Characterization of N₂O-producing *Xanthomonas*-like isolates from biofilters as *Stenotrophomonas nitritireducens* sp. nov., *Luteimonas mephitis* gen. nov., sp. nov. and *Pseudoxanthomonas broegbernensis* gen. nov., sp. nov.

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A group of yellow-pigmented isolates from ammonia-supplied biofilters showed an unusual denitrification reaction. All strains reduced nitrite but not nitrate without production of nitrogen (N₂). The only product found was nitrous oxide (N₂O). The strains were divided into two clusters and one separate strain by their fatty acid profiles, which were similar to the fatty acid profiles of the genera *Xanthomonas* and *Stenotrophomonas*. Analyses of the 16S rDNA sequences showed that these clusters and the separate strain form three independent lines within the *Xanthomonas* branch of the *Proteobacteria*. The evolutionary distances of the isolates to members of the related genera *Xanthomonas*, *Stenotrophomonas* and *Xylella* calculated by the 16S rDNA sequences led to the proposal of two new genera and three new species, *Stenotrophomonas nitritireducens* sp. nov., *Luteimonas mephitis* gen. nov., sp. nov. and *Pseudoxanthomonas broegbernensis* gen. nov., sp. nov. The type strains are *Stenotrophomonas nitritireducens* L2^T (= DSM 12575^T), *Luteimonas mephitis* B1953/27.1^T (= DSM 12574^T) and *Pseudoxanthomonas broegbernensis* B1616/1^T (= DSM 12573^T).

Keywords: *Stenotrophomonas*, *Xanthomonas*, nitrous oxide, denitrification, biofilter

INTRODUCTION

Bacterial strains which show chemotaxonomic characteristics similar to those of the genera *Stenotrophomonas* and *Xanthomonas* have been frequently isolated from biofilters for waste gas treatment (Lipski *et al.*, 1992; Lipski & Altendorf, 1997). The characteristic combination of markers were the presence of a branched chain fatty acid pattern and an ubiquinone with eight isoprenoid units (Q-8). This combination is restricted to these two genera so far. However, investigation of the physiological properties of these strains showed that the isolates were able to reduce nitrite.

The EMBL accession numbers for the 16S rDNA sequences of *Luteimonas mephitis* B1953/27.1^T, *Stenotrophomonas nitritireducens* L2^T, *Stenotrophomonas nitritireducens* B1910/29.1 and *Pseudoxanthomonas broegbernensis* B1616/1^T are AJ012228, AJ012229, AJ012230 and AJ012231, respectively.

This indicated the presence of a denitrification pathway, which at present, is unknown for strains of the genera *Stenotrophomonas* and *Xanthomonas*. The combination of chemotaxonomic and physiological markers suggested a different but closely related taxonomic position of these isolates to the genera *Stenotrophomonas* and *Xanthomonas*. This situation led us to investigate the phylogenetic position and the denitrification properties of the isolates in more detail.

The genera *Stenotrophomonas* and *Xanthomonas* were phylogenetically placed in the class *Proteobacteria*, where they form a deep branch located at the root of the gamma-subclass together with the genus *Xylella* (Moore *et al.*, 1997). This phylogenetic branch became of increasing interest since several strains with fundamentally different phenetic characteristics compared with the known plant- and human-pathogenic genera were isolated from different and sometimes extreme

environments and were assigned to this branch based on their 16S rDNA sequences. Chemolithoautotrophic Fe(II)-oxidizing strains were isolated by Buchholz-Cleven *et al.* (1997) and Emerson & Moyer (1997), an alkaliphilic *Stenotrophomonas*-like strain was isolated from a soda lake (Duckworth *et al.*, 1996), and Hoffmann *et al.* (1998) reported the isolation of three strains of *Stenotrophomonas maltophilia*-like strains from the gut and faeces of the arthropod *Folsomia candida*. The isolation, sequencing and phylogenetic analyses of 16S rRNA genes from a microbial mat at an active, deep-sea hydrothermal vent system indicated the presence of *Xanthomonas*-related strains in this microbial community (Moyer *et al.*, 1995). Beside these reports from extreme environments there are also reports about isolates assigned to the genus *Xanthomonas* from styrene- and H₂S-degrading biofilters (Arnold *et al.*, 1997; Cho *et al.*, 1992).

METHODS

Isolates and reference strains. Strains B1616/1^T, B1910/29.1, B1950/26, B1951a/26, B1953/27.1^T, B1956/27.1, B1957/27.1, B1959/26, B1962/27.1, B1964/27.1, B2060a/31.1, B2061a/31.1, B2067a/26, R514, R515, R516 and R517 were isolated from experimental biofilters used for the waste gas treatment of an animal-rendering plant (B. Bendinger and K. Reichert, personal communications).

