Classification of eight new species of ammonia-oxidizing bacteria:

Nitrosomonas communis sp. nov., *Nitrosomonas ureae* sp. nov.,
Nitrosomonas aestuarii sp. nov., *Nitrosomonas marina* sp. nov.,
Nitrosomonas nitrosa sp. nov., *Nitrosomonas eutropha* sp. nov.,
Nitrosomonas oligotropha sp. nov. and *Nitrosomonas halophila* sp. nov.

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A total of 13 species of lithotrophic ammonia-oxidizing bacteria assigned to the genus *Nitrosomonas* were characterized. DNA homologies, G+C content of the DNA, shape and ultrastructure of the cells, salt requirements, ammonia tolerance, utilization of urea as ammonia source, and whole-cell protein patterns were analysed. In addition to the described species *Nitrosomonas europaea* and '*Nitrosomonas cryotolerans*' eight new species are established. The names *Nitrosomonas communis* sp. nov., *Nitrosomonas ureae* sp. nov., *Nitrosomonas aestuarii* sp. nov., *Nitrosomonas marina* sp. nov., *Nitrosomonas nitrosa* sp. nov., *Nitrosomonas eutropha* sp. nov., *Nitrosomonas oligotropha* sp. nov. and *Nitrosomonas halophila* sp. nov. are proposed.

Introduction

With the exception of *Nitrosococcus oceanus* the species of all genera of the lithotrophic ammonia-oxidizing bacteria are grouped together phylogenetically in the so-called beta subdivision of the *Proteobacteria* (Woese *et al.*, 1984, 1985; Stackebrandt *et al.*, 1988). All are obligate lithotrophs oxidizing ammonia to nitrite as the sole energy source and assimilating carbon dioxide as the major carbon source, while they possess only a limited capacity to utilize organic compounds (Krümmel & Harms, 1982). The incorporation of carbon from organic compounds and its distribution among cell constituents are severely restricted in all species (Williams & Watson, 1968; Smith & Hoare, 1977; Martiny & Koops, 1982). Since there are no general differences in their metabolism the genera are exclusively defined by differences in the morphology and the ultrastructure of the cells (Watson *et al.*, 1981, 1989). However, it has subsequently been suggested on the basis of 16S rRNA (Woese *et al.*, 1984, 1985) and DNA homology analyses (Dodson *et al.*, 1983) that some species within the genera may not be phylogenetically closely related to each other. However, this is a general problem of many physiologically and

morphologically defined groups of the *Proteobacteria*. For example, the species of the lithotrophic sulphur-oxidizing genus *Thiobacillus* are widely spread among the different branches of the *Proteobacteria* (Lane *et al.*, 1985).

The present paper deals with *Nitrosomonas*, one of the five genera of the ammonia-oxidizers. This genus includes all species having ellipsoidal to rod-shaped cells with extensive intracytoplasmic membranes, arranged as flattened vesicles in the peripheral cytoplasm. At present only two species, *N. europaea* (Winogradsky, 1892; Watson, 1974) and the recently described '*N. cryotolerans*' (Jones *et al.*, 1988), are recognized, but the existence of diverse other species has been indicated by analysis of DNA homology (Watson & Mandel, 1971; Dodson *et al.*, 1983). DNA–DNA hybridization studies of 96 strains of ammonia-oxidizing bacteria suggested the existence of at least seven genospecies of *Nitrosomonas*, apart from *N. europaea* (Koops & Harms, 1985). These species formed six groups with different DNA G+C contents, ranging between 45.8 and 53.8 mol%.

Species of *Nitrosomonas* are by definition very similar in their morphological characteristics. The simple and uniform conception of the basic metabolism of the lithotrophic ammonia-oxidizers might be another reason why all species, even those of different genera, are

Abbreviation: LDS, lithium dodecyl sulphate.

very similar in many respects. For example, those chemotaxonomic markers which have been studied in detail are generally not helpful in species characterization. The lipid patterns were found to be similar in species of *Nitrosococcus*, *Nitrosomonas* and *Nitrosolobus* (Blumer *et al.*, 1969). Differences observed in these patterns were related more to the habitat (marine or terrestrial) of the respective species than to the genus it belonged to. The amino acid patterns were also very similar in all species of the different genera (Martiny & Koops, 1982). Furthermore, species of all genera show nearly identical dithionite-reduced-minus-oxidized difference spectra of living cells, with the major peaks at 423, 465, 522, 552, and 605 nm (Watson *et al.*, 1989).

The above-described difficulty in differentiating among species of the ammonia-oxidizing bacteria is the main reason why the new species of *Nitrosomonas* indicated by DNA analysis could not hitherto be defined taxonomically. However, the distribution of most of these species seems to be restricted to special environments (Watson & Mandel, 1971; Koops & Harms, 1985). Thus the existence of special physiological characteristics resulting from adaptations to these environments may be expected.

The purpose of the investigations described here was to search for properties allowing practical phenotypic differentiation of the genospecies of *Nitrosomonas*, and to define new species.

Methods

Bacterial strains. The 57 bacterial strains used in this investigation are listed in Table 1.

Culture conditions. Terrestrial and freshwater isolates were grown at 28 °C in a basal mineral salts medium containing (per litre): 0.535 g NH_4Cl , 0.054 g KH_2PO_4 , 0.074 g KCl , 0.049 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.147 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.584 g NaCl , 1 ml 0.05% cresol red solution, 1 ml trace elements solution (containing per litre: 0.1 M-HCl: 44.6 mg $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$, 49.4 mg H_3BO_3 , 43.1 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 37.1 mg $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 173 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 25.0 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). Isolates from brackish waters and from marine habitats were grown in the same medium but containing, respectively, 11.7 g and 23.4 g NaCl l^{-1} . Acidification changes the colour of the media from red to yellow. To maintain the pH between 7.0 and 8.0 (optimum around 7.8), 0.5 g CaCO_3 or 11.9 g HEPES l^{-1} was added to the media. In large-volume cultures the pH was adjusted manually using 10% (w/v) NaHCO_3 solution.

G + C content and DNA-DNA hybridization. DNA preparations were carried out as described by Koops & Harms (1985). G + C content of DNA and DNA-DNA homologies were estimated by photometric determination of thermal denaturation and renaturation rates (DeLey, 1970; DeLey *et al.*, 1970).

Morphological observations. Photomicrographs were taken of cells from exponentially growing cultures with a Zeiss microscope (Type Universal) using the method of Watson (1971*b*). Transmission electron microscopic studies were performed with a Philips EM 201 C. Preparation of the cells was as described by Koops *et al.* (1976).

Growth studies. The rate of oxidation of ammonia to nitrite was used as a growth parameter. Nitrite was measured by the method of Heubült (1929). Sodium requirement was examined in test tubes containing 10 ml portions of the basal mineral salts medium supplemented with 0–1000 mM-NaCl. Ammonia tolerance was tested in 10 ml portions of the basal medium with 10–600 mM- NH_4Cl . CaCO_3 was used as buffer in both of the above test series. Temperature characteristics were measured in 50 ml portions of the standard medium supplemented with 0.5 mM-HEPES as buffer in 100 ml Erlenmeyer flasks. The cultures were initially incubated at 28 °C with subsequent incubations at 14, 10, 5 and 0 °C. Urea utilization was determined in 10 ml portions of the basal mineral salts medium (with 2 mM- NH_4Cl) supplemented with 1 mM-urea, using HEPES as buffer. In addition to measuring nitrite, ammonia production was determined colorimetrically using Nessler's reagent. Generally the tests were read immediately after inoculation of the test tubes and subsequently once a week.

Polyacrylamide gel electrophoresis of whole-cell proteins. Polyacrylamide gel electrophoresis (PAGE) of cell proteins was performed using the procedure of Laemmli (1970), as modified by Francis & Becker (1984). Cells were harvested in the late exponential growth phase. Cell-free extracts were prepared according to Milde & Bock (1984) and separated using LDS (lithium dodecyl sulphate)-PAGE. Haem proteins were visualized by a peroxidase staining method (Francis & Becker, 1984), using dithioerythritol as reducing agent and dimethoxybenzidine as hydrogen donor. The gels were stained with Coomassie blue according to Weber & Osborn (1969). SDS-PAGE Molecular Weight Standards-Low (Bio-Rad) was used to provide size standards.

Results

DNA-DNA homologies

DNA reassociation currently represents the best procedure to define species (Wayne *et al.*, 1987).

