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# Moderately thermophilic nitrifying bacteria from a hot spring of the Baikal rift zone

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**Abstract**

Samples from three hot springs (Alla, Seya and Garga) located in the northeastern part of Baikal rift zone (Buryat Republic, Russia) were screened for the presence of thermophilic nitrifying bacteria. Enrichment cultures were obtained solely from the Garga spring characterized by slightly alkaline water (pH 7.9) and an outlet temperature of 75 °C. The enrichment cultures of the ammonia- and nitrite oxidizers grew at temperature ranges of 27–55 and 40–60 °C, respectively. The temperature optimum was approximately 50 °C for both groups and they thus can be designated as moderate thermophiles. Ammonia oxidizers were identified with classical and immunological techniques. Representatives of the genus *Nitrosomonas* and *Nitrosospira*-like bacteria with characteristic vibroid morphology were detected. The latter were characterized by an enlarged periplasmic space, which has not been previously observed in ammonia oxidizers. Electron microscopy, denaturing gradient gel electrophoresis analyses and partial 16S rRNA gene sequencing provided evidence that the nitrite oxidizers were members of the genus *Nitrospira*.


*Keywords:* Moderate thermophilic nitrification; Ammonia-oxidizing bacteria; Nitrite-oxidizing bacteria; *Nitrosomonas*; *Nitrosospira*; *Nitrospira*

## 1. Introduction

The biological oxidation of ammonia to nitrite and the oxidation of nitrite to nitrate, jointly called nitrification, are part of the global nitrogen cycle. Nitrification is accomplished by the metabolic activity of two groups of lithoautotrophic bacteria: the ammonia- and nitrite oxidizers [1–4]. These organisms are able to gain energy from the oxidation of inorganic nitrogen compounds and use carbon dioxide as their main carbon source. Nitrifying bacteria are widespread in biotopes such as sea and fresh waters, soils, rocks and stones of historical buildings [5].

Ammonia-oxidizing bacteria form two phylogenetically distinct groups based on their 16S rRNA gene sequences. The genus *Nitrosomonas* belongs to one cluster of the class *Betaproteobacteria* that includes *Nitrosomonas mobilis* (formerly *Nitrosococcus mobilis*). A second cluster formerly included the genera *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus*. Two marine species of

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the genus *Nitrosococcus* are affiliated to the class *Gammaproteobacteria* [6]. The separation of the genera of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) is based primarily on phenotypic characteristics. Irrespective of the gross morphological differences, the ammonia oxidizers *Nitrosovibrio*, *Nitrosospira* and *Nitrosolobus* were combined in the single genus *Nitrosospira* because of very high levels of homology in their 16S rRNA and their similarity with respect to the majority of phenotypic characteristics [7].

Nitrite-oxidizing bacteria are characterized by high phylogenetic diversity. The genera *Nitrobacter* and *Nitrococcus* belong to the *Alpha*- and *Gammaproteobacteria*, respectively. *Nitrospina* is currently allocated to the *Deltaproteobacteria* [8,9], although this phylogenetic positioning is still preliminary [10]. *Nitrospira* represents the deeply-branching phylum *Nitrospira* within the domain *Bacteria* [11,12].

Besides morphological characteristics, the presence or absence of intracytoplasmic membranes (ICMs) is traditionally used for differentiation among the genera of ammonia- and nitrite oxidizers [3]. ICMs occur in AOB of the genera *Nitrosomonas* and *Nitrosococcus* and in *Nitrobacter* and *Nitrococcus* among the NOB.

Today, modern molecular techniques and immunofluorescence (IF) labeling are suitable to identify nitrifying bacteria in environmental samples and in enrichment cultures. Whereas immunofluorescence assays with antibodies targeting components of the cell wall have a long tradition in ecological studies [13], a new approach focuses on antibodies recognizing two key enzymes of nitrification. For the detection of all AOB of the *Betaproteobacteria* and all known NOB, several polyclonal and monoclonal antibodies were developed targeting ammonia monooxygenase (AMO) and nitrite oxidoreductase (NOR) [14–16].

All cultured nitrifying bacteria known to date are mesophiles [3] with an upper growth temperature limit of 40 °C [11]. Thermophilic AOB, capable of growth at 55 °C, have been enriched from geothermal springs of Kamchatka [17]. However, the cultures were unstable and the organisms, in morphology similar to *Nitrosomonas*, not finally identified.

More recently, culture-independent 16S rRNA-based techniques have revealed the presence of *Nitrospira* relatives in subterranean hot springs of Iceland [18] and in a radon-containing hot spring in South Australia [19]. Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria thus belong, without a doubt, to the microflora of hot springs, however, cultured representatives have not been described.

In this study, enrichments of moderate thermophilic nitrifying bacteria from a hot spring in the northeastern part of Baikal rift zone were investigated. Classical, immunological and molecular techniques were applied for identification of AOB and NOB.

## 2. Materials and methods

### 2.1. Site description

In August 2001, three hot springs located in northeastern part of Baikal rift zone (Buryat Republic, Russia) were selected for sampling. The springs are named after the rivers they enter: Alla, Seya and Garga spring. Northeast of Lake Baikal is a geothermically active region with so-called nitric hydrotherms [20] that are characterized by a dominance of nitrogen in the gas phase.

Data regarding the environmental conditions and water analysis are presented in Table 1.

Microbial mats ranging from 0.5 to 13 cm in thickness had developed in the effluent channels and in a 20 $m^2$ pond of about 30 cm depth (Garga spring) at a water temperature of less than 60 °C. The mats were of the "translucent" type [22] and were dominated by a filamentous cyanobacterium *Phormidium* sp. and an anoxygenic phototrophic bacterium *Chloroflexus aurantiacus* [23]. In the Garga spring travertin of the calcium carbonate type was deposited near the outlet [24].

### 2.2. Sampling

Material was taken from microbial mats as well as from the surface layer of sediments located beneath the mats from Alla, Seya and Garga hot springs and transported to laboratories in Moscow and Hamburg.

### 2.3. Analyses

The temperature of water was measured with an electron thermometer from Prima (Singapore). The pH values were determined using an I-102 field pH meter (Russia). The measurement of mineralization was performed with a portable conductometer (TDS-4, Trans Instruments, Singapore). The alkalinity was determined in the field by titration of 1–10 ml water samples with

Table 1
Environmental data and water analysis of the Alla, Seya and Garga springs

| Parameter | Alla spring | Seya spring | Garga spring |
|---|---|---|---|
| Water temperature (°C) | 72 | 49 | 75 |
| pH | 9.7 | 9.6 | 7.9 |
| Sulfide (mg $l^{-1}$) | 15.6 | 5.0 | <0.1 |
| Sulfate (mg $l^{-1}$) | 80 | 98 | 256 |
| Ammonium (mg $l^{-1}$) | 0.1 | 0.1 | 0.1 |
| Nitrite (mg $l^{-1}$) | nd | nd | nd |
| Nitrate (mg $l^{-1}$) | nd | nd | nd |
| Mineralization (mg $l^{-1}$) | 500 | 320 | 130 |
| Silica (mg $l^{-1}$)[a] | nd | nd | 0.1 |
| Flow rate ($m^3$/day)[a] | 20 | 400 | 200 |
| Alkalinity (mg-eq $l^{-1}$) | 2.2 | 2.1 | 1.8 |
| Gas released (% $N_2$)[a] | 97.9 | 98.6 | 99.3 |

[a] Data reproduced from [21]. nd, not detected.