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Nitrosovibrio spp., the Dominant Ammonia-Oxidizing Bacteria in Building Sandstone

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In five historical buildings in the Federal Republic of Germany, ammonia-oxidizing bacteria of the genera *Nitrosovibrio*, *Nitrosospira*, and *Nitrosomonas* were detected in high cell numbers. In building stones, *Nitrosovibrio* was the most abundant ammonia-oxidizing organism. In the soil at the foot of each building, *Nitrosomonas* spp. were the most common ammonia oxidizers, whereas *Nitrosovibrio* spp. were not detected.

Buildings which are constructed of natural stones or concrete are endangered by corrosion. Pollutants, which are involved in the complex process of stone deterioration, are acids mainly derived from SO_2 and NO_x. The destructive activity of sulfuric acid has been demonstrated for limestone (5, 12), whereas the corrosive effect of nitrous and nitric acid is not well investigated.

Another compound in the anthropogenically influenced atmosphere is ammonia (7, 10, 11). The concentration of ammonia has significantly increased in the last decade. Sources of atmospheric ammonia are the decomposition of organic matter by microorganisms, the emission from coal combustion, and losses from the manufacture and use of fertilizers. Livestock production is one of the important sources of atmospheric ammonia in Europe (7). In areas with surface-applied animal manure, the volatilization of ammonia is on the order of hundreds of kilograms of nitrogen per hectare (8). The atmospheric ammonia probably reacts in an acid-aerosol neutralization with acidic components, forming ammonium salts such as ammonium sulfate and ammonium nitrate (6). These compounds form dry or wet deposits on the surfaces of buildings. High amounts of ammonia were detected in acidic building stones (3).

In nature, ammonia is converted to nitrate by the activity of nitrifying bacteria. These organisms were shown to be present in high cell numbers in sandstones of historic monuments in the Federal Republic of Germany (2). The oxidation of ammonia results in an acidification of the environment (10). On buildings with calcareous binding materials, acidification will result in a loss of binding material. Another consequence is the formation of nitrate, which is one major source of salt stress. This form of biodeterioration is correlated with the presence of ammonia-oxidizing bacteria.

For microbial investigation samples were transferred to the laboratory within 6 h. The material was pulverized into particles, suspended, diluted in 10-fold steps, and used as an inoculum in a three-tube most-probable-number technique. Positive most-probable-number tubes in the highest dilution steps were used as inocula for enrichment cultures. After the formation of about 7 mM nitrite, the enrichment cultures were examined for the presence of ammonia-oxidizing bacteria by phase-contrast microscopy. The genera of ammonia-oxidizing bacteria were distinguished by their morphology (1). Pure cultures were obtained by serial dilution. The cultures were considered pure when heterotrophic bacteria were not detectable. Purity of the cultures was checked by phase-contrast microscopy and by electron microscopy (4).

To summarize the experimental data, 13 pure cultures and 23 enrichment cultures of ammonia-oxidizing bacteria were obtained from five historical buildings. As is obvious from Table 1, ammonia-oxidizing bacteria were detectable at each sampling site. The population analysis on the genus level indicated that only bacteria of the genus *Nitrosovibrio* were always present. In enrichment and pure cultures, about 60% of the ammonia oxidizers belonged to the genus *Nitrosovibrio*. Besides *Nitrosovibrio* spp., *Nitrosospira* and *Nitrosomonas* spp. were found.

In control experiments, we enriched ammonia-oxidizing bacteria from soil at the foot of each historical monument. In all enrichment cultures, *Nitrosomonas* spp. were identified as the only ammonia-oxidizing organisms, except for *Nitrosospira* spp. in one enrichment culture. Bacteria of the genus *Nitrosovibrio* were not detectable.

Five *Nitrosovibrio* strains isolated in pure culture were studied by electron microscopy. The cells were slender, curved rods, 2 to 4 μm long and 0.3 to 0.4 μm in width (Fig. 1A). They were motile by means of a polar-to-subpolar flagellum. Strain K7.1 was characterized by intracytoplasmic membranes and carboxysomes (Fig. 1B) This strain was grown under lithoautotrophic conditions with substrate concentrations in the range of 3 to 160 mM ammonia. The formation of nitrite was used as a parameter for determining cell growth. The cells grew best at concentrations between 20 and 50 mM ammonia. The lag phases became longer at substrate concentrations above 50 mM. At 150 mM ammonia, growth of *Nitrosovibrio* spp. was detected only after a lag phase of 14 days.