Characteristic compounds of the waste gas were aldehydes, furans, alkyl sulfides and ammonia (Bendinger, 1992). Strains L2^T, L16, L53, L57 and L60 were isolated from laboratory-scale biofilters supplied with ammonia or dimethyl disulfide and ammonia (Lipski & Altendorf, 1997). All strains were maintained on NB-agar [5.0 g peptone l⁻¹ (from meat), 3.0 g meat extract l⁻¹ and 15.0 g agar l⁻¹]. Reference strains were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, and from Laboratorium Microbiologie Rijksuniversiteit Gent (LMG), Ghent, Belgium. Strain *Pseudomonas chlororaphis* ATCC 13985 (formerly *Pseudomonas aureofaciens*) was kindly provided by Heinz Körner, Karlsruhe.

Physiological tests. Denitrification reactions were tested in NB-medium (lacking agar) using screw-capped tubes. The medium was supplemented with either 10 mM K¹⁵NO₃ or 10 mM K¹⁵NO₂. The tubes were incubated for 5 d at 25 °C. The gas phase was checked for accumulation of the masses *m/z* 29 (^{14,15}N₂), *m/z* 30 (^{15,15}N₂), *m/z* 31 (¹⁵NO), *m/z* 45 (^{14,15}N₂O) and *m/z* 46 (^{15,15}N₂O). Gas samples were analysed by GC-MS with a Hewlett Packard model 5890 series II GC and a model 5972 mass selective detector. Helium was used as the carrier gas, the injection volume was 10 µl, the injector temperature was 120 °C, the column temperature was 50 °C and the GC-MS transfer line temperature was 280 °C. Reference strains for the denitrification tests are listed in Table 1. Additional physiological tests were performed according to Van den Mooter & Swings (1990).

Table 1. Denitrification reactions of biofilter isolates and reference strains based on the detection of gaseous products with *m/z* 46 (^{15,15}N₂O) and *m/z* 30 (^{15,15}N₂) from K¹⁵NO₂ and K¹⁵NO₃, respectively

	Reduction of:	Production of:	
		N ₂ O	N ₂
Biofilter isolates (<i>n</i> = 22)	Nitrite	+	—
	Nitrate	—	—
' <i>Corynebacterium</i> ' <i>nephridii</i> DSM 20150	Nitrite	+	—
	Nitrate	+	—
<i>Pseudomonas chlororaphis</i> ATCC 13985*	Nitrite	+	—
	Nitrate	+	—
<i>Alcaligenes faecalis</i> DSM 30033	Nitrite	—	+
	Nitrate	—	—
<i>Paracoccus denitrificans</i> DSM 65 ^T	Nitrite	—	+
	Nitrate	—	+
<i>Stenotrophomonas maltophilia</i> DSM 50170 ^T	Nitrite	—	—
	Nitrate	—	—
<i>Stenotrophomonas maltophilia</i> LMG 11114	Nitrite	—	—
	Nitrate	—	—
<i>Xylella fastidiosa</i> DSM 10026 ^T	Nitrite	—	—
	Nitrate	—	—
<i>Xanthomonas campestris</i> DSM 3586 ^T	Nitrite	—	—
	Nitrate	—	—
<i>Xanthomonas arboricola</i> LMG 747 ^T	Nitrite	—	—
	Nitrate	—	—
<i>Xanthomonas theicola</i> LMG 8684 ^T	Nitrite	—	—
	Nitrate	—	—

* Formerly *Pseudomonas aureofaciens*.

Fig. 1. Phylogenetic relationship of the isolates B1953/27.1^T, L2, B1910/29.1 and B1616/1^T to reference organisms of the Proteobacteria based on their 16S rDNA sequences. Numbers indicate the results of the bootstrap analysis (percentages). Scale bar, the distance in substitutions per nucleotide.

Fatty acid analyses. Reference strains and isolates were cultivated for 3 d on trypticase soy agar at 25 °C. *Xylella fastidiosa* DSM 10026^T was incubated for 14 d at 25 °C on buffered charcoal-yeast extract agar (Wells *et al.*, 1981). Harvesting, saponification, methylation and extraction of the fatty acid methyl esters were performed according to Sasser (1990). Identification of the fatty acid methyl esters was performed by GC-MS as described previously (Lipski & Altendorf, 1997).