In our earlier investigations (Koops & Harms, 1985) DNA analyses were carried out with 37 strains of *Nitrosomonas*. In that study eight genospecies were established which were distributed in six groups with different DNA G + C contents. An additional genospecies, containing the strains Nm 90 and Nm 91 and in that previous investigations placed as Nc 5 and Nc 6 in *Nitrosococcus*, was now allocated to *Nitrosomonas*. All of the 18 isolates (strains Nm 34, 41, 33, 88, 55, 62, 64, 69, 51, 63, 61, 71, 7, 72, 56, 87, 89) additionally examined in the present study belonged to the above-mentioned six G + C groups; however, they yielded four new genospecies on the basis of DNA-DNA hybridization (Table 2). Thus in the following investigations a total of 13 *Nitrosomonas* genospecies including 57 strains were studied to provide phenotypic characteristics for their differentiation.

It is important to note that the average background value of DNA homology is 25–30%, using the spectrophotometric method of DeLey *et al.* (1970). This was indicated by a study of Hus *et al.* (1983) and confirmed by our results in that the reference values measured between *Escherichia coli* and the different *Nitrosomonas* species

Table 1. *Strains of Nitrosomonas (Nm) used*

Strain no.	Genospecies no.	Isolated by*	Origin
Nm 2	1	N. Walker	Soil, Corfu
Nm 32	1		Soil, Buenos Aires
Nm 40	1		Soil, Hamburg
Nm 44	1		Soil, Sardinia
Nm 34	2		Soil, Japan
Nm 41	2		Soil, Leningrad
Nm 33	3		Soil, Japan
Nm 4	4		Soil, Sardinia
Nm 5	4		Fresh water, Sardinia
Nm 6	4		Pond, Hong Kong
Nm 9	4		Soil, Sardinia
Nm 10	4		Soil, Sardinia
Nm 13	4		Soil, Sardinia
Nm 42	4		Mud hole, Leningrad
Nm 88	4		Soil, Chile
Nm 55 (4W30)†	5	R. D. Jones/R. Y. Morita	Sea water, Kasitsna Bay
Nm 3	6		Sea water, North Sea
Nm 11	6		Sea water, North Sea
Nm 17	6		Sea water, North Sea
Nm 20	6		Sea water, North Sea
Nm 36	6		Sea water, North Sea
Nm 62 (C-52)	6		Sea water, Gulf of Maine
Nm 64 (C-121)	6		Sea water, off Joshida
Nm 69 (C-19)	6		Sea water, off Peru
Nm 51 (C-15)	7		Sea water, off Peru
Nm 63 (C-56)	7		Sea water, Gulf of Maine
Nm 22	8		Sea water, South Pacific
Nm 61	8		Sea water, off Senegal
Nm 71	8		Salt lake, Saudi Arabia
Nm 7	9		Pond, Hong Kong
Nm 72	9		Mud hole, Senegal
Nm 90 (Nc 5)	9	S. W. Watson	Industrial Sewage, Marl
Nm 91 (Nc 6)	9		Pond, Saudi Arabia
Nm 14	10		Municipal sewage, Gelsenkirchen
Nm 19	10		Municipal sewage, Gelsenkirchen
Nm 23	10		Municipal sewage, Gelsenkirchen
Nm 24	10		Municipal sewage, Gelsenkirchen
Nm 26	10		Municipal sewage, Gelsenkirchen
Nm 38	10		Industrial sewage, Marl
Nm 39	10		Industrial sewage, Marl
Nm 53	10		Mud, River Elbe
Nm 56	10		Soil, Germany
Nm 57 (C-91)	10		Municipal sewage, Chicago
Nm 8	11		Industrial sewage, Marl
Nm 37	11		Water, Leningrad
Nm 43	11		Soil, Hawaii
Nm 45	11		Soil, Hamburg
Nm 46	11		Industrial sewage, Marl
Nm 49	11		Sediment, River Elbe
Nm 27	12	N. Walker	Industrial sewage, Brunsbüttel
Nm 28	12		Industrial sewage, Brunsbüttel
Nm 35	12		Soil, USA
Nm 48	12		Industrial sewage, Brunsbüttel
Nm 50 (C-31)‡	12		Soil, USA
Nm 87	12		Water, River Elbe
Nm 89	12		Water, River Elbe, Cuxhaven
Nm 1	13		Sea water, North Sea

* Strains were isolated by H. Harms/H.-P. Koops unless otherwise indicated. Addresses: N. Walker (Rothamsted, UK), R. D. Jones/R. Y. Morita (Miami/Corvallis, USA), S. W. Watson (Woods Hole, USA).

† Type strain of '*N. cryotolerans*'.

‡ Neotype strain of *N. europaea* (ATCC 25978).

