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Growth at Low Ammonium Concentrations and Starvation Response as Potential Factors Involved in Niche Differentiation among Ammonia-Oxidizing Bacteria†

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In nature, ammonia-oxidizing bacteria have to compete with heterotrophic bacteria and plants for limiting amounts of ammonium. Previous laboratory experiments conducted with *Nitrosomonas europaea* suggested that ammonia-oxidizing bacteria are weak competitors for ammonium. To obtain a better insight into possible methods of niche differentiation among ammonia-oxidizing bacteria, we carried out a growth experiment at low ammonium concentrations with *N. europaea* and the ammonia oxidizer G5-7, a close relative of *Nitrosomonas oligotropha* belonging to *Nitrosomonas* cluster 6a, enriched from a freshwater sediment. Additionally, we compared the starvation behavior of the newly enriched ammonia oxidizer G5-7 to that of *N. europaea*. The growth experiment at low ammonium concentrations showed that strain G5-7 was able to outcompete *N. europaea* at growth-limiting substrate concentrations of about 10 μM ammonium, suggesting better growth abilities of the ammonia oxidizer G5-7 at low ammonium concentrations. However, *N. europaea* displayed a more favorable starvation response. After 1 to 10 weeks of ammonium deprivation, *N. europaea* became almost immediately active after the addition of fresh ammonium and converted the added ammonium within 48 to 96 h. In contrast, the regeneration time of the ammonia oxidizer G5-7 increased with increasing starvation time. Taken together, these results provide insight into possible mechanisms of niche differentiation for the ammonia-oxidizing bacteria studied. The *Nitrosomonas* cluster 6a member, G5-7, is able to grow at ammonium concentrations at which the growth of *N. europaea*, belonging to *Nitrosomonas* cluster 7, has already ceased, providing an advantage in habitats with continuously low ammonium concentrations. On the other hand, the ability of *N. europaea* to become active again after longer periods of starvation for ammonium may allow better exploitation of irregular pulses of ammonium in the environment.

Ammonia-oxidizing bacteria are chemolithoautotrophic bacteria, which generate their energy by oxidizing ammonia to nitrite for carbon fixation via the Calvin cycle (40). Ammonia oxidation is the first and usually rate-limiting step in the nitrification process and fulfills a critical link in the global nitrogen cycle.

With the exception of a few marine strains within the genus *Nitrosococcus* of the γ-subclass of the *Proteobacteria*, all ammonia-oxidizing bacteria known to date belong to a narrow clade within the β-subclass of the *Proteobacteria* (9). This clade comprises the genera *Nitrosospira* and *Nitrosomonas* and can be divided into a total of at least seven or eight subclusters (19, 27). A large number of studies have addressed the environmental niches of members of the different clusters by correlating the distributions of these bacteria across specific environmental gradients, with recent work making use of a number of culture-independent molecular methods (16). For example, *Nitrosomonas europaea* and other members of *Nitrosomonas* cluster 7 are often recovered from nitrogen-rich environments such as wastewater treatment plants (19, 20, 37). *Nitrosospira*-like ammonia-oxidizing bacteria appear to be more dominant in terrestrial ecosystems (14, 15, 27). *Nitrosomonas* cluster 6a and *Nitrosospira*-like bacteria have been found in freshwater environments (4, 8, 13, 23, 38).

The distribution of ammonia oxidizers in the environment strongly suggests that physiological differences are present between members of different clusters, but the nature of these differences remains poorly investigated. The ability to grow at low environmental ammonium concentrations and the response after deprivation for ammonium are thought to be important factors in the niche differentiation of ammonia-oxidizing bacteria. In natural environments, ammonia-oxidizing bacteria have to compete for limiting amounts of ammonium with heterotrophic bacteria and plants (3, 32, 33, 34, 35). Laboratory experiments have shown that *N. europaea* is a weak competitor for ammonium, being outcompeted by the heterotrophic bacteria *Arthrobacter globiformis* (33) and *Thiosphaera pantotropha* (32) at C/N ratios of higher than 10. Due to competition and the resulting periods without ammonium supply, ammonia-oxidizing bacteria are faced with conditions of starvation for ammonium. In retentostat and batch experiments, it has been observed that *N. europaea* can become active very rapidly after long periods of ammonia starvation (17, 30, 39).

