The phylogeny of autotrophic ammonia-oxidizing bacteria as determined by analysis of 16S ribosomal RNA gene sequences

IAN M. HEAD,[1]† WILL D. HIORNS,[1] T. MARTIN EMBLEY,[2] ALAN J. MCCARTHY[1] and JON R. SAUNDERS[1]*

[1]*Department of Genetics and Microbiology, Life Sciences Building, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK*
[2]*Department of Zoology, The Natural History Museum, London SW7 5BD, UK.*

(*Received 3 August 1992; revised 20 January 1993; accepted 24 February 1993*)

Partial sequences of the 16S ribosomal RNA genes of eleven autotrophic ammonia-oxidizing bacteria were determined by PCR amplification from small amounts of heat-lysed biomass followed by direct sequencing of PCR products. The sequences were aligned with those of representative Proteobacteria and phylogenetic trees inferred using both parsimony and distance matrix methods. This confirmed that the autotrophic ammonia-oxidizers comprise two major lines of descent within the Proteobacteria. *Nitrosomonas* spp., *Nitrosococcus mobilis*, and strains of *Nitrosovibrio*, *Nitrosospira* and *Nitrosolobus* were located in the beta-subdivision. The recovery of *Nitrosococcus oceanus* strains as a deep branch in the gamma-subdivision supported the RNA catalogue data which had indicated that the genus *Nitrosococcus* is polyphyletic. The autotrophic ammonia-oxidizing bacteria of the beta-Proteobacteria formed a coherent group which is interpreted as representing a single family. Within this clade, the genera *Nitrosovibrio*, *Nitrosospira* and *Nitrosolobus* exhibited very high levels of homology in their 16S ribosomal RNA gene sequences and can be accommodated within a single genus. Separation of these genera is currently based entirely on gross morphological differences and these can now be considered more appropriate for the identification of species within this group. It is therefore proposed that *Nitrosolobus*, *Nitrosovibrio* and *Nitrosospira* strains be reclassified in a single genus for which the name *Nitrosospira* has priority.

Introduction

Autotrophic ammonia-oxidizing bacteria are an ecologically important and physiologically specialized group. They are responsible for the oxidation of ammonia to nitrite, the reaction which drives the process of nitrification in a wide range of environments (Hall, 1986). The organisms are obligate chemoautotrophs and they all exhibit an essentially identical central metabolism. Carbon dioxide is fixed via the Calvin cycle, and ammonia is oxidized to nitrite to produce energy and reducing power. Their electron transport systems appear to be identical, as determined by difference spectroscopy (Giannakis *et al.*, 1985; Koops *et al.*, 1991).

Autotrophic ammonia-oxidizing bacteria are notoriously difficult to isolate in pure culture and, compared to chemoorganotrophs, grow to low cell densities and biomass yields *in vitro*. This, and their autotrophic physiology, means that they are largely intractable to traditional methods of phenotypic characterization (Koops & Möller, 1991). Consequently, ammonia-oxidizing bacteria are classified largely on the basis of morphological criteria such as cell shape and the arrangement of internal membranes (Watson *et al.*, 1989; Bock *et al.*, 1986). Chemosystematic studies of cellular lipids have provided little information of taxonomic utility, since all of the genera examined exhibit similar lipid profiles (Blumer *et al.*, 1969).

Data from 16S rRNA catalogues (Woese *et al.*, 1984*b*, 1985) first demonstrated that there are two phylogenetically distinct groups of autotrophic ammonia-oxidizing bacteria. One of these contained *Nitrosococcus oceanus*, and was within the gamma-subdivision of the Proteobacteria. The other contained *Nitrosococcus mobilis* and representatives of all of the other described genera of ammonia-oxidizers, and was located within the beta-subdivision of the Proteobacteria. The ammonia-oxidizing bacteria in the beta-subdivision formed three

* Author for correspondence. Tel. (051) 794 4416; fax (051) 794 4414.

† Present address: Newcastle Research Group in Fossil Fuels and Environmental Geochemistry, Drummond Building, The University, Newcastle-upon-Tyne NE1 7RU, UK.

Table 1. *Strains of autotrophic ammonia-oxidizing bacteria used in this study*

All strains were provided by Professor S. W. Watson, Woods Hole Oceanographic Institute, USA, except C-31, C-71 and C-91, which were provided by Dr G. H. Hall of the Institute for Freshwater Ecology, Windermere, UK.

