

Ottowia thiooxydans gen. nov., sp. nov., a novel facultatively anaerobic, N₂O-producing bacterium isolated from activated sludge, and transfer of *Aquaspirillum gracile* to *Hylemonella gracilis* gen. nov., comb. nov.

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Strain K11^T was isolated from activated sludge of a municipal wastewater-treatment plant. Phylogenetic analysis of the 16S rRNA gene sequence revealed that it represents a distinct line of descent within the *Comamonadaceae*. The novel strain was a Gram-negative, catalase- and oxidase-positive, non-motile, straight to slightly curved rod. Polyhydroxyalkanoate granules were stored intracellularly as reserve material. Colonies on agar plates were small, regular and characterized by a water-insoluble yellow pigment. Unbranched fatty acids 16:1 ω 7c, 16:0 and 18:1 ω 7c dominated the cellular fatty acid pattern and ubiquinone-8 (Q-8) was the major component of the respiratory lipoquinones, both traits typical of members of the *Comamonadaceae*. A distinguishing characteristic was the presence of the two hydroxy fatty acids 10:0 3-OH and 12:0 2-OH, each in significant amounts. The G + C content of the DNA was 59 mol%. Strain K11^T was capable of aerobic chemolithoheterotrophic growth using thiosulfate as an additional substrate, but could not grow autotrophically with thiosulfate or hydrogen. Facultative anaerobic growth was possible with nitrate and nitrite as electron acceptors, but not with ferric iron, sulfate or by fermentation. The sole end product of denitrification was N₂O; nitrite accumulated only transiently in small amounts. Based upon phylogenetic and phenotypic evidence, it is proposed to establish the novel taxon *Ottowia thiooxydans* gen. nov., sp. nov., represented by the type strain K11^T (= DSM 14619^T = JCM 11629^T). *Aquaspirillum gracile* was among the phylogenetically most closely related species to strain K11^T. This species has been wrongly classified, and it is proposed to reclassify it as *Hylemonella gracilis* gen. nov., comb. nov. The type strain is ATCC 19624^T (= DSM 9158^T).

In several recently published studies relying on cultivation-independent techniques, it has been demonstrated that β -proteobacteria represent a major fraction of the microbial community in the activated sludge of municipal

sewage-treatment plants (Wagner *et al.*, 1993; Manz *et al.*, 1994; Wallner *et al.*, 1995; Snaidr *et al.*, 1997; Wagner & Loy, 2002). On the other hand, nutrient-rich media (e.g. LB agar) traditionally applied for microbial analyses of wastewater samples favour the growth of γ -proteobacteria and suppress the isolation of members of β -Proteobacteria and other taxa (Wagner *et al.*, 1994b, 2002; Kämpfer *et al.*, 1996; Wagner & Amann, 1997). Hence, in contrast to the γ -proteobacteria, the diversity and abundance of β -proteobacteria in activated sludge has been widely underestimated for a long time and, as a consequence, detailed studies on their impact on the wastewater purification process have rarely been performed. *In situ* hybridization experiments with specific

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The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain K11^T is AJ537466.

A 16S rDNA-based neighbour-joining tree and characteristics that differentiate the novel taxon from some genera of the *Comamonadaceae* are available as supplementary material in IJSEM Online.

oligonucleotide probes revealed that, in activated sludge of the municipal wastewater-treatment plant München I and several other plants, members of the family *Comamonadaceae* were clearly dominant among the β -proteobacteria (Snaidr *et al.*, 1997).

To increase our knowledge of representatives of this important group of wastewater bacteria, an oligonucleotide-probe-assisted directed cultivation procedure was applied to activated sludge samples. In a previous report, a detailed description was given of such a cultivation strategy, based on screening with a probe specific for members of the genus *Acidovorax* (Schulze *et al.*, 1999). This approach resulted in the successful isolation of several potential novel species and the formal description of *Acidovorax defluvii*. In this study, we present the targeted isolation of a novel β -proteobacterium based on the screening of activated sludge isolates with an oligonucleotide probe specific for *Leptothrix discophora* and several related species within the *Comamonadaceae*.