To date, most of the data concerning starvation and competition of ammonia-oxidizing bacteria have been obtained with *N. europaea*, a species that may be of only limited importance in a number of natural environments. In order to gain insight into possible methods of niche differentiation among different groups of ammonia-oxidizing bacteria, we sought in this study

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to compare the growth and starvation properties of this species with a more environmentally relevant ammonia-oxidizing bacterium, namely, ammonia oxidizer G5-7. The ammonia oxidizer G5-7 was enriched from freshwater sediment at low ammonium concentrations and is closely related to *Nitrosomonas oligotropha*, belonging to *Nitrosomonas* cluster 6a (4). Growth experiments were conducted with chemostats, and the development of the populations was monitored by denaturing gradient gel electrophoresis (DGGE) of PCR products obtained with bacterium-specific primers (18, 41). Starvation behavior experiments were performed with batch cultures. The relative performance of these two strains in response to low ammonium concentrations and starvation conditions is discussed within the context of niche differentiation in the environment.

MATERIALS AND METHODS

Microorganisms. Two cultures of ammonia-oxidizing bacteria were used for the growth and starvation experiments: *N. europaea* ATCC 19718 and the ammonia oxidizer G5-7, present in an enrichment culture obtained by using continuous cultures to enrich ammonia-oxidizing bacteria at low ammonium concentrations from freshwater sediments (4). This enrichment culture contains the ammonia oxidizer G5-7, belonging to *Nitrosomonas* cluster 6a, closely related to *N. oligotropha*; an unknown nitrite oxidizer; and several unidentified heterotrophic bacteria. In the growth experiment, *Nitrobacter winogradskyi* ATCC 25391 was added to the *N. europaea* culture to remove nitrite, which might be toxic for members of the enrichment culture after mixing of the ammonia-oxidizing bacteria and therefore might affect the outcome of the growth experiment.

Media. Mineral salt medium containing 2.5 mM $(NH_4)_2SO_4$ (5 mM NH_4^+), 10 mM NaCl, 1 mM KCl, 0.2 mM $MgSO_4 \cdot 7H_2O$, 1 mM $CaCl_2 \cdot 2H_2O$, 0.4 mM KH_2PO_4, and 1 ml of trace element solution/liter (33) in distilled water was used for all experiments. For the batch incubations, 20 mM HEPES was added to keep the pH constant. The pH was adjusted to 7.8 with NaOH before autoclaving. The phosphate solution was autoclaved separately and added after the medium had cooled.

Growth experiment. The growth experiment was carried out in duplicate with Biostat M fermentors (B. Braun, Melsungen, Germany) as described by Verhagen and Laanbroek (33). All cultures of approximately 1 liter were kept at a temperature of 25°C and a stirrer speed of 300 rpm. The pH was adjusted continuously to 7.5 ± 0.2 by adding 5% Na_2CO_3. At the start, the chemostats were inoculated with actively growing batch cultures of a coculture of *N. europaea* and *N. winogradskyi* and the ammonia oxidizer G5-7, respectively. For a few days, the chemostats were run as batch cultures. When the cells were growing and had consumed 80 to 90% of the ammonium, the pumps were started to pump fresh medium containing 5 mM ammonium into the chemostats. The growth rate in both chemostats was adjusted to 0.014 h^{-1}. The chemostats were sampled daily to determine the ammonium, nitrite, and nitrate concentrations. Cultures were mixed only after the ammonium concentration was stable for 3 or 4 volume changes in both chemostats. Mixing involved the exchange of 300 ml of each culture. Different initial mixing ratios were applied to make sure that the outcome of the growth experiment was not influenced by the mixing ratio at the beginning of the experiment. After the cultures were mixed, daily samples were taken for ammonium, nitrite, and nitrate analysis, and additional samples were taken every third day (i.e., after every volume change) for molecular analysis.

Starvation experiment. *N. europaea* and the ammonia oxidizer G5-7 were grown in 50 ml of HEPES-containing mineral salt medium in 100-ml Erlenmeyer flasks. The starvation period started when the ammonium had been completely consumed. The cells were starved for 1, 2, 4, and 10 weeks at 25°C in the medium. At the end of the starvation period, ammonium was added to an initial concentration of 5 mM, and NaOH was used to restore the pH to approximately 7.8 after starvation. The Erlenmeyer flasks were incubated on a shaker at 120 rpm and 25°C. Samples were taken, cells were spun down at 15,000 × *g* for 10 min, and the supernatant was analyzed for nitrite and nitrate. The growth rates were calculated via ln transformation of the nitrite (*N. europaea*) and nitrite-plus-nitrate (ammonia oxidizer G5-7) contents (2). All of the different starvation treatments were tested in four replicates.

