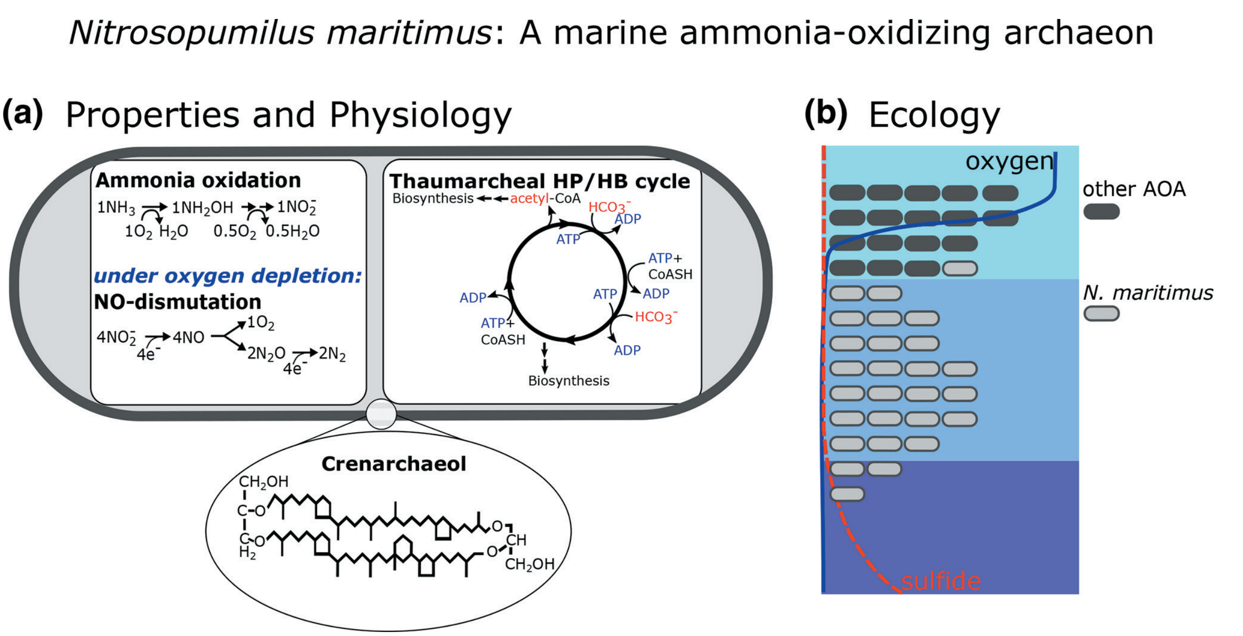
75 MICROBIOLOGY

MICROBE PROFILE
Kraft and Canfield†, *Microbiology* 2022;168:001207
DOI 10.1099/mic.0.001207

MICROBIOLOGY SOCIETY

Microbe Profile: *Nitrosopumilus maritimus*

Beate Kraft[1,*] and Donald E. Canfield†[1,2,3]



Grapical abstract

(a) *Nitrosopumilus maritimus* gains energy by ammonia oxidation coupled to oxygen consumption. When oxygen is depleted, it produces its own oxygen. NO-dismutation is the proposed oxygen production pathway. Carbon fixation operates via a modified hydroxypropionate/hydroxybutyrate cycle. Crenarchaeol is a membrane lipid unique to ammonia-oxidizing archaea (AOA). (b) Distribution of *N. maritimus*-related AOA in relation to oxygen concentrations in the example of the Black Sea [1].

Abstract

Nitrosopumilus maritimus is a marine ammonia-oxidizing archaeon with a high affinity for ammonia. It fixes carbon via a modified hydroxypropionate/hydroxybutyrate cycle and shows weak utilization of cyanate as a supplementary energy and nitrogen source. When oxygen is depleted, *N. maritimus* produces its own oxygen, which may explain its regular occurrence in anoxic waters. Several enzymes of the ammonia oxidation and oxygen production pathways remain to be identified.

Received 23 March 2022; Accepted 30 May 2022; Published 19 July 2022
Author affiliations: [1]Nordcee, Department of Biology, University of Southern Denmark, Odense, Denmark; [2]Key Laboratory of Petroleum Geochemistry, Research Institute of Petroleum Exploration and Development, China National Petroleum Corporation, Beijing 100083, PR China; [3]Danish Institute of Advanced Study, 5230 Odense, Denmark.
***Correspondence:** Beate Kraft, bkraft@biology.sdu.dk
Keywords: ammonia-oxidizing archaea; nitrification; NO-dismutation; *Nitrosopumilus*.
Abbreviations: AMO, ammonia mono-oxygenase; AOA, ammonia-oxidizing archaea; GDGT, glycerol dialkyl glycerol tetraether; HAO, hydroxylamine dehydrogenase; HP/HB, hydroxypropionate/hydroxybutyrate.
†Villum investigator

TAXONOMY

Domain *Archaea*, phylum *Nitrososphaerota* (*Thaumarchaeota*), class *Nitrososphaeria*, order *Nitrosopumilales*, family *Nitropumilaceae*, genus *Nitrosopumilus*, species *Nitrosopumilus maritimus*.