In 1994, grab samples of mixed liquor were taken from the high-load aeration basin of the wastewater-treatment plant München I (Großlappen, Germany). Activated sludge flocs in the sample were dispersed by treatment with a mechanical blender (Ultra-Turrax T25; IKA-Labortechnik Janke und Kunkel) for 4 min on ice. To facilitate dispersion, sodium pyrophosphate (2.8 g l^{-1}) was added as detergent. A dilution series was prepared and aliquots of the serial dilution were plated in duplicate on the solidified medium of Rouf & Stokes (1964), which was originally compounded for the enrichment and isolation of bacteria belonging to the *Sphaerotilus/Leptothrix* group of the β -Proteobacteria. After incubation for 5 days at room temperature, single colonies were picked randomly from dilution plates and purified by restreaking on agar plates of the same medium. A total of 24 isolates was obtained and screened by whole-cell hybridization with the oligonucleotide probe LDI23a (Wagner *et al.*, 1994a) as described previously (Wagner *et al.*, 1994b). Of two isolates that gave a positive hybridization result, one was chosen for detailed characterization and designated K11^T.

Standard bacteriological methods, as described previously for *Limnobacter thiooxidans* (Spring *et al.*, 2001), were used for the phenotypic characterization of strain K11^T. Unless otherwise stated, R2A medium (Reasoner & Geldreich, 1985) was used for growth experiments. Cells were Gram-negative, non-motile, straight to slightly curved rods with pointed ends. Motility was checked by phase-contrast microscopy in liquid mounts and by observation of potential colony spreading on the surface of solid water agar (Gerhardt *et al.*, 1994). A phase-contrast micrograph of cells of strain K11^T is shown in Fig. 1. In broth culture, especially in the early stationary growth phase, cells occasionally aggregated and formed flocs. Colonies on agar plates appeared after 2–3 days and displayed a cream to pale-yellow pigmentation. Production of the water-insoluble yellow pigment was stimulated by growth on peptone-rich media. The newly isolated strain grew well at temperatures ranging from 5 to 29 °C; only weak growth was detected

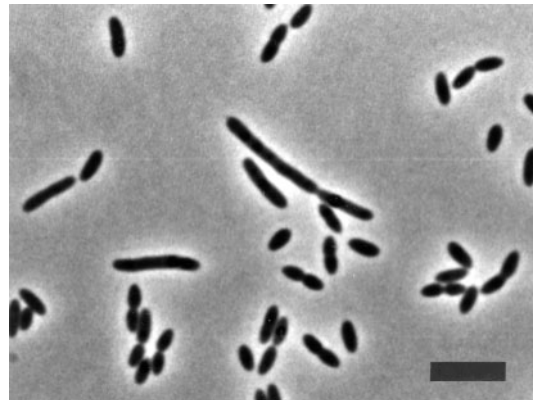


Fig. 1. Phase-contrast micrograph of cells of *Ottowia thiooxydans* gen. nov., sp. nov. Bar, 5 μm .

between 2 and 4 °C (temperatures below 2 °C were not tested). The pH range for growth in buffered R2A broth was pH 5.6–9.8. For aerobic growth under optimal conditions, the doubling time of K11^T was 3.5 h.

The spectrum of utilizable carbon sources was determined under aerobic conditions in a microplate assay as described by Kämpfer *et al.* (1991) with the modification that the final substrate concentration was 0.1% (w/v). The substrate spectrum of K11^T was restricted mainly to organic acids. None of the tested carbohydrates or sugar alcohols supported growth. A detailed description of the morphological and physiological characteristics of strain K11^T is given in Table 1 and the formal species description below.