PCR amplification of the 16S rDNA. A volume of 1 ml culture grown overnight was pelleted by centrifugation and resuspended in 100 µl distilled water. Cells were incubated at 100 °C for 5 min and pelleted again. The supernatant was diluted 10-fold and used as template for the PCR reaction. The 16S rDNA was amplified using the forward primer F1 (position 11–29) and the reverse primer R13 (position 1525–1544) (Dorsch & Stackebrandt, 1992). The positions for all primers refer to the *Escherichia coli* numbering system (Brosius *et al.*, 1978). For the PCR reactions, 5 µl of the

template solution was added to 3.5 µl primer F1 (10 µM), 3.5 µl primer R13 (10 µM), 75.5 µl double-distilled water, 10 µl 10-fold *Taq* polymerase buffer low salt (Gibco), 3 µl MgCl₂ (50 mM) and 2 µl dNTPs (10 mM). The reaction mixtures were incubated in a thermal cycler at 98 °C for 2 min and were then processed through 32 amplification cycles consisting of 1 min annealing at 52 °C, 2 min primer extension at 72 °C and 1 min denaturation at 93 °C. After the amplification process, a post-run of 1 min at 48 °C and 10 min at 72 °C was performed. The 16S rDNA was purified by agarose gel electrophoresis and extracted from the agarose with a QIAEX II kit.

Sequencing. The PCR products were sequenced by a ThermoSequenase fluorescent labelled primer cycle sequencing kit (Amersham) according to the manufacturer's instructions and an ALFExpress automatic sequencer. The nearly complete 16S rDNA sequences were determined by using the following sequencing primers [F, forward primer; R, reverse primer; numbers indicated the position of

Fig. 2. Phylogenetic relationship of the isolates B1953/27.1^T, L2^T, B1910/29.1 and B1616/1^T to reference organisms of the *Xanthomonas* branch of the *Proteobacteria* based on their 16S rDNA sequences. Numbers indicate the results of the bootstrap analysis (percentages). Scale bar, the distance in substitutions per nucleotide.

the 5'-base of the primer according to the *E. coli* numbering system (Brosius *et al.*, 1978): F9 (=PCR amplification primer F1), 5'-GAGTTTGATCCTGGCTCAG-3'; F337, 5'-ACTCCTACGGGAGGCA-3'; F511, 5'-TTCGTGCC-AGCAGCCG-3'; F929, 5'-CCCGCACAAAGCGGTGGA-3'; F1175, 5'-AGGAAGGTGGGGATGACGTC-3'; and R352, 5'-TGCCTCCCGTAGGAGTCTGG-3'.

Phylogenetic analyses. Sequences were compared by the FASTA program (Pearson & Lipman, 1988) with those in the EMBL database. The most similar sequences were included in the phylogenetic analysis. Sequences were aligned by the CLUSTAL X program (Thompson *et al.*, 1994) and edited manually. Phylogenetic analyses were restricted to those positions that were unambiguously aligned in all compared sequences. These were positions 49–74, 100–452, 461–835, 848–1132, 1141–1410 and 1415–1473 for the analysis presented in Fig. 1 and positions 49–77 and 93–1484 for the analysis presented in Fig. 2. These analyses were performed

with programs of the PHYLIP 3.5c program package (Felsenstein, 1993). Pairwise distances were calculated by the maximum-likelihood method with DNADIST. For bootstrap analyses, SEQBOOT, DNAPARS and CONSENSE were used. Phylogenetic dendrograms were calculated using DNAML with jumbled orders of the addition of sequences and allowed for global rearrangements of branches. The resulting trees were presented by means of the TREEVIEW program (Page, 1996). The reference sequences X67223 (*Agrobacterium tumefaciens* LMG 196), M22508 (*Alcaligenes faecalis* ATCC 8750^T), X57026 (*Bordetella bronchiseptica* S-1), M22510 (*Chromobacterium violaceum* ATCC 12472^T), M11224 (*Comamonas testosteroni* ATCC 11996^T), J01695 (*Escherichia coli*), M36023 (*Legionella pneumophila* strain Philadelphia 1^T), X07714 (*Neisseria gonorrhoeae* NCTC 83785), X06684 (*Pseudomonas aeruginosa* DSM 50071^T), U62646 (*Stenotrophomonas africana* MGB^T), X95923 (*Stenotrophomonas maltophilia* LMG 958^T), X95924 (*S.*