Taxon	Strain designation*	Origin	GenBank accession no.
Nitrosomonas			
N. europaea	C-31^T (Nm50)	Soil	M96399
N. eutropha	C-91^T (Nm57)	Sewage	M96402
Nitrosomonas sp.	C-56 (Nm63)	Seawater	M96400
Nitrosococcus			
N. mobilis	Nc2^T	Seawater	M96403
N. oceanus	C-27 (Nc9)	Seawater	M96398
N. oceanus	C-107^T (Nc10)	Seawater	M96395
Nitrosovibrio			
N. tenuis	Nv1^T	Soil	M96404
Nitrosovibrio sp.	Nv12	Soil	M96405
Nitrosovibrio sp.	C-141	Soil	M96397
Nitrosospira			
Nitrosospira sp.	C-128 (Nsp4)	Soil	M96396
Nitrosolobus			
N. multiformis	C-71^T (Nl13)	Soil	M96401

* ^{T}, Type strain. Alternative strain designations of Koops & Harms (1985) are given in parentheses.

deep branches; *Nitrosococcus mobilis*; *Nitrosomonas europaea*; and a third branch containing *Nitrosolobus*, *Nitrosovibrio* and *Nitrosospira* strains. Each genus was, however, represented by only a single strain and any taxonomic conclusions were further constrained by the fact that cataloguing methods only recover approximately 40% of the RNA sequence.

There have been a number of attempts at defining the taxonomic structure within the autotrophic ammonia oxidizers using DNA–DNA base pairing and mol% G+C determinations (Dodson *et al.*, 1983; Koops & Harms, 1985; Koops *et al.*, 1990, 1991). While groups of strains which could form the basis of species have been readily recognized using these approaches, they have proved of limited utility for the circumscription of genera. This is because the DNA pairing values between species are often very low and are close to the limits of resolution for DNA pairing methods (Huss *et al.*, 1983; Koops & Harms, 1985; Koops *et al.*, 1991).

The comparative analysis of complete or nearly complete 16S rRNA sequences provides greater resolution of higher level bacterial relationships than does DNA pairing or rRNA cataloguing (Wayne *et al.*, 1987; Stackebrandt, 1991). Furthermore, the development of the polymerase chain reaction (PCR; Saiki *et al.*, 1988) has made it possible to recover 16S rRNA gene sequences from uncultured and difficult to culture micro-organisms (Giovannoni *et al.*, 1990). The autotrophic ammonia-oxidizing bacteria are therefore excellent candidates for PCR-based phylogenetic analysis. In this paper, the higher level classification of ammonia-oxidizing bacteria was investigated by sequencing PCR-amplified 16S rRNA genes from representative strains of each of the currently recognized genera.

Methods

Organisms and growth conditions. The strains of ammonia-oxidizing bacteria used in this study are listed in Table 1. The organisms were grown autotrophically in the medium described by Watson (1971). Marine strains were grown in the same medium but containing 8.7 mg K_2HPO_4 l^{-1} and artificial sea water (Tropic Marin, Dr Biener, GmbH Aquarientechnik) (8·3 g l^{-1} *Nitrosococcus mobilis* Nc2; 30 g l^{-1} *Nitrosococcus oceanus* C-27, *Nitrosococcus oceanus* C-107; 25 g l^{-1} *Nitrosomonas marina* C-56). Erlenmeyer flasks (500 ml) containing 200 ml of medium were inoculated with 200 µl of stationary phase culture and incubated in the dark at 30 °C until the medium acidified due to oxidation of ammonia to nitrite. This was indicated by a colour change to yellow of the phenol red incorporated in the medium. The culture was then neutralized with filter-sterilized Na_2CO_3 (5%, w/v) and incubated again until acidification had occurred. Cells were harvested for DNA extraction or further subcultured.

DNA isolation, PCR amplification and sequencing of amplified 16S ribosomal RNA genes. Cells from 200 ml of culture were harvested by centrifugation in a Sorval RC-5 centrifuge (10000 r.p.m., 20 min, GSA rotor). The cells were transferred to a microcentrifuge tube, washed three times with 1 ml of TE buffer (10 mM-Tris/HCl, 1 mM-EDTA, pH 8·0), pelleted and resuspended in 100 µl of lysis buffer (TE containing 1%, v/v, Tween 80). The cell suspension was placed in a boiling water bath for 1 min and then snap-frozen in dry ice. This procedure was repeated three times and the cell lysate was extracted with chloroform (200 µl). The aqueous layer, separated during centrifugation, was used as a source of DNA for PCR amplification. If amplification was unsuccessful, the DNA preparation was further

purified by centrifugal ultrafiltration using a Centricon-100 Microconcentrator (Amicon). A 2–4 µl volume of the retentate was generally sufficient for PCR amplification of almost full-length 16S rRNA genes using primers pA and pH′ (Edwards *et al.*, 1989) and the PCR protocol of Embley (1991). The PCR products were sequenced using the linear PCR sequencing method of Embley (1991) and the sequencing primers described by Edwards *et al.* (1989).

Data analysis. The sequences from the ammonia-oxidizing bacteria were aligned by eye with published sequences from 41 strains chosen to represent the major proteobacterial subdivisions (Neefs *et al.*, 1991; Olsen *et al.*, 1991), and corrected for known secondary structure (Neefs *et al.*, 1991). Subsets of this alignment were used in all subsequent analyses. Positions which could not be aligned unambiguously were omitted. The final alignments comprised 1014 bases (all subdivisions of Proteobacteria, gamma-subdivision Proteobacteria) and 1236 bases (beta-subdivision Proteobacteria). The alignment for the beta-subgroup comprised positions 97–834, 849–1023, 1032–1125 and 1140–1372, and that for the gamma-subdivision covered positions 99–182, 243–451, 486–818, 862–997, 1042–1125, 1140–1254 and 1302–1361 (numbered as in *E. coli*; Woese et al., 1983). Distance values were calculated using the Jukes and Cantor (1969) correction in the DNADIST program from PHYLIP 3.4 (J. Felsenstein, 1991, University of Washington). Phylogenetic trees were constructed using the 'neighbor joining method' (Saitou & Nei, 1987). In order to evaluate the robustness of the inferred trees, a bootstrap analysis consisting of 100 resamplings of the data was performed using SEQBOOT (PHYLIP 3.4) and a consensus tree was generated using neighbor joining and the program CONSENSE (PHYLIP 3.4). Maximum parsimony analysis was carried out on the bootstrapped data using PAUP 3.0s (D. Swofford, 1990, Illinois Natural History Survey, Illinois).

Results and Discussion

The linear PCR method recovered almost full-length 16S rRNA gene sequences (1329–1448 nucleotides) for the eleven strains. The sequences have been deposited with GenBank and assigned the accession numbers given in Table 1. Preliminary analyses (data not shown) of the aligned sequences (1014 positions) from nine strains of ammonia-oxidizing bacteria, ten other Proteobacteria and *Bacillus subtilis* as an outgroup, confirmed the tree topology based on the 16S rRNA catalogue data of Woese *et al.* (1984*a*,*b*). *Nitrosococcus oceanus* C-27 and C-107 were recovered consistently as a deep branch within the gamma-Proteobacteria; all of the other strains, including *Nitrosococcus mobilis* Nc2, formed a clade in the beta-subdivision of the Proteobacteria. Signature nucleotide analysis (Woese, 1987) of the sequences supported these groupings, although in the case of *Nitrosococcus oceanus* C-27 and C-107, 8 of the 58 signature positions examined differed from the consensus base for the gamma-Proteobacteria. This may be a reflection of the depth of the branch containing these strains. The *Nitrosococcus oceanus* strains contained the sequence AAACUCAAAUG, which has only been found in organisms from the gamma-Proteobacteria (Woese *et al.*, 1985). The beta-subdivision diagnostic oligonucleotides AAAAACCUUACC and CYUUACACAUG (Woese *et al.*, 1984*b*) were present in all of the sequences from the beta-subdivision ammonia-oxidizers.

Gamma-subdivision ammonia-oxidizing bacteria

Only two *Nitrosococcus oceanus* strains were available for the present study. These organisms were found to be closely related to each other (96·4% identity over 1304 bp of unambiguously aligned sequence), thus confirming the results of DNA–DNA hybridization experiments (Koops & Harms, 1985; Koops *et al.*, 1990). The strains share a common ancestry with the photosynthetic organisms *Chromatium* and *Ectothiorhodospira* (Fig. 1), as previously suggested by 16S rRNA catalogue data (Woese *et al.*, 1985).