Table 2. Range of DNA-DNA homologies among strains of the genus *Nitrosomonas* [summary of published (Koops & Harms, 1985) and unpublished data]

G+C group (mol % species G+C) no.	Geno-species no.	Strains	% DNA homology to: Genospecies no.: Strain no.:												
I (45-8)	1	Nm 2, 32, 40, 44	62-100	36-49	39-50	31-44	34	33-43	39	39-40	34	39-46	32-41	31-38	37-44
I (45-8)	2*	Nm 34, 41	81-100	48-51	33-41	36	37-39	39	36	34	34	36-42	34	44	40
I (45-8)	3*	Nm 33	101		33	41	37	39	45	40	40	46	33	41	41
I (45-8)	4	Nm 4, 5, 6, 9, 10, 13, 42, 88	63-102		39	31-42	39	31-38	39	40	40	34-36	33-41	34-46	
I (45-8)	5*	Nm 55	100		41	35	30	35	33	33	33	40	42		
I (45-8)	6	Nm 3, 11, 17, 20, 36, 62, 64, 69	56-103		33-42	27-35	33	35	36	39	35-43				
II (47-7)	7*	Nm 51, 63	70-100		42	38	40	31	40	39					
II (47-7)	8	Nm 22, 61, 71	60-100		33	43	38	45	38						
III (47-9)	9†	Nm 7, 72, 90, 91	70-98		35	35	39	36							
III (48-2)	10	Nm 14, 19, 23, 24, 26, 38, 39, 53, 57	68-104		25-43	43	44								
IV (49-7)	11	Nm 8, 37, 43, 45	56-97		31-42	31-43									
V (51-0)	12	Nm 27, 28, 35, 48	81-98		31-44										
VI (53-8)	13	Nm 1	103												
Reference: <i>Escherichia coli</i>			31		31	31	32	25	25	34	31	30	34		

* New genospecies resulting from the present investigations.

† Genospecies placed in the genus *Nitrosococcus* in the earlier (Koops & Harms, 1985) investigation.

(Table 2) ranged between 25 and 34% homology. The values measured between strains of different species of *Nitrosomonas* were predominantly between 30 and 40% and thus near to the background. The lowest homology level between strains inside a genospecies was around 60%; generally the values were above 70%.

Morphological characteristics

The isolates in general were straight rods. However, several species tended to show coccoid forms and occasionally true cocci were observed (Fig. 1).

Cells of strains within the same genospecies were generally nearly identical in shape and size, with two exceptions. Cells of the strains Nm 90 (genospecies 9) and Nm 8 (genospecies 11) were real cocci (Fig. 1*i*₁ and 1*k*₂), while all the other isolates of these species were typical rods (Fig. 1*i*₂ and 1*k*₁). Motility was observed with only one strain of genospecies 7 and with the isolates of genospecies 10. On agar plates (standard medium plus 10 g agar l⁻¹) all species formed very similar small, brownish colonies of slimy consistency.

The ultrastructure of the cells was very similar in all genospecies. More or less extensive intracytoplasmic membrane systems were arranged as peripherally located flattened vesicles. Variations in details were not clearly species-specific. Polyhedral inclusions, however, were present in cells of all strains of the genospecies 7, 9, 10 and 13 but never in cells of the other genospecies. In genospecies 13 these inclusions are known to be carboxysomes (Harms *et al.*, 1981), whose presence is independent of the growth phase of the culture. Thus the possession of carboxysomes can be used as a species characteristic.

Physiological characteristics

Salt requirements, ammonia tolerance, utilization of urea as ammonia source, and the tolerance of low temperatures were found to be the most useful physiological characteristics to distinguish genospecies of *Nitrosomonas*.

Salt requirements were found to correspond with the conditions in the natural habitats of the respective species. Isolates from marine and brackish waters (genospecies 5, 6, 7, 8 and 13) showed an obligate salt requirement, with optimum values at around 300 mM-NaCl (genospecies 5, 6, 7 and 13) and 400 mM-NaCl (genospecies 8). Strains of the other genospecies showed no obligate salt requirement; they grew optimally at NaCl concentrations between 0 and 100 mM. However, strains isolated from strongly eutrophic environments (genospecies 9, 10 and 12) generally tolerated higher

levels of NaCl (maximum between 400 and 500 mM) than the other (genospecies 1–4 and 11) terrestrial and freshwater isolates (maximum between 200 and 300 mM). Significant differences between strains within a genospecies were not observed.

