The Lithoautotrophic Nitrite-Oxidizing Bacteria

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The lithotrophic nitrite oxidizers are Gram-negative eubacteria that are able to use nitrite as a sole source of energy and CO_2 as the main source of carbon. Some strains are able to grow mixotrophically. These organisms are obligate lithoautotrophs with the exception of *Nitrobacter*, which can grow heterotrophically. *Nitrobacter* has been shown to grow anaerobically by dissimilatory nitrate reduction (Freitag et al., 1987).

Morphological Characteristics

The nitrite oxidizers are a diverse group of rods, cocci, and spirilla. Historically, the classification of genera was founded primarily on cell shape and arrangement of intracytoplasmic membranes, and taxonomic categorization was based on the work of Sergei and Helene Winogradsky (Winogradsky, 1892). Four morphologically distinct genera (*Nitrobacter, Nitrococcus, Nitrospina*, and *Nitrospira*) have been described (Watson et al., 1989; Bock and Koops, 1992). Cells of *Nitrobacter* are pleomorphic short rods containing a polar cap of intracytoplasmic membranes. *Nitrococcus* occurs in form of coccoid cells with tubular intracytoplasmic membranes. Cells of *Nitrospina* appear as long rods, intracytoplasmic membranes in the form of flattened vesicles or tubes are missing. The genus *Nitrospira* is characterized by a spiral shape and the absence of intracytoplasmic membranes. Some strains are motile by means of a single polar or subpolar flagellum.

Phylogeny

Unlike the ammonia oxidizers, which are restricted to two lineages within the *Proteobacteria*, the nitrite oxidizers are more scattered phylogenetically (Fig. 1 of the chapter "Nitrifying Bacteria"). The genus *Nitrobacter* belongs to the *Alphaproteobacteria* (Woese et al., 1984a; Stackebrandt et al., 1988), whereas *Nitrococcus* is affiliated with the *Gammaproteobacteria* (Woese et al., 1985). The genus *Nitrospina* seems to be a member of the *Deltaproteobacteria* (Teske et al., 1994), although this assignment remains preliminary. *Nitrospira* was thought to be related to *Nitrospina*, but *Nitrospira* was later shown not to be a member of the *Proteobacteria*. Ehrich et al. (1995) demonstrated that this nitrite-oxidizing bacterium occupies a phylogenetically isolated position and represents a new phylum, *Nitrospirae*, of the domain *Bacteria*. *Nitrospira* was described in Volume I of the *Manual*; some data are also presented here to facilitate comparison.

The genus *Nitrobacter* is comprised of four described species (Bock and Koops, 1992; Sorokin et al., 1998), whereas one species is known for each of the genera *Nitrococcus* and *Nitrospina* (Watson and Waterbury, 1971). Two species of *Nitrospira* have been described in the literature (Watson et al., 1986; Ehrich et al., 1995),

FIGURE 1. Spiral-shaped cell of *Nitrospira marina* grown lithoautotrophically. Negative staining with uranylacetate. Bar = 500 nm.

but much higher phylogenetical diversity may be present in this genus (Schramm et al., 1999).

Ecology and Distribution

The best-investigated nitrite-oxidizing bacterium is *Nitrobacter*, which was believed to dominate in most natural environments except marine ones. This picture has changed significantly over the last few years, and current investigations, especially molecular ones, are focused on the occurrence of *Nitrospira*. Members of the genera *Nitrococcus* and *Nitrospina* have only been found in marine habitats to date.

Nitrobacter is a soil and freshwater organism that is tolerant of changing environmental conditions. Members of this genus also occur in sewage and marine environments. Other isolates were originally obtained from extreme environments such as concrete and natural stones, desert soils and sulfidic ore mines. One acidophilic strain with a pH optimum of 5.5 was isolated from an acidic forest soil (Hankinson and Schmidt, 1988). Facultatively alkalophilic strains of *Nitrobacter* were recently be isolated from soda lakes in Siberia and Kenya and described as a new species, *N. alkalicus* (Sorokin et al., 1998).