Chemical analysis. The samples taken for measuring the mineral nitrogen compounds were analyzed immediately or stored at −20°C. Ammonium (10) and nitrite (11) were measured colorimetrically. Nitrate was measured by high-pressure liquid chromatography as described by Bollmann and Laanbroek (4).

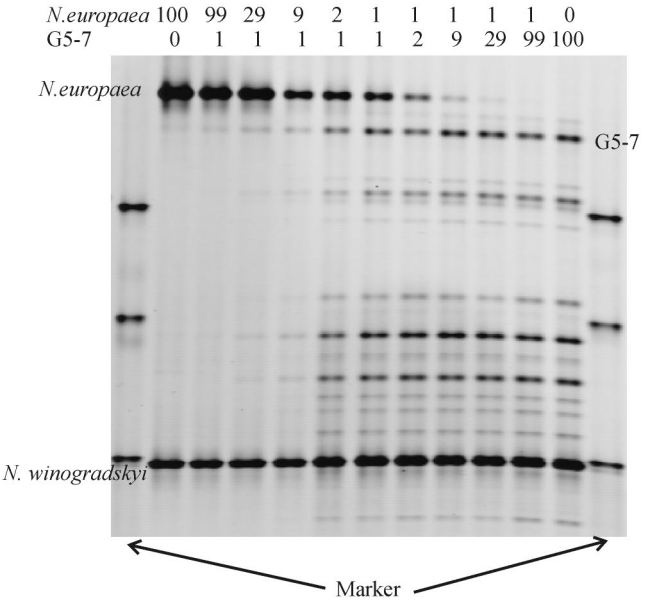

FIG. 1. Bacterium-specific DGGE of PCR products of DNA from cultures of *N. europaea* plus *N. winogradskyi* and of G5-7 mixed at different ratios. Cells had been grown in separate ammonium-limited chemostats at dilution rates of 0.014 h^{-1} and were mixed within 6 h after sampling from the effluents of the continuous cultures. DNA was isolated from 10 ml of the mixture. PCR was done with 100 ng of DNA in a 50-μl PCR mixture. The labeled bands (*N. europaea*, *N. winogradskyi*, and G5-7) were excised and sequenced. The other bands (not labeled) belong to the heterotrophic bacteria in the enrichment culture. The marker consists of PCR products from *Lactobacillus lactis*, *Escherichia coli*, and *Micrococcus luteus* (from top to bottom).

Molecular analysis. For the molecular analysis, 20 ml of chemostat culture liquid was filtered through a 0.2-μm-pore-size membrane filter (NC20; Schleicher & Schuell, Dassel, Germany). The filters were cut in half, placed in 2-ml screw-cap tubes, and stored at −80°C until further analysis. DNA was extracted as described by Bollmann and Laanbroek (4). After the DNA extraction, we determined the DNA concentration photometrically and diluted the DNA to obtain a final concentration of 5 or 100 ng of DNA in a 50-μl PCR mixture. The DNA was amplified with the bacterium-specific primers F357GC and R518 by using a *Taq* polymerase system. The PCR products obtained were analyzed by DGGE (18) with the modifications of Zwart et al. (41).

Statistical analysis. The two-sample *t* test to compare means was performed by using Microcal Origin, version 4.10.

RESULTS

Molecular analysis. DGGE of PCR products obtained with bacterium-specific primers was used to analyze the community composition during the growth experiment. To examine the sensitivity of the analysis and the potential influence of preferential amplification of one of the two ammonia-oxidizing bacteria, we performed a calibration by mixing culture liquids from both cultures at different ratios. DNA was isolated and amplified with bacterium-specific primers, and the PCR products were analyzed by DGGE (Fig. 1). When we mixed the two cultures at ratios of 2:1 and 1:1 (*N. europaea* and G5-7), both bands were visible, but the *N. europaea* band seemed to be more intense. At a mixing ratio of 1:2 (*N. europaea* and G5-7), the bands obtained from both cultures had almost the same intensity. These slight differences in the intensities of the bands could have been due to the fact that the two cultures, although

