Taxonomic Considerations of the Family *Nitrobacteraceae* Buchanan Requests for Opinions¹

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A revision of the family *Nitrobacteraceae* Buchanan is proposed. It is requested that the Judicial Commission issue an Opinion placing the generic names *Nitrosocystis* Winogradsky, *Nitrosogloea* Winogradsky, and *Nitrocystis* Winogradsky on the list of rejected names as *nomina dubia*. Organisms currently placed in these genera are to be transferred to other genera or their names are to be regarded as later, subjective synonyms of other names or as *nomina dubia*. Thus *Nitrosocystis oceanus* Watson will be transferred to *Nitrosococcus* as *Nitrosococcus oceanus* (Watson) *comb. nov.*; *Nitrosocystis coccoides* Starkey is to be regarded as a synonym of *Nitrosococcus nitrosus* (Migula) Buchanan, *Nitrosomonas monocella* Nelson, a synonym of *Nitrosomonas europaea* Winogradsky, *Nitrospira antarctica* Winogradsky and Winogradsky, a synonym of *Nitrospira briensis* Winogradsky and Winogradsky, and *Nitrocystis sarcinoides* Winogradsky, *Nitrocystis micropunctata* (Winogradsky) Winogradsky, and *Nitrobacter agilis* Nelson as later, subjective synonyms of *Nitrobacter winogradskyi* Winslow et al.; *Nitrosogloea merismoides* Winogradsky, *Nitrosogloea membranacea* Winogradsky, *Nitrosogloea schizobacteroides* Winogradsky, and *Nitrosocystis javanensis* (Winogradsky) Starkey are to be regarded as *nomina dubia*. The Judicial Commission is requested, therefore, to issue an Opinion placing these *nomina dubia* (names whose application is uncertain) on the list of rejected names. The type strain of *Nitrosolobus multiformis*, ATCC 25196, is described. The following strains are described and are here designated as type strains: *Nitrosococcus oceanus* ATCC 19707, *Nitrococcus mobilis* ATCC 25380, and *Nitrospina gracilis* ATCC 25379. The following strains are described and are here designated as neotypes: *Nitrosomonas europaea* ATCC 25978, *Nitrospira briensis* ATCC 25961, and *Nitrobacter winogradskyi* ATCC 25391.

The family *Nitrobacteraceae* Buchanan contains those chemoaerobic bacteria which oxidize either ammonia to nitrite or nitrite to nitrate to fulfill their primary energy needs. *Bergey's Manual of Determinative Bacteriology* (1) lists the genera of ammonia-oxidizing bacteria as *Nitrosomonas* Winogradsky, *Nitrosocystis* Winogradsky, *Nitrosogloea* Winogradsky, *Nitrospira* Winogradsky and Winogradsky, and *Nitrosococcus* Winogradsky, and the genera of nitrite-oxidizing bacteria as *Nitrobacter* Winogradsky and *Nitrocystis* Wino-

gradsky. All of these were originally described by Helene or Sergei Winogradsky (21, 22, 24, 26, 27). Many of the original descriptions were based on mixed cultures and were so brief that it is impossible to identify an organism from them. Some of the characteristics used for the separation of these bacteria into genera are variable and have resulted in the grouping of several morphologically unrelated strains into one genus. Moreover, none of the original strains were preserved in culture. The intent of this study was to update the specific and generic descriptions of the nitrifying bacteria and, where necessary, to designate type and neotype strains and to make taxonomic and

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nomenclatural recommendations concerning these organisms.

MATERIALS AND METHODS

Bacterial strains. The sources of the organisms used in this study are given below.

Methods. The methods used have been described previously (18, 19).

RESULTS AND DISCUSSION

Subsequent to the work of the Winogradskys, most investigators were able to isolate only members of the genus *Nitrosomonas* or *Nitrobacter*, and doubts were expressed as to the validity of the other genera (6, 7). Until recently, less than a dozen strains of nitrifying bacteria were preserved in culture, and all of these belonged either to the genus *Nitrosomonas* or *Nitrobacter*. These organisms have a generation time of 8 hr or more, making it difficult to isolate them in pure culture, thus few pure cultures existed. Although the taxonomy of the bacteria in the family *Nitrobacteraceae* has long been in need of revision, the lack of cultures has made such a task impossible.

Studies on nitrifying bacteria were initiated at the Woods Hole Oceanographic Institution in 1957 and are continuing at present. During this period, thousands of enrichment cultures were made, and over 200 nitrifying bacteria were isolated in pure culture. These studies showed that several morphologically different types of nitrifying bacteria existed and indicated that not all of the genera of nitrifying bacteria listed in *Bergey's Manual* were valid. The purpose of this present publication is to make specific recommendations concerning the taxonomy of the family *Nitrobacteraceae*.

The three genera of most questionable validity are *Nitrosogloea*, *Nitrosocystis*, and *Nitrocystis*. The first two genera are comprised of ammonia oxidizers and the latter of nitrite oxidizers. All of these genera are reserved for those bacteria which grow in aggregates forming either zoogloes or cysts. Based on these aggregation characteristics, several morphologically unrelated strains have been placed in the genus *Nitrosogloea*. Studies in this laboratory have demonstrated that most ammonia- and nitrite-oxidizing bacteria in mixed culture have a proclivity to grow in aggregates forming either zoogloes or cysts. Some organisms, e.g. *Nitrosocystis oceanus* Watson (17), no longer formed cysts when isolated in pure culture whereas others tended to aggregate only when cultural conditions were unfavorable. Other nitrifying bacteria, e.g. *Nitrococcus mobilis* Watson and

Waterbury (20), when grown in pure culture in a chemostat, grew as single cells freely suspended in the medium for 1 to 2 months but then started to grow in small aggregates suspended in the medium. Once a culture started to clump, it could not be induced to grow again as single cells even by varying the cultural conditions. The ability of cells to grow in clumps forming zoogloes or cysts is a characteristic shared by several morphological types of ammonia- and nitrite-oxidizing bacteria. Thus zoogloea and cyst formations are not reliable taxonomic criteria, and it is recommended that they should no longer be used to separate genera.

If the recommendation not to place organisms into taxa solely on the basis of their proclivity to aggregate is accepted, then disposition of those bacteria in the genera *Nitrosogloea*, *Nitrosocystis*, and *Nitrocystis* must be arranged. Recommendations concerning the organisms currently placed in all the ammonia-oxidizing genera will be discussed first. The disposition of the organisms in the genus *Nitrocystis* will be reserved until the nitrite oxidizers are reviewed.

The organisms in the genus *Nitrosogloea* include *N. merismoides* Winogradsky (21-23), *N. schizobacteroides* Winogradsky (21), and *N. membranacea* Winogradsky (21). The first of these three organisms is described as ellipsoidal rods, 0.5 to 1.5 μm , the second as elongated rods, 3 to 4 μm in length, and the last as ellipsoidal rods which often occur in pairs. The original descriptions of these three organisms consist of only a few short sentences, and except for the size and shape of these organisms no other information is available on the morphology of these cells. It is impossible to identify these organisms or to assign them to other genera from their original descriptions. Therefore, it is proposed to consider each of these three names as well as the generic name *Nitrosogloea* as *nomina dubia* (i.e., names whose applications are uncertain), and it is proposed that they should be included on the list of *nomina rejicienda* according to Rule 24f of the International Code of Nomenclature of Bacteria (8).

Since cyst formation can be observed in several morphologically unrelated strains in mixed but not in pure cultures, it is proposed that the organisms in the genus *Nitrosocystis* should be reassigned to other genera or that their names be considered as *nomina dubia*. Only one member of the genus *Nitrosocystis*, *N. oceanus*, is preserved in culture. This organism is a peritrichously flagellated large coccus (Fig. 1 and 19) and is characterized by exten-

FIG. 1. Phase-contrast photomicrograph of *Nitrosococcus oceanus* (formerly *Nitrosocystis oceanus*). $\times 2,500$; FIG. 2. Electron micrograph of *Nitrosococcus oceanus* showing centrally arranged cytomembranes. $\times 31,900$. FIG. 3. Phase-contrast photomicrograph of *Nitrosomonas europaea*. $\times 2,500$. FIG. 4. Electron micrograph of *Nitrosomonas europaea* showing peripheral cytomembranes. $\times 47,800$. FIG. 5. Electron micrograph of marine *Nitrosomonas* sp. showing rounded ends and peripheral cytomembranes. $\times 34,800$. FIG. 6. Frozen-etched preparation showing extra cell wall layer of a marine *Nitrosomonas* sp. $\times 95,700$.

sive cytomembranes arranged as flattened vesicles in the central region of the cell (Fig. 2). The size and shape of the vegetative cells of *Nitrosocystis oceanus* are similar to those of *Nitrosocystis coccoides* Starkey 1948 (15) and *Nitrosococcus nitrosus* (Migula 1900) Buchanan 1925 (4, 10). It is suggested that all three of these organisms belong to the genus *Nitrosococcus* and that this genus include spherical organisms having a diameter of 1.5 μm or greater. It is proposed that *Nitrosocystis coccoides* be regarded as a later, subjective synonym of *Nitrosococcus nitrosus*.

It is also proposed that *Nitrosocystis oceanus* be transferred to the genus *Nitrosococcus* as *Nitrosococcus oceanus* (Watson) comb. nov. Although *Nitrosococcus oceanus* is similar in size and shape to *Nitrosococcus nitrosus*, the former is an obligate marine organism and the latter is not. Since the fine structure of *Nitrosococcus nitrosus* has not been detailed, it is impossible to state whether the two organisms are similar. To avoid further taxonomic confusion, we recommend keeping *Nitrosococcus oceanus* as a separate species. Although no cultures of *Nitrosococcus nitrosus* are available, this organism must still be considered the type species of the genus.

The species *Nitrosocystis javanensis* (Winogradsky) Starkey (15, 26) is described as a small, ellipsoidal cell 0.5 to 0.6 μm in diameter. Since it is proposed that the genus *Nitrosococcus* contains only large, spherical, ammonia-oxidizing bacteria, *N. javanensis* cannot be transferred to this genus. This organism is poorly defined in the literature, and it is recommended that this species name as well as the generic name *Nitrosocystis* Winogradsky be considered as *nomina dubia* and placed on the list of *nomina rejicienda*.

The genus *Nitrosomonas* contains rod-shaped organisms which oxidize ammonia to nitrite, and it is recommended that this genus remain as described. Currently two species are listed in *Bergey's Manual* as belonging to this genus, *N. europaea* Winogradsky 1892 (26) and *N. monocella* Nelson 1931 (12). Comparison of the original descriptions of these two organisms reveals that they are morphologically similar. For this reason it is recommended that *Nitrosomonas monocella* be considered as a later, subjective synonym of *Nitrosomonas europaea*.

Since none of the original strains of *Nitrosomonas europaea* appear to be extant, it is recommended that an existing strain, filed with the American Type Culture Collection (ATCC) and assigned ATCC no. 25978, should be designated as the neotype strain according to

Rule 9d (1), Note 2(c) of the Bacteriological Code (8). Neither flagella nor motility has been observed in this designated neotype strain. In all other respects the characters of this designated neotype strain (see Table 1) agree with those recorded in the original species description (26). *Nitrosomonas europaea* was originally described as having a single, polar flagellum. Although we have not observed flagella in the designated neotype strain just mentioned, other strains of *Nitrosomonas* species were found to have one or two flagella with subterminal insertion (Fig. 20). The cells of the neotype strain are either ellipsoidal or short rods (0.8 by 1 to 2 μm) with pointed ends (Fig. 3), and their fine structure is characterized by cytomembranes (11) which are arranged in flattened vesicles in the peripheral regions (Fig. 4).

Although only one species of *Nitrosomonas*, *N. europaea*, will be listed in this taxonomic revision, other bacteria exist which should be placed in this genus. If this genus is to include rod-shaped organisms which have peripheral cytomembranes, then organisms which we have isolated from the marine environment and from the Chicago sewer system should also be placed in it as new species. For example, obligate marine ammonia oxidizers were isolated which were rod-shaped, with rounded ends, 1.0 to 1.3 μm wide and 2.0 to 2.5 μm long. All of these had membranes arranged as flattened lamellae in the peripheral regions of the cell (Fig. 5) and an additional cell wall layer (Fig. 6) not found in the strains of *Nitrosomonas europaea* that we examined.