Ammonia tolerance was also very different among the genospecies. All strains of genospecies 11 were remarkably sensitive to increasing concentrations and were severely inhibited even at 50 mM-NH₄Cl, the optimum concentration for all the other genospecies. Isolates of genospecies 9 tolerated concentrations of up to 100 mM, strains of species 4, 7 and 8 up to 200 mM, and those of species 1–3 up to 250 mM. Isolates of genospecies 5, 6, 12 (*N. europaea*) and 13 were totally inhibited only at 400 mM and those of genospecies 10 could grow at concentrations up to at least 600 mM. Within the latter genospecies the upper limit of tolerance was different between isolates, ranging from 600 to 800 mM, while differences between strains within the other genospecies were not significant.

Urea was hydrolysed by all strains of genospecies 4–9 and 11 with the exception of single isolates in genospecies 8 and 11. No strain of genospecies 1–3, 10, 12 and 13 was able to utilize this substrate (see Table 3). Thus utilization of urea is another useful species characteristic.

The ability of '*N. cryotolerans*' to grow at temperatures around 0 °C (Jones & Morita, 1985) was found to be unique among the *Nitrosomonas* species. Even at stepwise decreasing temperatures (28, 14, 10, 5 and 0 °C) the lowest limit for growth was 5 °C for all species except '*N. cryotolerans*'. This is important in distinguishing the latter species from genospecies 6, since both were very similar in all other physiological properties.

Ecological observations

In our laboratory the enrichment and isolation of ammonia-oxidizing bacteria from environmental samples is carried out under standard conditions. Generally three different media (composed for terrestrial, brackish water and seawater strains) are simultaneously used for enrichment and finally drops of the enrichment cultures are streaked out on agar plates (media used for enrichment supplemented with 1% agar). Strain obtained from other laboratories, thus far known, were isolated under similar but not identical conditions.

All strains of genospecies 5, 6, 7, 8 and 13 originated from marine and brackish waters and they were all obligately halophilic. The isolates of genospecies 9, 10 and 12 were common in eutrophic habitats. Most of them were isolated from municipal and industrial sewage disposal systems. The isolates of genospecies 11 were remarkably often dominant in enrichments from samples strongly contaminated with heavy metals. Strains of

Fig. 1. Phase-contrast photomicrographs of the different *Nitrosomonas* species. (a) *N. communis*, strain Nm 2; (b) genospecies 2, strain Nm 41; (c) genospecies 3, strain Nm 33; (d) *N. ureae*, strain Nm 10; (e) *N. cryotolerans*, strain Nm 55; (f) *N. aestuarii*, strain Nm 36; (g) genospecies 7, strain Nm 51; (h) *N. marina*, strain Nm 22; (i₁) *N. nitrosa*, strain Nm 90; (i₂) *N. nitrosa*, strain Nm 91; (j) *N. eutropha*, strain Nm 57; (k₁) *N. oligotropha*, strain Nm 45; (k₂) *N. oligotropha*, strain Nm 8; (l) *N. europaea*, strain Nm 50; (m) *N. halophila*, strain Nm 1. (k₁). Bar, 5 μ m.

Fig. 2. LDS-PAGE (12.5% polyacrylamide) of whole-cell proteins from the different *Nitrosomonas* species (type strains). (a) Complete patterns, stained with Coomassie blue; (b) selective presentation of the high- M_r haem proteins, peroxidase stained. Lane 1, *N. communis*; lane 2, genospecies 2; lane 3, genospecies 3; lane 4, *N. ureae*; lane 5, *N. cryotolerans*; lane 6, *N. aestuarii*; lane 7, genospecies 7; lane 8, *N. marina*; lane 9, *N. nitrosa*; lane 10, *N. eutropha*; lane 11, *N. oligotropha*; lane 12, *N. europaea*; lane 13, *N. halophila*. Standard protein molecular masses are indicated on the left.

genospecies 1, 2 and 3, were common in soils; those of genospecies 4 were isolated from soils and from fresh waters. These results indicate that a predominant distribution in special environments may be a characteristic of the *Nitrosomonas* species.

Protein patterns

In LDS-PAGE analyses of whole-cell proteins, the genospecies of *Nitrosomonas* exhibited significantly different band patterns (Fig. 2a). This was most striking for the high- M_r haem proteins (Fig. 2b), which are believed to be components of hydroxylamine oxidoreductase (Miller & Wood, 1983). This is of special interest, because hydroxylamine oxidoreductase is known to be a highly complex key enzyme of the energy-generating system of ammonia oxidizers. Thus it must be present in every strain and should be a useful chemotaxonomic marker.

Figs 3(a) and 3(b) show the variability of protein patterns obtained from the two strains of the various genospecies which showed the lowest DNA-DNA homologies of the respective species. Despite the fact that the DNA-DNA homologies between these strain

pairs were mostly at the lower limit of the species level (60%), the patterns obtained (especially for the high- M_r haem proteins) were very similar within each species.

Discussion

In our investigations most of the genospecies of *Nitrosomonas* could be distinguished phenotypically. The DNA G+C content, the banding patterns of the high- M_r haem proteins, the utilization of urea as ammonia source, salt requirements, the tolerance of increasing ammonia concentrations and the possession of carboxysomes turned out to be the most suitable properties for practical differentiation of the species. With some limitations, the shape and size of the cells may be used as a further characteristic.

The phenotypic data were without exception in congruence with the genomic relationships. However, genospecies 1-3 and 7-8, respectively, resembled each other in so many respects that they were not distinguishable by phenotypic characteristics alone. The genospecies 1 and 8 were chosen to represent these two groups. Thus a total of 10 species could be defined by phenotype from the 13 genospecies studied. Two of the 10 defined species, *N. europaea* (the type species of the genus *Nitrosomonas*) and '*N. cryotolerans*', have already been described. Based on characteristic properties the names *Nitrosomonas communis* (genospecies 1), *Nitrosomonas ureae* (genospecies 4), *Nitrosomonas aestuarii* (genospecies 6), *Nitrosomonas marina* (genospecies 8), *Nitrosomonas nitrosa* (genospecies 9), *Nitrosomonas eutropha* (genospecies 10), *Nitrosomonas oligotropha* (genospecies 11), and *Nitrosomonas halophila* (genospecies 13) are proposed for the eight new species.

The distinguishing properties of the *Nitrosomonas* species, except the high- M_r haem proteins, are summarized in Table 3.

Emended description of the genus *Nitrosomonas* Winogradsky 1892

The emended description summarizes results of the present investigation and earlier publications (Watson, 1971a; Watson *et al.*, 1981, 1989).

Ni. tro. so. mo' nas. ML adj. *nitrosus* nitrous; Gr. fem. n. *monas*, a unit, monad; ML fem. n. *Nitrosomonas* nitrite monad, i.e. the monad producing nitrite.

Gram-negative organisms; marine forms can possess an additional outer protein cell wall layer. Shape and size of cells are variable between species; generally rod-shaped to coccoid, with rounded or pointed ends.

Fig. 3. LDS-PAGE (12.5% polyacrylamide) of whole-cell proteins from different *Nitrosomonas* species showing the variability of patterns of the two strains with the lowest DNA-DNA homologies of the respective species. (a) Complete patterns, stained with Coomassie blue; (b) selective presentation of the high-*M_r* haem proteins, peroxidase stained. Lanes 1a/1b, *N. communis*, strains Nm 2/Nm 40; lanes 2a/2b, genospecies 2, strains Nm 41/Nm 34; lane 3, genospecies 3, strain Nm 33; lanes 4a/4b, *N. ureae*, strains Nm 10/Nm 42; lane 5, *N. cryotolerans*, strain Nm 55; lanes 6a/6b, *N. aestuarii*, strains Nm 36/Nm 11; lanes 7a/7b, genospecies 7, strains Nm 51/Nm 63; lanes 8a/8b, *N. marina*, strains Nm 22/Nm 61; lanes 9a/9b, *N. nitrosa*, strains Nm 90/Nm 91; lanes 10a/10b, *N. eutropha*, strains Nm 57/Nm 23; lanes 11a/11b, *N. oligotropha*, strains Nm 45/Nm 8; lanes 12a/12b, *N. europaea*, strains Nm 50/Nm 28; lane 13, *N. halophila*, strain Nm 1. Standard protein molecular masses are indicated on the left.