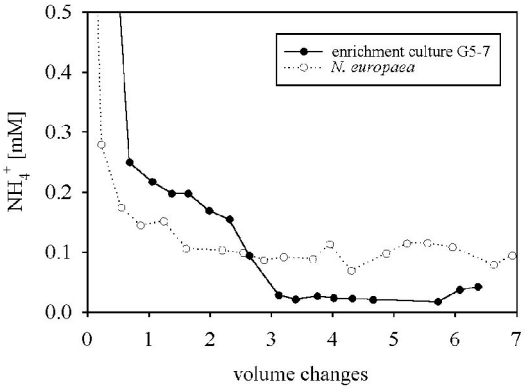

FIG. 2. Ammonium dynamics of the continuous cultures of the ammonia oxidizer G5-7 and of *N. europaea* plus *N. winogradskyi* before mixing of both cultures for the growth experiment.

grown under the same conditions, may have had differences in cell numbers.

The bacterium-specific primer set was the best one to use in the PCR-based method, because amplification with ammonia oxidizer-specific 16S rRNA primers and *amoA*-specific primers caused a clear preferential amplification of *N. europaea* (results not shown). With the bacterium-specific primers, DGGE revealed visible bands for the less numerous species of ammonia oxidizers when they were present at 10% of the total or more. This experiment was performed with DNA concentrations of 5 ng (results not shown) and 100 ng per 50-μl reaction volume. No influence of the initial DNA concentration was observed. For amplification of the samples from the chemostats, we used a DNA concentration of 100 ng per 50-μl reaction volume. *N. europaea* was grown together with *N. winogradskyi* in the chemostats to prevent inhibitory effects of nitrite. The DGGE pattern from the enrichment culture showed a band at the same height as the band for *N. winogradskyi*. This band was present in all samples at almost the same intensity. Therefore, we assume that the nitrite oxidizer in the enrichment culture is closely related to *N. winogradskyi*.

Growth in continuous cultures at low ammonium concentrations. We cultured *N. europaea* in a coculture with *N. winogradskyi* and the enrichment culture containing the ammonia oxidizer G5-7 in separate chemostats (Fig. 2). Both cultures started to consume ammonium at once, and fresh medium was pumped into the chemostats after 3 to 5 days of batch culturing. The *N. europaea*-*N. winogradskyi* coculture consumed ammonium to a growth-limiting substrate concentration of about 100 μM, which remained stable after 2 volume changes. The growth-limiting ammonium concentration in the enrichment culture of the ammonia oxidizer G5-7 was about 200 μM for 2 volume changes and then decreased to 10 to 20 μM. The relatively high ammonium concentration was observed when nitrite was still present in the culture and decreased after the nitrite had been completely consumed (results not shown), indicating that ammonia oxidation by G5-7 is sensitive to nitrite.

When the growth-limiting substrate concentration had been stable for 3 to 4 volume changes, the cultures were mixed. After the cultures were mixed, the nitrite concentrations remained near the detection limit (<1 μM), indicating that all the consumed ammonium was further oxidized to nitrate (results not shown).

