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Abundance and Composition of Epiphytic Bacterial and Archaeal Ammonia Oxidizers of Marine Red and Brown Macroalgae

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Ammonia-oxidizing bacteria (AOB) and archaea (AOA) are important for nitrogen cycling in marine ecosystems. Little is known about the diversity and abundance of these organisms on the surface of marine macroalgae, despite the algae's potential importance to create surfaces and local oxygen-rich environments supporting ammonia oxidation at depths with low dissolved oxygen levels. We determined the abundance and composition of the epiphytic bacterial and archaeal ammonia-oxidizing communities on three species of macroalgae, *Osmundaria volubilis*, *Phyllophora crispa*, and *Laminaria rodriguezii*, from the Balearic Islands (western Mediterranean Sea). Quantitative PCR of bacterial and archaeal 16S rRNA and *amoA* genes was performed. In contrast to what has been shown for most other marine environments, the macroalgae's surfaces were dominated by bacterial *amoA* genes rather than those from the archaeal counterpart. On the basis of the sequences retrieved from AOB and AOA *amoA* gene clone libraries from each algal species, the bacterial ammonia-oxidizing communities were related to *Nitrosospira* spp. and to *Nitrosomonas europaea* and only 6 out of 15 operational taxonomic units (OTUs) were specific for the host species. Conversely, the AOA diversity was higher (43 OTUs) and algal species specific, with 17 OTUs specific for *L. rodriguezii*, 3 for *O. volubilis*, and 9 for *P. crispa*. Altogether, the results suggest that marine macroalgae may exert an ecological niche for AOB in marine environments, potentially through specific microbe-host interactions.

Macroalgae and other marine eukaryotes, such as corals and sponges, harbor complex epiphytic microbial communities (39). These communities are distinct from the planktonic ones, indicating that macroalgae are specific and unique habitats for microorganisms (7, 25, 26). The composition and diversity of bacterial communities in macroalga-related biofilms have been surveyed (7, 15, 25, 26, 47, 53) and have in some cases been shown to depend on the specific attraction that algal exudates may exert on certain bacteria but also on the antimicrobial effect of some compounds produced by the algae (27). Macroalgal epiphytic bacterial communities have been reported to be host specific (25, 29), and their composition can even differ between the algal rhizoid, cauloid, meristem, and phyloid, indicating a close host-bacterium interaction (47). However, although the bacterial community composition of the green alga *Ulva australis* was unique compared to that of the planktonic community, there was a high variability among individuals (7), which suggests that algal surface colonization is driven by selection processes in combination with stochastic recruitment of bacteria from the planktonic community.

Algal surfaces are important microbial habitats in the marine environments, but most experimental studies have focused on bacterial communities and no attention has been paid to archaea as members of the epiphytic communities. Conversely, archaea have been widely studied in marine environments, especially after the discovery of the ammonia-oxidizing archaea (AOA). The AOA are key players in nitrogen cycling in marine systems by performing the first step of nitrification, a process that the ammonia-oxidizing bacteria (AOB) also carry out (13, 56). AOB and AOA belong to the phyla proteobacteria and thaumarchaeota, respectively (6, 24). Phylogenetic studies on ammonia oxidizers are mainly based on the analysis of the *amoA* gene, coding for the α subunit of the ammonia monooxygenase (20, 44). The ammonia oxidizers are present in marine environments either as planktonic populations (21, 34) or on host-associated marine microbial communities of corals (3) and sponges (35, 48). In all examples of marine environments, the AOA outnumber the AOB. For algal species, the ammonia oxidizers have not been described. However, we hypothesize that macroalgae may offer a selective habitat for ammonia oxidizers and hot spots for nitrification since algae produce oxygen, which is needed for ammonia oxidation. This may be important, especially at depths with low dissolved oxygen levels. However, epiphytic communities in general, let alone the AOA and AOB within these communities, are basically unknown at mesophotic depths (30 to 200 m), since all the studies have focused on macroalgae inhabiting shallower depths (25, 26, 47, 53).

To address the importance of macroalgae as habitats for AOA and AOB in the mesophotic zone, we determined their composition and abundance on the surface of three dominant seascape-building macroalgae in the northwestern Mediterranean Sea. Host specificity was analyzed by sequencing bacterial and archaeal *amoA* genes from the surface of *Osmundaria volubilis*, *Phyllophora crispa*, and *Laminaria rodriguezii*.