Analysis of the respiratory lipoquinones of the novel isolate by HPLC (Tindall, 1990) gave only one characteristic peak, which corresponded to ubiquinone-8 (Q-8). This quinone system is a characteristic feature of the β -Proteobacteria (Yokota *et al.*, 1992). The cellular fatty acid composition was analysed after growth on tryptic soy broth agar (BD BBL) for 72 h at 25 °C. Fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification System (MIDI; Microbial ID). Extracts were analysed by GLC as described previously (Kämpfer & Kroppenstedt, 1996; Kämpfer *et al.*, 1997). The fatty acid profile of K11^T was dominated by only a few components: 42.7% 16:1 ω 7c, 20.4% 18:1 ω 7c and 19.9% 16:0. Fatty acids present in minor amounts included 17:0 cyclo (3.5%), 10:0 3-OH (2.8%), 12:0 (2.8%), 16:1 ω 5c (2.6%), 12:0 2-OH (2.1%), 14:0 (2.1%) and iso 15:1 and/or 13:0 3-OH (0.7%). The software and database of the MIDI system identified similar fatty acid profiles in species belonging to the genera *Hydrogenophaga* and *Acidovorax*, both taxa being affiliated to the family *Comamonadaceae*. Differentiation of the newly isolated strain K11^T was however achieved easily by the presence of the hydroxylated fatty acid 12:0 2-OH, which does not occur in combination with 10:0 3-OH in other known members of the *Comamonadaceae*. This characteristic may be also

Table 1. Differential characteristics of the genus *Ottowia* and related genera in the family *Comamonadaceae*

All genera listed are unable to grow phototrophically, reduce nitrate to nitrite and are oxidase-positive. Symbols: +, present in all type strains; -, absent from all type strains; w, weak reaction; d, 11–89% of type strains positive; ND, not determined; RQ, rhodoquinone. Data for genera other than *Ottowia* were taken from Blümel *et al.* (2001), Chang *et al.* (2002), Etchebere *et al.* (2001), Gardan *et al.* (2000), Gumaelius *et al.* (2001), Heulin *et al.* (2003), Hylemon *et al.* (1973), Mechichi *et al.* (2003) and Sakane & Yokota (1994).

Characteristic	<i>Ottowia</i>	<i>Hylemonella</i>	<i>Ramlibacter</i>	<i>Xenophilus</i>	<i>Acidovorax</i>	<i>Comamonas</i>	<i>Alicyclophilus</i>	<i>Brachymonas</i>
Species (<i>n</i>)	1	1	2	1	9	5	1	1
Cell shape	Rods	Spirilla	Rods, cysts	Rods	Rods	Rods or spirilla	Rods	Cocccobacilli
Flagellation	None	Bipolar tufts	None	None*	One polar or none	d	+	None
Non-diffusible pigments	+	-	+	+	-	-	-	w
Autotrophic growth with H ₂	-	-*	ND	-*	d	-	-	-
Anaerobic growth with nitrate	+	-	-	-*	d	d	+	+
Thiosulfate→sulfate	+	-*	ND	-*	ND†	ND‡	ND	-*
Growth factors required	+	+	+	-	-	d	-	-
Growth at 30 °C	-	+	+	+	+	+	+	+
Utilization of:								
D-Fructose	-	-	-	+	d	-	+	-
D-Glucose	-	+	d	-	d	d	+	-
Fumarate	+	-	ND	+	d	+	+	+
DL-Lactate	+	-	-	+	d	+	+	+
Malonate	-	-	-	ND	d	-	+	-
Glycerol	-	-	-	w	d	d	+	-
D-Mannitol	-	-	-	+	d	d	ND	-
Major 3-OH fatty acids	10:0	10:0, 12:0	ND	8:0, 10:0	10:0	10:0	10:0	10:0
Major 2-OH fatty acids	12:0	-	ND	16:0, 16:1, 18:1	-	(16:0)§	-	-
Major quinone system	Q-8	Q-8	ND	Q-8	Q-8	Q-8	ND	Q-8+RQ-8
DNA G+C content (mol%)	59	65	67–70	70	62–70	61–66	66	63–65

*Results obtained in this study.