Although nitrite oxidizers do not form endospores, they can survive long periods of starvation and dryness. One survival strategy used by these organisms may be the formation and accumulation of extracellular compatible solutes. *Nitrobacter* was found to produce trehalose and was able to accumulate glycine

betaine and sucrose from the medium. An increase in the amounts of compatible solutes was reproducibly found in cultures exposed to salt stress and dryness. *Nitrobacter vulgaris* can survive a period of 24 months without water (L. Lin, personal communication). Diab and Shilo (1988) found that adhesion to particles had a positive effect on both the activity and the survival of *Nitrobacter* cells.

When the habitat-specific distribution of three species of *Nitrobacter* was examined using automated pattern matching of proteins, it was found that *Nitrobacter vulgaris* was the dominant species in building stone (T. Krause-Kupsch, personal communication). *Nitrobacter hamburgensis* was only found in soil, whereas *Nitrobacter winogradskyi* occurred in various habitats such as soils, fresh water, sewage and concrete. According to Both et al. (1992) *Nitrobacter winogradskyi* out-competes *Nitrobacter hamburgensis* in well-aerated soils under nitrite-limiting conditions, since the former has a lower K_m for nitrite under autotrophic as well as mixotrophic conditions. However, the activity of *Nitrobacter hamburgensis* increases when oxygen tension decreases.

Immunological and molecular investigations of *Nitrobacter* populations demonstrated that several strains of this genus can coexist (Stanley and Schmidt, 1981; Degrange et al., 1997). Navarro et al. (1992) characterized natural populations of *Nitrobacter* by PCR/RFLP (restriction fragment length polymorphism). These authors differentiated several coexisting strains in various soils and a lake; the coexistence of several strains may reflect the existence of local niches. Genetic distances obtained by amplified ribosomal DNA restriction analysis (ADRA) of the 16S-23S rRNA intergenic spacer regions and partial sequences of the 23S rRNA gene enable comparison of *Nitrobacter* species in soil (Grundmann and Normand, 2000). Two 16S rRNA-targeted oligonucleotide probes specific for *Nitrobacter* have been developed for the *in situ* analysis of nitrite oxidizers (Wagner et al., 1996). Although this genus has been regarded as the most abundant nitrite oxidizer in various environments, it could not be detected in activated sludge samples and reactor biofilms. The authors suggested that still unknown organisms might be responsible for nitrification in these habitats. This hypothesis was confirmed recently when several groups reported that *Nitrospira*-like bacteria seem to be the dominant nitrite oxidizers in freshwater aquaria, biofilms and activated sludge (Burrell et al., 1998; Hovanec et al., 1998; Juretschko et al., 1998). Schramm et al. (1998) found in a nitrifying reactor organisms that formed two phylogenetically distinct groups affiliated with *Nitrospira moscoviensis*. The novel genus *Nitrospira marina* was first isolated by Watson et al. (1986) from the Gulf of Maine (Fig. 1). *Nitrospira moscoviensis* was first isolated from a heating system in Moscow (Ehrich et al., 1995). Similar nitrite-oxidizing organisms have been enriched from soil samples, sediments, beach sands, and salt marshes (Watson et al., 1989). It seems that although the genus *Nitrospira* is ubiquitous, it is outcompeted by *Nitrobacter* when standard isolation procedures are used (Johnson and Sieburth, 1976). In studies using monoclonal antibodies that recognize the nitrite oxidoreductase (NOR) enzyme of *Nitrobacter*, Bartosch et al. (1999) demonstrated that different genera of nitrite oxidizers were enriched from activated sludge depending on the substrate concentration of the media. When enrichments were made in accordance with the instructions of Watson et al. (1989), *Nitrospira* was the most abundant nitrite oxidizer in enrichment cultures grown in mixotrophic medium containing 0.2 g $NaNO_2$ per liter. In contrast, cells of *Nitrobacter* dominated when the medium contained 2 g $NaNO_2$ per liter. Although *Nitrospira* from wastewater

treatment plants was postulated to be "unculturable," microcolonies of *Nitrospira* from wastewater samples from Dradenau in Hamburg were highly enriched in laboratory cultures (Fig. 2). *Nitrospira* can be regularly enriched using adapted cultivation techniques that include the avoidance of turbulence, and cultures originating from a wide range of habitats such as permafrost soil (Bartosch et al., 2002), caves, and hot springs are being investigated.