MATERIALS AND METHODS

Site description and sampling. Samples were collected from the MEDITS_ES05 2008 bottom trawl survey (4), conducted in June 2008

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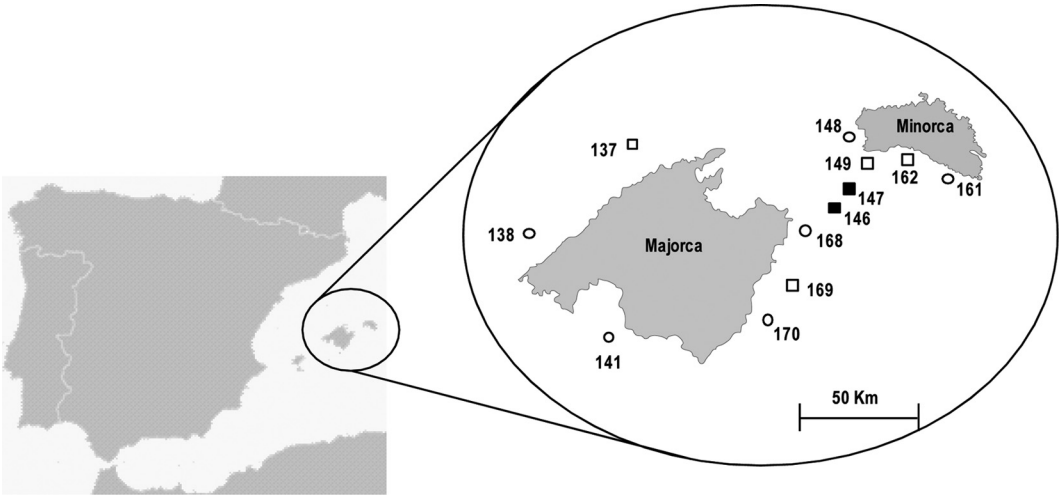

FIG 1 Geographic situation of sampling locations in the Balearic Islands. Seascapes dominated by *Laminaria rodriguezii* are shown with closed symbols. Seascapes dominated by *Osmundaria-Phyllophora* communities are shown with open symbols. Different shapes indicate the depth intervals for each sample: ○, 50 to 64 m; □, 65 to 81 m.

along the shelf and slope (50 to 100 m depth) of Majorca and Minorca Islands, on board the R/V *Cornide de Saavedra* (Fig. 1). Estimation of the physical and chemical parameters of water masses around the Balearic Islands is regularly performed by the Spanish Institute of Oceanography following the European Framework Directive (5, 31) with the monitoring system RADMED (54). Water around the Balearic Islands has an Atlantic origin, with homoeothermic winter and summer stratification (13 to 26°C). Surface salinity values vary from 37.5 to 38.2 practical salinity units (psu). These waters are oligotrophic, with low nitrate concentrations (0.75 μM), and show maximum fluorescence values of 1.5 mg/m^3 in spring and summer at 60 to 80 m depth. Fluorescence is generated by photosynthetic pigments, organic matter, and suspended particles. The maximum dissolved oxygen concentration in the mixed water mass is reached in winter, with about 6.5 ml/liter. During summer, these values are maintained at the thermocline and decrease down to 4.5 ml/liter at 200 m depth. The water masses in the area are slightly alkaline, with the pH ranging from 8.1 to 8.3.

The sampling scheme and gear were the same as those used throughout the northern Mediterranean coast in the international MEDITS surveys (4). This sampling strategy is appropriate for screening of algae at depths that cannot be reached by scuba diving (19). A total of 12 samplings were obtained, resulting in 26 samples of algal thalli from the red algae *Osmundaria volubilis* (Linnaeus) R. E. Norris and *Phyllophora crispa* (Hudson) P. S. Dixon and the brown alga *Laminaria rodriguezii* Bornet, which are important seascape builders in this area (1, 19). When possible, we separated three replicates with intact phyloids of similar size for each species and sampling point. The specimens were gently cleaned in seawater and stored at −20°C until further analysis.

DNA extraction and quantification. Clean phyloids of several individuals were cut in pieces of approximately 2 cm^2 and distributed in three subsamples of 1 g of fresh material. For biofilm detachment from the algal surfaces, the cut algae were immersed in 2 ml of 0.1 M sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$). Biofilm was dislodged by two rounds of sonication for 20 s at maximum intensity (Selecta, Barcelona, Spain), followed by 30 s on ice (23). Algal suspensions were centrifuged at 10,000 × *g* for 2 min, and supernatants were discarded. Cell pellets were resuspended in 0.6 ml of sodium phosphate buffer. Nucleic acids were extracted using a FastDNA Spin kit for soil (MP, Biomedicals) and stored at −20°C.