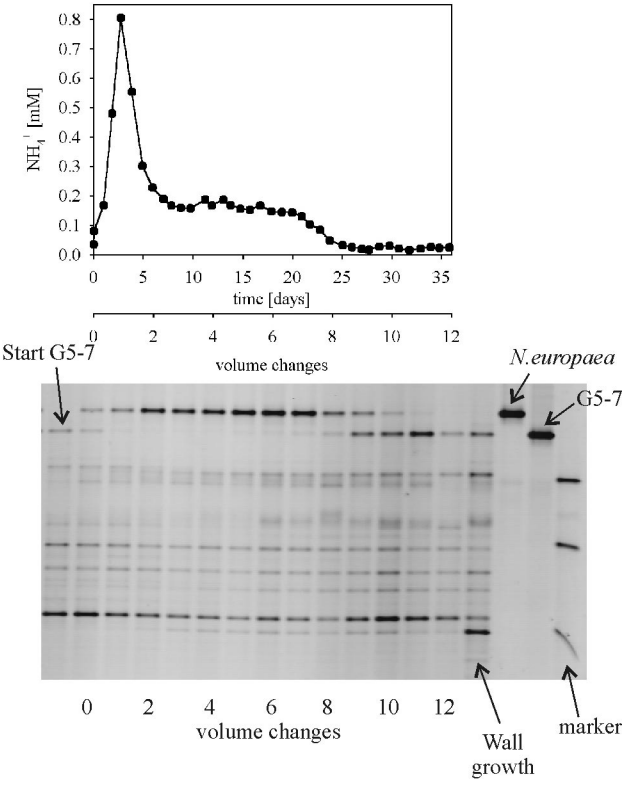

FIG. 3. Ammonia oxidizer G5-7 in coculture with *N. europaea* and *N. winogradskyi* in an ammonium-limited chemostat at a dilution rate of 0.014 h^{-1}. At the start of the experiment, 300 ml of the coculture of *N. europaea* and *N. winogradskyi* was inoculated into 700 ml of the culture of the ammonia oxidizer G5-7. (Upper panel) Ammonium dynamics. (Lower panel) Population dynamics indicated by DGGE of PCR products obtained with bacterium-specific primers. The marker consists of PCR products from *L. lactis*, *E. coli*, and *M. luteus* (from top to bottom).

In the chemostat with the low *N. europaea* ratio (Fig. 3), the ammonium concentration increased up to 800 μM within 1 volume change after mixing; then decreased to 200 μM, where it remained constant for 5 to 6 volume changes; and finally decreased at the end of the experiment to about 10 μM. The molecular analysis showed that the level of the ammonia oxidizer G5-7 declined after mixing and that *N. europaea* became dominant in the culture liquid. When the ammonium concentration dropped from 200 to 10 μM, the dominant population shifted back from *N. europaea* to the ammonia oxidizer G5-7. The second chemostat culture (Fig. 4) showed similar behavior, except that the ammonium concentration did not increase at the beginning of the experiment. After 12 volume changes, the pH started to fluctuate and increased up to 9.5. The ammonium concentration increased for a short time, indicating that ammonia oxidation was partly inhibited by the high pH. The pH fluctuation had no influence on the observed population shift. At the end of the growth experiment, we analyzed the bacterial biofilm on the wall of the vessel as well and found that it was dominated by the ammonia oxidizer G5-7.

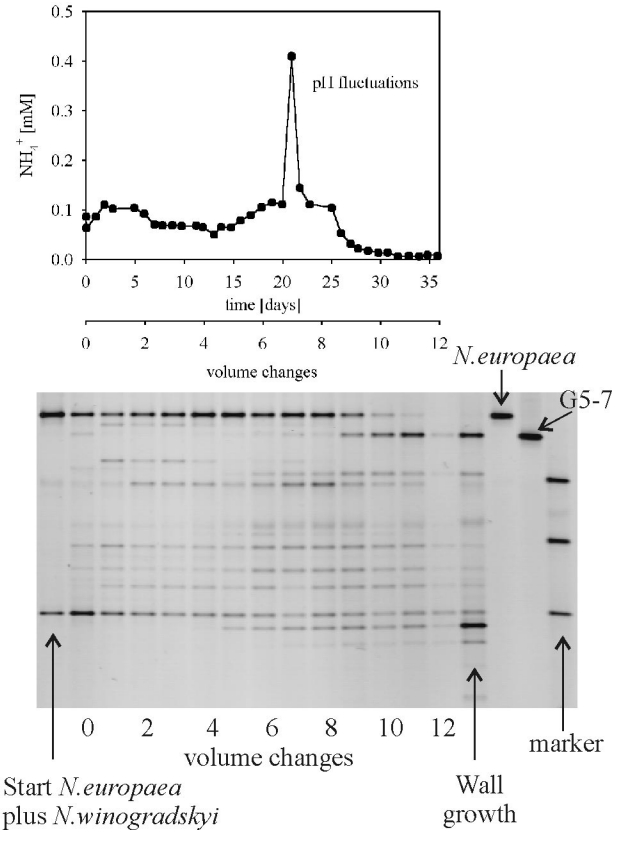

FIG. 4. *N. europaea* and *N. winogradskyi* in the coculture with ammonia oxidizer G5-7 in an ammonium-limited chemostat at a dilution rate of 0.014 h^{-1}. At the start of the experiment, 300 ml of the enrichment culture G5-7 was inoculated into 700 ml of the coculture of *N. europaea* and *N. winogradskyi*. (Upper panel) Ammonium dynamics. (Lower panel) Population dynamics indicated by DGGE of PCR products obtained with bacterium-specific primers. The marker consists of PCR products from *L. lactis*, *E. coli*, and *M. luteus* (from top to bottom).

