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Elevated salinity selects for a less diverse ammonia-oxidizing population in aquarium biofilters

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Abstract

The activity and changes in the structure of the community of the ammonia-oxidizing bacteria belonging to the Betaproteobacteria were monitored in freshwater and artificial seawater biofilters for two months after inoculation with a commercial nitrifying consortium. Both in freshwater and artificial seawater, ammonium oxidation proceeded immediately after addition of the inoculum, although initial activity in artificial seawater was lower than in freshwater. Denaturing gradient gel electrophoresis of the ammonia-oxidizing bacterial community of the inoculum and the freshwater and the artificial seawater aquaria as a function of time showed that initially only one dominant ammonia-oxidizer, closely related to *Nitrosomonas marina*, was detectable in all the systems. The fingerprint of the ammonia-oxidizing bacterial community in the artificial seawater biofilters continued to be dominated by this single band. In the freshwater aquaria, in contrast, the composition of the ammonia-oxidizer community became more diverse after one month, with 4–7 new bands appearing in the denaturing gradient gel fingerprint. Since the inoculum is cultivated at an average salinity of 11 g l^{-1}, it is argued that the elevated salinity selects for a less diverse ammonia-oxidizer community in the inoculum and the artificial seawater aquaria.

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1. Introduction

In aquaria and recirculating aquaculture systems, accumulation of ammonia, an end product of protein metabolism in fish, must be prevented as it is toxic to fish and other aquatic organisms even at low concentrations in the order of 0.5–1 mg NH_3–N l^{-1} [1–3]. Through the process of nitrification, ammonia can be oxidized to nitrite, which can be further oxidized to nitrate. While the intermediate nitrite is also toxic to certain aquatic organisms at low concentrations [4], the reported LC_{50} values for nitrate for fish are in the order of 1300–1500 mg N l^{-1} [5,6].

The nitrification process is catalysed by two phylogenetically unrelated groups of chemolithoautotrophic bacteria: the ammonia-oxidizing bacteria (AOB) and the nitrite-oxidizing bacteria (NOB). AOB form two monophyletic groups within the Betaproteobacteria and Gammaproteobacteria [7], while the NOB belong to four different genera within the Alphaproteobacteria, Gammaproteobacteria and Deltaproteobacteria and the phylum *Nitrospira* [8]. The Betaproteobacteria contains the highest diversity of AOB, encompassing the genera *Nitrosomonas*, *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio*. The latter three genera are phylogenetically closely related and form the *Nitrosospira* lineage. A further phylogenetic classification of betaproteobacterial AOB describes 6 stable lineages: '*Nitrosomonas cryotolerans*', *Nitrosomonas oligotropha*, *Nitrosomonas marina*, *Nitrosomonas europaea*/'*Nitrosococcus mobilis*',

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Nitrosomonas communis, *Nitrosomonas* sp. Nm143 and *Nitrosospira* [9]. The Gammaproteobacteria, in contrast, contains only the genus *Nitrosococcus*, comprising two described species *Nitrosococcus oceani* and *Nitrosococcus halophilus* [10,11].

Ammonia-oxidizing bacteria are ubiquitous in soils, freshwater, estuarine and marine environments [12]. Ecophysiological differences exist between representatives of the phylogenetic groups of AOB and urease activity and salt requirement are two ecophysiologically relevant discrimination factors [13]. Cultured representatives of the *Nitrosomonas marina* lineage and *Nitrosococcus* genus are obligate halophilic. Within the group of the nitrosomonads, other species have no salt requirements or are salt sensitive [14].

To promote the growth of nitrifying bacteria in aquaria and recirculating aquaculture systems, biofilters are used [15]. Nitrifying bacteria are notorious for their low growth rate, resulting in long start-up periods for new biofilters, during which both ammonia and nitrite can accumulate to toxic concentrations [16]. To shorten the time of the start-up period, it is possible to seed the biofilters with an inoculum of nitrifying bacteria [17].