Beta-subdivision ammonia-oxidizing bacteria

In order to analyse in more detail the relationships between the ammonia-oxidizing genera recovered within the beta-Proteobacteria, longer stretches of 16S rRNA sequences from nine strains representing the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrosovibrio*, *Nitrosolobus* and *Nitrosospira*, were examined. Alignment against eight other beta proteobacterial sequences and *E. coli* (gamma-subdivision) as an outgroup enabled 1236 sequence positions to be aligned unambiguously. The phylogenetic tree (Fig. 2) demonstrated that the autotrophic ammonia-oxidizing bacteria form a clade (100% support from bootstrap resampling) within the beta-subdivision. A single tree with identical topology was also recovered using parsimony analysis of the bootstrapped sequence data (not shown). No other beta-Proteobacteria were ever recovered within the radiation of nitrifying organisms.

The depth of branching within the cluster of autotrophic ammonia-oxidizing bacteria is comparable to that within the family *Neisseriaceae* (Dewhirst *et al.*, 1989), which is represented in this study (Fig. 2) by the genera *Eikenella*, *Neisseria*, *Vitreoscilla* and *Chromobacterium*. It could therefore be suggested that the beta-subdivision, autotrophic ammonia-oxidizers should form the basis of a single family. The family *Nitrobacteraceae*, which currently contains these taxa, is polyphyletic since it includes ammonia-oxidizing bacteria from both the gamma- and beta-subdivisions of the Proteobacteria, plus the nitrite-oxidizing organisms from the alpha-subdivision (Woese *et al.*, 1984*a*). However, the published data are not exhaustive and we advocate the acquisition of more sequences from the gamma- and alpha- members of the family *Nitrobacteraceae* before a formal taxonomic revision at the family level is undertaken.

The beta-subdivision ammonia-oxidizers could be

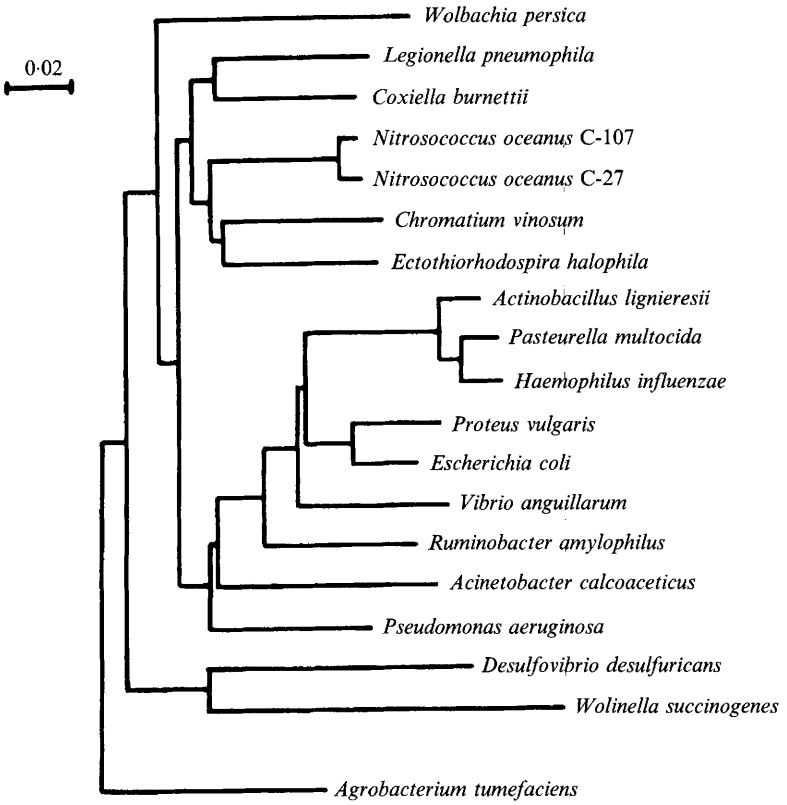

Fig. 1. Phylogenetic tree of representatives of the Proteobacteria showing the position of *Nitrosococcus oceanus* within the gamma-subdivision. The tree was generated using the method of Saitou & Nei (1987). *Agrobacterium tumefaciens* (alpha) was used as an outgroup. The scale bar indicates 0·02 substitutions per nucleotide position.