maltophilia LMG 11087), X95925 (*S. maltophilia* LMG 11114), AF017749 (*S. maltophilia* N4-15), Y13836 (*Stenotrophomonas* sp. strain NCTR), AJ002814 (*Stenotrophomonas* sp. S3), AJ002806 (*Stenotrophomonas* sp. R2), AJ002807 (*Stenotrophomonas* sp. R3), Z29975 (*Thiobacillus caldus* DSM 8584^T), M90662 (*Thiobacillus hydrothermalis* strain R3), X95918 (*Xanthomonas albilineans* LMG 494^T), Y10757 (*Xanthomonas arboricola* LMG 747^T), X95917 (*Xanthomonas campestris* LMG 568^T), AF000946 (*Xanthomonas campestris* pv. *campestris* strain 17), X95920 (*Xanthomonas fragariae* LMG 708^T), Y10754 (*Xanthomonas hyacinthi* LMG 739^T), X95921 (*Xanthomonas oryzae* LMG 5047^T), X95922 (*Xanthomonas populi* LMG 5743^T), Y10766 (*Xanthomonas sacchari* LMG 471^T), Y10763 (*Xanthomonas theicola* LMG 8684^T), X99299 (*Xanthomonas translucens* LMG 876^T), Y10761 (*Xanthomonas vesicatoria* LMG 911^T), M26601 (*Xylella fastidiosa* ATCC 35880), U51103 [denitrifying Fe(II)-oxidizing bacterium BrG3], AF012541 (iron-oxidizing lithotroph ES-1), U15111 (hydrothermal vent eubacterium PVB 4), U15113 (hydrothermal vent eubacterium PVB 3), U89956 (Gamma Proteobacterium N4-7), X92132 (Lake Natron isolate 97NT4) were obtained from the ribosomal database of Maidak *et al.* (1994).

DNA–DNA hybridization. Isolation of DNA of *S. maltophilia* LMG 11114, and strains L2^T and B1910/29.1 followed the method described by Cashion *et al.* (1977). DNA–DNA similarity was performed by the renaturation method (Escara & Hutton, 1980; Huß *et al.*, 1983) using a Gelford 2600 spectrophotometer and by the method of De Ley *et al.* (1970) using a Kontron Uvikon 933 spectrophotometer. Similarity values were calculated according to Jahnke (1992).

RESULTS

Denitrification reactions

The characteristic property of the isolates was the degradation of nitrite while nitrate was not degraded. The degradation of nitrate and nitrite was also investigated for several phylogenetically related reference strains and, in addition, for several denitrifying reference strains (Table 1). Incubation of the biofilter isolates in the presence of K¹⁵NO₂ and K¹⁵NO₃, respectively, with subsequent MS analyses of the products revealed ^{15,15}N₂O (*m/z* 46) to be the only product found. Only nitrite was used as an electron acceptor; nitrate was not reduced. All 22 isolates showed the same reaction pattern. No denitrification reaction was found for the reference strains of the genera *Stenotrophomonas*, *Xylella* and *Xanthomonas*. The denitrifying reference strains ‘*Corynebacterium nephridii*’ DSM 20150 and *Pseudomonas chlororaphis* ATCC 13985 produced N₂O from nitrate and nitrite, whereas *Alcaligenes faecalis* DSM 30033 produced N₂ from nitrite but not from nitrate. *Paracoccus denitrificans* DSM 65^T produced N₂ from both nitrate and nitrite.

Fatty acid analyses

The isolates were grouped according to their fatty acid profiles. Irrespective of previous chemotaxonomic analyses of most isolates, all strains were re-examined to guarantee identical incubation and preparation

conditions. Two clusters and one strain with a different profile could be defined according to these profiles. Cluster 1 consisted of the isolates B1950/26, B1951a/26, B1953/27.1^T, B1956/27.1, B1957/27.1, B1959/26, B1962/27.1, B1964/27.1, B2060a/31.1, B2061a/31.1, B2067a/26, R514, R515, R516 and R517. Cluster 2 consisted of the isolates L2^T, L16, L53, L57, L60 and B1910/29.1. The isolate B1616/1^T differed from isolates in clusters 1 and 2 (Table 2). Cluster 1 was characterized by high amounts of 17:1 iso *cis*9 and a high ratio of 15:0 iso to 15:0 anteiso. The isolates of cluster 2 were characterized by the presence of 17:0 cyclo and 13:0 iso 3-OH, and the fatty acid 14:0 iso was present in high amounts. The isolate B1616/1^T was characterized by high amounts of 15:0 anteiso. A remarkable profile was found for the reference strain *Xylella fastidiosa* DSM 10026^T. Instead of branched-chain fatty acids, we found saturated and unsaturated straight-chain fatty acids, whereas the unsaturated fatty acids had unusual double-bond positions (15:1 *cis*5, 15:1 *cis*7, 16:1 *cis*7 and 17:1 *cis*7).

Physiological tests

The isolates were also characterized by a range of physiological tests, which confirmed the differentiation of the isolates into three clusters (Table 3). Cluster 1 and isolate B1616/1^T were differentiated from the isolates of cluster 2 by their resistance against streptomycin and nalidixic acid. Characteristic properties of cluster 2 were resistance against ZnCl₂ and tetracycline and the susceptibility to nalidixic acid. None of the strains tested, except *S. maltophilia* DSM 50170^T, reduced nitrate to nitrite.