†Reaction of *Acidovorax defluvii* DSM 12644^T is negative.

‡Reaction of *Comamonas nitratorans* DSM 13191^T is negative.

§Not present in all strains.

useful for the identification of K11^T, because both hydroxylated fatty acids were found in significant amounts independent of the medium composition.

The G+C content of total DNA was determined from the midpoint value of the thermal denaturation profile obtained with a Gilford 2600 spectrophotometer (Mandel & Marmur, 1968). The G+C content of K11^T DNA was calculated to be 59.3 mol% by using the equation of De Ley (1970) and *Escherichia coli* ATCC 11775^T DNA as a reference (G+C content, 51 mol%).

The 16S rRNA gene was amplified and sequenced as reported previously (Spring *et al.*, 2001). A continuous stretch of 1531 nt of the 16S rRNA gene of strain K11^T was determined and analysed phylogenetically using the ARB software package (Ludwig & Strunk, 1997). A comparison of the sequence of the oligonucleotide probe LDI23a with the complementary region of the 16S rRNA gene revealed one

terminal mismatch at the 3' end of the probe sequence. However, the probe mismatch did not prevent a positive signal under the hybridization conditions used, leading to the selection of this strain for further characterization. An ARB parsimony analysis of the 16S rRNA gene sequence obtained, based on the complete SSU rRNA database of ARB (release June 2002), placed strain K11^T within the family *Comamonadaceae* of the β -*Proteobacteria*. Sequence similarity values obtained with members of the *Comamonadaceae* indicated no close relationship of strain K11^T to any validly published species known to date. The nearest relatives, based on 16S rRNA gene sequence similarity values, were *Aquaspirillum gracile* (95.4%), *Acidovorax avenae* subsp. *avenae* (95.3%), *Ramlibacter tataouinensis* (95.2%) and *Xenophilus azovorans* (95.1%). The position of K11^T in various phylogenetic trees reconstructed by using neighbour-joining, maximum-likelihood or maximum-parsimony methods was not stable. In trees including representatives of the family *Comamonadaceae* (as defined by

Wen *et al.*, 1999), K11^T formed a common branch with either *Aquaspirillum gracile* or the two *Ramlibacter* species. In every case, the resulting branching pattern was not supported by high bootstrap values and was largely dependent on the dataset or algorithm used for tree reconstruction. The highest bootstrap value was 58 %, for a common branch with *Aquaspirillum gracile* obtained by resampling of the maximum-parsimony tree shown in Fig. 2. A phylogenetic tree including a wider selection of species, including all established representatives of the *Comamonadaceae*, was reconstructed by using the neighbour-joining method and is available as supplementary material in IJSEM Online.

Aquaspirillum gracile, which shared the highest 16S rRNA gene similarity with K11^T, is regarded as misclassified, because it is phylogenetically only distantly related to *Aquaspirillum serpens*, the type species of the genus *Aquaspirillum* (Ding & Yokota, 2002).

The metabolic versatility of strain K11^T was determined in a supplemented freshwater (SFW) medium of the following composition (l⁻¹ distilled water): 0.8 g K₂HPO₄, 0.3 g KH₂PO₄, 0.4 g MgSO₄·7H₂O, 0.5 g yeast extract, 0.5 g Trypticase peptone (BD BBL), 2 ml trace elements solution