Table 3. Phenotypic differentiation of the species of the genus *Nitrosomonas* (results obtained with the type strains)

Species	No. of strains	G+C content (mol %)	Salt requirement	Maximum ammonia tolerance (mM)	Use of urea	Carboxy-somes	Growth at 0 °C
<i>N. communis</i>	4	45.8	—	250	—	—	—
<i>N. ureae</i>	8	45.8	—	200	+	—	—
<i>N. cryotolerans</i>	1	45.8	+	400	+	—	+
<i>N. aestuarii</i>	8	45.8	+	400	+	—	—
<i>N. marina</i>	3	47.7	+	200	+	—	—
<i>N. nitrosa</i>	4	47.9	—	100	+	+	—
<i>N. eutropha</i>	10	48.2	—	600	—	+	—
<i>N. oligotropha</i>	6	49.7	—	50	+	—	—
<i>N. europaea</i>	7	51.0	—	400	—	—	—
<i>N. halophila</i>	1	53.8	+	400	—	+	—

Proclivity to grow in aggregates in mixed but not in pure cultures. Motility rarely established, and seems to depend on the stage of culture development; motile cells bear polar flagella. Extensive intracytoplasmic membranes arranged as flattened vesicles in the peripheral protoplasm; sometimes intrusions into the inner protoplasm. Carboxysomes present in some but not all species. Cells reddish coloured due to cytochromes. Aerobic organisms; can grow at reduced oxygen concentration with denitrifying activities. Obligately lithotrophic with ammonia as energy source; urea used as ammonia source by some not all species. Organisms generally grow autotrophically, carbon dioxide being assimilated via the Calvin cycle; can grow mixotrophically but not heterotrophically. Terrestrial and marine forms occur; marine species are obligately halophilic. Optimum growth at pH values between 7.5 and 8.0. Optimum temperature for growth around 30 °C, minimum temperature in general about 5 °C; one species grows at below 0 °C. Optimum ammonia concentration for growth 10–50 mM, variable between species. G + C content of the DNA 45.8–53.8 mol%; variable between species.

Type species: *Nitrosomonas europaea* Winogradsky 1892, Watson 1971a.

Species descriptions

Description of *Nitrosomonas communis* sp. nov.

Nitrosomonas communis (com.mu'nis. L adj. *communis*, common).

The description is based on four isolates. Cells 1.0–1.4 by 1.7–2.2 µm in size with rounded ends. Motility not observed. Carboxysomes not present. No salt requirement. Utilization of urea not observed. The G + C content of the DNA is 45.6–46.0 mol% (T_m).

Habitat: common in soils.

Type strain: Nm 2 – culture collection of the Institut für Allgemeine Botanik der Universität Hamburg, Mikrobiologische Abteilung, FRG.

At least two further species exist that are phenotypically very similar. Species designation will await the recognition of additional phenotypic characteristics.

Description of *Nitrosomonas ureae* sp. nov.

Nitrosomonas ureae (u're.ae. ML n. *urea*, urea; ML gen. n. *ureae*, of urea).

The description is based on eight isolates. Cells 0.9–1.1 by 1.5–2.5 µm with rounded ends. Motility not observed. Carboxysomes not present. Urea can be used as ammonia source. No salt requirement. The G + C content of the DNA is 45.6–46.0 mol% (T_m).

Habitat: common in soils and fresh waters.

Type strain: Nm 10 – culture collection of the Institut für Allgemeine Botanik der Universität Hamburg, Mikrobiologische Abteilung, FRG.

Description of *Nitrosomonas aestuarii* sp. nov.

Nitrosomonas aestuarii (ae.stu.a'ri.i. L n. *aestus*, tides; ML gen. n. *aestuarii*, of the estuary).

The description is based on eight isolates. Cells 1.0–1.3 by 1.4–2.0 µm in size with rounded ends. Motility not observed. Carboxysomes not present. Cells have an obligate salt requirement, with optimum growth around 300 mM-NaCl. Urea can be used as ammonia source. The G + C content of the DNA is 45.7–46.3 mol% (T_m).

Habitat: common in marine and estuarine waters.

Type strain: Nm 36 – culture collection of the Institut für Allgemeine Botanik der Universität Hamburg, Mikrobiologische Abteilung, FRG.

Description of *Nitrosomonas marina* sp. nov.

Nitrosomonas marina (ma.ri'na. L. fem. adj. *marina*, of the sea, marine).

The description is based on three isolates. Cells 0.7–0.9 by 1.4–2.3 µm in size with rounded ends. Motility not observed. Carboxysomes not present. Obligatory salt requirement, optimum growth at around 350 mM-NaCl. Urea can be used as ammonia source. The G + C content of the DNA is 47.4–48.0 mol% (T_m).