So far, phylogenetic analysis of *Nitrospira* has revealed four sublineages based on environmental sequences from various aquatic environments (summarized by Daims et al., 2001). Two of the sublineages include the described species *N. moscoviensis* and *N. marina*. A third species, originating from a Moscow heating system, will be described in the future (Lebedeva, personal communication). A thermophilic culture derived from a hot spring at Lake Baikal differed from known *Nitrospira* isolates based on DGGE (Alawi and Lebedeva, personal communication). It is likely that the phylogenetic tree of *Nitrospira* will become more complex as new representatives are isolated and sequence analysis of environmental samples is carried out.

GROWTH CHARACTERISTICS

Lithotrophic growth of nitrite oxidizers is slow. The generation time varies from 8 h to several days. Growth rates are controlled by substrate concentration, temperature, pH, light, and oxygen concentration. Most nitrite oxidizers grow best at nitrite concentrations of 2–30 mM at a pH of 7.5–8.0 and at temperatures of 25–30°C. Some strains are able to grow mixotrophically; the cell yield from mixotrophically grown cultures can be ten-fold greater than that from lithotrophically grown cultures.

BIOCHEMISTRY

Most biochemical investigations have been performed on the genus *Nitrobacter*. Initial biochemical studies of *Nitrospira* revealed several significant differences between *Nitrospira* and *Nitrobacter* (Watson et al., 1986). Little is known about the biochemistry of *Nitrococcus* and *Nitrospina*. The genera *Nitrobacter* and *Nitrococcus* are similar in cytochrome content and in the location and molecular masses of the nitrite-oxidizing enzymes, whereas the genera *Nitrospina* and *Nitrospira* differ from *Nitrobacter* and *Nitrococcus* but are similar to each other with respect to these characteristics.

Nitrite, the substrate for aerobic nitrification, is thought to be transported into the bacteria by a nitrite/nitrate antiport system (Wood, 1986). Nitrate, the electron acceptor for the reverse reaction, is assumed to be transferred by the same transporter.