Phylogenetic analyses

The isolates B1953/27.1^T (representative of cluster 1), L2^T and B1910/29.1 (representatives of cluster 2), and B1616/1^T were subjected to a phylogenetic analysis based on their 16S rDNA sequences. Two representatives of cluster 2 were analysed since these strains were isolated from different locations. All isolates were members of the *Xanthomonas*-branch of the *Proteobacteria* (Fig. 1). Fig. 2 shows the result of a detailed analysis including additional sequences of this branch but omitted reference sequences which had larger phylogenetic distances except the *E. coli* sequence, which was used as an outgroup. The analysis shows a separation of the isolates into three lines. The 16S rDNA sequence of isolate B1953/27.1^T was remotely related to a group of highly similar sequences (group A) (94.2–94.4% similarity), which included two cloned sequences obtained from a microbial mat at a deep-sea hydrothermal vent system (PVB 3 and PVB 4) (Moyer *et al.*, 1995), one sequence of the Fe-oxidizing lithotrophic isolate ES-1 (Emerson & Moyer, 1997), and one sequence from strain N4-7 for which no further information was available. The sequences of the isolates L2^T and B1910/29.1 were almost identical

Table 2. Fatty acid profiles (%) of the isolates and reference strains

Isolates were grouped according to the similarity of their profiles. Strains: 1, *Luteimonas mephitis* (cluster 1, $n = 15$); 2, *Stenotrophomonas nitritireducens* (cluster 2, $n = 6$); 3, *Pseudoxanthomonas broegbernensis* B1616/1^T; 4, *Stenotrophomonas maltophilia* LMG 11114; 5, *Stenotrophomonas maltophilia* DSM 50170^T; 6, *Xanthomonas campestris* DSM 3586^T; 7, *Xylella fastidiosa* DSM 10026^T.

Fatty acid	1		2		3	4	5	6	7
	Range	Mean	Range	Mean					
10:0 iso			0.2–0.3	0.3				0.2	
10:0			0.1–0.3	0.2		0.2	0.4	0.6	1.3
10:0 2-OH									0.3
11:0 iso	3.8–5.1	4.5	3.1–4.1	3.6	5.1	4.0	3.8	3.9	
11:0 anteiso	0.2–0.2	0.2	0.2–0.3	0.3				0.3	
11:0 iso 2-OH					0.9				
11:0 iso 3-OH	2.0–2.7	2.3	0.3–0.5	0.4	2.2	0.6		0.3	
11:0 3-OH									0.2
13:0 iso	0.2–0.6	0.4	0.4–0.8	0.6					
12:0 iso 3-OH			0.3–0.6	0.4					
12:0 3-OH			0.3–0.3	0.3		0.6	0.6		0.3
14:0 iso	0.5–1.3	0.8	7.2–14.5	10.3	1.1	0.4	0.8		
14:0	0.3–0.5	0.4	1.0–2.4	1.7		1.8		0.7	
13:0 iso 3-OH			0.3–0.6	0.5		1.9	1.5	1.5	
15:1 iso 7	0.8–4.1	2.4	2.1–3.0	2.6	0.9	0.4		1.2	
15:0 iso	38.1–57.3	47.1	29.1–38.5	33.8	33.0	44.3	49.2	31.7	
15:0 anteiso	2.0–3.8	3.1	6.3–10.6	7.7	22.9	12.6	19.0	13.8	
15:1 <i>cis</i> 5									0.6
15:1 <i>cis</i> 7									0.7
15:0			0.8–4.0	2.3				1.0	7.3
16:0 iso	1.6–10.6	4.0	8.5–16.2	10.6	2.4	1.0	1.7	0.9	
16:1 <i>cis</i> 7			0.6–1.4	1.0		1.8	1.0	2.2	36.6
16:1 <i>cis</i> 9	0.7–1.2	0.9	1.1–4.8	2.5	9.4	11.6	7.7	13.8	
16:0	0.9–2.1	1.5	3.3–7.6	5.3	9.9	4.9	4.1	4.2	24.4
17:1 iso <i>cis</i> 9	18.2–26.4	23.5	9.5–11.0	10.2	9.4	5.7	3.3	13.9	
17:0 iso	3.7–11.0	8.3	1.5–2.3	1.8	2.4	4.7	3.4	7.7	
17:0 anteiso						0.3		0.6	
17:1 <i>cis</i> 7									3.9
17:1 <i>cis</i> 9			0.4–1.4	0.9				1.6	6.2
17:0 cyclo			0.5–4.1	2.8			2.0		
17:0			0.0–0.3	0.1					13.7
18:1 <i>cis</i> 9						1.3			2.8
18:1 <i>cis</i> 11						1.2			

(99.7% similarity) and formed a subcluster within the genus *Stenotrophomonas* including the sequences of *S. maltophilia* LMG 11114, two sequences of *Stenotrophomonas*-like isolates from the gut and faeces of a microarthropod (Hoffmann *et al.*, 1998) and *Stenotrophomonas* sp. strain NCTR, which was isolated from aquaculture pond slurry (Wang *et al.*, 1998). The sequence similarity within this subcluster was 98.7–100.0%. The similarity of the isolates L2^T and B1910/29.1 to the type strains *S. maltophilia* LMG 958^T and *S. africana* MGB^T was 96.7–97.3%. Isolate B1616/1^T was positioned moderately close to the cluster of type strains of the genus *Xanthomonas* (96.3–97.5% similarity). The phylogenetic position of

Xylella fastidiosa was not stable during several phylogenetic analyses; the relationship of this strain to isolate B1616/1^T thus could not be determined unambiguously. The sequence similarity between *Xylella fastidiosa* and strain B1616/1^T was 95.0%.