As shown for five historical buildings at heights of 10 m and more, ammonia-oxidizing bacteria were living endolithically in weathered sandstone. A population analysis indi-

TABLE 1. Distribution of ammonia-oxidizing genera at different historical monuments

Sampling site	No. of pure and enrichment cultures with the most abundant organism belonging to the genus:		
	Nitrosovibrio	*Nitrosospira*	*Nitrosomonas*
Kölner Dom	5	0	0
Regensburger Dom	3	6	1
Alte Pinakothek	6	4	0
Gelnhausen	3	1	2
Pommersfelden	4	1	0

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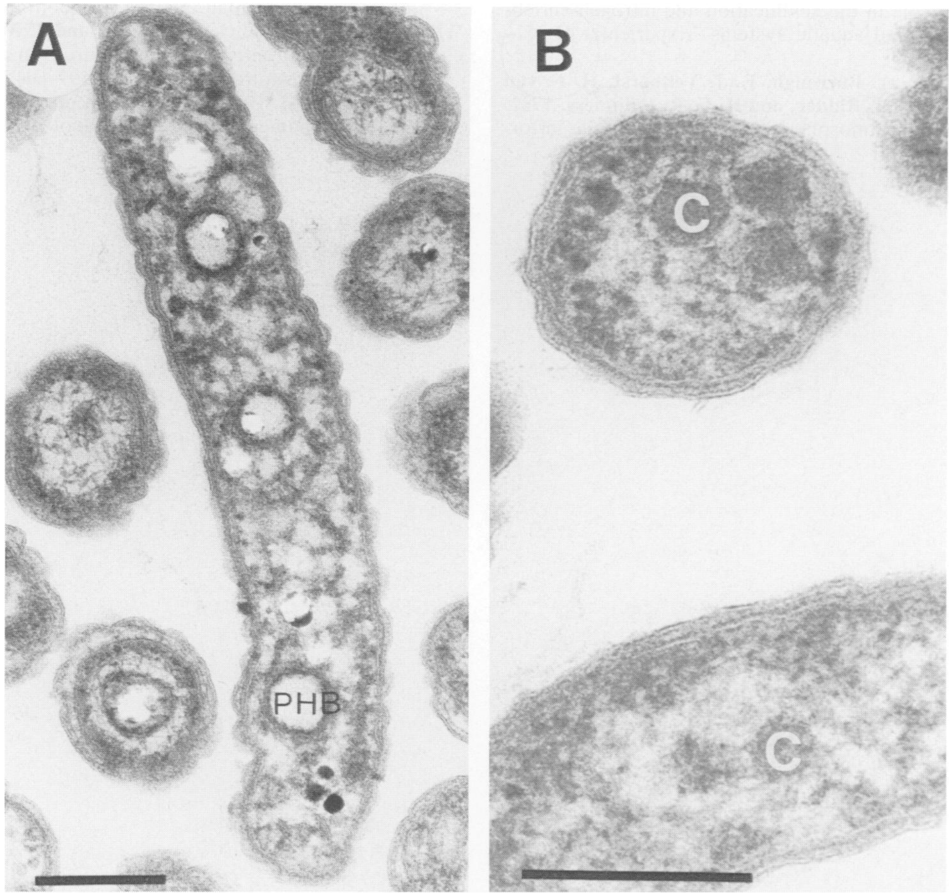

FIG. 1. Electron micrograph of thin sections of *Nitrosovibrio* spp. (A) Strain isolated from the Regensburger Dom. (B) Strain isolated from the Alte Pinakothek in Munich, Federal Republic of Germany. PHB, Poly-β-hydroxybutyrate granules; C, carboxysomes. Bar, 0.3 μm.

cated that *Nitrosovibrio* spp. were the most abundant ammonia-oxidizing organisms. Although other ammonia-oxidizing bacteria of the genera *Nitrosomonas* and *Nitrosospira* were present, *Nitrosovibrio* spp. predominated at each sampling site. However, in soil taken at the foot of the monuments, *Nitrosovibrio* spp. were never detected. This control experiment is in accordance with the finding that *Nitrosomonas* and *Nitrosospira* spp. are the most abundant soil organisms for the first step of nitrification (1). In addition, this result clearly indicates that the media used for the isolation were not selective for *Nitrosovibrio* spp.

The production of nitric acid causes corrosion of sandstone. This report is the first to show that *Nitrosovibrio* spp. are the most abundant organisms in building sandstone. This organism might be an indicator for biologically induced nitric acid production and biodeterioration of calcareous sandstone.

The endolithic *Nitrosovibrio* spp. exhibited tolerance to high substrate concentrations and grew optimally at pH values of 7.2 to 7.6. However, the slight decrease in the pH optimum and the tolerance to high substrate concentrations compared with those of other ammonia oxidizers do not completely explain why *Nitrosovibrio* spp. are the dominant ammonia oxidizers. Investigations are in progress to answer this question.

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