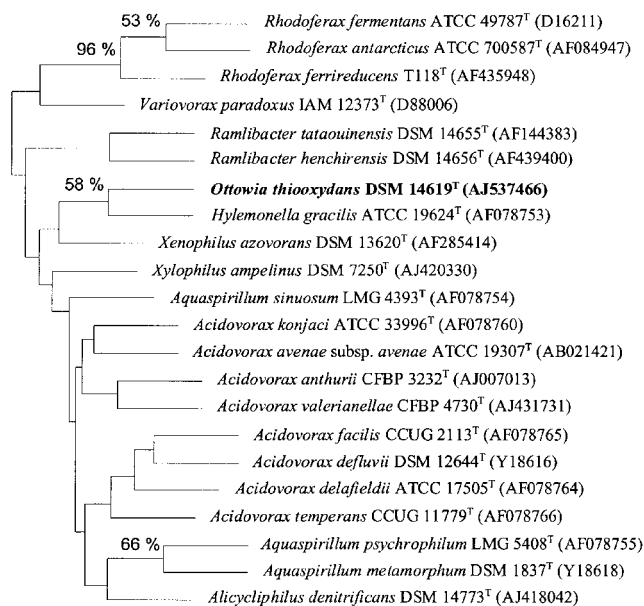


Fig. 2. Phylogenetic dendrogram illustrating the position of *Ottowia thiooxydans* and some closely related taxa within the *Comamonadaceae*. The tree is based on a collection of almost complete 16S rRNA gene sequences representing members of the *Comamonadaceae* as defined by Wen *et al.* (1999). GenBank/EMBL accession numbers are shown in parentheses. The tree was reconstructed using the maximum-parsimony method as implemented in the ARB package. Only bootstrap values above 50 % (1000 bootstrap resamplings for each node) are shown. The sequence of *Escherichia coli* (L10328) was used as an outgroup (not shown).

(Vishniac & Santer, 1957), 1 ml selenite/tungstate solution (Tschech & Pfennig, 1984) and 1 ml seven vitamins solution (Platen *et al.*, 1990), final pH 7.2–7.4. Substrates were added after autoclaving at the concentrations indicated below. Anoxic media were prepared by applying the anaerobe culture technique of Hungate (1950) with the modifications introduced by Bryant (1972).

Facultative anaerobic growth of the novel isolate was tested with pyruvate (20 mM) as carbon source and nitrate (10 mM), nitrite (5 mM), sulfate (20 mM) or ferric iron (50 mM) as alternative electron acceptors. Only nitrate and nitrite allowed anaerobic growth of K11^T by denitrification, whereas sulfate or ferric iron were not reduced. Yeast extract and peptone were essential for the growth of K11^T in SFW medium under anaerobic as well as aerobic conditions. It was not possible to replace these complex organic nutrients by vitamins or amino acids. Fermentation of carbohydrates or peptones was not observed. No phototrophic growth was detected under aerobic or anaerobic conditions.

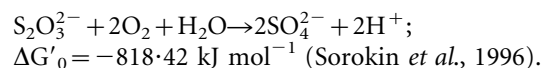
Growth of strain K11^T by denitrification was characterized by an extended lag phase (generally 2–3 weeks), a slow growth rate and a low maximum cell density (OD₄₃₀ approx. 0.3) compared with cultures that were grown aerobically in the same medium (OD₄₃₀ approx. 0.7). The end products of denitrification were analysed with cultures grown in SFW medium supplemented with 20 mM pyruvate and 10 mM nitrate. The concentration of nitrate and nitrite in spent culture media was determined colorimetrically using Griess–Ilosvay reagent (Merck); Nessler’s reagent (Merck) was used for the quantification of ammonium. A peculiarity of the studied strain was the production of nitrous oxide (N₂O) as the sole end product of denitrification. The amount of N₂O was quantified by gas chromatography as described previously by Weiske *et al.* (2001) after heating samples for 80 min at 80 °C. At the end of growth, the total amount of nitrate was converted nearly stoichiometrically to N₂O. Due to the high solubility of N₂O in water, almost no visible gas accumulation was observed during nitrate reduction. Nitrite was detected during denitrification only transiently in very small amounts, whereas the concentration of ammonium remained constant. In contrast, most other known facultatively anaerobic, heterotrophic bacteria produce dinitrogen or nitrite as the major end products of nitrate respiration. Strain K11^T represents the first example of a bacterium isolated from activated sludge that produces N₂O by incomplete denitrification. The previously characterized denitrifiers that are known to produce N₂O were isolated from soil, natural freshwater, solar salterns or hot springs. Examples include representatives from the β -*Proteobacteria* (*Chromobacterium violaceum*; Bazylinski *et al.*, 1986), γ -*Proteobacteria* [*Pseudomonas chlororaphis* (Christensen & Tiedje, 1988) and *Pseudoxanthomonas taiwanensis* (Chen *et al.*, 2002)], *Firmicutes* (*Bacillus halodenitrificans*; Denariuz *et al.*, 1989) and *Actinobacteria* (*Streptomyces nitrosporeus*; Wenzhofer *et al.*, 1997).