Starvation experiment. Parallel to the growth experiment at low ammonium concentrations, we also performed a starvation experiment with the ammonia oxidizer G5-7 and *N. europaea*. Both cultures were inoculated into mineral salt medium containing 5 mM ammonium. The starvation period started when the cultures had consumed all the ammonium. Independent of the length of the starvation period, *N. europaea* started to produce nitrite again at 1 to 2 h after the addition of fresh ammonium (Fig. 5). After starvation for 1 to 4 weeks, the applied ammonium was consumed within 2 days of incubation. Only after a starvation period of 10 weeks did the oxidation of fresh ammonium proceed more slowly, taking 4 days for the ammonium to be consumed. The ammonia oxidizer G5-7 started to oxidize fresh ammonium almost immediately after a starvation period of 1 to 2 weeks (Fig. 5). However, after 4 weeks of starvation, a lag of several hours was observed prior to detectable ammonia oxidation. After 10 weeks, 5 days were needed before ammonia oxidation started, and an additional 5 days were needed before all the ammonium was consumed. The ammonium concentration in the starvation medium of the ammonia oxidizer G5-7 remained constant at ca. 0.02 mM, but it increased from 0.11 to 0.19 mM in the *N. europaea* culture over the whole starvation period (Table 1). The growth rates of both cultures decreased with increasing starvation time, but *N. europaea* grew faster at all time points than the ammonia oxidizer G5-7 (Table 2).

DISCUSSION

The different ammonia oxidizers in this study can apparently carve out separate niches due to their differential responses to low ammonium concentrations and starvation conditions. The ammonia oxidizer G5-7 was able to outcompete *N. europaea* at low ammonium concentrations (Fig. 3 and 4), but after starvation for ammonium, *N. europaea* recovered faster than the ammonia oxidizer (Fig. 5). The ability to grow at low ammonium concentrations has been observed for other members of *Nitrosomonas* cluster 6a (4, 12, 24, 28, 29).

Directly after mixing of the two populations of ammonia-oxidizing bacteria in the growth experiment, the ammonia oxidizer G5-7 was not detectable anymore in the culture liquid, indicating that *N. europaea* had become dominant. Given that the low ammonium concentrations, 80 and 63 μM, at that time were below the growth-limiting substrate concentration for *N. europaea* (>100 μM before mixing), one would expect ammonia oxidizer G5-7 to be favored. When the ammonia oxidizer G5-7 reappeared, the ammonium concentration decreased simultaneously. Although small populations of the ammonia oxidizer G5-7 may have remained in the culture liquid, we assume that most of these organisms survived on the wall of the vessel. At the end of the growth experiment, the biofilm on the wall was dominated by the ammonia oxidizer G5-7, and in earlier enrichment experiments at low ammonium concentrations, these bacteria were also surviving and growing on the wall of the vessel (4). The apparent repression of the ammonia oxidizer G5-7 at the beginning of the experiment could have been due to an inhibitory compound produced by *N. europaea* cells (A. Bollmann, unpublished results). By sticking to the wall, the ammonia oxidizer G5-7 might have been protected against potential inhibitory effects. The protection of ammonia-oxidizing bacteria against the toxic effects of heavy metals by the production of extracellular polymeric substances and growth in aggregates has been observed for relatives of *Nitrosomonas ureae* and *N. oligotropha* isolated from the Elbe River (25).

In the chemostat cultures after mixing, *N. europaea* grew at growth-limiting substrate concentrations of 170 and 82 μM. The rest of the ammonium was available for the growth of other ammonia-oxidizing bacteria. After the release from inhibition, the ammonia oxidizer G5-7 became active and further decreased the available ammonium in the chemostat to levels not available anymore for *N. europaea*. Finally, *N. europaea* was displaced from the culture by the ammonia oxidizer G5-7. In the end, it was the difference in the ammonium threshold concentration that was the selective factor determining the outcome of the growth experiment.