PROPERTIES

Nitrosopumilus maritimus SCM1, isolated from a tropical marine fish tank at the Seattle Aquarium, USA, is a chemolithoautotroph, gaining energy from aerobic ammonia oxidation to nitrite [2]. The cells are non-motile, straight rods with a diameter of 0.17–0.22 µm and a length of 0.5–0.9 µm. The core membrane lipids of *N. maritimus* consist of glycerol dialkyl glycerol tetraethers (GDGTs), including crenarchaeol. Since crenarchaeol is unique to *Nitrososphaerota*, it is used as a biomarker for this archaeal phylum including *Nitrosopumilus* spp. *N. maritimus* produces methylphosphonate esters that can release methane when degraded, potentially contributing to methane production in marine oxic waters [3].

PHYLOGENY

Ammonia-oxidizing archaea (AOA) form the monophyletic class *Nitrososphaeria*, which divides into the group of *Candidatus* Nitrosocaldales and a second group that splits into two major lineages: the order *Nitrososphaerales*, with representatives mostly in soils, and a second major clade. This second clade consists of the two orders: *Candidatus* Nitrosotaleales and *Nitrosopumilales*. The *Nitrosopumilales*, including the species *Nitrosopumilus maritimus*, are mostly found in marine environments.

ECOLOGY

Members of the order *Nitrosopumilales* are ubiquitous and abundant in the environment. They are key players in the nitrogen cycle of the world's oceans and make up approximately 20% of the microbial community in the oxic water column. They are abundant in oxygenated environments, but are also found in oxygen-depleted environments such as marine oxygen-minimum zones and anoxic basins such as the Black Sea, even though ammonia-oxidation requires oxygen [1].

KEY FEATURES AND DISCOVERIES

Ammonia oxidation

N. maritimus SCM1 has an extremely high specific affinity for ammonia (defined here as ammonia +ammonium) with an apparent half-saturation constant (*Km*) of 0.132 µM [4]. A high ammonia affinity allows members of the genus *Nitrosopumilius* to thrive in the open ocean, for example, where ammonium is present at low nanomolar concentrations.

The biochemical pathway of ammonia oxidation in *N. maritimus* or other AOA is not fully resolved. With current understanding, the first step of ammonia oxidation by *N. maritimus* is the oxidation of ammonia to hydroxylamine, catalysed by a putative ammonia mono-oxygenase (AMO) [5]. The gene coding for the alpha subunit of AMO, *amoA*, is typically used to assess the diversity and abundance of AOA in the environment. The enzyme responsible for hydroxylamine oxidation is unclear and genes coding for a homologue to the bacterial hydroxylamine dehydrogenase (HAO) are absent in the *Nitrososphaeria* including *N. maritimus*. The *N. maritimus* genome, however, encodes several blue copper-containing plastocyanin-like electron carriers. Some of them have been proposed to be involved in electron transfer from hydroxylamine or other potential intermediates to the terminal oxidase [5].

Utilization of alternative nitrogen substrates

Marine *Nitrosospumilales* often live with vanishingly low ammonium concentrations and they can supplement their nitrogen requirements with simple organic nitrogen compounds that are ubiquitous in marine systems [6]. Pure cultures of *N. maritimus* SCM1 can convert cyanate to ammonia, using it as an energy and nitrogen source even though no known cyanases are present in its genome nor any other marine *Nitrosopumilales*. While some *Nitrosopumilales* can use urea, *N. maritimus* SCM1 cannot, and its genome does not encode any known ureases [6].

Carbon fixation pathway

N. maritimus assimilates inorganic carbon via a modified hydroxypropionate/hydroxybutyrate (HP/HB) cycle distinct from the cycle operating in *Crenarchaeota* [7]. In the HP/HB cycle of *Nitrososphaeria*, ADP (and not AMP as in the *Crenarchaetoa*) is produced during the activation of 3-hydroxypropionate and 4-hydroxybutyrate. Furthermore, some enzymes catalyse multiple reactions, which reduces the cost of protein biosynthesis. Therefore, the HP/HB cycle in *Nitrososphaeria* is the most energy-efficient carbon fixation pathway found in aerobes, helping *Nitrososphaeria* to attain high numbers in oligotrophic environments of low energy supply and ammonia limitation.

