Linking crenarchaeal and bacterial nitrification to anammox in the Black Sea

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Active expression of putative ammonia monooxygenase gene subunit A (*amoA*) of marine group I *Crenarchaeota* has been detected in the Black Sea water column. It reached its maximum, as quantified by reverse-transcription quantitative PCR, exactly at the nitrate maximum or the nitrification zone modeled in the lower oxic zone. Crenarchaeal *amoA* expression could explain 74.5% of the nitrite variations in the lower oxic zone. In comparison, *amoA* expression by γ-proteobacterial ammonia-oxidizing bacteria (AOB) showed two distinct maxima, one in the modeled nitrification zone and one in the suboxic zone. Neither the *amoA* expression by crenarchaea nor that by β-proteobacterial AOB was significantly elevated in this latter zone. Nitrification in the suboxic zone, most likely microaerobic in nature, was verified by $^{15}NO_2^-$ and $^{15}N^{15}N$ production in $^{15}NH_4^+$ incubations with no measurable oxygen. It provided a direct local source of nitrite for anammox in the suboxic zone. Both ammonia-oxidizing crenarchaea and γ-proteobacterial AOB were important nitrifiers in the Black Sea and were likely coupled to anammox in indirect and direct manners respectively. Each process supplied about half of the nitrite required by anammox, based on ^{15}N-incubation experiments and modeled calculations. Because anammox is a major nitrogen loss in marine suboxic waters, such nitrification–anammox coupling potentially occurring also in oceanic oxygen minimum zones would act as a short circuit connecting regenerated ammonium to direct nitrogen loss, thus reducing the presumed direct contribution from deep-sea nitrate.

ammonia-oxidizing bacteria | *amoA* gene expression | marine group | *Crenarchaeota* | marine nitrogen loss

Nitrification, the stepwise oxidation of ammonium to nitrite and then nitrate, is a key process in marine nitrogen cycling. It is responsible for the formation of the large deep-sea nitrate reservoir. It connects the recycling of organic nitrogen to the ultimate nitrogen loss from the oceans, because its products are substrates for denitrification and anaerobic ammonium oxidation (anammox), the only two presently known nitrogen loss processes. In productive waters such as upwelling regions, high fluxes of organic matter and thus remineralization create strong subsurface oxygen minima, enabling denitrification (1–4) or anammox (5–8) to occur. Nitrogen losses from these oxygen minimum zones (OMZs) are estimated to account for 30–50% of total nitrogen loss from the oceans (9, 10). Because remineralization also releases large amounts of ammonium, high nitrification rates are often associated with these OMZs (11), implying that nitrification may play an important role in promoting marine nitrogen loss.

The Black Sea is the largest marine anoxic basin in the world. A 20- to 40-m-thick suboxic transitional zone, characterized by low oxygen (<5 μM) and undetectable sulfide, persists throughout the basin between the surface oxic layer and the sulfidic anoxic deep water (≥100 m) (12, 13). The exact depth zonation varies according to the location within the basin because of circulation and gyre formation, but similar concentrations of chemical species can be traced along isopycnals or density (σ_t) surfaces throughout the basin (12). Therefore, the Black Sea provides an ideal model system to study nitrogen cycling processes along oxygen gradients. Nitrification has been reported in the lower oxic zone (14) and so has nitrogen loss via anammox in the suboxic zone (15). Nevertheless, the identity and abundance of the responsible nitrifiers, or any coupling between nitrification and nitrogen losses, remain poorly documented.

The first and rate-limiting step of nitrification is aerobic ammonia oxidation. It is a microbially mediated reaction. For decades, only specific groups of β- and γ-proteobacteria have been found to exhibit this capability. However, recent metagenomic studies in the Sargasso Sea (16, 17) and later of a marine sponge symbiont (18) have identified in marine group I (MGI) *Crenarchaeota* genes encoding proteins resembling ammonia monooxygenase (AMO), the key enzyme in aerobic ammonia-oxidizing bacteria (AOB). This chemoautotrophic ammonia-oxidizing potential was confirmed in *Candidatus* "Nitrosopumilus maritimus," an MGI crenarchaeon isolated from a marine aquarium (19). This crenarchaeon is highly similar to the Sargasso Sea phylotypes based on their 16S rRNA (>98% sequence identity) and putative AMO (93–98% amino acids homology) sequences. This putative AMO is, however, only 38–51% (amino acids) homologous to those of AOB. Since then, similar sequences of crenarchaeal AMO gene subunit A (*amoA*) (69–99% amino acid homology) have been detected in various marine water columns and sediments, including the Black Sea (20). Because nonthermophilic MGI *Crenarchaeota* constitute a significant portion of oceanic picoplankton (up to 30%) (21, 22) and a considerable fraction are likely autotrophic (23, 24), it is speculated that these MGI *Crenarchaeota* could be more important nitrifiers in the oceans than the usually less abundant AOB (18, 19). Indeed, crenarchaeal *amoA* genes were reported to be more abundant than bacterial *amoA* in a North Atlantic study (25). However, no published data to date have shown crenarchaeal *amoA* expression in marine environments.

In this study, we provide direct evidence of crenarchaeal *amoA* activities in the Black Sea water column. Its expression was com-

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Abbreviations: AOB, ammonia-oxidizing bacteria; βAOB, β-proteobacterial AOB; γAOB, γ-proteobacterial AOB; MGI, marine group I; *amoA*, ammonia monooxygenase gene subunit A; OMZ, oxygen minimum zone; AMO, ammonia monooxygenase; CARD, catalyzed reporter deposition; qPCR, quantitative PCR; OTU, operational taxonomic unit.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. EF414229–EF414283).

See Commentary on page 6881.

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