Microbe profile

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Microbe Profile: *Nitrosopumilus maritimus*

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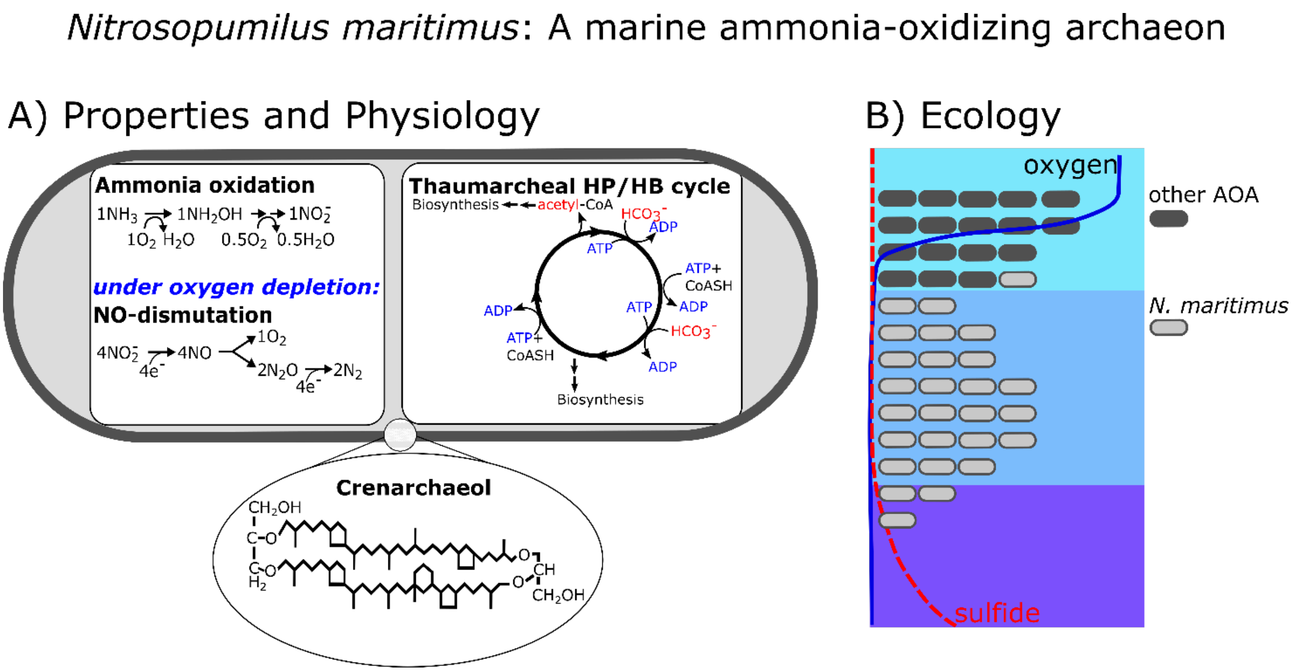
Graphical abstract



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 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.

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, is a chemolithoautotroph, gaining energy from aerobic ammonia oxidation to nitrite [2]. The cells are non-motile straight rods with a diameter of 0.17–0.22μm and a length of 0.5–0.9μm. 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 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 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 also found in oxygen-depleted environments such as marine oxygen-minimum zones and anoxic basins like t the Black sea, even though ammonia-oxidation requires oxygen [1].

Key features and discoveries:

1. 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, catalyzed by a putative ammonia mono oxygenase (AMO) [5]. The gene encoding 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 encoding 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].

2. 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 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].

3. 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 catalyze 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.

4. NO-dismutation and oxygen production

N. maritimus SCM1 has a relative low affinity for oxygen with a relatively high apparent half saturation constant of K_m= 3.9 µM [4]. With such a high K_m, *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 metabolic 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 catalyzing steps in the ammonia-oxidation and NO-dismutation pathways remain unidentified. The identification of these missing enzymes is a 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?

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

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