subdivided into two major lineages (Fig. 2). One lineage encompassed the strains of *Nitrosovibrio*, *Nitrosospira* and *Nitrosolobus* (100% support in bootstrap replicates), and the other contained the three *Nitrosomonas* species and *Nitrosococcus mobilis* (95% support). The taxonomy of the genus *Nitrosomonas* has recently been comprehensively overhauled and the two established species, *Nitrosomonas europaea* and '*Nitrosomonas cryotolerans*', were joined by eight new species of *Nitrosomonas* on the basis of DNA–DNA pairing, supported by a small number of phenotypic tests on type strains (Koops *et al.*, 1991). Based on these criteria, *Nitrosomonas* strain C-56 was designated *Nitrosomonas marina* and strain C-91, *Nitrosomonas eutropha* (Koops *et al.*, 1991). The separate taxonomic status of these two species was verified by our analyses (Fig. 2). Furthermore, on the basis of our data, *Nitrosococcus mobilis* Nc2 should also be transferred to the genus *Nitrosomonas*. The genus *Nitrosomonas* has recently been shown to contain both rods and true cocci (Koops *et al.*, 1991). Our data confirm that these two different morphologies occur in closely related bacteria, and provide further evidence that simple morphological traits are not reliable indicators of higher relationships (van Niel, 1946; Stackebrandt & Woese, 1981).

The low level of sequence variation between the representatives of *Nitrosolobus*, *Nitrosospira* and *Nitrosovibrio* (96·1% identity between all five strains over 1335 bases of unambiguously aligned sequence) suggests that they are very closely related indeed. The DNA pairing data of Dodson *et al.* (1983) demonstrated that there was slightly higher DNA homology between the genera *Nitrosospira* and *Nitrosolobus* than there was between some species classified within the genus *Nitrosomonas*. Subsequent DNA pairing measurements, determined from renaturation kinetics (De Ley *et al.*, 1970), gave mean hybridization values between representatives of these three genera of 33%, compared to average values within the genera of 33–42% (Koops & Harms, 1985). However, the renaturation method gives high background similarity values (typically 20 to 30%), because of the high baseline reassociation rate (Huss *et al.*, 1983; Johnson, 1991). Values in this region should therefore be treated with caution and cannot be reliably used to resolve the generic status of *Nitrosovibrio*,

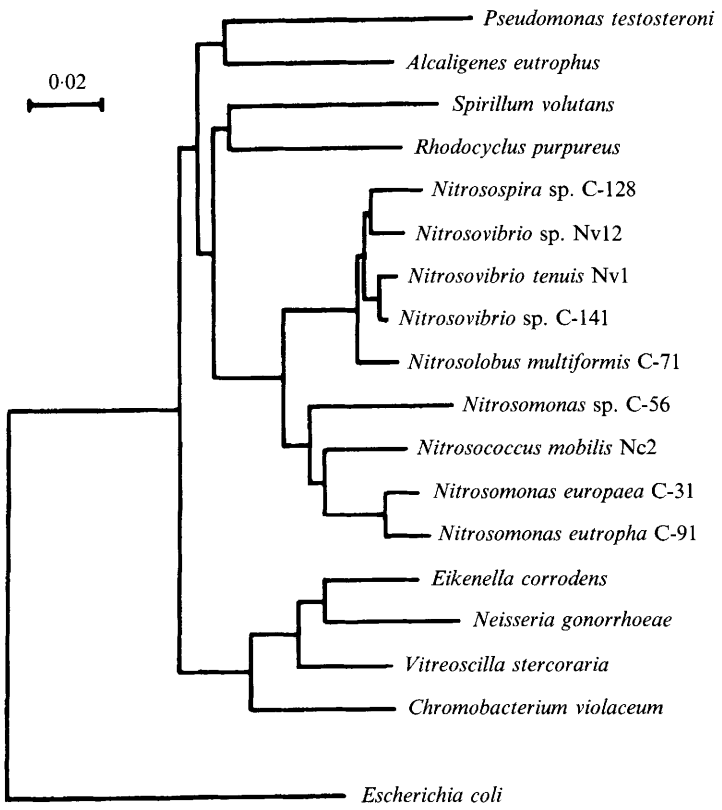

Fig. 2. Phylogenetic tree of autotrophic ammonia-oxidizing bacteria from the beta-Proteobacteria derived from the neighbor joining method of Saitou & Nei (1987). *Escherichia coli* (gamma) was used as the outgroup. The scale bar indicates 0·02 substitutions per nucleotide position.