The emission of N₂O in the environment has recently

become a major concern due to the potential harmful effects of this trace gas to the atmosphere (Dickinson & Cicerone, 1986). N_2O is a very potent greenhouse gas and is more effective than carbon dioxide or methane. In particular, an increase in microbial denitrification in conjunction with anthropogenic activities such as fertilization of soil or wastewater purification may lead to a steady rise in N_2O release. For instance, a large number of wastewater-treatment plants will be equipped in the future with nitrification/denitrification units for the effective removal of dissolved nitrogen compounds. In this respect, the novel strain may be an interesting model organism for studying incomplete denitrification in wastewater-treatment units. However, it has to be noted that recent investigations on the exhaust gas of wastewater-treatment plants claim that a large part of the N_2O released is produced by aerobic nitrifiers, which perform incomplete denitrification of nitrite to N_2O under oxygen limitation (Sommer *et al.*, 1998; Burgess *et al.*, 2002).

Chemolithoautotrophic growth of strain K11^T with hydrogen was tested under the conditions described by Malik & Schlegel (1981) for the cultivation of various Knallgas bacteria. As a trait that distinguishes the novel strain from the closely related genus *Hydrogenophaga*, no aerobic autotrophic growth with hydrogen as substrate was detected. However, thiosulfate could be utilized by K11^T as an additional substrate for chemolithoheterotrophic growth (Fig. 3), but only under aerobic conditions. Growth stimulation by the addition of 10 mM thiosulfate as cosubstrate was most effective in SFW medium with pyruvate, and was less pronounced in R2A medium. The concentrations of thiosulfate and tetrathionate were quantified in spent culture media by the cyanolysis method as described by Kelly *et al.* (1969) and the amount of sulfate was estimated using the barium sulfate turbidity method of Berglund & Sörbo (1960). The end product of thiosulfate oxidation was

identified as sulfate. Therefore, it can be concluded that K11^T is able to gain energy according to the following equation:



The effect of thiosulfate on growth of K11^T in SFW-pyruvate medium was quantified by determination of the cellular dry weight in cultures grown to stationary phase. In two independent experiments, a mean increase of cellular dry weight of $31.2 \text{ mg l}^{-1} (\pm 7 \text{ mg l}^{-1})$ or 17.6% was obtained for cultures grown in medium with thiosulfate compared with cultures grown without thiosulfate. The correlation of thiosulfate consumption with growth behaviour of K11^T is shown in Fig. 3.

To date, thiosulfate oxidation has not been tested routinely in the description of newly isolated species. To find out whether it would be possible to use this trait for the differentiation of species within the *Comamonadaceae*, we performed tests with species that are phenotypically similar to K11^T (i.e. non-motile, heterotrophic denitrifiers), *Acidovorax defluvi* (DSM 12644^T), *Comamonas nitrivorans* (DSM 13191^T) and *Brachymonas denitrificans* (JCM 9216^T), as well as with two species that were only phylogenetically closely related, *Aquaspirillum gracile* (DSM 9158^T) and *Xenophilus azovorans* (DSM 13620^T).

