Description of *Pseudaminobacter* gen. nov. with two new species, *Pseudaminobacter salicylatoxidans* sp. nov. and *Pseudaminobacter defluvii* sp. nov.

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An aerobic bacterium, strain BN12^T, which degrades substituted naphthalenesulfonates and substituted salicylates was isolated from a 6-aminonaphthalene-2-sulfonate-degrading microbial consortium originating from the River Elbe, Germany. Chemotaxonomic investigations of quinones, polyamines and polar lipids allowed allocation of this strain to the α -subclass of the *Proteobacteria* and revealed similarity to species of the genera *Aminobacter*, *Chelatobacter* and *Mesorhizobium*. This was confirmed by typing with 16S rRNA-targeted oligonucleotide probes and 16S rDNA sequencing and phylogenetic analysis, indicating that BN12^T clusters most closely with a strain 'Thiobacillus' THI 051^T and with the above genera but comprising a separate branch. DNA–DNA hybridizations demonstrated that strain BN12^T is different from all species of *Aminobacter* currently described and recognized. The fatty acid patterns, substrate utilization profile and biochemical characteristics displayed no obvious similarity to the characteristics of *Aminobacter* and *Chelatobacter* species. 'Thiobacillus' THI 051^T, however, revealed phenotypic similarities to BN12^T. Furthermore, 16S rRNA sequences of *Chelatobacter heintzii* showed a high similarity to the 16S rRNA sequences of all currently recognized *Aminobacter* species. On the basis of these and previously published results, the new genus *Pseudaminobacter* is proposed, harbouring the two new species *Pseudaminobacter salicylatoxidans* sp. nov. and *Pseudaminobacter defluvii* sp. nov. The type strains are BN12^T (= DSM 6986^T) and THI 051^T (= IFO 14570^T), respectively.

Keywords: *Pseudaminobacter* gen. nov., *Pseudaminobacter salicylatoxidans* sp. nov., *Pseudaminobacter defluvii* sp. nov.

INTRODUCTION

Pure cultures of the genus *Pseudomonas* and members of the α -subclass of *Proteobacteria* are able to mineralize sulfonated aromatic compounds (Zürner *et al.*, 1987; Busse *et al.*, 1989). Plasmid localization and

conjugational transfer of this trait have also been reported (Jahnke *et al.*, 1993). Often, microbial degradation of sulfonated aromatic compounds is accomplished by mixed bacterial cultures (Nörtemann *et al.*, 1986; Nörtemann, 1987; Feigel & Knackmuss, 1988, 1993; Thurnheer *et al.*, 1988; Jiménez *et al.*, 1991; Contzen *et al.*, 1996; Dangmann *et al.*, 1996). One intensively studied example of the microbial interactions within mixed bacterial cultures is the degradation of amino- and hydroxynaphthalene-2-sulfonates. A member of this co-culture, *Sphingomonas* sp. BN6 (Nörtemann *et al.*, 1986), converts these substrates into the corresponding salicylates basically

Abbreviations: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PDE, phosphatidyl-dimethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid; PME, phosphatidyl-monomethylethanolamine.

The GenBank/EMBL accession numbers for the sequences reported in this paper are AF072542, AJ011759, AJ011760, AJ011761 and AJ011762.

via the same catabolic pathway as described for naphthalene degradation (Nörtemann *et al.*, 1986, 1994; Kuhm *et al.*, 1991, 1993). The salicylates resulting from the degradation of the naphthalene-sulfonates are not further metabolized by strain BN6. They are, therefore, excreted in stoichiometric amounts and can be degraded by bacteria harbouring complementary catabolic pathways (Stolz *et al.*, 1992; Stolz & Knackmuss, 1993a, b).

The mixed culture containing strain BN6 and various (substituted) salicylate(s)-degrading bacteria was maintained for several years by continuous transfers in liquid media with 6-aminonaphthalene-2-sulfonate as the sole source of carbon and energy. After approximately 40 months cultivation, a new strain (BN12^T) was observed which was able, on solid media, to degrade 6-aminonaphthalene-2-sulfonate in a pure culture without excreting 5-aminosalicylate (Nörtemann, 1987). Results of partial 16S rRNA sequencing indicated that the organism belongs within the α -subclass of the *Proteobacteria* and, initially, it was presumed that the strain could belong to either the genus *Aminobacter* or *Chelatobacter*. Here, we report the results of a comprehensive taxonomic characterization of strain BN12^T and the related strain THI 051^T in comparison with representative strains of all *Aminobacter* species and *Chelatobacter heintzii*. As a result of this polyphasic approach, we propose the new genus *Pseudaminobacter* with the two new species *Pseudaminobacter salicylatoxidans* and *Pseudaminobacter defluvi*.

METHODS

Bacterial strains. Strain BN12^T was isolated as a 6-aminonaphthalene-2-sulfonate-mineralizing isolate from a microbial consortium originating from the River Elbe, Germany. Strain THI 051^T was obtained from A. Yokota. *Aminobacter aminovorans* DSM 7048^T, *Aminobacter aganoensis* DSM 7051^T, *Aminobacter niigataensis* DSM 7050^T and *C. heintzii* DSM 6450^T were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). All strains were maintained on nutrient agar (Oxoid) at 25 °C.

Morphological and physiological characterization. The Gram reactions of BN12^T and THI 051^T were tested as described by Gerhardt *et al.* (1994). Cell morphologies of BN12^T and THI 051^T were observed under a light microscope (1.250 $\times$) with cells grown for 24 h at 25 °C on nutrient agar. Physiological tests were performed with all strains as described previously (Kämpfer *et al.*, 1997).

Cellular fatty acids. Fatty acid methyl esters were extracted from BN12^T and THI 051^T and prepared by the standard protocol of the Microbial Identification system (MIDI; Microbial ID). Extracts were analysed by using a Hewlett-Packard model HP6890A gas chromatograph equipped with a flame-ionization detector, an automatic sampler, an integrator and a computer, as described previously (Kämpfer & Kroppenstedt, 1996; Kämpfer *et al.*, 1997).

Polyamines. Polyamines of strains BN12^T and THI 051^T were extracted and analysed according to Busse *et al.* (1997).

Polar lipids and quinones. The quinone system of strain BN12^T and the polar lipids were determined by TLC as described previously (Kroppenstedt, 1982; Auling *et al.*, 1986, 1993; Tindall, 1990).

Analysis of the G + C content. DNAs of BN12^T and THI 051^T were extracted according to Auling *et al.* (1986) and the G + C content was determined by HPLC analyses of the nucleosides (Kaneko *et al.*, 1986). The HPLC apparatus was equipped with a Waters pump (model 510), a model U6K injector, a column oven, a UV detector (Waters 484) and a reverse phase column (250 $\times$ 4 mm) hypersil C18, 5 μ m particles. The analyses were performed at 30 °C and nucleosides were measured at 270 nm. The values were calibrated using digested lambda (49.8 mol% G + C) as a standard.

DNA-DNA hybridizations. Isolation and hybridization of DNA was carried out by using the spectrophotometric method of De Ley *et al.* (1970) according to Auling *et al.* (1986).

Whole cell hybridizations. Exponentially growing cells of strain BN12^T were cultivated in nutrient bouillon, after harvesting (10000 g, 10 min) suspended in PBS solution (130 mM NaCl, 10 mM sodium phosphate buffer, pH 7.4) and fixed with 4% paraformaldehyde as described by Amann *et al.* (1990). Hybridizations were performed on coated slides as described by Manz *et al.* (1992) with rhodamine-labelled probes RHI1247 (Ludwig *et al.*, 1998) and ALF1b (Manz *et al.*, 1992). Microscopy was performed with a Zeiss Axioplan 2 using filter set no. 15.

16S rDNA analysis. 16S rDNA sequences were determined as described previously (Kämpfer *et al.*, 1997) using the 'universal primers' 27f, 357f, 530f, 704f, 926f, 1242f, 321r, 685r, 907r, 1069r, 1220r and 1522r (Gerhardt *et al.*, 1994). rDNA sequences were compared against those in the GenBank database using FASTA (Pearson & Lipman, 1988) to identify related sequences. Sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994). Evolutionary distances were calculated by pairwise comparison of the aligned sequences (Jukes & Cantor, 1969), excluding all positions where there was a gap in any sequence in the alignment. A neighbour-joining tree (Saitou & Nei, 1987) was constructed and bootstrap values (1000 replicates) were calculated by the method of Felsenstein (1985). Phylogenies were also constructed by parsimony using the DNAPARS program in PHYLIP (Felsenstein, 1993).

Nucleotide sequence accession numbers. 16S rDNA sequences were deposited in the EMBL/GenBank database with the following accession numbers: BN12^T (AF072542), *A. aminovorans* (AJ011759), *A. aganoensis* (AJ011760), *A. niigataensis* (AJ011761) and *C. heintzii* (AJ011762).

RESULTS AND DISCUSSION

Morphological and cultural characteristics

The obligate aerobic heterotrophic strains BN12^T and THI 051^T grew as circular, entire, slightly convex and smooth, glistening beige colonies with a diameter of 1–3 mm on nutrient agar (Oxoid). BN12^T was able to grow on nutrient agar (Oxoid), R2A agar (Oxoid) and TSB agar (BBL) at temperatures of 20–40 °C. No growth was observed after 7 d incubation at 10 and 45 °C. The cells were Gram-negative, non-spore-

Fig. 1. Phase-contrast micrographs of BN12^T (a) and THI 051^T (b). Bar, 5 µm.

forming, motile, coccoid to rod-shaped with cell dimensions of 1–2 µm (length) and 0.5–0.8 µm (width). Fig. 1 shows photomicrographs of BN12^T and THI 051^T, respectively.

Physiological and biochemical characteristics

BN12^T and THI 051^T were oxidase- and catalase-positive. A large number of organic compounds can be used as sole source of carbon (Table 1). BN12^T produced acid aerobically from D-glucose, D-mannitol, dulcitol and melibiose, but only very weak positive results could be observed. THI 051^T produced acid only from D-glucose. Table 1 shows that BN12^T and THI 051^T can be clearly differentiated from one another. Far more differentiating characters can be found for differentiation of the two strains from all *Aminobacter* species and *C. heintzii*. In addition, several characters differentiated the two strains from *Mesorhizobium* (Table 3). Where possible, a detailed comparison was made with all previously published data (Auling *et al.*, 1993; Urakami *et al.*, 1992; Jarvis *et al.*, 1997; de Lajudie *et al.*, 1998). Table 1 shows that nearly all test results which could be compared directly with data from previous papers were confirmed. Acid formation from various sugars often resulted in very weak positive results; these test results should be interpreted with care. A numerical analysis of the data

were performed by using the simple matching coefficient (S_{SM}) and the Jaccard coefficient (S_J) (Sneath & Sokal, 1973), and phenograms were generated by using the unweighted pair group with mathematical mean (UPGMA) algorithm. Similar phenograms were generated with the two coefficients. The results of the UPGMA/ S_{SM} analysis are shown in Fig. 2.

Chemotaxonomy

BN12^T contained a ubiquinone with 10 isoprenoid units in the side chain (ubiquinone Q-10), which is in agreement with the situation in *Aminobacter* species (Urakami *et al.*, 1992) and *C. heintzii* (Auling *et al.*, 1993). For THI 051^T, the presence of ubiquinone Q-10 was reported by Katayama-Fujimura *et al.* (1984).

The overall polar lipid patterns of BN12^T and THI 051^T were similar to the patterns determined for the other *Aminobacter* species and *C. heintzii*. Nearly the same amounts of the polar lipids phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidyl-dimethylethanolamine (PDE), phosphatidylmonomethylethanolamine (PME), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) were detected in BN12^T (Fig. 3) as well as an unidentified phospholipid (PL). THI 051^T had the same pattern,

Table 1. Physiological characteristics of the type strains of *Pseudaminobacter*, *Chelatobacter* and *Aminobacter* species

Strains: 1, *P. salicylatoxidans* BN12^T; 2, *P. defluvi* THI 051^T; 3, *C. heintzii* DSM 6450^T; 4, *A. aminovorans* DSM 7048^T; 5, *A. niigataensis* DSM 7050^T; 6, *A. aganoensis* DSM 7051^T. +, Positive; -, negative; (+), weak positive; pNP, *para*-nitrophenyl; pNA, *para*-nitroanilide. Test results given in the table were read after 72 h incubation at 30 °C. All strains were negative for: acid production from adonitol, rhamnose, methyl D-glucoside and erythritol; hydrolysis of aesculin, pNP- β -D-galactopyranoside, pNP- β -D-glucuronide, 2-deoxythymidine-5'-pNP-phosphate and L-glutamate- γ -3-carboxy-pNA; and assimilation of *p*-arbutin, α -D-melibiose, salicin, maltitol, putrescine, *trans*-aconitate, adipate, azelate, fumarate, itaconate, mesaconate, suberate, L-tryptophan, 3-hydroxybenzoate and phenylacetate. All strains were positive for: hydrolysis of L-alanine-pNA and L-proline-pNA; and assimilation of *N*-acetyl-D-glucosamine, 4-aminobutyrate, DL-3-hydroxybutyrate, DL-lactate, oxoglutarate, L-alanine, L-histidine, L-leucine and L-proline.

Test	1	2	3	4	5	6
Acid produced from:*						
Glucose	(+)	(+)	(+)	(+) [†]	(+) [†]	(+) [†]
Lactose	-	-	-	- [†]	- [†]	- [†]
Sucrose	-	-	(+)	(+) [†]	(+) [†]	(+) [†]
D-Mannitol	(+)	-	(+)	(+) [†]	(+) [†]	(+) [†]
Dulcitol	(+)	-	(+)	(+)	(+)	-
Salicin	-	-	(+)	(+)	(+)	-
Inositol	-	-	-	-	(+) [†]	(+) [†]
Sorbitol	-	-	(+)	-	(+) [†]	(+) [†]
L-Arabinose	-	-	(+)	(+) [†]	(+)	- [†]
Raffinose	-	-	-	(+)	-	-
Maltose	-	-	(+)	(+)	(+) [†]	(+) [†]
D-Xylose	-	-	(+)	(+) [†]	(+) [†]	(+) [†]
Trehalose	-	-	(+)	(+) [†]	(+) [†]	- [†]
Cellobiose	-	-	(+)	(+)	-	-
Melibiose	(+)	-	-	-	-	-
D-Arabitol	-	-	(+)	(+)	(+) [†]	(+) [†]
D-Mannose	-	-	(+)	(+)	(+)	(+)
Hydrolysis of:						
pNP- α -D-glucopyranoside	-	-	+	+	+	+
pNP- β -D-glucopyranoside	-	-	(+)	(+)	(+)	(+)
Bis-pNP-phosphate	+	(+)	+	+	+	+
pNP-phenyl-phosphonate	+	+	+	+	(+)	+
pNP-phosphorylcholine	-	-	+	+	-	(+)
Assimilation of:						
L-Arabinose	-	-	+	+ [†]	(+) [†]	+ [†]
D-Cellobiose	-	-	+	+	+	+
D-Fructose	-	-	-	+ [†]	+ [†]	(+) [†]
D-Galactose	-	-	+	+ [†]	+ [†]	+ [†]
Gluconate	-	-	- [‡]	-	-	-
D-Glucose	+	+	+	+ [†]	+ [†]	+ [†]
D-Mannose	-	-	+	+ [†]	+ [†]	+ [†]
D-Maltose	+	-	+	+ [†]	+ [†]	+ [†]
L-Rhamnose	-	-	+ [‡]	+	+	-
D-Ribose	+	+	+	+	+	+
Sucrose	-	-	+	+ [†]	+ [†]	+ [†]
D-Trehalose	+	-	-	-	+ [†]	+ [†]
D-Xylose	+	+	+	+ [†]	+ [†]	+ [†]
Adonitol	+	-	- [‡]	-	-	-
i-Inositol	-	-	+	+ [†]	+ [†]	+ [†]
D-Mannitol	+	-	+	+ [†]	+ [†]	+ [†]
D-Sorbitol	+	+	+	+ [†]	+ [†]	+ [†]
Acetate	+	-	+	+ [†]	(+) [†]	(+) [†]
Propionate	+	+	+ [‡]	+	-	+
<i>cis</i> -Aconitate	+	-	-	-	-	-
Citrate	-	-	-	- [†]	- [†]	- [†]
Glutarate	+	-	+	+	-	+
L-Malate	+	-	-	-	-	+
Pyruvate	+	+	-	-	+	+
β -Alanine	+	+	+	-	+	+
L-Aspartate	+	-	+	+	+	+
L-Ornithine	-	+	+	+	+	+
L-Phenylalanine	-	-	(+) [‡]	-	-	-
L-Serine	-	+	+	+	+	+
4-Hydroxybenzoate	+	-	-	-	+	+

* Acid formation from carbohydrates in most cases was very weak (even after prolonged incubation). These tests cannot be recommended for differentiation.

[†] Data are in line with those of Urakami *et al.* (1992).

[‡] Data are in line with those of Auling *et al.* (1992).

Fig. 2. Phenogram of the type strains of the genera *Aminobacter*, *Chelatobacter* and *Pseudaminobacter* by using the UPGMA analysis and S_{SM} .

Fig. 3. Polar lipids of BN12^T and *A. aminovorans* DSM 7048^T after separation by two-dimensional TLC. Abbreviations: PC, phosphatidylcholine; PG, phosphatidylglycerol; PDE, phosphatidylmethylethanolamine; PME, phosphatidylmonomethylethanolamine; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol; PL, unidentified phospholipid.

but only minor amounts of PDE differentiated the two strains. All three type strains of *Aminobacter* species and the type strain of *C. heintzii* were very similar with respect to their polar lipid patterns. PME was the sole predominant polar lipid in these four species in contrast to BN12^T and THI 051^T. PC, PG, PDE, PE, DPG and an unidentified phospholipid are present in minor amounts.

Both strains BN12^T and THI 051^T were also similar in their polyamine patterns. BN12^T contained spermidine [32.2 $\mu\text{mol (g dry wt)}^{-1}$], *sym*-homospermidine [18.6 $\mu\text{mol (g dry wt)}^{-1}$] and putrescine [15.6 $\mu\text{mol (g dry wt)}^{-1}$] as predominant polyamines. Cadaverine [0.1 $\mu\text{mol (g dry wt)}^{-1}$], spermine [0.3 $\mu\text{mol (g dry wt)}^{-1}$] and 1,3-diaminopropane [0.5 $\mu\text{mol (g dry wt)}^{-1}$] were detected in low amounts.

THI 051^T also contained spermidine [16.0 $\mu\text{mol (g dry$

wt)^{-1}], *sym*-homospermidine [15.5 $\mu\text{mol (g dry wt)}^{-1}$] and putrescine [23.9 $\mu\text{mol (g dry wt)}^{-1}$] as major polyamines. The organism also contained cadaverine [1.0 $\mu\text{mol (g dry wt)}^{-1}$], spermine [1.3 $\mu\text{mol (g dry wt)}^{-1}$] and 1,3-diaminopropane [0.5 $\mu\text{mol (g dry wt)}^{-1}$]. Both strains were similar with respect to polyamine patterns. BN12^T and THI 051^T could be clearly distinguished by their polyamine patterns from the three *Aminobacter* species (Hamana & Kishimoto, 1996) and from *C. heintzii* (Auling *et al.*, 1993). These species contain polyamine patterns which are characterized by the predominant compound *sym*-homospermidine, high amounts of putrescine and low amounts of spermidine. Strains BN12^T, THI 051^T, all type strains of *Aminobacter* species and *C. heintzii* contained the fatty acids 16:0, 18:0, 17:0iso, summed feature 4 (16:1 7c and/or 15:0iso 2-OH), summed feature 7 (18:1 7c, 18:1 9t and/or 18:1 12t) and an unknown fatty acid not identified from the MIDI

Table 2. Major fatty acid compositions (%) of type strains of species of the genera *Pseudaminobacter*, *Chelatobacter* and *Aminobacter*

Strain 1, *P. salicylatoxidans* BN12^T; 2, *P. defluvii* THI 051^T; 3, *C. heintzii* DSM 6450^T; 4, *A. aminovorans* DSM 7048^T; 5, *A. niigataensis* DSM 7050^T; 6, *A. aganoensis* DSM 7051^T. All strains were grown on Trypticase soy broth agar at 28 °C for 48 h prior to fatty acid analysis. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) group of the carbon chain; *cis* is indicated by the suffix *c*. Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 4 contained one or more of the following fatty acids: 16:1 ω 7*c* and 15:0iso 2-OH. Summed feature 7 contained one or more of the following isomers: 18:1 ω 7*c*, 18:1 ω 9*t* and/or 18:1 ω 12*t* (*cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively). The unknown fatty acids have no name listed in the Peak library file of the MIDI system and therefore cannot be identified.

Compound	1	2	3	4	5	6
Saturated fatty acids:						
9:0		0.7	1.0			
16:0	2.3	3.7	6.3	4.9	6.0	6.2
17:0	9.1	3.0	0.7	0.7		
18:0	2.6	1.5	2.1	1.5	2.0	2.0
Unsaturated fatty acids:						
15:1 ω 8 <i>c</i>						1.3
17:1 ω 8 <i>c</i>	3.4	3.0	0.5	0.7		
17:1 ω 6 <i>c</i>	1.2	2.0				
19:1 ω 12 <i>t</i>	1.3					
20:0 ω 6,9 <i>c</i>	0.6					
20:1 ω 9 <i>t</i>	0.6	1.8	0.8	0.8	0.7	
Branched fatty acids:						
15:0iso	1.2	0.7			3.1	2.9
17:0iso	1.5	2.0	2.6	1.0	3.0	4.8
Hydroxy fatty acids:						
12:0 3-O H			0.4	0.3	0.4	0.4
15:0iso 3-OH	0.5	0.5				
Summed features:						
Summed feature 4	0.4	0.8	0.4	0.5	1.0	1.1
Summed feature 7	43.4	58.5	46.2	46.4	48.6	64.8
Cyclo propane acids:						
17:0 cyclo				0.5		
19:0 cyclo ω 8 <i>c</i>	25.4	21.6	14.7	13.7	10.3	4.7
Unknown 14-966		0.7	0.6	0.7		
Unknown 18-081	0.8		24.5	24.8	21.0	10.9
Unknown 18-597	1.2		0.8	0.5	1.8	0.8
Unknown 18-804	4.7		0.9	0.4	0.7	

database (Table 2). These fatty acids have also been detected in previous studies and are characteristic of all validly described species of the genus *Aminobacter* (Urakami *et al.*, 1992). The major hydroxy fatty acid in *Aminobacter* species could be confirmed as 12:0 3-OH. This fatty acid was also detected in *C. heintzii* (Table 2), but not in strains BN12^T and THI 051^T. In contrast, BN12^T and THI 051^T contained the hydroxylated fatty acid 15:0iso 3-OH, which could not be detected in any *Aminobacter* and *Chelatobacter* strains (Table 2). Some additional differences in fatty acid patterns between *Aminobacter*, *Chelatobacter*, BN12^T and THI 051^T are shown in Table 2.

The G+C content of BN12^T was estimated to be 63.9 mol %. This was very similar to the G+C content of THI 051^T, which was reported to be 62.9 mol % (Katayama-Fujimuri *et al.*, 1983).

DNA-DNA similarity

No close similarities between strain BN12^T and the type strains of the species of *Aminobacter* or *Chelatobacter* were detected in two series of hybridization experiments with DNA preparations isolated from two batches grown independently. The following similarity values [given as *D* values (%)]

Table 3. Main differential characters of the genera *Pseudaminobacter*, *Aminobacter*/*Chelatobacter* and *Mesorhizobium*

+, 90% or more strains are positive; –, 90% or more strains are negative; +/–, some strains are positive; ND, not determined or data are not available for all species (*M. loti*, *M. huakuii*, *M. mediterraneum*, *M. ciceri*, *M. tianshanense* and *M. plurifarum*).

Characteristic	<i>Pseudaminobacter</i>	<i>Aminobacter</i> / <i>Chelatobacter</i> *	<i>Mesorhizobium</i> †
Assimilation of:			
L-Arabinose	–	+	+
D-Cellobiose	–	+	ND
D-Fructose	–	+ / –	+
Fumarate	–	–	+ ‡
D-Galactose	–	+	ND
D-Mannose	–	+	+ §
Sucrose	–	+	+ §
Fatty acids:			
12:0 3-OH	Absent	Present	Present
13:0 iso 3-OH	Absent	Absent	Present
15:0 iso 3-OH	Present	Absent	Absent
Major polyamines	Spermidine, sym-homospermidine, putrescine	sym-Homospermidine, putrescine	sym-Homospermidine, putrescine ¶

* Differentiating characters between *Chelatobacter heintzii* and *Aminobacter* species are given in Table 1.

† Data of physiological tests from Jarvis *et al.* (1997) and de Lajudie *et al.* (1998).

‡ *M. tianshanense* is negative (Jarvis *et al.*, 1996; de Lajudie *et al.*, 1998).

§ Some strains of *M. tianshanense* are negative (de Lajudie *et al.*, 1998).

|| Data are available only for *M. loti* and *M. huakuii* (from Jarvis *et al.*, 1996).

¶ Data are only available for *M. loti* (Hamana *et al.*, 1990).

according to De Ley *et al.* (1970)] were determined between DNA of strain BN12^T and *C. heintzii* ATCC 29600^T (= DSM 6450^T) (13.4%), DNA of *A. aminovorans* NCIB 9039^T (= DSM 7048^T) (18.9%), and DNA of *A. aganoensis* DSM 7051^T (22.6%) and *A. niigataensis* DSM 7050^T (25.2%). The *D* values obtained represent background levels of the optical method of De Ley *et al.* (1970) and do not support an allocation of strain BN12^T into either of the above genera. Between BN12^T and THI 051^T, a DNA–DNA similarity of 36% was found, indicating that the two strains do not belong to the same species.

Typing with 16S rRNA-targeted oligonucleotides

Fixed cells of strains BN12^T and THI 051^T showed strong hybridization signals with probes RHI1247 and ALF1b. Binding of these two oligonucleotides supports their taxonomic position as members of the family *Rhizobiaceae*.

16S rDNA sequencing

The nearly complete 16S rRNA gene sequences of strains BN12^T, DSM 7048^T, DSM 7050^T, DSM 7051^T and DSM 6450^T were determined. FASTA analysis of

the BN12^T sequence showed this strain to be most closely related (98% similarity across 1406 bp) to THI 051^T.

The 16S rDNA sequences of *A. aganoensis* and *A. niigataensis* were identical across 1466 bp. