

# Diversity of nitrifying bacteria in microbial communities from water and epilithic biofilms of the Lake Baikal littoral zone

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**ABSTRACT.** Using the metagenomics approach and high throughput sequencing of the 16S rRNA gene amplicons, we studied the taxonomic diversity of nitrifying bacteria in microbial communities from epilithic biofilms and plankton of the littoral zone of Lake Baikal. The family Nitrosomonadaceae represents the diversity of ammonia-oxidizing bacteria; the genera *Nitrospira* (Nitrospirae) and *Candidatus Nitrotoga* (Gammaproteobacteria) – nitrite-oxidizing bacteria; and the phyla Nitrospinae – unidentified bacteria. Microbial communities from epilithic biofilms and the near-bottom water layer showed the highest diversity and those from the surface water layer – the lowest one. Among ammonia oxidizers, bacteria of the uncultured genus Ellin6067 (27 phylotypes) dominated and were ubiquitous, and among nitrite oxidizers – bacteria of the genus *Nitrospira* (7 phylotypes).

**Keywords:** Lake Baikal, nitrification, ammonia-oxidizing bacteria, nitrite oxidizing bacteria, plankton, epilithic biofilm.

## 1. Introduction

Nitrification is microbial oxidation of ammonia to nitrate through nitrite. Chemolithoautotrophic ammonia-oxidizing bacteria (AOB) and archaea (AOA) perform the first phase of nitrification, ammonia oxidation to nitrite. Chemolithoautotrophic and photolithoautotrophic nitrite-oxidizing bacteria (NOB) perform the second phase of nitrification, nitrite oxidation to nitrate. The genera *Nitrosomonas*, *Nitrospira*, *Nitrosovibrio* (Betaproteobacteria), and *Nitrosococcus* (Gammaproteobacteria) represent the phylogenetic diversity of AOB; the genera *Nitrobacter* (Alphaproteobacteria), *Nitrococcus* (Gammaproteobacteria), *Nitrospina* (Nitrospinae), *Nitrospira* (Nitrospirae), *Nitrotoga* (Betaproteobacteria), *Nitrolancea* (Chloroflexi), and “*Candidatus Nitromaritima*” (Nitrospinae) – NOB (Daims et al., 2016). All known AOA are the members of the phylum Thaumarchaeota (Taylor and Kurtz, 2020).

For many decades, it was believed that fundamentally different groups of microorganisms perform ammonia oxidation and nitrite oxidation. In recent years, nitrifiers of the genus *Nitrospira* have been detected, which can perform the complete oxidation of ammonia to nitrate, “comammox” (van Kessel et al., 2015). Comammox by *Nitrospira* is often found in its oligotrophic habitats where it competes for a substrate

with AOB and AOA, having a higher affinity for ammonium (Kits et al., 2017).

The diversity of nitrifiers in oligotrophic Lake Baikal has not been studied so far and is of doubtless interest.

## 2. Materials and methods

Samples of epilithic biofilms (15 samples), as well as surface (14 samples) and near-bottom (12 samples) water, were taken in June, August and September 2017 from the littoral zone of three basins of Lake Baikal.

Total DNA of the samples was extracted through a phenol-chloroform extraction technique. The V3-V4 16S rRNA gene fragment was amplified using the 343F and 806R primers according to the previously described protocol (Martemyanov et al., 2016). Sequencing was carried out on the Illumina platform at Sintol company (Russia).

Sequence sets were clustered in OTUs using the mothur v.1.39.5 software package according to the standard processing protocol ([https://mothur.org/wiki/miseq\\_sop](https://mothur.org/wiki/miseq_sop)) (Schloss, 2009). The sequences were aligned using the SILVA database (v.132 release, <https://www.arb-silva.de>). Homologous sequences were searched for using BLAST analysis (<http://blast.ncbi.nlm.nih.gov>). All calculations were performed

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with HPC-cluster «Akademik V.M. Matrosov», Irkutsk Supercomputer Center of SB RAS, Russia (<http://hpc.icc.ru>).

### 3. Results and discussion

In 60% of the investigated samples, we detected a small amount of AOB, comprising less than 1% of the total number of sequences. We did not detect AOB in the surface water in August and the near-bottom water in September. The family Nitrosomonadaceae (Betaproteobacteria) represents the taxonomic composition of AOB and includes 47 phylotypes (OTU, cluster distance 0.03). This family, in turn, includes the genera *Nitrosospira* (3 OTUs), uncultured Ellin6067 (27 OTUs), MND1 (5 OTUs), 966-1 (2 OTUs), mle1-7 (2 OTUs), GOUTA6 (1 OTU), oc32 (1 OTU), cr616 (1 OTU), IS-44 (1 OTU), and DSSD61 (1 OTU) as well as unidentified bacteria (3 OTUs). The members of the genus Ellin6067 dominated and comprised a significant proportion of ammonia oxidizers in all investigated microbial communities, and two phylotypes were present both in water and epilithic biofilms. We detected bacteria of the genus *Nitrosospira* only in the water column.

The highest diversity of AOB was typical of microbial communities from epilithic biofilms (28 OTUs) and near-bottom water (19 OTUs); the lowest diversity – of those from the surface water (13 OTUs). Aerated near-bottom water contacting with the bottom sediments is the most favourable ecological niche for biological ammonia oxidation. On the contrary, in the surface water, ammonia oxidizers have to compete for the substrate ( $\text{NH}_4^+$ ) with photoautotrophs (Smith et al., 2014). Additionally, light has a substantial inhibitory effect on the activity of the ammonia monooxygenase enzyme (Merbt et al., 2017).

Based on the results of BLAST analysis, the closest relatives of AOB from Lake Baikal were uncultured bacteria detected in the association with the deep-sea Baikal sponges, *Baikalospingia intermedia*, in the waters of lakes Geneva, Mystic, Michigan, Ontario, Yellowstone, etc.

In addition to bacteria, the primers used in the study detected some archaea. Owing to this, we identified the members of the family Nitrosopumilaceae (Thaumarchaeota): *Candidatus Nitrosopumilus* (1 OTU) and *Candidatus Nitrosoarchaeum* (2 OTUs) in the near-bottom water at one of the stations in the southern basin. The closest relatives of the detected phylotypes were archaea from the *Thioploca* sp. microbial community (area of the Posolsk Bank cold methane seep, Lake Baikal), the water of Lake Maggiore, the bottom sediments of an oligotrophic lake of the Andes, etc. Previously, the members of Thaumarchaeota were found in the bottom sediments of Southern Baikal; in different layers, their proportion varied from 1 to 100% (Zemskaya et al., 2018). In the surface sedimentary layer of the Posolsk Bank methane seep, the sequences of the phylum Thaumarchaeota were numerically dominant in the microbial community (Chernitsyna et al., 2016).

NOB detected in 38% of the investigated samples were minor, comprising less than 0.5% of the total number of sequences. We did not identify NOB in any investigated sample of the surface water of August and the near-bottom water of September. The genera *Nitrospira* (Nitrospirae), *Candidatus Nitrotoga* (Gammaproteobacteria) and unidentified bacteria of the phylum Nitrospinae represented the general diversity of NOB. Bacteria of the genus *Nitrospira* (7 OTUs) dominated and were ubiquitous. At the same time, we did not identify common OTUs for the communities from biofilms and water. The biofilm fouling was dominated by the phylotype, which closest homologue was the uncultured bacteria found in the association with the Baikal endemic sponges, *Lubomirskia baicalensis*. The water column was dominated by the phylotype associated with the uncultured bacteria from the sediments of the Arctic oligotrophic Lake Char (Canada) and freshwater Lake Ibaraki (Japan). The closest cultured homologue of these phylotypes (96% similarity), the type *Nitrospira japonica* (NR\_114396) strain, was isolated from the activated sludge of a wastewater treatment plant. Perhaps, some of our detected phylotypes can completely oxidize ammonia to nitrate.

In biofilms and near-bottom water at the stations of Listvennichny Bay and Severobaikalsk town, we detected unidentified bacteria of the phyla Nitrospinae. Bacteria of the genus *Nitrospina* are dominant nitrite oxidizers in the World Ocean (Pachiadaki et al., 2017). In bottom sediments of Lake Baikal (Posolsk Bank methane seep), also identified a significant amount of molecular markers of the family Nitrospinae; in the surface layers, their proportion was from 4.9 to 8.8 % (Chernitsyna et al., 2016).

In the biofilm fouling from the station of Elokhin Cape, were detected sequences homologous to the candidate genus *Nitrotoga*. The similarity to the closest homologue, «Ca. Nitrotoga arctica» (DQ839562), isolated from the permafrost soils of Siberia was 97-99%. To date, it is known that bacteria of the genus *Nitrotoga* can adapt to low temperatures, and an increase in the nitrite concentration in the environment stimulates their growth and predominance over the genus *Nitrospira* (Kinnunen et al., 2017). The presence of the members of the genus *Candidatus Nitrotoga* untypical of the Baikal ecosystem in the biofilms sampled at the station near Elokhin Cape indicates the high nitrite load likely caused by the fires that occurred in the previous year.

### 4. Conclusions

Therefore, our studies have revealed that the family Nitrosomonadaceae represents the main diversity of AOB in microbial communities of epilithic biofilms and plankton of the littoral zone of Lake Baikal. The genera *Nitrospira* (Nitrospirae) and *Candidatus Nitrotoga* (Gammaproteobacteria), as well as unidentified bacteria of the phylum Nitrospinae, represent the taxonomic composition of NOB.

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