Habitat: marine waters and salt lakes.

Type strain: Nm 22 – culture collection of the Institut für Allgemeine Botanik der Universität Hamburg, Mikrobiologische Abteilung, FRG.

At least one further species exists that is very similar to *N. marina* in many characteristics. However, cells of this species are more coccoid and possess carboxysomes. Species designation will await the recognition of additional phenotypic characteristics.

Description of *Nitrosomonas nitrosa* sp. nov.

Nitrosomonas nitrosa (ni.tro'sa. ML. fem. adj. *nitrosa*, nitrous).

The description is based on four isolates. Spheres or rods with rounded ends, 1.3–1.5 by 1.4–2.2 µm in size. Motility not observed. Carboxysomes are present. Urea can be used as ammonia source. No salt requirement. The G + C content of the DNA is 47.9 mol% (T_m).

Habitat: isolated from eutrophic environments.

Type strain: Nm 90 (= Nc 5) – culture collection of the Institut für Allgemeine Botanik der Universität Hamburg, Mikrobiologische Abteilung, FRG.

Description of *Nitrosomonas eutropha* sp. nov.

Nitrosomonas eutropha (eu.troph'a. Gr. pref. *eu-*, good; Gr. n. *trophos*, one who feeds; ML fem. n. *eutropha*, good nutrition).

The description is based on 10 isolates. Cells tend to be pleomorphic, rod to pear-shaped (sometimes coccoid). One or both ends pointed. Cells 1.0–1.3 by 1.6–2.3 µm in size, occasionally in short chains. Cells are motile.

Carboxysomes present. Utilization of urea not observed. No salt requirement, high tolerance of increasing ammonia concentrations. The G+C content of the DNA is 47.9–48.5 mol % (T_m).

Habitat: common in municipal and industrial sewage disposal systems; seems to be distributed generally in strongly eutrophic environments.

Type strain: C-91 (= Nm 57) – culture collection of the Institut für Allgemeine Botanik der Universität Hamburg, Mikrobiologische Abteilung, FRG.

Description of *Nitrosomonas oligotropha* sp. nov.

Nitrosomonas oligotropha (o.li.go.tro'pha. Gr. adj. *oligos*, little; Gr. n. *trophos*, one who feeds; ML fem. n. *oligotropha*, little nutrition).

The description is based on six isolates. Cells rod-shaped with rounded ends or spherical, 0.8–1.2 by 1.1–2.4 μ m in size. Motility not observed. Cell aggregates are present after exponential growth has ceased. Carboxysomes not present. Utilization of urea as ammonia source observed in five of the six strains. No salt requirement. Sensitive to increasing ammonia concentrations > 50 mM. The G+C content of the DNA is 49.4–50.0 mol % (T_m).

Habitat: common in industrial sewage disposal systems; most of the isolates originate from water samples contaminated with chemicals.

Type strain: Nm 45 – culture collection of the Institut für Allgemeine Botanik der Universität Hamburg, Mikrobiologische Abteilung, FRG.

Emended description of *Nitrosomonas europaea* Winogradsky 1892, Watson 1971a

The description is based on characteristics of the neotype strain (Watson, 1971a) and six genetically related strains. Cells short rods with pointed ends, 0.8–1.1 by 1.0–1.7 μ m in size. Motility not observed. Carboxysomes not present. Utilization of urea not observed. No salt requirement. The G+C content of the DNA is 50.6–51.4 mol % (T_m).

Habitat: common in soils and fresh waters.

Neotype strain: ATCC 25978 (= C-31, = Nm 50 – culture collection of the Institut für Allgemeine Botanik der Universität Hamburg, Mikrobiologische Abteilung, FRG).

Description of *Nitrosomonas halophila* sp. nov.

Nitrosomonas halophila (hal.o'phi.la. Gr. n. *halos*, salt; Gr. adj. *philos*, loving; L fem. adj. *halophila*, salt-loving).

The description is based on only one isolate. Cells 1.1–1.5 by 1.5–2.2 μ m in size. Motility not observed. Carboxysomes present. Cells have an obligate salt requirement, with optimum growth around 300 mM-NaCl. Utilization of urea not observed. The G+C content of the DNA is 53.8 mol % (T_m).

Habitat: the only strain was isolated from the North Sea.

Type strain: Nm 1 – culture collection of the Institut für Allgemeine Botanik der Universität Hamburg, Mikrobiologische Abteilung, FRG.

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