DNA–DNA hybridization experiments

The phylogenetic relationship between the highly related strains L2^T, B1910/29.1 and *S. maltophilia* LMG 11114 was resolved by DNA–DNA reassociation experiments. These experiments, performed with two different spectrophotometers, revealed high similarity values among the isolates L2^T

Table 3. Physiological properties of the isolates and reference strains

For *Luteimonas mephitis* and *Stenotrophomonas nitritireducens*, the number of positive isolates are given as a percentage. All isolates and reference strains were positive for growth in the presence of 0.001 % malachite green. Strains: 1, *Luteimonas mephitis* (cluster 1, $n = 15$); 2, *Pseudo-xanthomonas broegbernensis* B1616/1^T; 3, *Stenotrophomonas nitritireducens* (cluster 2, $n = 6$); 4, *Stenotrophomonas maltophilia* DSM 50170^T; 5, *Xanthomonas campestris* DSM 3586^T.

Character	1*	2	3†	4	5
Nitrate reduction to nitrite	0	—	0	+	—
Tween 80 hydrolysis	100	+	33	+	+
Aesculin hydrolysis	0	—	0	+	—
Lecithinase	0	—	0	+	+
Growth at 37 °C	0	—	67	+	—
Growth in the presence of:					
Cadmium acetate (0.001 %)	20	—	83	+	—
ZnCl ₂ (0.01 %)	0	—	100	+	—
Lead acetate (0.01 %)	73	—	83	+	+
Erythromycin (10 µg)	100	+	83	+	+
Streptomycin (10 µg)	100	+	0	+	—
Nalidixic acid (30 µg)	100	+	0	+	+
Kanamycin (30 µg)	7	+	0	—	—
Ampicillin (10 µg)	100	+	83	+	+
Penicillin G (10 U)	100	+	83	+	+
Gentamicin (10 µg)	73	+	67	+	+
Fucidin (10 µg)	80	+	100	+	+
Tetracycline (30 µg)	13	+	100	—	—
Novobiocin (30 µg)	80	+	100	+	—
Neomycin (30 µg)	73	—	50	+	+

* For variable tests, isolate 1953/27.1^T was positive for growth in the presence of lead acetate, gentamicin, fucidin, novobiocin and neomycin.

† For variable tests, isolate L2^T was positive for growth in the presence of cadmium acetate, lead acetate, erythromycin, ampicillin, penicillin G, gentamicin and neomycin.

and B1910/29.1 (78 and 85 %). In contrast, DNA of both isolates revealed only remote similarity with *S. maltophilia* LMG 11114 (34–38 % similarity). These values clearly indicate that both isolates should be considered as strains of the same species, whereas *S. maltophilia* LMG 11114 represents a separate species.

DISCUSSION

Aspects of denitrification properties

The denitrification properties of the biofilter isolates revealed the remarkable position of these strains within the *Xanthomonas* branch of the *Proteobacteria*, where these isolates represent the first denitrifying strains of this phylogenetic branch. Also, the truncated denitrification pathway, restricted to nitrite reduction to nitrous oxide, gives these strains an outstanding position within the denitrifying organisms. As well as

the majority of denitrifiers which possess the complete denitrification pathway and were able to reduce NO₃[−], NO₂[−], NO and N₂O to N₂, there is a minority of organisms which lack the first or last reaction of the pathway. The formation of N₂O as the end-product of NO₃[−] reduction is reported for several bacteria such as *Chromobacterium violaceum* (Bazylnski *et al.*, 1986), *Zavarzinia compransoris*, *Pseudomonas carboxydohydrogena*, ‘*Pseudomonas gazotropha*’ (Frunzke & Meyer, 1990), ‘*Corynebacterium*’ *nephridii* (Hart *et al.*, 1965), for some strains of the species *Pseudomonas fluorescens* and *Pseudomonas chlororaphis* (Greenberg & Becker, 1977), for several strains of the genus *Propionibacterium* (Kaspar, 1982), *Roseobacter denitrificans* (Shiba, 1991) and *Bacillus halodenitrificans* (Denariáz *et al.*, 1989). However, all of these strains were able to reduce NO₃[−]. The lack of nitrate-reducing activity is known for several other denitrifiers like *Neisseria sicca*, *Neisseria flavescens* and *Neisseria subflava* (Grant & Payne, 1981), *Sphingobacterium mizutae* (Yabuuchi *et al.*, 1983), *Oligella urethralis* (Rossau *et al.*, 1987), *Alcaligenes faecalis* (Rubin *et al.*, 1985), *Myroides odoratus* and *Myroides odoratimimus* (Vancanneyt *et al.*, 1996). Although the end-product was not identified for all of these species, none of these organisms was reported to terminate the denitrification sequence with the production of N₂O. To our knowledge, the restriction of the denitrification pathway to the central reaction, nitrite reduction to nitrous oxide, has been reported only for *Nitrosomonas europaea* which reduces nitrite to nitrous oxide under oxygen limitation (Poth & Focht, 1985).