Nitrosospira and *Nitrosolobus*. It is also well established that DNA hybridization values can be very low between organisms that otherwise share many phenotypic properties. Examples of this phenomenon can be found in *Streptomyces* (Mordarski *et al.*, 1986), *Staphylococcus* (Freney *et al.*, 1988) and *Legionella* (Fry *et al.*, 1991). Additional data (to the rRNA sequences) that support a close relationship between *Nitrosolobus*, *Nitrosovibrio* and *Nitrosospira* include similar fatty acid and cytochrome compositions and the limited spread of mol% G+C values (52·0 to 56·3%) (Blumer *et al.*, 1969; Giannakis *et al.*, 1985; Watson *et al.*, 1989).

The separation of *Nitrosolobus*, *Nitrosospira* and *Nitrosovibrio* into distinct genera is currently based solely on cell shape (Watson *et al.*, 1989; Koops & Möller, 1991). We suggest that simple morphological differences are useful here for species classification and identification, but are insufficient to retain separate generic status for bacteria which on the weight of other phenotypic and genotypic evidence appear to be so closely related. Our assertion that cell shape alone is a poor criterion for the differentiation of autotrophic ammonia-oxidizing bacteria at the genus level, is supported by considering the taxonomy of *Nitrosomonas*, where cell shape varies between species. We therefore propose that *Nitrosolobus*, *Nitrosovibrio* and *Nitrosospira* be reclassified in a single genus for which the name *Nitrosospira* has nomenclatural priority. The genera *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus* each contain a single validly described nomenspecies and representative and/or type strains were examined in this study. Transfer of species from *Nitrosovibrio* and *Nitrosolobus* to the genus *Nitrosospira* results in the new combinations *Nitrosospira tenuis* and *Nitrosospira multiformis*. The species descriptions have been published previously (Watson *et al.*, 1971, 1989; Harms *et al.*, 1976).

Emended description of the genus Nitrosospira

Nitrosospira Winogradsky and Winogradsky 1933 emend. Cells are spiral, curved or lobate containing either no intracytoplasmic membranes or membranes which partially compartmentalize the cell. Spherical forms are present in early stationary phase cultures.

Gram-negative. Motile by flagella or non-motile. Aerobic. The major source of energy and reducing power is from the oxidation of ammonia to nitrite. Chemolithotrophs. May grow mixotrophically, but never heterotrophically. Carboxysomes and other inclusions are not observed. Occur in terrestrial and freshwater environments. Optimal growth occurs in mineral salts medium containing ammonium salts. Optimum temperature: 25–30 °C. Optimum pH 7·5. The mol% G+C of the DNA is 52·2 to 56·3 (Bd, T_m).

Type species: *Nitrosospira briensis*

We are greatly indebted to Professor Stanley Watson of the Woods Hole Oceanographic Institute for providing the majority of strains used in this study and Dr Grahame Hall of the Institute for Freshwater Ecology, Windermere for providing the remainder. This work was supported by grants GR3/7160 and GST/02/569 from the Natural Environment Research Council. W. D. Hiorns was supported by a NERC postgraduate studentship.

References

BLUMER, M., CHASE, T. & WATSON, S. W. (1969). Fatty acids in the lipids of marine and terrestrial nitrifying bacteria. *Journal of Bacteriology* **99**, 366–370.

BOCK, E. KOOPS, H.-P. & HARMS, H. (1986). Cell biology of nitrifying bacteria. In *Nitrification*, Special Publication of the Society for General Microbiology, vol. 20, pp. 17–38. Edited by J. I. Prosser. Oxford: IRL Press.

DE LEY, J., CATTOIR, H. & REYNAERTS, A. (1970). The quantitative measurements of DNA hybridization from renaturation rates. *European Journal of Biochemistry* **12**, 133–142.