Thiosulfate oxidation was tested for each strain in R2A medium and SFW-pyruvate medium supplemented with 10 mM thiosulfate. Interestingly, all tested strains showed a reaction different from K11^T. *Acidovorax defluvi* DSM 12644^T, *Aquaspirillum gracile* DSM 9158^T and *Xenophilus azovorans* DSM 13620^T were not able to oxidize thiosulfate, whereas *Comamonas nitrivorans* DSM 13191^T and *Brachymonas denitrificans* JCM 9216^T oxidized thiosulfate, but to tetrathionate rather than sulfate, leading to an increase of the pH of spent medium. Consequently, the straightforward test of thiosulfate oxidation may be a valuable tool for differentiation of closely related taxa that are otherwise difficult to distinguish.

From the results presented, it can be concluded that isolate K11^T represents a novel taxon within the *Comamonadaceae*, for which we propose the name *Ottowia thiooxydans* gen. nov., sp. nov. This conclusion is based on a polyphasic approach including biochemical, physiological and genotypic methods. Characteristics that differentiate *Ottowia thiooxydans* gen. nov., sp. nov. from the most closely related taxa of the *Comamonadaceae* are listed in Table 1. Differentiation from all remaining genera of the *Comamonadaceae* is set out in a supplementary table available in IJSEM Online.

Based on phylogenetic evidence presented by Ding & Yokota (2002), it is further proposed to transfer the species *Aquaspirillum gracile* to a novel genus, *Hylemonella* gen. nov., as *Hylemonella gracilis* comb. nov.

Description of *Ottowia* gen. nov.

Ottowia (Ot.to'wi.a. N.L. fem. n. *Ottowia* of Ottow, named after Johannes C. G. Ottow, who made several interesting

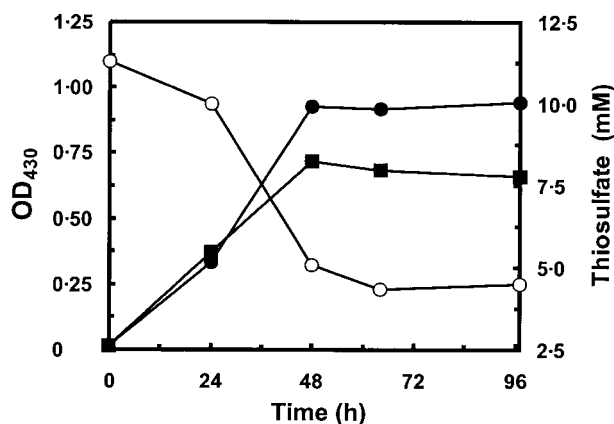


Fig. 3. Effect of thiosulfate on the growth of *Ottowia thiooxydans* in SFW-pyruvate medium: ■, growth in medium without thiosulfate; ●, growth in medium supplemented with thiosulfate; ○, concentration of thiosulfate over time of incubation.

contributions to our knowledge of denitrification in soil and activated sludge).

Cells are Gram-negative, non-motile and rod-shaped. No spores or cysts are formed. Polyhydroxyalkanoate granules are stored as intracellular reserve material. Yellow, water-insoluble pigment is produced. Oxidase- and catalase-positive. Mesophilic. Respiratory chemoheterotrophic metabolism. Growth factors required. Generally, carboxylic acids and amino acids are utilized as substrates. Facultatively anaerobic growth by nitrate reduction. No aerobic chemolithoautotrophic growth with hydrogen as substrate. Major respiratory lipoquinone is ubiquinone 8 (Q-8). Major cellular fatty acids are 16:1 ω 7c, 18:1 ω 7c and 16:0. Phylogenetically affiliated to the family *Comamonadaceae* within the β -*Proteobacteria*. The type species is *Ottowia thiooxydans*.

Description of *Ottowia thiooxydans* sp. nov.