A different rod-shaped, ammonia-oxidizing bacterium was isolated from the Chicago sewer system. The cells of this organism were 1.2 μm wide and 2.0 to 3.5 μm long with pointed ends, and there were prominent connections between dividing cells (Fig. 7). Electron microscopic observations (Fig. 8) showed that, although two or more cells were often interconnected, no separating cross wall was visible. I would like to postpone judgment of the species designation of these strains until they are studied in greater detail. These strains are mentioned only to illustrate that not all strains of *Nitrosomonas* are morphologically identical.

Another genus of ammonia-oxidizing bacteria listed in *Bergey's Manual* is *Nitrospira* (27), which includes two species, *N. briensis* Winogradsky and Winogradsky 1933 (28) and *N. antarctica* Winogradsky and Winogradsky 1933 (28). Both organisms have spiral-shaped cells and apparently have a similar morphology. The only difference noted was that the cells of

FIG. 7. Phase-contrast photomicrograph of a *Nitrosomonas* sp. isolated from the Chicago sewer system, showing pointed ends of cells and interconnections between cells. $\times 25,000$. FIG. 8. Electron micrograph of *Nitrosomonas* sp. in Fig. 7, showing the lack of cross walls between cells. $\times 18,800$. FIG. 9. Phase-contrast photomicrograph of *Nitrospira briensis* showing that the spiral nature of cells is barely visible. $\times 2,500$. FIG. 10. Negatively stained preparation of *Nitrospira briensis* showing spiral nature of cells and flagella. $\times 28,700$. FIG. 11. Phase-contrast photomicrograph of *Nitrosolobus multiformis* showing lobular nature of cells. $\times 2,500$. FIG. 12. Electron micrograph of *Nitrosolobus multiformis* showing how the lobular cells are partially compartmentalized by cytomembranes. $\times 26,600$.

Nitrosospira antarctica form more compact spirals than do the cells of *Nitrosospira briensis*. *Nitrosospira briensis* was isolated recently in this laboratory and described in detail (18). It was observed that the degree of compactness of the spirals depended upon the mounting technique. If heated, the cells would often partially uncoil. It does not seem justified to use this criterion for the separation of these organisms into two species. It is recommended that the name *Nitrosospira antarctica* be considered a later, subjective synonym of *Nitrosospira briensis*.

It is further recommended that the genus *Nitrosospira* contain the ammonia-oxidizing bacteria whose cells are tightly wound (Fig. 9), so much so that the spiral nature may not be obvious under a phase-contrast microscope. However, the spiral nature of these cells should be clearly evident when negatively stained preparations (Fig. 10) are examined with an electron microscope. In thin sections, several sections of a single cell will be seen lying side-by-side due to the spiral nature of the organism. *N. briensis* does not possess a well-developed cytomembrane system that is characteristic of the other ammonia oxidizers. Since the original strain of *N. briensis* was not preserved in culture, ATCC 25961 is here designated as the neotype strain of this species (Table 1). This designated neotype strain of *Nitrosospira briensis* is peritrichous (Fig. 21), but in all other respects the characters of this strain agree with those recorded in the original species description (28).

Recently a peritrichous, lobular-shaped, ammonia-oxidizing bacterium (Fig. 11 and 22) was isolated in this laboratory and named *Nitrosolobus multiformis* Watson et al. (19). It was proposed that the genus *Nitrosolobus* contain the lobular-shaped organisms with cytomembranes which partially compartmentalize the cells (Fig. 12). The type strain of this species is ATCC 25196 (reference 19; incorrectly cited herein as ATCC 2519), and its characters are summarized in Table 1.

Watson and Mandel (19a) analyzed the deoxyribonucleic acid (DNA) base compositions of 19 strains of ammonia-oxidizing bacteria; these were found to form two homogenous groups with respect to their guanine plus cytosine (GC) compositions. The GC compositions of members of *Nitrosomonas* and *Nitrosocystis* ranged from 47.7 to 51.0 moles per cent and those of the members of *Nitrosolobus* and *Nitrosospira* from 53.6 to 55.1 moles per cent.

According to the recommendations made

here, *Nitrosomonas* would contain the rod-shaped organisms, *Nitrosococcus* the large, spherical organisms, *Nitrosospira* the spiral-shaped organisms, and *Nitrosolobus* the lobular-shaped organisms.

The genera of nitrite-oxidizing bacteria listed in the 7th edition of *Bergey's Manual* include *Nitrobacter* and *Nitrocystis*. Winogradsky (26) described the first nitrite-oxidizing bacterium and proposed the genus *Nitrobacter* for it. This generic name, with *Nitrobacter winogradskyi* Winslow et al. 1917 (31) as the type species, was conserved by the Judicial Commission (9). The cells of this species were described as short rods, 0.6 to 0.8 μm wide and 1.0 to 1.2 μm long, often pear- or wedge-shaped, and reproducing by budding (5, 13, 15, 30). The typical morphology of *Nitrobacter winogradskyi* cells is illustrated in Fig. 13. The fine structure of *Nitrobacter* species is characterized by a polar cap of cytomembranes arranged as flattened lamellae (Fig. 14).

In the present studies two strains of *Nitrobacter winogradskyi* received from August Van Gool, who designated these strains Engel strain No. 1 and Lees strain No. 2, were examined. Strains of *Nitrobacter agilis* were obtained from Helen Funk and Alvin Nason. In addition, over 15 other strains of *Nitrobacter* species were isolated and studied in our own laboratory.

The second species of *Nitrobacter*, *N. agilis*, was described by Nelson (12). The chief difference between *N. agilis* and *N. winogradskyi* was that the former organism was observed to be motile and the latter was not. In studies conducted in our laboratory, all strains were found to be motile when cultivated in a chemostat, but none of the strains were motile when grown in batch culture. Separation of *N. winogradskyi* and *N. agilis* into two species based on their motility or lack of motility does not appear to be justified.

The cells of all strains were similar in size and shape and all appeared to reproduce by budding. The fine structure of all the strains was similar and all had a polar cap of cytomembranes (11, 16). The GC compositions of five strains of *Nitrobacter* sp. varied from 61.7 to 60.2 moles per cent. These strains formed a homogenous group with respect to their GC compositions. Since these two species seem to be similar in all respects, it is recommended that *N. agilis* be considered a synonym of *N. winogradskyi*.

The genus *Nitrocystis* (21) was proposed for ellipsoidal to rod-shaped cells which grew em-

TABLE 1. Descriptions of type and neotype strains of nitrifying bacteria

Characters	Type strains				Neotype strains		
	<i>Nitrosolobus multififormis</i> ATCC 25196 ^a	<i>Nitrosococcus oceanus</i> ATCC 19707	<i>Nitrococcus mobilis</i> ATCC 25380	<i>Nitrospina gracilis</i> ATCC 25379	<i>Nitrospira briensis</i> ATCC 25961	<i>Nitrobacter winogradskyi</i> ATCC 25391	<i>Nitrosomonas europaea</i> ATCC 25978
Shape	Lobular	Spherical	Spherical	Slender rod	Spiral	Pearlike	Rod
Size (µm)	1.0 × 1.5	1.8 × 2.2	1.5 × 1.8	0.3-0.4 × 2.7-6.5	0.3-0.4 × 1.0-5.0	0.6-0.8 × 1.0-2.0	0.8-0.9 × 1.0-2.0
Method of reproduction	Binary fission	Binary fission	Binary fission	Binary fission	Binary fission	Budding	Binary fission
Nature of cytomembranes	Internal, compartmentalizing the cell	Internal, lamellar	Internal, tubular	Cytomembranes absent	Cytomembranes absent	Peripheral, lamellar	Peripheral, lamellar
Flagellation	Peritrichous	Peritrichous	Two subpolar	None observed	Peritrichous	Single subpolar	None observed
Gram reaction	-	-	-	-	-	-	-
Endospores	-	-	-	-	-	-	-
Oxidation of nitrite to nitrate	-	-	+	+	-	+	-
Oxidation of ammonia to nitrite	+	+	-	-	+	-	+
Obligately aerobic	+	+	+	+	+	+	+
Optimum temp (°C)	25-30	25-30	25-30	25-30	25-30	25-30	25-30
Optimum pH	7.5-8.0	7.5-8.0	7.5-8.0	7.5-8.0	7.5-8.0	7.5-8.0	7.5-8.0
Moles %GC ^b in DNA	54.6	50.5	61.2	57.7	54.1	60.7	51.0

^aATCC, American Type Culture Collection, Rockville, Md.^b% GC, Per cent guanine plus cytosine.

FIG. 13. Phase-contrast photomicrograph of *Nitrobacter winogradskyi* showing wedge-like shape of cells. $\times 2,500$. FIG. 14. Electron micrograph of *Nitrobacter winogradskyi* showing polar cap of cytomembranes. $\times 51,300$. FIG. 15. Phase-contrast photomicrograph of *Nitrospina gracilis* showing the long and slender morphology of the cells. $\times 2,500$. FIG. 16. Electron micrograph showing longitudinal and cross sections of *Nitrospina gracilis*. $\times 15,900$. FIG. 17. Phase-contrast photomicrograph showing spherical nature of *Nitrococcus mobilis*. $\times 2,500$. FIG. 18. Electron micrograph of *Nitrococcus mobilis* showing tubular cytomembrane system. $\times 31,900$.

bedded in a slime and united to form compact zoogloeal aggregates. The two species described were *N. sarcinoides* Winogradsky 1937 (23), whose cells were small rods, 0.5 by 1.0 μm , and *N. micropunctata* (Winogradsky 1935) Winogradsky 1937 (21), whose cells were described as ellipsoidal rods about 0.5 μm in diameter. A clear-cut morphological distinction between these two species is not obvious from their original descriptions.

Studies in this laboratory of over 200 strains of nitrite-oxidizing bacteria revealed that most of these strains grew in compact clumps in enrichment cultures. The clumps were composed of cells similar in shape and size to those of *Nitrobacter winogradskyi* as illustrated in Fig. 13, and the fine structure of all strains was similar to or identical with that of strains of *Nitrobacter winogradskyi* (Fig. 14). When these strains were isolated in pure culture, aggregates were no longer formed. For these reasons it is recommended that *Nitrocystis sarcinoides* and *Nitrocystis micropunctata* be considered as later, subjective synonyms of *Nitrobacter winogradskyi* Winslow et al. 1917 and that the name *Nitrocystis* be declared a *nomen dubium* and placed on the list of *nomina rejicienda*.

Since the original strain of *Nitrobacter winogradskyi* was not preserved in culture, ATCC 25391 is here designated as the neotype strain. This strain was obtained from August Van Gool and was designated as Lees strain No. 1 of *Nitrobacter winogradskyi*. The characters (Table 1) of this designated neotype strain agree with those recorded in the original species description (31). The cells of this strain were rarely motile, and electron micrographs of their flagella were not obtained. In another strain a single, subterminally inserted flagellum was observed (Fig. 23).

Two additional nitrite-oxidizing bacteria, *Nitrospina gracilis* Watson and Waterbury and *Nitrococcus mobilis* Watson and Waterbury, were described recently (20). *Nitrospina gracilis* is a long, slender, nonmotile rod, 0.3 to 0.4 μm wide and 2.7 to 6.5 μm long (Fig. 15), and reproduces by binary fission. Its fine structure is characterized by the absence of the extensive cytomembrane system found in most other ammonia- and nitrite-oxidizing bacteria (Fig. 16). *N. gracilis* was isolated from a marine environment and has an obligate salt requirement. The GC composition of this organism was found to be 57.7 moles per cent. The type strain (20) of *Nitrospina gracilis* is ATCC 25379.

Nitrococcus mobilis is a large coccus which measures 1.5 to 1.8 μm in diameter just after division and 1.8 by 3.5 μm just prior to division

(Fig. 17). When motile, the cells exhibited a rolling, spinning action but rarely moved in a given direction for more than a few micrometers. The cells were propelled by one or two flagella which were inserted close together and subterminally in dividing cells (Fig. 24). The flagella were 12.5 to 15.0 μm in diameter and 3 to 4 μm long. The fine structure of *Nitrococcus mobilis* is unique in that this organism has a tubular cytomembrane system (Fig. 18). *N. mobilis* was isolated from a marine environment and has an obligate salt requirement. The GC composition of this bacterium was 61.2 moles per cent. The type strain (20) of *N. mobilis* is ATCC 25380.

Thus it is recommended that only three genera of nitrite-oxidizing bacteria, *Nitrobacter*, *Nitrospina*, and *Nitrococcus*, be considered as valid genera. *Nitrobacter* should include the short, ellipsoidal to pear-shaped rods which reproduce by budding, *Nitrospina* the long, slender rods, and *Nitrococcus* the large, spherical organisms. Although morphologically three different types of nitrite oxidizers exist, they could be segregated into only two groups with respect to their GC compositions.