The aim of this study was to determine whether AOB strains present in an inoculum were able to colonize and persist in freshwater and artificial seawater biofilters over a period of 2 months. The influence of increased salinity on the activity and the population dynamics of betaproteobacterial AOB was also investigated as a function of time in biofilters in artificial seawater and compared with freshwater aquaria systems, seeded with a nitrifying inoculum. Gammaproteobacterial AOB were not monitored in this study, as they could not be detected in a clone library containing 16S rRNA gene fragments from the inoculum [18].

2. Materials and methods

2.1. Test system

The test system consisted of six aquaria with a volume of 50 l. Each aquarium contained two internal, submerged biofilters (Aquaball, EHEIM, Germany) with a total volume of 0.4 l and filled with polyester cotton. Three aquaria (freshwater; f1, f2 and f3) were filled with 50 l tap water and three (s1, s2 and s3) with 50 l artificial seawater (Instant Ocean®, Aquarium Systems, France). The salinity of the artificial seawater was adjusted to 35 ppt and contains 10.6 g l^{-1} Na^+ and 19.1 g l^{-1} Cl^-. The exact composition of Instant Ocean® is given by Atkinson and Bingman [19] The biofilters were inoculated with 10 mg l^{-1} volatile suspended solids of a standard commercial nitrifying consortium (ABIL, AVECOM, Belgium) at the start of experiments. Therefore, 200 ml of the consortium, containing 2.5 g volatile suspended solids l^{-1}, was added directly to the aquarium water. The inoculum is standardised in terms of its production and has a guaranteed specific nitrification activity of 0.3 g N (g VSS)$^{-1}$ d^{-1} [17]. Regular doses of NH_4Cl were added to all aquaria as indicated in Table 1. Water changes were performed as a measure against increasing Cl^- and NO_3^- concentrations and to prevent a drop in pH due to alkalinity consumption during the nitrification process. On days 9 and 12, 50% of the water in all aquaria was replaced with tap water and artificial seawater, respectively. On days 22, 33 and 44, 75% of the water was replaced.

2.2. Chemical analysis

Water samples were taken regularly and analysed for TAN (total ammoniacal nitrogen), NO_2^-–N and NO_3^-–N. TAN in freshwater was determined spectrophotometrically with Nessler's reagent [20]. NO_2^-–N and NO_3^-–N in freshwater were determined with an ion chromatograph (IC 761 Compact, Metrohm, Switzerland) with a metrosep A supp 5 column (Metrohm, Switzerland). The eluent was 3.2 mM Na_2CO_3 and 1 mM $NaHCO_3$ at a flow of 0.7 ml min^{-1}. TAN in seawater was determined with a modified method using an Aquamerck® Kit (Merck, Germany). Briefly, reagent 2 was dissolved in distilled water (0.2 g ml^{-1}) for more accurate dosing and after addition of reagents 1 and 3, the absorbance was measured at a wavelength of 690 nm. NO_2^-–N in seawater was determined spectrophotometrically according to [21]. To determine NO_3^-–N in seawater, NO_3^-–N was reduced to NO_2^-–N with nitrate reductase according to the manufacturer's instructions (NECi, USA; [22]). The salinity was measured with a refractometer (ATAGO S-10E, Japan). The pH and dissolved oxygen concentration were measured respectively with a handheld pH meter (CONSORT C532) and a membrane covered amperometric electrode (COS 381 oxygen probe with a COM 381 meter) (Endress + Hauser, Belgium).

2.3. Calculations

The average TAN and NO_2^-–N oxidizing activity in the freshwater and artificial seawater biofilters were cal-

Table 1
TAN feeding regime used in this study

Day	TAN dosage
1	5 mg N l^{-1}
4	5 mg N l^{-1}
6–11	5 mg N l^{-1} d^{-1}
12–17	2.5 mg N l^{-1} d^{-1}
18–64	1 mg N l^{-1} d^{-1}

On days 1 and 4, a pulse of 5 mg TAN l^{-1} was added to all aquaria. From day 6 onwards, daily doses of TAN were provided as indicated.

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