The key enzyme of nitrite oxidation has been studied in *Nitrobacter* and *Nitrospira*; this enzyme is called the nitrite oxidoreductase (NOR) in the genus *Nitrobacter* and nitrite-oxidizing system (NOS) in the genera *Nitrococcus*, *Nitrospina* and *Nitrospira*. The occurrence of membrane-bound particles containing the enzyme is a general characteristic of all members of nitrite-oxidizing bacteria; these particles are densely packed on the surface of the cytoplasmic and intracytoplasmic membranes. The location of the particles is coincident with immunolabeling of the NOR and NOS enzymes (Spieck et al., 1996a). In *Nitrobacter* and *Nitrococcus* the key enzyme is located on the inner side of the cytoplasmic and intracytoplasmic membranes (Watson and Waterbury, 1971; Sundermeyer and Bock, 1981a). In cells of *Nitrospina* and *Nitrospira*, which do not possess intracytoplasmic membranes, the nitrite-oxidizing system is found in the periplasmic space and is associated with the outer surface of the cell membrane in *Nitrospira* (Spieck et al., 1998). This location of the
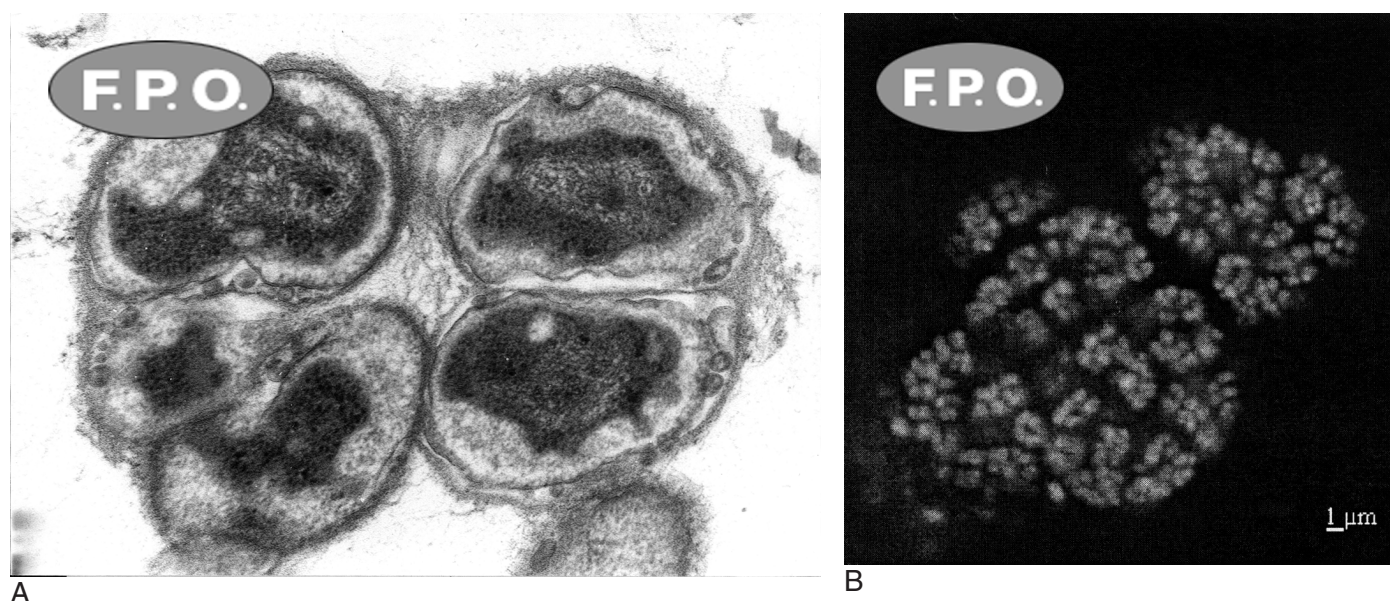
FIGURE 2. Microcolonies of *Nitrospira*-like bacteria in activated sludge from waste water treatment plant in Dradenau, Hamburg. *A*) Ultrathin section of activated sludge. Cells are similar in ultrastructure to those of the genus *Nitrospira* with respect to the extended perimplasmic space and lack of intracytoplasmic membranes. Bar = 250 nm. *B*) Fluorescence *in situ* hybridization (FISH) of a nitrite-oxidizing enrichment culture with oligonucleotide probe S-*Ntspa-1026-a-A-18 specific for *N. moscoviensis* (Juretschko et al., 1998). Cells were grown in mixotrophic medium containing 0.2 g NaNO$_2$/l. Picture courtesy of S. Bartosch

enzyme may explain the higher sensitivity of *Nitrospina* and *Nitrospira* to nitrite in comparison to *Nitrobacter* and *Nitrococcus*. The molecular masses of the β-NOR of *Nitrobacter* and the β-NOS of *Nitrococcus* are identical (65 KDa), whereas the β-NOSs of *Nitrospina* (48 KDa) and *Nitrospira* (46 KDa) differ (Bartosch et al., 1999). Images showing the location and arrangement of the NOR can be found in the chapters describing the genera *Nitrobacter*, *Nitrococcus*, and *Nitrospina*. For *Nitrospira*, see Volume 1.

The NOR of *Nitrobacter* forms a periodic arrangement in paired rows. Tsien and Laudelout (1968) provided the first evidence that a minimum of four particles had to remain associated in order to retain enzymatic activity. The integrity of a structure extending between neighboring particles was assumed to be necessary for conservation of activity. The molecular weight of a single particle was 186 KDa; this result suggests that each particle is an αβ-heterodimer (Spieck et al., 1996b).

The biochemistry of *Nitrobacter* has been reviewed by several authors (Wood, 1986; Yamanaka and Fukumori, 1988; Hooper, 1989; Bock et al. 1991, 1992; Bock and Wagner, 2001.). Two electrons are released during the oxidation of nitrite to nitrate as shown in the following equation. The third oxygen atom in the nitrate molecule is derived from water (Aleem et al., 1965).