DEWHIRST, F. E., PASTER, B. F., BRIGHT, P. L. (1989). *Chromobacterium*, *Eikenella*, *Kingella*, *Neisseria*, *Simonsiella* and *Vitreoscilla* species comprise a major branch of the beta group of *Proteobacteria* by 16S ribosomal ribonucleic acid sequence comparison: transfer of *Eikenella* and *Simonsiella* to the family *Neisseriaceae* (emend.). *International Journal of Systematic Bacteriology* **39**, 258–266.

DODSON, M. S., MANGAN., J. & WATSON, S. W. (1983). Comparison of deoxyribonucleotide acid homologies of six strains of ammonia-oxidizing bacteria. *International Journal of Systematic Bacteriology* **33**, 521–524.

EDWARDS, U., ROGALL, T., BLÖCKER, H., EMDE, M. & BÖTTGER, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Research* **17**, 7843–7853.

EMBLEY, T. M. (1991). The linear PCR reaction: a simple and robust method for sequencing amplified rRNA genes. *Letters in Applied Microbiology* **13**, 171–174.

FRENEY, J., BRUN, Y., BES, M., MEUGNIER, H., GRIMONT, F., GRIMONT, P. A. D., NERVI, C. & FLEURETTE, J. (1988). *Staphylococcus lugdunensis* sp. nov. and *Staphylococcus schleiferi* sp. nov., two species from human clinical specimens. *International Journal of Systematic Bacteriology* **38**, 168–172.

FRY, N. K., WARWICK, S., SAUNDERS, N. A. & EMBLEY, T. M. (1991). The use of 16S ribosomal RNA analyses to investigate the phylogeny of the family *Legionellaceae*. *Journal of General Microbiology* **137**, 1215–1222.

GIANNAKIS, C., MILLER, D. J. & NICHOLAS, D. J. D. (1985). Comparative studies on redox proteins from ammonia-oxidizing bacteria. *FEMS Microbiology Letters* **30**, 81–85.

GIOVANNONI, S. J., BRITSCHGI, T. B., MOYER, C. L. & FIELD, K. G. (1990). Genetic diversity in Sargasso Sea bacterioplankton. *Nature, London* **345**, 60–63.

HALL, G. H. (1986). Nitrification in lakes. In *Nitrification*, Special Publication of the Society for General Microbiology, vol. 20, pp. 127–156. Edited by J. I. Prosser. Oxford: IRL Press.

HARMS, H., KOOPS, H.-P. & WEHRMANN, H. (1976). An ammonia-oxidizing bacterium, *Nitrosovibrio tenuis* nov. gen. nov. sp. *Archives of Microbiology* **108**, 105–111.

HUSS, V. A. R., FESTL, H. & SCHLEIFER, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Systematic and Applied Microbiology* **4**, 184–192.

JOHNSON, J. L. (1991). DNA reassociation experiments. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 205–248. Edited by E. Stackebrandt & M. Goodfellow. Chichester: John Wiley.

JUKES, T. H. & CANTOR, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

KOOPS, H.-P. & HARMS, H. (1985). Deoxyribonucleic acid homologies among 96 strains of ammonia-oxidizing bacteria. *Archives of Microbiology* **141**, 214–218.

KOOPS H.-P. & MÖLLER, U. C. (1991). The lithotrophic ammonia-oxidizing bacteria. In *The Prokaryotes*, 2nd edn, pp. 2625–2637. Edited by A. Balows, H. G. Truper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Fischer-Verlag.

KOOPS, H.-P., BÖTTCHER, B., MÖLLER, U. C., POMMERENING-RÖSER, A. & STEHR, G. (1990). Description of a new species of *Nitrosococcus*. *Archives of Microbiology* **154**, 244–248.

KOOPS, H.-P., BÖTTCHER, B., MÖLLER, U. C., POMMERENING-RÖSER, A. & STEHR, G. (1991). 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., *Nitrosomonas halophila* sp. nov. *Journal of General Microbiology* **137**, 1689–1699.

MORDARSKI, M., GOODFELLOW, M., WILLIAMS, S. T. & SNEATH, P. H. A. (1986). Evaluation of species groups in the genus *Streptomyces*. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes*, pp. 517–526. Edited by G. Szabo, S. Biro & M. Goodfellow. Budapest: Akademiai Kiado.