Denitrification *senso stricto* is defined as the reduction of nitrogen oxide with the production of N₂O or N₂ which is coupled with the generation of an electrochemical gradient across the cytoplasmic membrane (Zumft, 1997). Our data do not show whether the nitrite reduction of our isolates has any bioenergetic significance. The lack of two energy-providing reaction steps suggests that nitrite reduction can be viewed as a detoxification reaction. This was also discussed for *Chromobacterium violaceum* (Bazylnski *et al.*, 1986) and several strains of the genus *Propionibacterium* (Kaspar, 1982). Further, the accumulation of non-volatile nitrification products and the resulting acidification of biofilters and similar systems can be reduced by such mechanisms. However, the advantages of nitrite reduction were linked to the formation of the greenhouse gas, nitrous oxide. Considering the problematic climatic effect of this gas, the isolation and investigation of nitrous oxide-producing microorganisms and, in a future step, the investigation of their distribution and activity under *in situ* conditions is an important field for the understanding of global nitrous oxide sources.

Classification of the isolates

The detailed phylogenetic analysis presented in Fig. 2 shows that the isolates represented three phylogenetic lines within the *Xanthomonas* branch of the *Proteo-*

bacteria. The 16S rDNA sequence of isolate B1953/27.1^T had a similarity of 94.2–94.4% to the sequences of members of group A, representing the most closely related taxon. This phylogenetic distance is significantly larger than the distance between the genera *Stenotrophomonas* and *Xanthomonas* (95.2–97.3% similarity) and therefore justifies the description of the isolates of cluster 1 as a member of a new genus for which the name *Luteimonas* is proposed. Apart from the 16S rDNA sequence, phenetic data for strains of group A were available only for the iron-oxidizing strain ES-1. This strain was reported to grow exclusively in the presence of Fe²⁺ and under micro-aerophilic conditions. Moreover, this strain does not grow on heterotrophic media (Emerson & Moyer, 1997). These properties are different from the properties of the isolates of cluster 1 which grow on several heterotrophic media in the absence of Fe²⁺ and under fully aerobic conditions. The physiological differences between the iron-oxidizing strain ES-1 and the isolates of cluster 1 support the differentiation of these strains on the genus rank.

The 16S rDNA sequences of the isolates L2^T and B1910/29.1 are similar to four other sequences deposited in the EMBL database which formed a subgroup within the genus *Stenotrophomonas* (Fig. 2). These are the sequences of the strain *S. maltophilia* LMG 11114, the two *Stenotrophomonas*-like strains R2 and R3, isolated from gut and faeces of the microarthropod *Folsomia candida*, and strain *Stenotrophomonas* sp. NCTR, isolated from aquaculture pond slurry. The phylogenetic position of the isolates L2^T and B1910/29.1 is clearly different from the positions of the type strains of *S. maltophilia* and *S. africana*, since the similarity of the 16S rDNA sequences between the isolates and the type strains was 96.7–97.3%. The next related strain, which was available from a culture collection, was *S. maltophilia* LMG 11114. However, according to the differences in the 16S rDNA sequences to the type strain of this species (96.9%), this strain should be excluded from the species *S. maltophilia* as suggested by Nesme *et al.* (1995), who found that *S. maltophilia* LMG 11114 had a significantly different 16S rDNA restriction pattern compared to those of representative strains of the genera *Xanthomonas* and *Stenotrophomonas*. The DNA–DNA hybridization analyses, which separated the isolates L2^T and B1910/29.1 from *S. maltophilia* LMG 11114 on the species level, correlated with the fatty acid pattern, showing similar significant differences (Table 2). Differentiating fatty acids were 14:0 iso, 17:0 cyclo, 18:1 *cis*9 and 18:1 *cis*11. Moreover, no denitrification activity was found for *S. maltophilia* LMG 11114 in contrast to the isolates L2^T and B1910/29.1.