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$$
$$2H^+ + 2e^- + 0.5\ O_2 \rightarrow H_2O$$
$$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$$
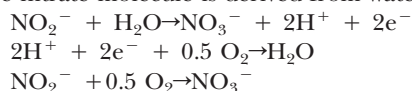
The electron flux from nitrite to oxygen is thought to flow through the following electron carriers.

nitrite→molybdopterin→iron-sulfur-clusters→cytochrome a_1→cytochrome c→cytochrome aa_3→O$_2$
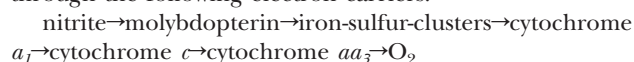
The electron transfer from nitrite to cytochrome a_1 is catalyzed by the enzyme nitrite oxidoreductase (NOR) which contains molybdopterin and iron-sulfur clusters. Cytochrome a_1 is necessary to channel electrons from nitrite to cytochrome c (Yamanaka and Fukumori, 1988), where the electrons enter the respiratory chain (Cobley, 1976; Aleem and Sewell, 1981). The reduction of cytochrome c is a thermodynamically unfavorable step because the NO_2^-/NO_3^- couple has a redox potential of $E_0' = +420$ mV. Nitrite-oxidizing cells of *Nitrobacter winogradskyi* have a very low energy charge of 0.37 during the logarithmic growth phase (Eigener, 1975). The inefficiency of energy generation in *Nitrobacter* may be compensated for by high levels of NOR, which may comprise 10–30% of total protein (Bock et al., 1991). The primary energy product is NADH (Sundermeyer and Bock, 1981b), which is used for ATP synthesis (Freitag and Bock, 1990). It is not clear how energy conservation occurs because the postulated reverse electron flow for the generation of NADH has not yet been demonstrated. The nitrite oxidase system of *Nitrobacter winogradskyi* was reconstituted in proteoliposomes with isolated nitrite oxidoreductase, cytochrome c oxidase and the substrate nitrite. In this system oxygen was consumed in the presence of membrane-bound cytochrome c_{550} (Nomoto et al., 1993). A purified ATPase from *N. winogradskyi* has been characterized by Hara et al. (1991).

Cells of *Nitrobacter hamburgensis* seem to utilize different terminal oxidases in response to different growth conditions. During nitrite oxidation, cytochrome aa_3 is active, whereas a *b*-type cytochrome is used as a terminal oxidase for heterotrophic growth (Kirstein et al., 1986). *Nitrobacter* and *Nitrococcus* are rich in cytochromes c and a; dense cell suspensions exhibit a typical red to brownish color. Characteristic peaks occur at 420, 440, 550, 587 and 600 nm in oxidized/dithionite-reduced difference spectra. The other two genera of nitrite-oxidizing bacteria, *Nitrospina* and *Nitrospira*, apparently lack type *a* cytochromes (Watson et al., 1989).

Lithoautotrophic nitrite oxidizers fix carbon dioxide via the Calvin cycle. About 80% of the energy generated by nitrite oxidation is used for CO$_2$ fixation. In *Nitrobacter* ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) is responsible for

this reaction. In *Nitrobacter* (Shively et al., 1977) and *Nitrococcus*, the enzyme may be soluble as well as carboxysome-bound; carboxysomes are found in most but not all species of *Nitrobacter*. In *Nitrobacter winogradskyi* the soluble form of RubisCO has a molecular mass of 480 KDa. In *Nitrobacter hamburgensis* X14 the enzyme occurs in two forms with different molecular masses— 480 KDa and 520 KDa; both forms have an L8S8 quaternary structure. In this species two different genes and gene products for the large subunit of RubisCO have been identified; one is located on the chromosome and the other on a plasmid (Harris et al., 1988). The Calvin cycle genes are located in two separate clusters on the chromosome in *Nitrobacter vulgaris* (Strecker et al., 1994).