Emended description of the family Nitrobacteraceae Buchanan 1917

Ni.tro.bac.te.ra'ce.ae. M.L. *Nitrobacter* name of type genus of the family; -aceae ending to denote a family; M.L. plural noun *Nitrobacteraceae* the *Nitrobacter* family.

Rod-shaped, ellipsoidal, spherical, spirillar, and lobular cells without endospores. Flagella subpolar or peritrichous and often absent. Gram-negative. Cells derive energy from the oxidation of ammonia or nitrite and satisfy their carbon needs by the fixation of CO_2 . Only one species, *Nitrobacter winogradskyi*, has been shown to be facultatively autotrophic. Not parasitic. Commonly found in soil, fresh water, and seawater.

The type genus of the family is *Nitrobacter Winogradsky*.

Key to the genera of the family Nitrobacteraceae

- I. Nitrite oxidized to nitrate.
 - A. Cells rod-shaped.
 1. Short rods, often wedge- or pear-shaped, with a polar cap of cytomembranes.

FIG. 19. Shadowed preparation of *Nitrosococcus oceanus* showing peritrichous cells. $\times 10,000$. FIG. 20. Negatively stained preparation of *Nitrosomonas europaea* showing paired flagella inserted subpolarly. $\times 14,500$. FIG. 21. Negatively stained preparation of *Nitrospira briensis* showing peritrichous cells. $\times 22,300$. FIG. 22. Negatively stained preparation of *Nitrosolobus multiformis* showing peritrichous cells. FIG. 23. Negatively stained preparation of *Nitrobacter winogradskyi* showing single flagellum with subpolar insertion. FIG. 24. Negatively stained preparation of *Nitrococcus mobilis* showing paired flagella. $\times 17,500$.

Genus I. *Nitrobacter*.

2. Long, slender rods with no extensive cytomembrane system.

Genus II. *Nitrospina*.

- B. Cells spherical (diameter 1.54 μm or more) with cytomembranes forming a branched, tubular network in the cytoplasm.

Genus III. *Nitrococcus*.

II. Ammonia oxidized to nitrite.

- A. Cells are straight rods with peripheral membranes occurring as flattened lamellae.

Genus IV. *Nitrosomonas*.

- B. Cells are not straight rods and do not contain flattened lamellae in the peripheral regions.

1. Cells are spirals with no evident cytomembranes.

Genus V. *Nitrosospira*.

2. Cells are not spirals but do contain cytomembranes.

- a. Cells spherical (diameter 1.5 μm or more) with cytomembranes forming flattened lamellae in center of cells.

Genus VI. *Nitrosococcus*.

- b. Cells lobular and partially compartmentalized by cytomembranes.

Genus VII. *Nitrosolobus*.Emended description of the genus *Nitrobacter*
Winogradsky 1892

Nitro.bac'ter. Gr. noun *nitrum* nitre, M.L. nitrate; M.L. noun *bacter* the masculine form of the Gr. neut. n. *bactrum* a rod; M.L. mas. n. *Nitrobacter* nitrate rod.

Cells are short rods, often wedge- or pear-shaped, 0.6 to 1.0 by 1.0 to 2.5 μm . Reproduce by budding. Gram-negative. Cells possess a polar cap of cytomembranes arranged to form flattened vesicles. Usually not motile unless grown in continuous culture; when motile, cells are propelled by a single, subterminal flagellum. Cells are rich in cytochromes, imparting a yellowish color to cell suspensions; void of other pigments.

Some strains are obligate chemoautotrophs which oxidize nitrite to nitrate and fix CO_2 to fulfill energy and carbon needs. The autotrophic medium consists of fresh water or seawater enriched with nitrite and other inorganic salts, no organic growth factor being required. Some strains can be grown heterotrophically, but the growth rate is slower than that when the cells are grown autotrophically. Strictly aerobic, using oxygen as a terminal electron acceptor. Optimum pH, 7.5 to 8.0.

Optimum growth temperature, 25 to 30 $^{\circ}\text{C}$.

Range of DNA base ratios: 60.7 to 61.7 moles % GC (buoyant density; one species, six strains).

Habitat: Soils, fresh water, and seawater.

Type species: *N. winogradskyi* Winslow et al.

Emended description of *Nitrobacter*
winogradskyi Winslow et al. 1917

winograd'skyi. Named for S. Winogradsky, the microbiologist who first isolated these bacteria; M.L. mas. gen. n. *winogradskyi* of Winogradsky.

Short rods, often pear- or wedge-shaped, 0.6 to 0.8 by 1.0 to 2.0 μm , gram-negative. Cells have a polar cap of cytomembranes arranged as flattened vesicles. Motile or non motile. Cells reproduce by budding. Grow either free in a liquid medium or in small clumps of 100 or more cells embedded in a slime matrix.

Cells are obligate chemoautotrophs or facultative autotrophs. When grown autotrophically, cells oxidize nitrite to nitrate and fix CO_2 to fulfill their energy and carbon needs. Strictly aerobic, using oxygen as a terminal electron acceptor. Temperature range of growth, 14 to 40 $^{\circ}\text{C}$; optimum temperature for growth, 25 to 30 $^{\circ}\text{C}$; optimum pH for growth, 7.5 to 8.0. Many strains will grow either in a fresh water or a seawater medium. Marine strains are morphologically indistinguishable from terrestrial strains.

Cell suspensions show characteristic oxidized minus dithionite reduced absorption peaks at 420, 440, 522, 550, 587, and 600 nm. These absorption peaks represent cytochromes which impart a brownish-yellow color to cell suspensions and a reddish-brown color to a pellet of cells.

Storage materials: Glycogen, poly- β -hydroxybutyrate, and polyphosphates.

Range of DNA base ratios: 60.7 to 61.7 moles % GC (buoyant density; six strains).

Habitat: Widely distributed in soils.

Designated neotype strain: ATCC 25391 (isolated from soil; see Table 1 for description).

Description of the genus *Nitrospina*
Watson and Waterbury 1971

Nitro.spina. Gr. noun *nitrum* nitre, M.L. nitrate; L. noun *spina* spine; M.L. mas. n. *Nitrospina* nitrate spine.

