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Variability of the transporter gene complement in ammonia-oxidizing archaea

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Ammonia-oxidizing archaea (AOA) are a widespread and abundant component of microbial communities in many different ecosystems. The extent of physiological differences between individual AOA is, however, unknown. Here, we compare the transporter gene complements of six AOA, from four different environments and two major clades, to assess their potential for substrate uptake and efflux. Each of the corresponding AOA genomes encode a unique set of transporters and although the composition of AOA transporter complements follows a phylogenetic pattern, few transporter families are conserved in all investigated genomes. A comparison of ammonia transporters encoded by archaeal and bacterial ammonia oxidizers highlights the variance among AOA lineages as well as their distinction from the ammonia-oxidizing bacteria, and suggests differential ecological adaptations.

Ammonia-oxidizing archaea: a widespread and diverse group of organisms

Ammonia-oxidizing archaea (AOA) were discovered approximately a decade ago [1,2] and are now a major research focus in environmental microbiology. These organisms belong to a recently described phylum, the Thaumarchaeota [3,4], and can be found in almost every aquatic and terrestrial habitat, including extreme environments such as hot springs and Antarctic waters [5–7]. Owing to their high abundance in the oceans and soils, AOA might be among the most abundant groups of organisms in the biosphere [8–10]. AOA are able to grow autotrophically by oxidizing ammonia (NH_3) to nitrite (NO_2^-) as their main source of electrons and energy (see [6] and references therein). Together with their long-known bacterial counterparts, the aerobic autotrophic ammonia-oxidizing bacteria (AOB) [11], AOA might play a significant role in the microbial oxidation of ammonia. Ammonia oxidation represents the first and rate-limiting step of the nitrification process, which is an essential component of the nitrogen cycle [12], and recent studies indicate that AOA might also play significant roles in the production of two greenhouse gases, nitrous oxide [13–15] and methane [16].

Cultivated AOA belong to four lineages [group 1.1a, group 1.1a-associated, group 1.1b, and thermophilic AOA (ThAOA)], which form a well-supported monophyletic clade in many 16S rRNA gene phylogenies (e.g., [17]). Representatives of group 1.1a and 1.1b account for most AOA-related 16S rRNA gene sequences obtained in molecular surveys and are particularly prominent in marine waters and soils, respectively [6,7,18]. Both groups are also represented in other environments and include many sub-clades that can share as little as ~92% 16S rRNA gene sequence identity, which reflects their considerable phylogenetic breadth. Cultivation and environmental studies have shown that AOA are functionally heterogeneous [17,19,20], as indicated by their different ammonia concentration and pH preferences [17,19,21–23], their variable ability to grow on urea as an ammonia source [22,24], and their variable requirements for small amounts of organic compounds to sustain growth [22]. The number and extent of physiological differences among individual AOA have, however, not been systematically investigated and the different environmental resources supporting their growth remain largely unknown.

The set of transport systems encoded in an organism's genome define, to a large extent, its potential to take up environmental resources (e.g., nutrients, water, and ions), resist toxic substances, and sense intercellular signaling compounds. Therefore, the transporter gene complement of an organism also contains information on this organism's ecological preferences. Accordingly, comparative analyses of archaeal and bacterial genomes have indicated that transporter-coding genes are over-represented among recently acquired (horizontally transferred) genes [25–27], which enable microorganisms to sustain activity in their local environment. To explore the metabolic versatility and interspecies variations within AOA, in this study, we compare the transporter gene complements from six AOA strains and discuss both common and unique characteristics of these organisms (lists of the fully annotated transporter genes are available at the Archaea Biology and Ecogenomics Division, University of Vienna website: http://genetics-ecology.univie.ac.at/aoa_transport_systems.html). The analyzed AOA genomes include members of group 1.1a and

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Table 1. Number of transport systems and transporter families in selected AOA genomes