Enrichment and Isolation Procedures

Nitrite oxidizers can be isolated using a mineral medium containing nitrite; the compositions of media for lithotrophic, mixotrophic, and heterotrophic growth are given in Table 1. Serial dilutions of enrichment cultures must be incubated for one to several months in the dark. Since nitrite oxidizers are sensitive to high partial pressures of oxygen, cell growth on agar surfaces is limited. Pure cultures of *Nitrobacter alkalicus* were obtained by multiple passages in liquid medium of colonies from nitrite agar (Sorokin et al., 1998). Nitrite oxidizers like *Nitrospira* can be separated from heterotrophic contaminants by Percoll gradient centrifugation and subsequent serial dilution (Ehrich et al., 1995).

Maintenance Procedures for Stock Cultures

Nitrifying organisms can survive starvation for more than one year when kept at 17°C in liquid media. Nevertheless, cells should be transferred to fresh media every four months. In Table 1 three different growth media for nitrite oxidizers are listed. Freezing in liquid nitrogen is a suitable technique for maintenance of stock cultures that are suspended in a cryoprotective buffer containing sucrose and histidine. When freeze-dried on lavalite or polyurethane, about 0.5% of *Nitrobacter* cells survive for one year (L. Lin, personal communication). Another possibility for the storage of *Nitrobacter* for several years is cultivation in 1l-bottles filled to the top with complex medium and closed by a screw top. Glycerol should be used instead of pyruvate to keep the pH stable for a long period. Since the bacteria are able to oxidize nitrite to nitrate aerobically and subsequently able to reduce the nitrate anaerobically, a high cell yield can be obtained using this method (Freitag et al., 1987).

Differentiation of the four genera of nitrite-oxidizing bacteria

Morphological, genotypic, and chemotaxonomic characteristics that can be used to differentiate the four genera of nitrite-oxidizing bacteria are given in Tables 2, 3, and 4.

Specific reaction patterns of a set of three monoclonal antibodies (MAbs) that recognize the nitrite-oxidizing system (Aamand et al., 1996) were shown to be useful for taxonomic investigations of pure and enrichment cultures by western blot analysis and immunofluorescent labelling (Bartosch et al., 1999). The three different MAbs have different degrees of specificity that permit classification to the genus level. MAb Hyb 153-2 recognizes the α-NOR of the described species of *Nitrobacter*. MAb Hyb 153-1 recognizes the β-NOS of *Nitrobacter* and *Nitrococcus*, whereas MAb Hyb 153-3 reacts with the β-NOS of all known nitrite-oxidizing bacteria. The differing molecular masses of the β-NOSs enable differentiation of the four genera.

The results summarized in Table 2 indicate that the epitope of the β-subunit recognized by MAb Hyb 153-3 is highly conserved. The finding of such conserved regions in the key enzyme of nitrite oxidation does not support the hypothesis of Teske et al. (1994) that the nitrifiers arose independently multiple times, possibly from different photosynthetic ancestors. The specific reactions of the MAbs suggest a close correlation between phylogeny and function and underscore the utility of investigation of the comparative biochemistry of proteins involved in energy metabolism as an approach to the study of bacterial evolution (Brock, 1989).

TABLE 1. Three different media for lithoautotrophic (medium A for terrestrial strains; medium B for marine strains), mixotrophic (medium C), and heterotrophic (medium C without NaNO₂) growth of nitrite oxidizers

Ingredient	Culture medium		
	A[a]	B[b]	C[c, d]
Distilled water (ml)	1000	300	1000
Seawater (ml)		700	
NaNO$_2$ (mg)	200–2000	69	200–2000
MgSO$_4$·7H$_2$O (mg)	50	100	50
CaCl$_2$·2H$_2$O (mg)		6	
CaCO$_3$ (mg)	3		3
KH$_2$PO$_4$ (mg)	150	1.7	150
FeSO$_4$·7H$_2$O (mg)	0.15		0.15
Chelated iron (13%, Geigy) (mg)		1	
Na$_2$MoO$_4$·2H$_2$O (μg)		30	
(NH$_4$)$_2$Mo$_7$O$_{24}$·4H$_2$O (μg)	50		50
MnCl$_2$·6H$_2$O (μg)		66	
CoCl$_2$·6H$_2$O (μg)		0.6	
CuSO$_4$·5H$_2$O (μg)		6	
ZnSO$_4$·7H$_2$O (μg)		30	
NaCl (mg)	500		500
Sodium pyruvate (mg)			550
Yeast extract (Difco) (mg)			1,500
Peptone (Difco) (mg)			1,500
pH adjusted to[e]	8.6	6	7.4

[a]For terrestrial strains from Bock et al. (1983).

[b]For marine strains modified from Watson and Waterbury (1971).

[c]For terrestrial strains from Bock et al. (1983).

[d]For heterotrophic growth medium C without NaNO₂ is used.

[e]After sterilization pH should be 7.4–7.8.

TABLE 2. Differentiation of the four genera of nitrite-oxidizing bacteria

Characteristic	*Nitrobacter*	*Nitrococcus*	*Nitrospina*	*Nitrospira*
Phylogenetic position	*Alphaproteobacteria*	*Gammaproteobacteria*	*Deltaproteobacteria* (preliminary)	Phylum *Nitrospirae*
Morphology	Pleomorphic short rods	Coccoid cells	Straight rods	Curved rods to spirals
Intracytoplasmic membranes	Polar cap	Tubular	Lacking	Lacking
Size (µm)	0.5–0.9 × 1.0–2.0	1.5–1.8	0.3–0.5 × 1.7–6.6	0.2–0.4 × 0.9–2.2
Motility	+	+	−	−
Reproduction:	Budding or binary fission	Binary fission	Binary fission	Binary fission
Main cytochrome types[a]	*a, c*	*a, c*	*c*	*b, c*
Location of the nitrite oxidizing system on membranes	Cytoplasmic	Cytoplasmic	Periplasmic	Periplasmic
MAb-labeled subunits (KDa)[b]	130 and 65	65	48	46
Crystalline structure of membrane-bound particles	Rows of particle dimers	Particles in rows	Hexagonal pattern	Hexagonal pattern

[a]Lithoautotrophic growth.

[b]MAbs, monoclonal antibodies.

TABLE 3. Properties of the nitrite-oxidizing bacteria

Characteristic	*Nitrobacter winogradskyi*	*Nitrobacter alkalicus*	*Nitrobacter hamburgensis*	*Nitrobacter vulgaris*	*Nitrococcus mobilis*	*Nitrospina gracilis*	*Nitrospira marina*	*Nitrospira moscoviensis*
Mol% G + C of the DNA	61.7	62	61.6	59.4	61.2	57.7	50	56.9
Carboxysomes	+	−	+	+	+	−	−	−
Habitat:								
Fresh water	+			+				
Waste water	+			+				
Brackish water				+				
Oceans	+				+	+	+	
Soda lakes		+						
Soil	+		+	+				
Soda soil		+						
Stones	+			+				
Heating system								+

TABLE 4. Primary fatty acids of the described species of nitrite-oxidizing bacteria[a,b]

Fatty acid	*Nitrobacter winogradskyi* Engel	*Nitrobacter alkalicus* AN4	*Nitrobacter hamburgensis* X14	*Nitrobacter vulgaris* Z	*Nitrococcus mobilis* 231	*Nitrospina gracilis* 3	*Nitrospira marina* 295	*Nitrospira moscoviensis* M1
$C_{14:1cis9}$						+		
$C_{14:0}$	+		+		+	+ + +	+	+
$C_{16:1cis7}$							+ + +	+ +
$C_{16:1cis9}$	+	+	+	+	+ + +	+ + +		
$C_{16:1cis11}$							+ + +	+ + +
$C_{16:0}3OH$						+		+
$C_{16:0}$	+ +	+ +	+ +	+ +	+ + +	+ +	+ + +	+ + +
$C_{16:0\ 11methyl}$							+	+ + +
$C_{18:1cis9}$	+		+			+	+	
$C_{18:1cis11}$	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	+	+	+
$C_{18:0}$	+	+	+		+	+	+ +	+
$C_{19:0cyclo11-12}$	+	+	+	+	+			

[a]Symbols: +, <5%; + +, 6–15%; + + +, 16–60%; + + + +, >60%.

[b]Stirred cultures were grown autotrophically at 28°C (*Nitrospira moscoviensis* at 37°C) and collected at the end of exponential growth. Modified from Lipski et al., (2001).