Quantitative PCR. Selective quantification of AOA and AOB was done by targeting the *amoA* genes using the primer pairs amoA1F-amoA2R (44) and crenamoA23F-crenamoA616R (51), while the primers 341F (37) and 534R (30) and crenar771F and crenar957R (38) were used for quantification of the 16S rRNA genes of the domain bacteria and the archaeal phyla crenarchaeota and thaumarchaeota, respectively. Quantitative PCR (qPCR) was performed as described previously with minor modifications (17, 38). Quantifications were carried out in a Bio-Rad IQ5 thermal cycler (Bio-Rad, Richmond, CA), using a Dynamo Flash SYBR green qPCR kit (Finnzymes, Oy, Espoo, Finland). Twenty-microliter reaction mixtures contained 1× SYBR green master mix, 1 μg/μl bovine serum albumin (BSA), and 10 ng of DNA. Primer concentrations used were 1 μM for 16S rRNA and 0.5 μM for *amoA*. The standard curves were obtained from serial dilutions of linearized plasmids (pGEM-T Easy; Promega, Madison, WI) containing standard sequences. The PCR efficiency ranged between 80 and 100%. Negative controls resulted in undetectable values in all cases. To detect possible inhibitory effects, 10^5 copies of the pGEM-T Easy plasmid (Promega) were mixed with sample DNA and quantified with plasmid-specific primers T7 and SP6. The obtained cycle thresholds (C_Ts) were not significantly different from those obtained when quantifying the plasmid alone.

Statistical analysis of gene abundances. Sampling locations were grouped into two different seascape categories according to the dominant algal species, in agreement with previous results that statistically tested the presence and dominance of different algal species around the islands (19). These categories corresponded to *Laminaria*-dominated assemblages in samplings 146 and 147 and *Osmundaria-Phyllophora*-dominated assemblages at the rest of the locations. Additionally, samples were grouped into two depth categories (above 65 m and below 65 m) (Fig. 1). Differences in gene abundances in relation to the depth, algal species, and algal seascape categories were calculated using the Kruskal-Wallis test for nonnormal distributions. Pairwise comparisons of the means of the 4 genes quantified were analyzed using a Mann-Whitney U test; and linear regressions, significance, and Spearman correlation coefficients among gene abundances were calculated. All the statistical analyses were performed with SPSS for Windows (version 15.0; SPSS, Inc.).

PCR, cloning, and sequencing of *amoA* genes. All the DNA extractions obtained from the same algal species were pooled to minimize individual diversity and to determine the host-specific AOA and AOB *amoA* gene diversity. Clone libraries of archaeal and bacterial *amoA* genes were constructed for the three algal species. PCR amplifications were performed in a GenAmp PCR system 9700 (Applied Biosystems, Foster City, CA) using the same primers used for qPCR. In both cases, reaction mixtures contained 2.5 U of DreamTaq DNA polymerase (Fermentas, Hanover, MD), 1× PCR buffer, 0.2 μM each primer, 0.2 mM deoxynucleoside triphosphates, 0.1 μg/μl BSA (New England BioLabs, Beverly, MA), and

TABLE 1 Sampling dates, depths, locations, seascape types, algal species, and abundance of bacterial and thaumarchaeal 16S rRNA genes and bacterial and archaeal *amoA* genes

Sampling	Sampling date (day/mo/yr)	Depth (m)	Location	Seascape[a]	Species	No. of copies/g (dw) of algae[b]			
						16S rRNA gene		*amoA* gene	
						Bacteria (10^9)	Archaea[c] (10^6)	AOB (10^7)	AOA (10^6)
137	09/06/2008	72	Ponent Mallorca (Sóller)	*Osmundaria-Phyllophora*	*P. crispa*	1.70 ± 0.86	2.10 ± 0.52	1.83 ± 0.68	4.50 ± 2.27
138	09/06/2008	62	Ponent Mallorca (Dragonera)	*Osmundaria-Phyllophora*	*P. crispa*	1.84 ± 0.05	2.22 ± 0.56	2.61 ± 1.52	2.37 ± 0.68
141	10/06/2008	60	Migjorn Mallorca (Badia)	*Osmundaria-Phyllophora*	*P. crispa*	1.51 ± 0.17	1.55 ± 1.48	1.53 ± 1.54	1.68 ± 1.20
					O. volubilis	0.57 ± 0.14	0.12 ± 0.12	1.07 ± 1.05	1.24 ± 1.22
					L. rodriguezii	1.27 ± 0.21	1.59 ± 1.46	1.92 ± 0.16	3.21 ± 1.44
146	11/06/2008	81	Canal Menorca	*Laminaria*	*O. volubilis*	1.84 ± 0.92	2.61 ± 0.76	1.88 ± 0.90	12.70 ± 9.21
					L. rodriguezii	0.42 ± 0.20	0.54 ± 0.03	0.64 ± 0.15	1.24 ± 0.67
147	11/06/2008	77	Canal Menorca	*Laminaria*	*O. volubilis*	2.50 ± 1.29	2.85 ± 1.21	4.55 ± 1.71	5.20 ± 3.43
					L. rodriguezii	69.20 ± 11.60	6.03 ± 2.17	30.50 ± 4.90	5.00 ± 3.84
148	11/06/2008	57	Migjorn Menorca (Ciutadella)	*Osmundaria-Phyllophora*	*P. crispa*	3.60 ± 1.99	2.26 ± 3.22	3.03 ± 3.46	1.31 ± 0.64
					O. volubilis	2.35 ± 1.20	2.11 ± 0.84	2.36 ± 1.02	3.72 ± 0.89
					L. rodriguezii	2.22 ± 1.72	1.18 ± 0.96	2.21 ± 1.61	4.25 ± 3.42
149	11/06/2008	77	Migjorn Menorca (Ciutadella)	*Osmundaria-Phyllophora*	*P. crispa*	10.90 ± 2.48	2.77 ± 1.57	7.39 ± 2.59	2.21 ± 0.65
					O. volubilis	37.70 ± 28.70	7.98 ± 6.10	17.10 ± 10.50	7.38 ± 6.80
					L. rodriguezii	44.70 ± 44.10	3.17 ± 3.12	17.60 ± 15.40	1.14 ± 0.99
161	15/06/2008	58	Migjorn Menorca (Maó)	*Osmundaria-Phyllophora*	*P. crispa*	5.86 ± 4.07	4.36 ± 2.32	4.81 ± 2.48	6.57 ± 6.06
					O. volubilis	1.69 ± 0.98	2.06 ± 1.67	2.23 ± 1.07	2.81 ± 2.07
162	15/06/2008	65	Migjorn Menorca (Maó)	*Osmundaria-Phyllophora*	*P. crispa*	3.95 ± 1.66	3.55 ± 0.09	6.35 ± 2.65	3.17 ± 1.02
					O. volubilis	4.54 ± 2.68	7.49 ± 3.98	6.72 ± 2.19	7.45 ± 3.85
168	17/06/2008	57	Llevant Mallorca (Capdepera)	*Osmundaria-Phyllophora*	*P. crispa*	3.93 ± 2.59	3.17 ± 2.28	5.93 ± 4.14	1.28 ± 1.25
					O. volubilis	4.35 ± 1.08	2.75 ± 0.80	5.32 ± 0.94	1.64 ± 0.58
169	17/06/2008	75	Llevant Mallorca (Portocolom)	*Osmundaria-Phyllophora*	*P. crispa*	2.21 ± 0.76	2.11 ± 1.04	1.79 ± 0.83	4.77 ± 2.85
					O. volubilis	3.41 ± 1.71	3.85 ± 3.37	5.18 ± 3.90	6.37 ± 5.05
					L. rodriguezii	2.04 ($n = 1$)	1.19 ($n = 1$)	2.42 ($n = 1$)	1.63 ($n = 1$)
170	17/06/2008	52	Llevant Mallorca (Portocolom)	*Osmundaria-Phyllophora*	*P. crispa*	5.81 ± 5.13	3.81 ± 2.51	6.59 ± 4.83	1.95 ± 1.73
					O. volubilis	6.43 ± 2.13	5.87 ± 3.47	9.50 ± 3.47	4.35 ± 3.28

[a] Two seascape types were found: one dominated by *Osmundaria-Phyllophora* and one dominated by *Laminaria*.

[b] Means of three replicates and standard deviations are shown, unless stated otherwise.

[c] Thaumarchaeota and crenarchaeota.

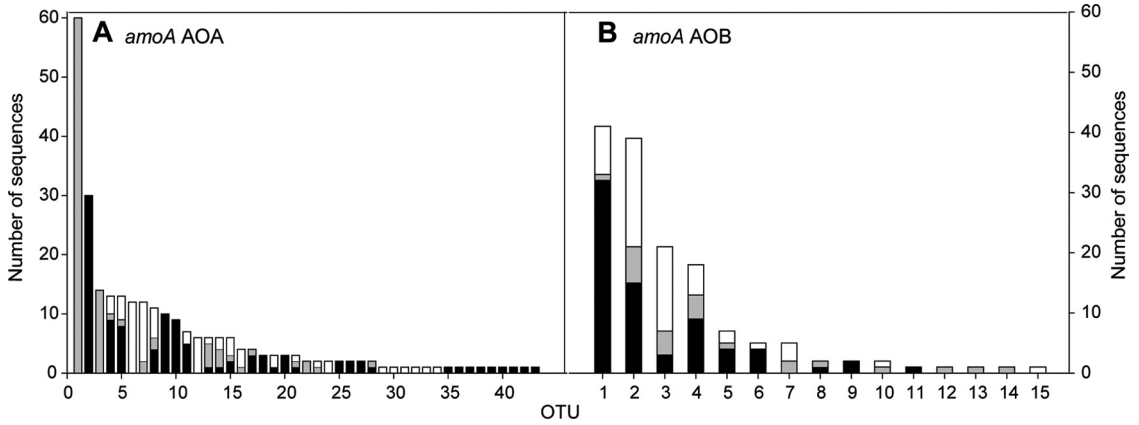

FIG 2 Rank-abundance curves showing the number of OTUs obtained for archaeal (A) and bacterial (B) *amoA* sequences corresponding to three different algal species. OTUs were obtained using a cutoff distance of 5% for each algal species; gray bars, *O. volubilis*; white bars, *P. crispa*; black bars, *L. rodriguezii*.

2.2 mM $MgCl_2$ in a total volume of 50 μl. Thermal conditions used were 5 min at 94°C, 42 cycles of 30 s at 94°C, 30 s at 55°C, and 60 s at 72°C, and a final extension of 10 min at 72°C. PCR products were excised from an agarose gel, purified using an Ultraclean DNA purification kit (MoBio, Carlsbad, CA), and then cloned using the pGEM-T Easy vector and *Escherichia coli* JM109 competent cells (Promega). Clones were screened for correct insert size by PCR with primers T7 and SP6 and sequenced using T7 primer (Macrogen, Seoul, South Korea). In total, 411 sequences were recovered.

Sequences analysis and phylogeny. Sequences were checked for chimeras and manually refined by using the BioEdit (version 7.0) package (16). Alignment of sequences and clustering (cutoff = 0.05) were done using the platform mothur (version 1.11.0) (46). Operational taxonomic units (OTUs) were defined at the same cutoff value and used to construct rarefaction curves, to estimate richness (Chao1), and to calculate diversity indices (Shannon) (9, 33). This procedure was repeated using single clone libraries for each algal species and for all the sequences retrieved from the three algal species to compare for shared OTUs between clone libraries.

Representative sequences for each OTU were identified as the sequences having the maximum average similarity to the other sequences in the same OTU and were used for a phylogenetic tree reconstruction by neighbor joining (NJ). Phylogenetic trees with 1,000 bootstrap replicates were obtained using the MEGA program (version 4.0) (50). Gene clusters were named according to Purkhold et al. (42) and Stopnišek et al. (49). To compare the AOA or AOB community diversity in the three algae, weighted and unweighted UniFrac analyses were performed (32).

Nucleotide sequence accession numbers. Partial *amoA* gene sequences obtained from the AOB and AOA were deposited in GenBank under accession numbers JF715503 to JF715649 and JF715650 to JF715913, respectively.

RESULTS

Abundance of 16S rRNA and *amoA* genes. For the bacterial 16S rRNA genes, abundances ranged from 5×10^8 to 7×10^{10} gene copies/g (dry weight [dw]) of algae, whereas for the thaumarchaeota and crenarchaeota, values were significantly lower ($P = 0.005$) and ranged from 1×10^5 to 3×10^6 (Table 1). Similarly to 16S rRNA genes, *amoA* abundances were significantly higher for bacteria than for archaea ($P < 0.001$), with 6×10^6 to 3×10^8 and 4×10^5 to 1×10^7 copies/g dw of algae, respectively (Table 1).

Algal seascapes were the only *a priori*-defined groups displaying significant differences of the abundances according to algal species. AOA *amoA* gene abundances were significantly lower on the surface of *O. volubilis* than on the surface of the other two algal species in the *Laminaria* seascapes ($P = 0.005$). Pairwise correlations between bacterial 16S rRNA and bacterial *amoA* gene abundances were highly significant regardless of the algae studied in the *Osmundaria-Phyllophora* seascapes (see Table S1 in the supplemental material), supporting the idea that the proportion of AOB in relation to total bacteria was stable (about 0.01). Significant correlations between 16 rRNA and *amoA* genes were also found, with a proportion of 1. In contrast, significant correlations between AOA and AOB *amoA* genes were found only for *O. volubilis* in *Osmundaria-Phyllophora* seascapes, suggesting a clear differentiation between the two groups of ammonia oxidizers (see Table S1 and Fig. S1 in the supplemental material).

Effects of algal species on ammonia-oxidizing community diversity and composition. The AOA community was more diverse than the AOB counterpart (see the text and Fig. S2 in the supplemental material). Moreover, the AOA presented higher host specificity, with 29 out of 43 OTUs containing sequences from one algal epiphytic community (Fig. 2A). The three most abundant OTUs were specific for *O. volubilis* and *L. rodriguezii*. For bacterial *amoA* genes, host specificity was less pronounced. Out of the 15 OTUs, only 6 were specific, and all specific OTUs contained only 1 or 2 sequences (Fig. 2B). The observed differences at the OTU level were confirmed by the UniFrac analysis comparing the AOB and AOA communities (Table 2). The

TABLE 2 UniFrac scores and *P* values when comparing bacterial and archaeal ammonia oxidizer communities in the three algal species *O. volubilis*, *P. crispa*, and *L. rodriguezii*

UniFrac test	Groups compared	Score	P^a
Unweighted	Bacterial *L. rodriguezii-O. volubilis-P. crispa*	0.54	*
	Archaeal *L. rodriguezii-O. volubilis-P. crispa*	0.74	***
Weighted	Bacterial *P. crispa-L. rodriguezii*	1.52	***
	Bacterial *O. volubilis-P. crispa*	0.83	NS
	Bacterial *L. rodriguezii-O. volubilis*	2.14	***
	Archaeal *P. crispa-L. rodriguezii*	1.72	***
	Archaeal *O. volubilis-P. crispa*	2.00	***
	Archaeal *L. rodriguezii-O. volubilis*	0.76	***

[a] NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

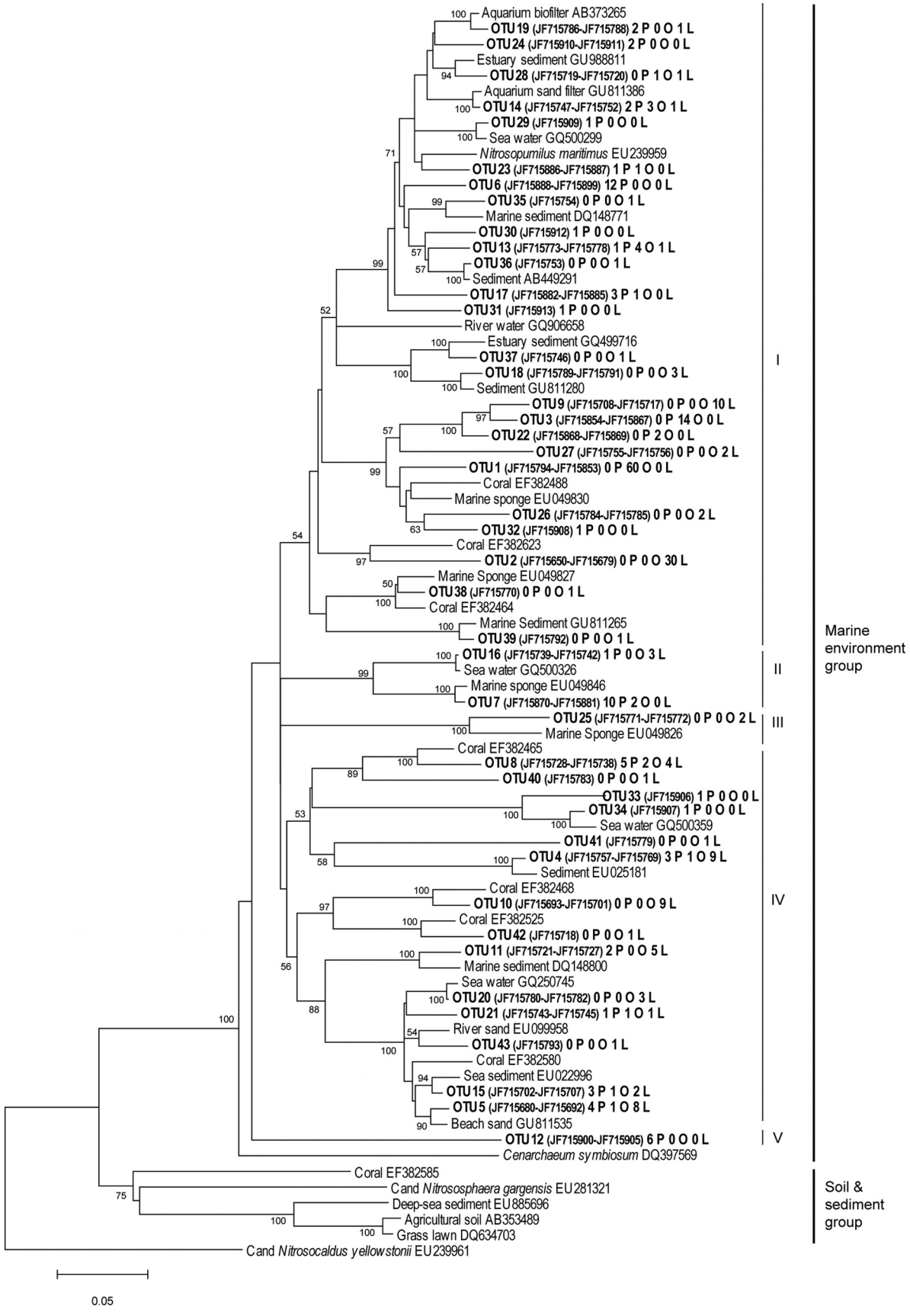

FIG 3 Neighbor-joining phylogenetic tree of archaeal *amoA* sequences from epiphytic communities of three different algal species. Representative sequences of each OTU (cutoff distance, 5%) are shown in bold, followed by the GenBank accession numbers of all the sequences in the same OTU. Origins of the sequences in each OTU are indicated with letter codes (O, *O. volubilis*; P, *P. crispa*; and L, *L. rodriguezii*). Bootstrap values higher than 50% are indicated at branch points (1,000 replicates). Reference sequences from the GenBank database are added.

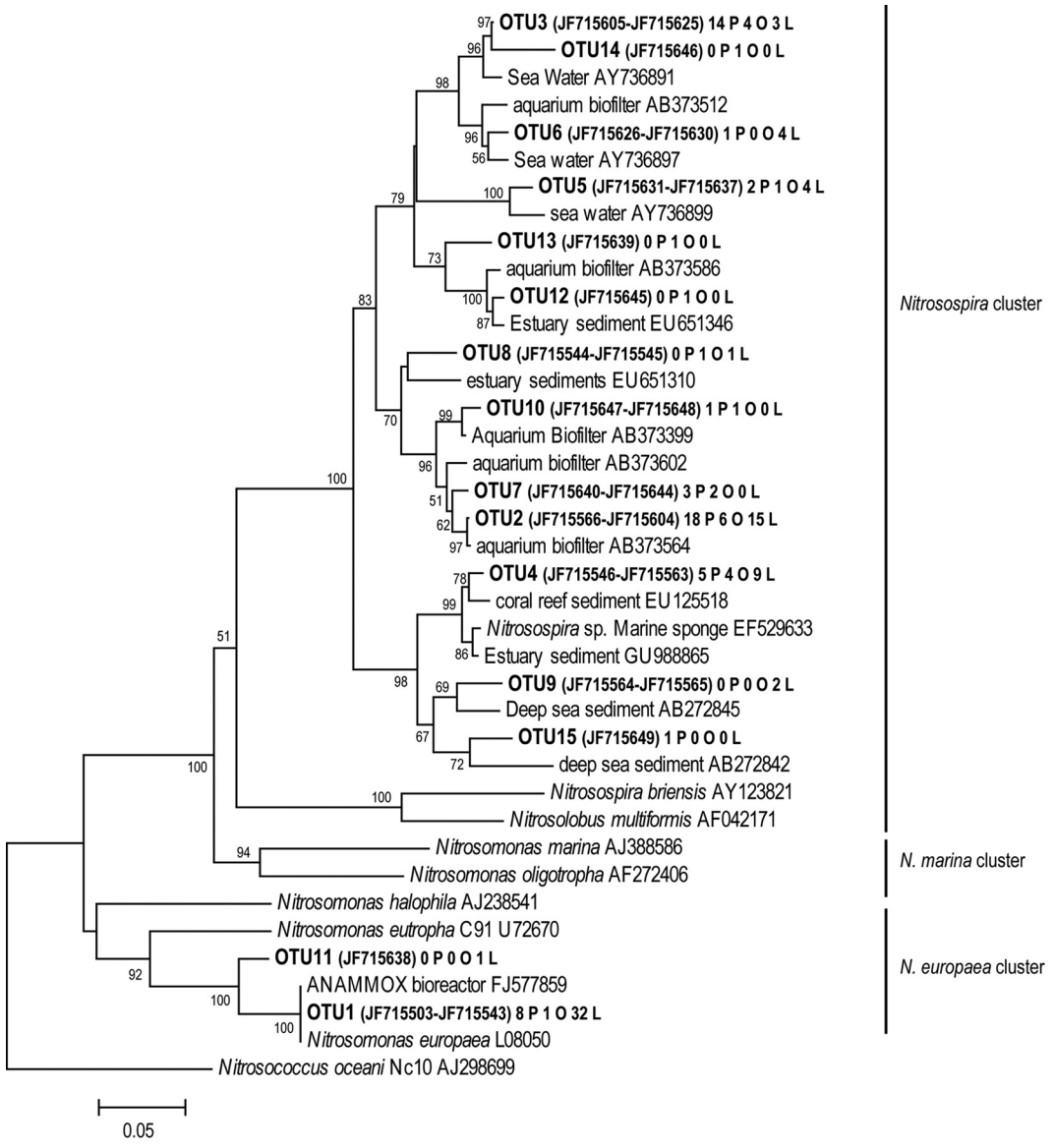

FIG 4 Neighbor-joining phylogenetic tree of bacterial *amoA* sequences from epiphytic communities of three different algal species. Representative sequences of each OTU (cutoff distance, 5%) are shown in bold, followed by the GenBank accession numbers of all the sequences in the same OTU. Origins of the sequences in each OTU are indicated with letter codes (O, *O. volubilis*; P, *P. crispa*; and L, *L. rodriguezii*). Bootstrap values higher than 50% are indicated at branch points (1,000 replicates). Reference sequences from the GenBank database are added.

weighted analysis showed that the specific selection of the macroalgae was stronger on the AOA than the AOB. For the AOA, significant differences in all pairwise comparisons between algal species were found, but no significant differences for the bacterial communities associated with *O. volubilis* and *P. crispa* were detected (Table 2).