The isolate B1616/1^T showed a moderate 16S rDNA sequence similarity to the genus *Xanthomonas* of 96.3–97.5%. As this similarity is lower than within the genus *Xanthomonas*, the formation of a new genus *Pseudoxanthomonas* is justified. This was supported by

fatty acid analysis, showing the lack of the *Xanthomonas*-characteristic fatty acid 13:0 iso 3-OH (Yang *et al.*, 1993) for the isolate B1616/1^T.

The descriptions of the species are based on these and previously published investigations (Lipski *et al.*, 1992; Lipski & Altendorf, 1997).

Description of *Luteimonas* gen. nov.

Luteimonas (Lu.te.i.mo'nas. M.L. adj. *luteus* yellow; Gr. n. *monas* a unit; M.L. fem. n. *Luteimonas* a yellow unit).

Cells are aerobic, Gram-negative rods. The quinone type is Q-8, fatty acids are of the iso/anteiso-type with 15:0 iso predominating. The strains have a yellow colour on trypticase soy agar and nutrient agar. The type species is *Luteimonas mephitis*.

Description of *Luteimonas mephitis* sp. nov.

Luteimonas mephitis (me.phi'tis. L. fem. n. *mephitis* harmful odour; L. gen. fem. n. *mephitis* of harmful odour).

Predominant fatty acids are 15:0 iso, 17:1 iso *cis*9 and 17:0 iso. 17:0 cyclo and 13:0 iso 3-OH are absent. Strains reduce nitrite to nitrous oxide, nitrate is not reduced and nitrous oxide is the end-product. Tween 80 is hydrolysed. Aesculin is not hydrolysed and lecithin is not degraded. Strains are resistant to erythromycin, streptomycin, nalidixic acid, ampicillin and penicillin G. The strains were isolated from experimental biofilters supplied with the waste gas of an animal-rendering plant. The type strain is *Luteimonas mephitis* B1953/27.1^T (= DSM 12574^T).

Description of *Pseudoxanthomonas* gen. nov.

Pseudoxanthomonas (Pseu.do.xan.tho.mo'nas. Gr. *pseudes* false; M.L. n. *Xanthomonas* a bacterial generic name; M.L. fem. n. *Pseudoxanthomonas* false *Xanthomonas*).

Cells are aerobic, Gram-negative rods. The quinone type is Q-8, fatty acids are of the iso/anteiso-type with 15:0 iso and 15:0 anteiso predominating. The strains have a yellow colour on trypticase soy agar and nutrient agar. The type species is *Pseudoxanthomonas broegbernensis*.

Description of *Pseudoxanthomonas broegbernensis* sp. nov.

Pseudoxanthomonas broegbernensis [broeg.ber.nen'sis. M.L. fem. adj. *broegbernensis* pertaining to Brögbern (location near Lingen/Germany, where the organism was isolated)].

Predominant fatty acids are 15:0 iso, 15:0 anteiso, 16:0, 16:1 *cis*9 and 17:1 iso *cis*9. 17:0 cyclo and 13:0 iso 3-OH are absent. The strain reduces nitrite to

nitrous oxide, nitrate is not reduced and nitrous oxide is the end-product. Tween 80 is hydrolysed. The strain is resistant to erythromycin, streptomycin, nalidixic acid, kanamycin, ampicillin, penicillin G, gentamicin, fucidin, tetracycline and novobiocin. The strain was isolated from an experimental biofilter supplied with the waste gas of an animal-rendering plant. The type strain is *Pseudoxanthomonas broegbernensis* B1616/1^T (= DSM 12573^T).

Description of *Stenotrophomonas nitritireducens* sp. nov.

Stenotrophomonas nitritireducens (ni.tri.ti.re.du'cens. M.L. *nitritis* nitrite; L. *reducere* to reduce; L. part. pres. *reducens* reducing; M.L. part. pres. *nitritireducens* reducing nitrite).

Predominant fatty acids are 15:0 iso, 17:0 iso *cis*9, 16:0 iso, 14:0 iso and 15:0 anteiso. 17:0 cyclo and 13:0 iso 3-OH are present. The strains reduce nitrite to nitrous oxide, nitrate is not reduced and nitrous oxide is the end-product. Aesculin is not hydrolysed and lecithin is not degraded. The strains grow in the presence of 0.01% ZnCl₂. The strains are resistant to fucidin, tetracycline and novobiocin, but susceptible to streptomycin, nalidixic acid and kanamycin. The strains show a strong hydrolytic activity for *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide. The strains were isolated from experimental biofilters supplied with artificial waste gases containing ammonia or ammonia and dimethyl disulfide. One strain (B1910/29.1) was isolated from an experimental biofilter supplied with the waste gas of an animal-rendering plant. The type strain is *Stenotrophomonas nitritireducens* L2^T (= DSM 12575^T).

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