Cells are straight, slender rods, 0.3 to 0.4 μm by 2.7 to 6.5 μm ; spherical forms, 1.35 to 1.45 μm in diameter, are found in senescent cultures. There is no extensive cytomembrane

system. Cell division is by binary fission. Gram-negative. Nonmotile. Cells have cytochromes but no other pigments.

Obligately chemoautotrophic bacteria which oxidize nitrite to nitrate and fix CO₂ to fulfill energy and carbon needs. Grow in seawater enriched with nitrite and inorganic salts; no organic growth factors are required. Strictly aerobic, using oxygen as a terminal electron acceptor. Optimum growth temperature, 25 to 30 °C. Optimum pH range, 7.5 to 8.0. Optimum growth in 70 to 100% seawater; no growth in distilled water mineral salts medium even if NaCl is included.

DNA base ratio: 57.7 moles % GC (buoyant density; one species, one strain).

Habitat: South Atlantic Ocean.

Type species: *N. gracilis* Watson and Waterbury.

Description of *Nitrospina gracilis* Watson and Waterbury 1971

gracilis. L. adj. *gracilis* slender.

Cells are long, slender rods, 0.30 to 0.40 µm by 2.7 to 6.5 µm; spherical forms 1.35 to 1.45 µm in diameter are found in old cultures. Cells lack an extensive cytomembrane system, but occasional bleb-like invaginations of the plasma membrane occur. Occur singly or in pairs. Multiplication is by binary fission. Gram-negative. Nonmotile. Grow free in liquid media but will adhere to culture vessel walls in old cultures.

Cells are obligate chemoautotrophs using nitrite and carbon dioxide as major energy and carbon sources. Will not grow on organic media, and no organic growth factors are required; some organic compounds inhibit growth. Grow in 70 to 100% seawater enriched with nitrite and other mineral salts. Strictly aerobic, using oxygen as a terminal electron acceptor. Optimum growth temperature, 25 to 30 °C. Optimum pH, 7.5 to 8.0.

Cell suspensions show characteristic oxidized minus dithionite reduced absorption peaks at 425, 532, and 553 nm. These absorption peaks represent cytochromes which impart a brownish-yellow color to cell suspensions and to pellets of cells.

Storage material: Glycogen.

DNA base ratio: 57.7 moles % GC (buoyant density).

Designated type strain: ATCC 25379 (isolated from the South Atlantic Ocean; see Table 1 for description).

Description of the genus *Nitrococcus* Watson and Waterbury 1971

Nitrococcus. Gr. noun *nitrum* nitre, M.L. nitrate; Gr. *coccus* berry; M.L. mas. n. *Nitrococcus* nitrate sphere.

Cells spherical, 1.5 µm or larger. Gram-negative. Motile by means of one or two subterminally inserted flagella. Division by binary fission. Cells are rich in cytochromes imparting a yellowish to reddish color to cell suspensions; void of other pigments.

Obligately chemoautotrophic bacteria which oxidize nitrite to nitrate and fix CO₂ to fulfill energy and carbon needs. Growth medium is seawater enriched with nitrite and other inorganic salts, no organic growth factor being required. Strictly aerobic, using oxygen as a terminal electron acceptor. Optimum pH, 7.5 to 8.0. Optimum growth temperature, 25 to 30 °C.

DNA base ratio: 61.2 moles % GC (buoyant density; one species, one strain).

Habitat: South Pacific Ocean.

Type species: *N. mobilis* Watson and Waterbury.

Emended description of *Nitrococcus mobilis* Watson and Waterbury 1971

mobilis. L. adj. *mobilis* moveable, motile.

Cells spherical, 1.5 to 1.8 µm in diameter after division, but elongating with dimensions of 1.8 by 3.5 µm just before division. Cells occur singly or in pairs and divide by binary fission. Gram-negative. Cells have an extensive cytomembrane system which forms tubes randomly arranged in the cytoplasm. Motile by means of one or two flagella inserted subpolarly in elongated cells; the flagella are 12.5 nm wide and 3 to 4 µm long. When motile, cells have a turning, twisting, erratic motion. Grow either free in liquid media or in small clumps of 100 or more cells embedded in a slime matrix; this aggregate of cells is not surrounded by any type of limiting membrane.

Cells are obligate chemoautotrophs oxidizing nitrite to nitrate and fixing CO₂ to fulfill their energy and carbon needs. Strict aerobes using oxygen as a terminal electron acceptor. Temperature range of growth, 14 to 40 °C; optimum temperature for growth, 25 to 30 °C. Optimum pH for growth, 7.5 to 8.0. Optimum growth in 70 to 100% seawater; will not grow in fresh water even if NaCl is included.

Cell suspensions show characteristic oxidized minus dithionite reduced absorption peaks at

420, 440, 522, 550, 587, and 600 nm. These absorption peaks represent cytochromes which impart a brownish-yellow color to cell suspensions and a reddish-brown color to a pellet of cells.

Storage materials: Glycogen and poly- β -hydroxybutyrate.

DNA base ratio: 61.2 moles % GC (buoyant density).

Designated type strain: ATCC 25380 (isolated from South Pacific Ocean; (see Table 1 for description).

Emended description of the genus *Nitrosomonas* Winogradsky 1890

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

Cells ellipsoidal or short rods, 0.8 to 1.0 by 1.0 to 3.0 μ m, motile or nonmotile, occurring singly, in pairs, or as short chains. Gram-negative. Possess cytomembranes which occur as flattened vesicles in the peripheral regions of the cytoplasm. Cells grow free in the medium or are embedded in a slime matrix. Cells are rich in cytochromes, which impart a yellowish to reddish color to cell suspensions; void of other pigments.

Obligately chemoautotrophic bacteria which oxidize ammonia to nitrite and fix CO₂ to fulfill energy and carbon needs. Grow in fresh water or seawater enriched with ammonia and inorganic salts, no organic growth factors being required. Strictly aerobic, using oxygen as a terminal electron acceptor. Optimum pH range, 7.5 to 8.0. Optimum growth temperature, 25 to 30 C.

Range of DNA base ratios: 47.4 to 51.0 moles % GC (buoyant density; thirteen strains).

Habitat: Soils, fresh water, and seawater.

Type species: *N. europaea* Winogradsky.

Emended description of *Nitrosomonas* *europaea* Winogradsky 1892

Eu.ro.pae'a. Gr. adj. *europaeus* of Europe, European.