NEEFS, J.-M., VAN DE PEER, Y., DE RIJK, P., GORIS, A. & DE WACHTER, R. (1991). Compilation of small ribosomal subunit RNA sequences. *Nucleic Acids Research* **19** (Suppl.), 1987–2015.

OLSEN, G. J., LARSEN, N. & WOESE, C. R. (1991). The ribosomal RNA database project (RDP). *Nucleic Acids Research* **19** (Suppl.), 2017–2021.

SAIKI, R. K., GELFAND, D. H., STOFFEL, S., SCHARF, S. J., HIGUCHI, G. T., HORN, G. T., MULLIS, K. B. & ERLICH, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491.

SAITOU, N. & NEI, M. (1987). The neighbor joining method: a new method for constructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406–425.

STACKEBRANDT, E. (1991) Unifying phylogeny and phenotypic diversity. In *The Prokaryotes*, 2nd edn, pp. 19–48. Edited by A. Balows, H. G. Truper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Fischer-Verlag.

STACKEBRANDT, E. & WOESE, C. R. (1981). Towards a phylogeny of the actinomycetes and related organisms. *Current Microbiology* **5**, 197–202.

SWOFFORD, D. (1991). PAUP: Phylogenetic analysis using parsimony, version 3.0. Computer program distributed by Illinois Natural History Survey, Champaign, Illinois, USA.

VAN NIEL, C. B. (1946). The classification and natural relationships of bacteria. *Cold Spring Harbor Symposia on Quantitative Biology* **11**, 285–301.

WATSON, S. W. (1971). Reisolation of *Nitrosospira briensis* S. Winogradsky and H. Winogradsky 1933. *Archiv für Mikrobiologie* **75**, 179–188.

WATSON, S. W., GRAHAM, L. B., REMSEN, C. C. & VALOIS F. W. (1971). A lobular ammonia-oxidizing bacterium, *Nitrosolobus multiformis* nov. gen. nov. sp. *Archiv für Mikrobiologie* **76**, 183–203.

WATSON, S. W., BOCK, E., HARMS, H., KOOPS, H.-P. & HOOPER, A. B. (1989). Nitrifying bacteria. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 1808–1834. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.

WAYNE, L. G., BRENNER, D. J., COLWELL, R. R., GRIMONT, P. A. D., KANDLER, O., KRICHEWSKY, M. I., MOORE, L. H., MOORE, W. E., MURRAY, E., STARR, M. P. & TRUPER, H. G. (1987). Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. *International Journal of Systematic Bacteriology* **37**, 463–464.

WINOGRADSKY, S. & WINOGRADSKY, H. (1933). Études sur la microbiologie du sol. VII. Nouvelles recherches sur les organismes de la nitrification. *Annals de l'Institut Pasteur* **50,** 350–432.

WOESE, C. R. (1987). Bacterial evolution. *Microbiological Reviews* **51**, 221–271.

WOESE, C. R., GUTELL, R., GUPTA, R. & NOLLER, H.R (1983). Detailed higher order structure of 16S-like ribosomal ribonucleic acids. *Microbiological Reviews* 47, 621–669.

WOESE, C. R., STACKEBRANDT, E., WEISBURG, W. G., PASTER, B. J., MADIGAN, M. T., FOWLER, V. J., HAHN, C. M., BLANZ, P., GUPTA, R., NEALSON, K. H., & FOX, G. E. (1984*a*). The phylogeny of purple bacteria: the alpha subdivision. *Systematic and Applied Microbiology* **5**, 315–326.

WOESE, C. R., WEISBURG, W. G., PASTER, B. J., HAHN, C. M., TANNER, R. S., KRIEG, N. R., KOOPS, H.-P., HARMS, H. & STACKEBRANDT, E. (1984*b*). The phylogeny of the purple bacteria: the beta subdivision. *Systematic and Applied Microbiology* **5**, 327–336.

WOESE, C. R., WEISBURG, W. G., HAHN, C. M., PASTER, B. J., ZABLEN, L. B., LEWIS, B. J., MACKE, T. J., LUDWIG, W. & STACKEBRANDT, E. (1985). The phylogeny of the purple bacteria: the gamma subdivision. *Systematic and Applied Microbiology* **6**, 25–33.