aquatic ecosystems are subjected to increased eutrophication, AOB community succession may affect the amount of nitrogenous greenhouse gases, such as nitric oxide or nitrous oxide that are released from that environment. Therefore, the cluster-specific physiological and genomic adaptations detailed in

this study highlight how AOB communities will differentially respond to environmental changes such as eutrophication and impact global nitrous oxide emissions.

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