Rods, 0.8 to 0.9 by 1.0 to 2.0 μ m, occurring singly, rarely in chains. Gram-negative. When motile, possess one to two subpolar flagella which measure three to four times the length of the rod. Cells divide by binary fission and have cytomembranes which form flattened lamellae in the peripheral regions of the cytoplasm.

Cells are obligate chemoautotrophs oxidizing

ammonia and hydroxylamine to nitrite. Growth occurs with ammonia but not with hydroxylamine. Fix CO₂ to fulfill their carbon needs. Grow in particulate-free liquid salts media as individual cells or in small aggregates embedded in a slime. Strict aerobes which use oxygen as a terminal electron acceptor but which can oxidize ammonia at reduced oxygen tensions. Temperature growth range, 15 to 30 C; optimum temperature for growth, 25 to 30 C; optimum pH for growth, 7.5. No sodium chloride requirement. Cannot be grown in seawater.

Cell suspensions show characteristic oxidized minus dithionite reduced absorption peaks at 423, 465, 522, 552, and 605 nm. These absorption peaks represent cytochromes which impart a brownish to straw color in cultures and a reddish color to a pellet of cells.

Storage materials: Polyphosphates.

Range of DNA base ratios: 50.5 to 51.0 moles % GC (buoyant density; three strains).

Habitat: Widely distributed in soils.

Designated neotype strain: ATCC 25978 (isolated from soils in the United States; see Table 1 for description).

Emended description of the genus *Nitrosospira* Winogradsky 1931

Ni.tro.so.spi'ra. M.L. *nitrosus* nitrous; Gr. *spira* a coil, spiral; M.L. fem. n. *Nitrosospira* nitrous spiral.

Cells spiral shaped. Gram-negative. Cells lack cytomembranes. Motile, the cells being peritrichous. Grow free in liquid medium. Cells are rich in cytochromes, which impart a yellowish to reddish color to cell suspensions; void of other pigments.

Obligately chemoautotrophic bacteria which oxidize ammonia to nitrite and fix CO₂ to fulfill energy and carbon needs. Grow in fresh water enriched with ammonia and inorganic salts; no organic growth factors are required. Strictly aerobic using oxygen as a terminal electron acceptor. Optimum pH range, 7.5 to 8.0. Optimum growth temperature, 25 to 30 C.

DNA base ratio: 54.1 moles % GC (buoyant density; one species, one strain).

Habitat: Soils from Brie, France; Island of Crete; the summit of Mt. Pilatus in Switzerland; and the Parthenon in Athens, Greece.

Type species: *N. briensis* Winogradsky and Winogradsky.

**Emended description of *Nitrosospira briensis*
Winogradsky and Winogradsky 1933**

bri.en'sis. French Brie, place name; M.L. adj. *briensis* of Brie.

Cells are tightly wound spirals with 3 to 20 turns. Short spirals have the appearance of short rods and ellipsoidal cells; the spiral nature of the cells may not be evident when examined with a light-field or a phase-contrast microscope. Width of spiral filament, 0.3 to 0.4 μm ; amplitude of the spiral, 0.8 to 1.0 μm . Small pseudococci are observed in senescent cultures. Gram-negative. Motile or nonmotile; when motile, cells are peritrichous, containing 1 to 6 flagella 3 to 5 μm in length. Grow in particulate-free liquid mineral salts medium as individual cells.

Cells are obligate chemoautotrophs oxidizing ammonia and hydroxylamine to nitrite and fixing CO_2 . Growth occurs with ammonia but not with hydroxylamine. Strict aerobes, but ammonia can be oxidized at reduced oxygen tensions. Temperature range of growth, 15 to 30 $^{\circ}\text{C}$; optimum temperature for growth, 25 to 30 $^{\circ}\text{C}$; optimum pH for growth, 7.5. No sodium chloride requirement; cannot be grown in seawater.

Cell suspensions show characteristic oxidized minus dithionite reduced absorption peaks at 423, 465, 522, 552, and 605 nm. These absorption peaks represent cytochromes which impart a brownish straw like color to cultures and a reddish color to a pellet.

DNA base ratio: 54.1 moles % GC (buoyant density).

Habitat: Widely distributed in soils.

Designated neotype strain: ATCC 25961 (isolated from soil from the Island of Crete; see Table 1 for description).

**Emended description of the genus
Nitrosococcus Winogradsky 1892**

Ni.tro.so.coc'cus. M.L. *nitrosus* nitrous; Gr. *coccus* grain, berry; M.L. mas.n. *Nitrosococcus* nitrous sphere.

Cells spherical, 1.5 μm or larger in diameter. Motile or nonmotile. When motile, the cells are peritrichous with 1 to 12 flagella. Gram-negative. Grow singly, in pairs or in tetrads either suspended free in liquid medium or embedded in slime to form aggregates which are either attached to vessel walls or are suspended in the liquid medium.

Obligately chemoautotrophic bacteria which oxidize ammonia to nitrite and fix CO_2 to

fulfill energy and carbon needs. Grow in fresh water or seawater enriched with ammonia and inorganic salts, no organic growth factors being required. Strictly aerobic, using oxygen as a terminal electron acceptor. Optimum pH range, 7.5 to 8.0. Optimum growth temperature, 25 to 30 $^{\circ}\text{C}$.

Range of DNA base ratios: 50.5 to 51.0 moles % GC (buoyant density; one species, three strains).

Habitat: Soils and seawater.

Type species: *N. nitrosus* (Migula) Buchanan.

Emended description of *Nitrosococcus nitrosus* (Migula) Buchanan

ni.tro'sus. M.L. adj. *nitrosus* nitrous.

Large spheres, 1.5 to 1.7 μm in diameter, with thick cell membranes. Motility has not been demonstrated. Stain readily with aniline dyes. Zoogloea formation not observed.

Growth medium consists of fresh water, inorganic salts and ammonia. Aerobic cells use oxygen as a terminal electron acceptor. Optimum temperature, 20 to 25 $^{\circ}\text{C}$.

Source: Soils from Quito, Ecuador; Campinas, Brazil; and Melbourne, Australia.

Habitat: Presumably widely distributed in soil.

Emended description of *Nitrosococcus oceanus* (Watson) comb. nov.

o.ce.an'us. L. mas. n. *oceanus* ocean.