Organism		*Nitrosopumilus maritimus* SCM1		*Nitrosoarchaeum limnia* SFB1		*Nitrosoarchaeum koreensis* MY1		*Cenarchaeum symbiosum* A		*Nitrososphaera gargensis*		*Nitrososphaera viennensis* EN76	
Affiliation		Group 1.1a (Alpha I-clade)[a]		Group 1.1a (Alpha II-clade)[a]		Group 1.1a (Alpha II-clade)[a]		Group 1.1a (Beta-clade)[a]		Group 1.1b		Group 1.1b	
Habitat		Marine water aquarium sediment		Brackish water sediment		Rhizosphere soil of *Caragana sinica*		Tissues of the sponge *Axinella mexicana*		Microbial mat (Garga spring outflow pond)		Garden soil	
Ammonia sources[b]	Ammonia	+		+		+		n.d.		+		+	
	Urea	n.d.		n.d.		n.d.		n.d.		+		+	
Carbon sources[b]	Bicarbonate	+		+		+		n.d.		+		+	
	Organic acids	n.d.		n.d.		n.d.		n.d.		n.d.		+[c]	
Genome size in Mb (Nb. contigs)		1.65 (1)		1.77 (76)		1.61 (1)		2.05 (1)		2.83 (1)		2.53 (1)	
Refs		[44]		[87]		[88]		[45]		[43]		This study [d]	
Number of transport systems and relative abundance of transporter classes [e, f, g]													
Total		51		60		50		33		83		78	
Channels/pores		11	21.6%	9	15.0%	8	16.0%	7	21.2%	15	18.1%	11	14.1%
Carrier-type transporters		21	41.2%	32	53.3%	25	50.0%	10	30.3%	42	50.6%	36	46.2%
Primary active transporters		10	19.6%	9	15.0%	8	16.0%	9	27.3%	14	16.9%	19	24.4%
Uncharacterized transporters		9	17.6%	10	16.7%	9	18.0%	7	21.2%	12	14.5%	12	15.4%
Number and distribution of transporter families within the main classes of transporters [g]													
Total		35		42		36		27		47		43[h]	
Channels/pores		6	17.1%	5	11.9%	5	13.9%	5	18.5%	8	17.0%	6	14.0%
Carrier-type transporters		15	42.9%	21	50.0%	17	47.2%	8	29.6%	22	46.8%	20	46.5%
Primary active transporters		8	22.9%	8	19.0%	7	19.4%	9	33.3%	9	19.1%	10	23.3%
Uncharacterized transporters		6	17.1%	8	19.0%	7	19.4%	5	18.5%	8	17.0%	7	16.3%

[a]Clade designations are according to [89].

[b]Positive signs ''+'': evidence for growth on the corresponding substrate. n.d.: not determined on the basis of growth experiments.

[c]Pyruvate, oxaloacetate, 2-oxoglutarate and glyoxylate stimulate *N. viennensis* EN76 growth on ammonia and bicarbonate [22,42].

[d]The genome of *N. viennensis* EN76 has been submitted to GenBank under Accession Number CP007536.

[e]In the case of heteromultimeric transporters, only complete transport systems were counted and not every open reading frames encoding the protein subunits.

[f]Fragmented open reading frames were considered as pseudogenes and transport systems encoded by fragmented open reading frames were therefore excluded from the counts.

[g]Several proteins and protein complexes referenced in the transporter classification database (TCDB) [28] and present in all six AOA genomes were not taken in account in this analysis, i.e., proton-pumping complexes of the respiratory electron-transfer chain, the general and twin-arginine secretory pathways for protein export, a putative group-translocating polysaccharide exporter, transmembrane electron carriers and accessory factors involved in transport.

[h]One transport system in *N. viennensis* EN76 could not be assigned to a transporter family (Figure 1) and was excluded from this count.

1.1b (Table 1). Two of the organisms investigated are pelagic marine (*Nitrosopumilus maritimus* SCM1) or brackish water (*Nitrosoarchaeum limnia* SFB1) organisms, one is a sponge symbiont (*Cenarchaeum symbiosum* A), two are from soil (*Nitrosoarchaeum koreensis* MY1 and *Nitrososphaera viennensis* EN76) and one is from a moderately thermophilic biofilm (*Nitrososphaera gargensis*).

The transport systems of AOA

Transporters are classified into four major classes according to the mechanism and energy source driving the transport process: (i) channels that facilitate the diffusion of substrates, usually by a passive mechanism; (ii) carriers that include both passive and active transporters, the latter using chemiosmotic energy; (iii) primary active transporters (PAT), which use primary energy sources (e.g., ATP) to transport solutes; and (iv) group translocators (e.g., phosphotransferase systems (PTS)), which chemically modify the substrate during the transport process [28]. Results from a genome study [29] suggest that channels, carriers, and PAT are well represented in the Crenarchaeota and Euryarchaeota phyla, whereas group translocators and PTS in particular appear less common in archaea, which is in stark contrast to their wide occurrence in bacteria. Thus far, the genomes of haloarchaeal euryarchaea, including *Haloferax volcanii*, *Haloarcula marismortui*, *Haloarcula hispanica*, *Halalkalicoccus jeotgali*, and *Haloterrigena turkmenica* [30,31], are the sole archaeal genomes known to encode PTS. The composition, relatedness, and adaptive function of archaeal transporter complements remain however largely unknown, especially in recently discovered archaeal lineages.

The genomes of AOA encode different numbers of transport systems

All six of the examined AOA genomes encode channels, carriers, and PAT, whereas PTS are absent (Table 1). In addition, several partially characterized and putative transport systems, classified here as uncharacterized transporters, are also present. The relative abundance patterns of the four classes of transporters (channels, carriers, PAT, and uncharacterized transporters) are similar in the free-living