In the starvation experiment, *N. europaea* became almost immediately active after the addition of fresh ammonium, indicating that long-term starvation did not affect the ammonia oxidation capacity of *N. europaea* (6, 17, 30, 39). The ammonia oxidizer G5-7 in the enrichment culture also responded quickly

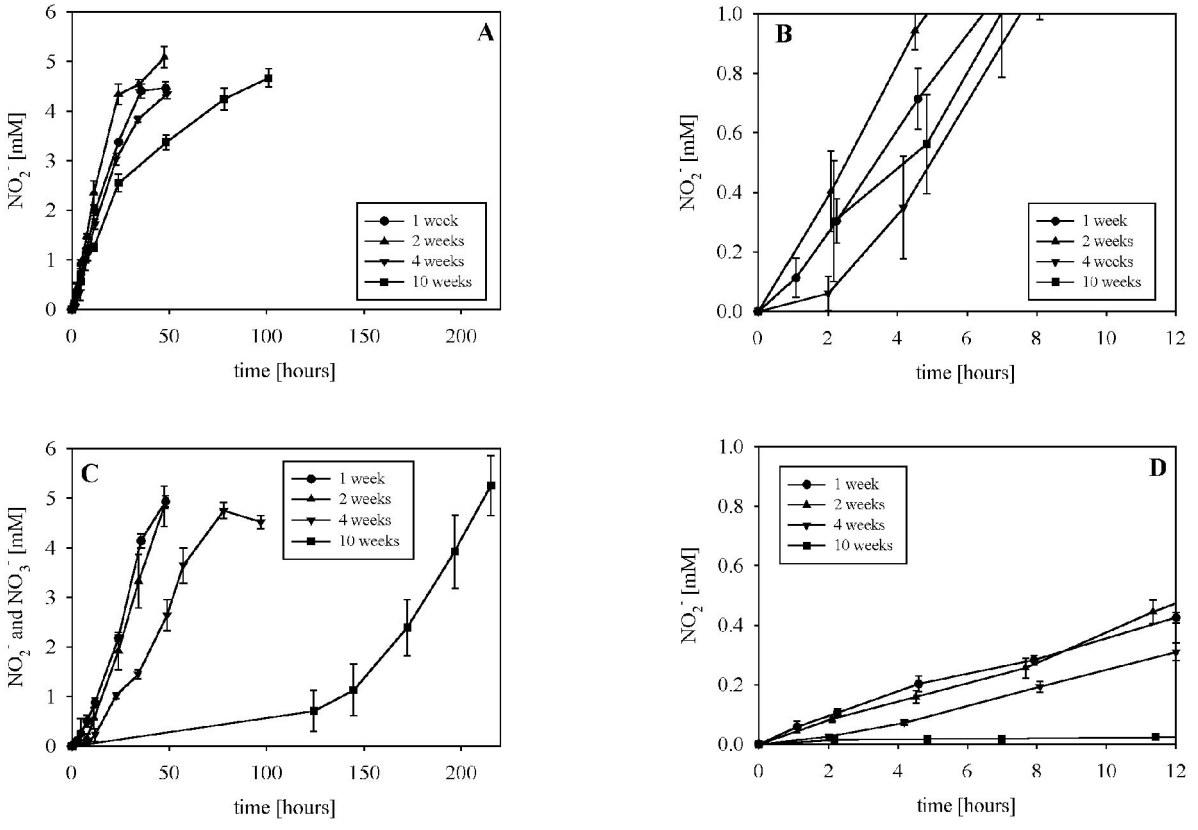

FIG. 5. Nitrite and nitrite plus nitrate production by *N. europaea* and the ammonia oxidizer G5-7 present in the enrichment culture after different periods of starvation (mean and standard deviation for four replicates). (A) Nitrite production by *N. europaea* over more than 200 h. (B) Nitrite production by *N. europaea* during the first 12 h. (C) Nitrite plus nitrate production by the enrichment culture containing the ammonia oxidizer G5-7 over more than 200 h. (D) Nitrite production by the ammonia oxidizer G5-7 during the first 12 h.

to freshly added ammonium after 1 to 2 weeks of starvation, but longer starvation periods resulted in an increase in the lag phase. The time required by cells to use a substrate again after starvation periods has an important effect on selection; there was a clear advantage for *N. europaea* under fluctuating N availability. This effect could have been caused by the presence of ammonium even at a relatively low concentration in the starvation medium of *N. europaea* (Table 1), which was ca. 10 times higher than the ammonium concentration in the medium of the ammonia oxidizer G5-7. Thus, ammonium or ammonia could have had a protective effect on the ammonia-oxidizing enzyme system, as has been shown previously for ammonium (27 mM; pH 5.6; equivalent to 6 μM NH_3) and short-chain alkanes (26). The presence of nitrite during starvation had no effect on the maintenance of the ammonia-oxidizing potential during the starvation of cells of *N. europaea* (17). Thus, the different starvation responses of the two investigated cultures were probably not caused by the presence of nitrite in the starvation medium of *N. europaea* and its absence (<0.1 μM) in the medium of the ammonia oxidizer G5-7 due to the presence of a nitrite oxidizer.