Cells spherical to ellipsoidal, 1.8 to 2.2 μm in size. Gram-negative. Occur singly, in pairs, and occasionally as tetrads either growing free in a liquid medium or as aggregates suspended in the medium. Cells divide by binary fission. Cysts were occasionally observed in mixed cultures but have not been found in pure cultures. Motile or nonmotile; when motile, the cells are peritrichous, with a single flagellum or a small tuft. Cells have cytomembranes which are arranged as flattened lamellae in the central region of the cell.

Cells are obligate chemoautotrophs oxidizing ammonia and hydroxylamine to nitrite and fixing CO_2 to fulfill their energy and carbon needs. Growth occurs with ammonia but not with hydroxylamine. Strict aerobes, using oxygen as a terminal electron acceptor. Temperature range of growth, 2 to 30 $^{\circ}\text{C}$; optimum temperature for growth, 25 to 30 $^{\circ}\text{C}$; optimum pH for growth, 7.5 to 8.0; optimum growth in 70 to 100% seawater; will not grow in fresh water even when NaCl is included.

Cell suspensions show characteristic oxidized minus dithionite reduced absorption peaks at 423, 465, 522, 552, and 605 nm. These absorption peaks represent cytochromes which impart a brownish color to cultures and a reddish color to a pellet of cells. No other pigments are present.

Storage materials: Glycogen and polyphosphates.

Range of DNA base ratios: 50.5 to 51.0 moles % GC (buoyant density; three strains).

Habitat: Atlantic and Pacific Oceans.

Designated type strain: ATCC 19707 (isolated from North Atlantic seawater; see Table 1 for description).

Description of the genus *Nitrosolobus*

Watson et al. 1971

Ni.tro.so.lob'us. M.L. *nitrosus* nitrous; M.L. noun *lobus* a lobe; M.L. mas. noun *Nitrosolobus* nitrous lobe, i.e., a lobe producing nitrite.

Cells pleomorphic and lobate, 1.0 to 1.5 μ m in diameter; division by constriction. Gram-negative. Cells partially compartmentalized by the invagination of the plasma membrane and other segments of the cell envelope into the cytoplasm forming vesicular regions. Motile, the cells being peritrichous. Grow free in liquid medium. Cells are rich in cytochromes, which impart yellowish to reddish color to cell suspensions; void of other pigments.

Obligately chemoautotrophic bacteria which oxidize ammonia to nitrite and fix CO₂ to fulfill energy and carbon needs. Grow in fresh water enriched with ammonia and inorganic salts; no organic growth factors are required. Strictly aerobic, using oxygen as a terminal electron acceptor. Optimum pH range, 7.5 to 8.0. Optimum growth temperature, 25 to 30 C.

Range of DNA base ratios: 53.6 to 55.1 moles % GC (buoyant density; one species, three strains).

Habitat: Soils from Surinam, South America; Galapagos Archipelago; Southwest Africa; and Russia.

Type species: *N. multiformis* Watson et al.

Description of *Nitrosolobus multiformis*

Watson et al. 1971

mul.ti.for.'mis. L. adj. *multus* many; L. noun *forma* shape. M.L. adj. *multiformis* many shapes.

Cells composed of multiple ellipsoidal units, randomly arranged, forming lobular, pleomorphic cells which are internally compartmentalized, having one to five membrane-bounded central areas surrounded by numerous smaller membrane-bounded areas rich in glycogen deposits. Cells brain-like in appearance and 1.0 to 1.5 μ m by 1.0 to 2.5 μ m. Gram-negative. Motile by means of 1 to 20 flagella randomly arranged; the flagella are 15.0 nm wide and 2.2 to 5.0 μ m long. Grow in particulate-free liquid mineral salts medium as individual cells.

Cells are obligate chemoautotrophs oxidizing ammonia, urea, hydroxylamine, and biuret to nitrite and fixing CO₂. Growth occurs with ammonia and urea but not with hydroxylamine or biuret. Strict aerobes, although cells can oxidize ammonia at reduced oxygen tensions. Temperature range of growth, 15 to 30 C; optimal temperature for growth, 25 to 30 C; optimum pH for growth, 7.5. No sodium chloride requirement; cannot be grown in seawater.

Cell suspensions show characteristic oxidized minus dithionite reduced absorption peaks at 423, 465, 522, 552, and 605 nm. These absorption peaks represent cytochromes, which impart a brownish color to cultures and a reddish color to a pellet of cells.

Storage materials: Glycogen and polyphosphates.

DNA base ratio: 54.6 moles % GC (buoyant density).

Habitat: Widely distributed in soils.

Type strain (original designation; originally cited in reference 19 as ATCC 2519): ATCC 25196 (isolated from soils from Surinam, South America; see Table 1 for description).

REQUESTS FOR OPINIONS

1. The generic names *Nitrosocystis* Winogradsky, *Nitrosogloea* Winogradsky, and *Nitrocystis* Winogradsky are here regarded as *nomina dubia*, and the Judicial Commission is requested to issue an Opinion placing these names on the list of *nomina rejicienda*.

2. The specific names *Nitrosogloea merismoides* Winogradsky, *Nitrosogloea membranacea* Winogradsky, *Nitrosogloea schizobacteroides* Winogradsky, and *Nitrosocystis javanensis* (Winogradsky) Starkey are regarded as *nomina dubia*, and the Judicial Commission is asked to issue an Opinion placing these names on the list of *nomina rejicienda*.

SPECIFIC PROPOSALS

1. The genus *Nitrosococcus* Winogradsky will contain the large spherical organisms which oxidize ammonia to nitrite; the fact that the type species, *N. nitrosus*, has not been cultivated does not affect the validity of this genus.

2. *Nitrosocystis coccoides* Starkey is regarded as a later, subjective synonym of *Nitrosococcus nitrosus* (Migula) Buchanan.

3. *Nitrosocystis oceanus* Watson is transferred to the genus *Nitrosococcus*; the name of this organism therefore is *Nitrosococcus oceanus* (Watson) comb. nov.

4. *Nitrosomonas monocella* Nelson is a later, subjective synonym of *Nitrosomonas europaea* Winogradsky.

5. *Nitrosospira antarctica* Winogradsky and Winogradsky is a later, subjective synonym of *Nitrosospira briensis* Winogradsky and Winogradsky.

6. *Nitrocystis sarcinoides* Winogradsky, *Nitrocystis micropunctata* (Winogradsky) Winogradsky, and *Nitrobacter agilis* Nelson are later, subjective synonyms of *Nitrobacter winogradskyi* Winslow et al.

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