The archaeal *amoA* sequences showed from 82 to 99% similarity to previously published sequences, with a majority assigned to *amoA* genes from seawater, marine sediments, and symbionts of corals or marine sponges. All clones distributed in five clusters in the marine environment group 1.1a (41, 49) (Fig. 3). Cluster I contained sequences from 25 OTUs, most of them species specific. Clusters III and V contained sequences specific for *L. rodriguezii* and *P. crispa*, respectively, and clusters II and IV contained non-species-specific OTUs. The bacterial *amoA* sequences showed 93 to 100% similarity to members of the *Nitrosomonas europaea* and the *Nitrosospira* clusters (42) (Fig. 4). The majority of sequences belonged to the *Nitrosospira* cluster and distributed in 13 OTUs. These sequences were closely related to sequences found in seawater and sediments, aquarium filters, and coral reef sediments. The *Nitrosospira* cluster contained one specific OTU for *P. crispa*, three for *O. volubilis*, and one for *L. rodriguezii*. The *Nitrosomonas europaea* cluster contained sequences belonging to two OTUs, with one being specific for *L. rodriguezii*.

DISCUSSION

In all algal samples, AOB *amoA* genes were about 10 times more abundant than those from the AOA. This contrasts with the ma-

jority of marine environments, in which AOA dominate (11, 13), with a few exceptions (8, 36). The relative proportions of AOB and AOA differ significantly between compartments of the sea. For example, on marine sponges, the abundance of AOA assessed by fluorescent *in situ* hybridization measurements was only twice as high as that of AOB (3), whereas the relative proportion of AOA increased up to 90 to 99% in open ocean waters (55), and AOA completely outnumbered AOB in corals (3). An estimation of the cell number cannot be translated directly from gene abundances since differences in the number of copies of a gene in a genome may exist between phylotypes. However, a mean value of 2.5 *amoA* gene copies per genome has been proposed for the AOB, whereas the information available for AOA describes a single *amoA* gene copy per genome (18, 20, 22, 28). Using these estimations for our data, the cell number of the AOB was six times higher than that of the AOA, showing that surfaces of macroalgae favor the presence of AOB instead of AOA. If we compare the estimated number of total bacterial cells (average 16S rRNA gene copy number, 3.6 per cell) to the estimated number of AOB, the bacterial ammonia oxidizers comprise 1% of the total bacterial community. In comparison, only 0.1% of the total bacteria found on marine sponges were *Nitrosospira* spp. (2). Our results suggest that the three macroalgae studied here are important and selective habitats for AOB in the epiphytic biofilms. However, the reasons for this enrichment remain elusive. Possible explanations could be favorable physicochemical conditions over the algal surface or the fact that enrichment is a result of a combination of specific positive and negative epiphyte-host interactions. Further experiments are needed to determine the local environmental conditions that determine selection for AOB rather than AOA.

In general, the host organism had a greater influence than depth and geographical location on both AOB and AOA community composition. Interestingly, the UniFrac analyses revealed that both AOA and AOB communities specific for *L. rodriguezii* were significantly different from those specific for the other macroalgal species. *L. rodriguezii* is a brown seaweed that differs from the red algae *O. volubilis* and *P. crispa* in several ways. *L. rodriguezii* assemblages are found in areas with unidirectional currents and low water temperatures (12, 14) and are normally restricted to deeper areas, those below 60 m (40). At our sampling locations, *O. volubilis* and *P. crispa* formed codominant assemblages, and they are known to spread over rocky or detrital bottoms at depths of between 50 to 70 m (1, 19). Moreover, the cell wall composition and the main storage carbohydrates are different between the three algal species considered (10, 43). Availability of organic compounds for mixotrophic nitrification has recently been considered a selective factor for AOA (45, 52). Unfortunately, no information about exudates produced by these macroalgae exists, but the exudates produced could potentially be a key factor that explains the greater host specificity observed among the AOA than the AOB community members. In agreement with our study, others have shown that a core epiphytic bacterial community can be defined at the algal species level, indicating a specific selection for particular microbial epiphytes (25, 26, 53). However, whether this applies to other macroalgal species is not known. All our obtained archaeal *amoA* sequences grouping in the marine environment group (49, 51) were closely related to sequences retrieved from corals and sponges, indicating that high similarities exist among these epiphytic microbial communities. The AOB communities were mainly represented by *Nitrosospira* sp.-related sequences, and, in agreement, *Nitrosospira* spp. have also been described to be the most abundant AOB present in sponges (2, 35). Among our algal species, the differences in the AOA communities in particular indicate host specificity, but larger sample sets are needed to establish if a defined host-specific core community exists.

In summary, we have shown that algal species may exert a specific ecological niche for AOB in the marine environment, traditionally considered to be dominated by AOA. We calculated that AOB account for about 1% of the total bacterial community on the algal surfaces and can be up to 6 times more abundant than the AOA. Similar proportions were found for the three algal species dominating in the mesophotic zone in the western Mediterranean Sea (Balearic Islands). Despite their lower abundance, the AOA showed a higher host specificity, indicating a closer relationship between the algal species and the AOA compared to the AOB. Further studies should aim at deciphering potential interactions between algal species and ammonia-oxidizing bacteria and archaea and determining if the higher abundance of AOB on algal surfaces equals a hot spot for nitrification in marine environments.

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