TABLE 1. Ammonium concentrations measured in the medium of *N. europaea* and in the medium of the ammonia oxidizer G5-7 after different periods of starvation

Starvation time (wk)	Ammonium concn (mM) in the medium of[a]:	
	N. europaea	Ammonia oxidizer G5-7
1	0.11 ± 0.00 (0.62)[b]	0.02 ± 0.01 (0.11)[b]
2	0.13 ± 0.01 (0.73) A	0.02 ± 0.00 (0.11) A
4	0.15 ± 0.01 (0.84) A	0.03 ± 0.00 (0.16) B
10	0.19 ± 0.02 (1.07) B	0.01 ± 0.01 (0.06) A

[a] Data are means and standard deviations for four replicates, unless otherwise indicated. Values in parentheses are the ammonia concentrations at pH 7 (in micromolar). Different characters following values indicate significant ($P < 0.05$) differences between the means for the cultures.
[b] Data are means and ranges for two replicates.

TABLE 2. Growth rates of *N. europaea* and the ammonia oxidizer G5-7 after the addition of fresh ammonium in relation to different periods of starvation

Starvation time (wk)	Growth rate (h^{-1}) of[a]:	
	N. europaea	Ammonia oxidizer G5-7
1	0.138 ± 0.015 A	0.071 ± 0.005 A
2	0.133 ± 0.020 A	0.080 ± 0.009 A
4	0.065 ± 0.004 B	0.037 ± 0.004 B
10	0.063 ± 0.013[b]	0.023 ± 0.004 B

[a] Data are means and standard deviations for four replicates, unless otherwise indicated. Different characters following values indicate significant ($P < 0.05$) differences between the means for the cultures.
[b] Data are means and ranges for two replicates.

From an ecological point of view, *N. europaea* may represent the r-strategist among the ammonia-oxidizing bacteria, with a relatively high growth rate. The ammonia oxidizer G5-7 may be more of a K-strategist, with a lower growth rate but also a lower ammonium threshold concentration for growth (Table 2 and Fig. 3 and 4) (1). Similar observations have been reported for comparisons between the pairs *Nitrosomonas* and *Nitrosospira* and *Nitrobacter* and *Nitrospira* (5, 21, 22). The conclusion that *N. europaea* and other members of *Nitrosomonas* cluster 7 can be assigned as r-strategists among the ammonia-oxidizing bacteria is supported by the fact that their main habitats have high N input and turnover, such as wastewater treatment plants (12, 19). *Nitrosomonas* cluster 6a and *Nitrosospira*-like bacteria may both represent K-strategists. Both groups have been found mainly in more oligotrophic places, such as soils and sediments (12, 23, 31).

Our results showed that ammonia-oxidizing bacteria belonging to *Nitrosomonas* cluster 6a are better adapted to growth at low ammonium concentrations than *N. europaea*. This observation could have important consequences for the interpretation of previous experiments examining competition for limiting amounts of ammonium among ammonia-oxidizing bacteria, plants, and heterotrophic bacteria. These experiments showed that *N. europaea* is a weak competitor for ammonium (3, 32, 33, 34). Taking into account the different habitats of *N. europaea* and *Nitrosomonas* cluster 6a (12, 19, 23) and a comparison of K_m values for ammonium between *Nitrosomonas* cluster 6a (50 to 100 μM ammonium at pH 7.8) and the high-affinity ammonium uptake system of higher plants (10 to 170 μM ammonium at a neutral pH) (7, 12, 36), it is highly likely that the ammonia-oxidizing bacteria belonging to *Nitrosomonas* cluster 6a are better competitors with plant roots for limiting amounts of ammonium than *N. europaea*. This hypothesis warrants further investigations.

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