While the growth of *N. maritimus* is stimulated by the small organic molecules pyruvate, oxaloacetate and α-ketoglutarate, mixotrophy has not been confirmed [8]. α-Keto acids abiotically scavenge hydrogen peroxide. *N. maritimus* lacks the hydrogen peroxide-detoxifying enzyme catalase [9], and by removing hydrogen peroxide, α-keto acids may enhance growth.

NO-dismutation and oxygen production

N. maritimus SCM1 has a relatively low affinity for oxygen with a relatively high apparent half saturation constant of Km=3.9 µM [4]. With such a high Km, *N. maritimus* should have difficulties competing with other aerobes utilizing high-affinity oxidases in oxygen-limited environments. We recently showed, however, that *N. maritimus* SCM1 can produce its own oxygen when exposed to anoxia [10], and the oxygen produced is partly used for ammonia oxidation. In the proposed oxygen-production pathway (see graphical abstract), *N. maritimus* reduces nitrite to NO via a NirK-nitrite reductase. We proposed that NO is then dismutated to oxygen and nitrous oxide, which is further reduced to N_2. Producing one oxygen molecule requires four nitrite molecules, and the coupling of NO-dismutation to ammonia oxidation would lead to a net loss of nitrite. The proposed NO-dismutation pathway in *N. maritimus* would constitute the only known oxygen production pathway in the archaeal domain. The proposed pathway in *N. maritimus* is similar to the NO-dismutation pathway proposed for the methanotroph *Candidatus* Methylomirabilis oxyfera [11] in that each organism produces oxygen for the aerobic oxidation of their key electron donor (ammonia or methane). However, intermediates of the pathways seem to differ, and oxygen accumulates in the medium of *N. maritimus*, with the potential to support other aerobes in the environment.

OPEN QUESTIONS

- Several enzymes catalysing steps in the ammonia-oxidation and NO-dismutation pathways remain unidentified. The identification of these missing enzymes is a step crucial for elucidating the pathways and their intermediates.
- So far, oxygen production has only been shown in pure-culture incubations of *N. maritimus*. Is the dark oxygen production pathway present in other AOA? What is the ecological relevance of NO-dismutation and oxygen production by AOA?
- What are the interactions of *N. maritimus* with other microbes in the environment? For example, can the oxygen released by *N. maritimus* support other aerobes in anoxic environments?

Funding information
B.K. and D.E.C acknowledge support from the Villum Foundation, Denmark (Villum Young Investigator Grant 25491 to B.K. and Villum Investigator Grant 16518 to D.E.C.).

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Sollai M, Villanueva L, Hopmans EC, Reichart GJ, Sinninghe Damsté JS. A combined lipidomic and 16S rRNA gene amplicon sequencing approach reveals archaeal sources of intact polar lipids in the stratified Black Sea water column. *Geobiology* 2019;17:91–109.
2. Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, *et al*. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 2005;437:543–546.
3. Metcalf WW, Griffin BM, Cicchillo RM, Gao J, Janga SC, *et al*. Synthesis of methylphosphonic acid by marine microbes: a source for methane in the aerobic ocean. *Science* 2012;337:1104–1107.
4. Martens-Habbena W, Berube PM, Urakawa H, de la Torre JR, Stahl DA. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature* 2009;461:976–979.
5. Walker CB, de la Torre JR, Klotz MG, Urakawa H, Pinel N, *et al*. Nitrosopumilus maritimus genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. *Proc Natl Acad Sci U S A* 2010;107:8818–8823.
6. Kitzinger K, Padilla CC, Marchant HK, Hach PF, Herbold CW, *et al*. Cyanate and urea are substrates for nitrification by Thaumarchaeota in the marine environment. *Nat Microbiol* 2019;4:234–243.
7. Könneke M, Schubert DM, Brown PC, Hügler M, Standfest S, *et al*. Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO2 fixation. *Proc Natl Acad Sci U S A* 2014;111:8239–8244.
8. Qin W, Amin SA, Martens-Habbena W, Walker CB, Urakawa H, *et al*. Marine ammonia-oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation. *Proc Natl Acad Sci U S A* 2014;111:12504–12509.
9. Kim J-G, Park S-J, Sinninghe Damsté JS, Schouten S, Rijpstra WIC, *et al*. Hydrogen peroxide detoxification is a key mechanism for growth of ammonia-oxidizing archaea. *Proc Natl Acad Sci U S A* 2016;113:7888–7893.
10. Kraft B, Jehmlich N, Larsen M, Bristow LA, Könneke M, *et al*. Oxygen and nitrogen production by an ammonia-oxidizing archaeon. *Science* 2022;375:97–100.
11. Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, *et al*. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* 2010;464:543–548.