Ottowia thiooxydans (thi.o.ox'y.dans. Gr. n. *thion* sulfur; N.L. v. *oxydo* to make acid, oxidize; N.L. part. adj. *thiooxydans* oxidizing sulfur).

Cells are straight to slightly curved rods with pointed ends, 0.8 μ m wide and 2–6 μ m long, occurring singly, in pairs or short chains. Colonies are small (diameter approx. 1 mm), circular, smooth, slightly raised and translucent with a cream to yellow pigmentation. Temperature and pH ranges for growth are 2–29 °C and pH 5.6–9.8. Optimal conditions for growth are pH 6.8–7.8 and 25–27 °C. Facultatively anaerobic growth with nitrate and nitrite. Produces N₂O as sole end product of nitrate respiration. Substrates assimilated under aerobic conditions (0.1 % w/v): acetate, aconitate, fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, propionate, pyruvate, suberate, L-aspartate, L-leucine, L-phenylalanine, L-proline and phenylacetate. Carbon sources not assimilated under aerobic conditions (0.1 % w/v): N-acetyl-D-glucosamine, L-arabinose, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, α -D-melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-trehalose, D-xylose, adonitol, i-inositol, maltitol, D-mannitol, D-sorbitol, adipate, 4-aminobutyrate, azelate, citrate, itaconate, mesaconate, oxoglutarate, L-alanine, L-histidine, L-ornithine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and putrescine. Negative for aesculin hydrolysis (β -glucosidase). Thiosulfate can be utilized as cosubstrate for aerobic chemolithoheterotrophic growth and is oxidized to sulfate. The G+C content of the DNA is 59 mol% (T_m).

The type strain, K11^T (= DSM 14619^T = JCM 11629^T), was isolated from activated sludge of a high-load basin of a municipal wastewater-treatment plant.

Description of *Hylemonella* gen. nov.

Hylemonella (Hy.le.mo.nel'la. L. dim. ending -*ella*; N.L. fem. dim. n. *Hylemonella* of Hylemon, named after Phillip B. Hylemon, who made important contributions to the taxonomy of freshwater spirilla).

The description is essentially based on the work of Hylemon *et al.* (1973) on *Aquaspirillum gracile*. It is supplemented by results obtained in this study and data taken from Sakane & Yokota (1994) and Hamana *et al.* (1994).

Cells are Gram-negative spirilla of small diameter. Motile by bipolar tufts of flagella. No spores or cysts are produced and no coccoid bodies are formed in older cultures. Pigments are not produced. Polyhydroxyalkanoates are not stored intracellularly. Oxidase- and catalase-positive. No growth at temperatures below 15 or above 42 °C. Respiratory chemoheterotrophic metabolism. Growth factors required. Generally, only carbohydrates are utilized as sole carbon sources. No anaerobic growth with nitrate. No aerobic chemolithoautotrophic growth with hydrogen. Thiosulfate is not oxidized to sulfate. Major respiratory lipoquinone is ubiquinone 8 (Q-8). Major cellular fatty acids are 16:1, 16:0, 18:0, 10:1 and 17:0. Hydroxylated fatty acids 10:0 3-OH and 12:0 3-OH are present. Cells contain the polyamines 2-hydroxyputrescine and putrescine. Phylogenetically affiliated to the family *Comamonadaceae* within the β -*Proteobacteria*. The type species is *Hylemonella gracilis*.

Description of *Hylemonella gracilis* comb. nov.

Hylemonella gracilis (gra'ci.lis. L. fem. adj. *gracilis* slender).

Basonym: '*Spirillum gracile*' Canale-Parola *et al.* 1966.

The description of this species is as given above for the genus with additional characteristics presented by Hylemon *et al.* (1973). The G+C content of DNA is 65 mol% (T_m). Isolated from pond water. The type strain is ATCC 19624^T (= DSM 9158^T).

Acknowledgements

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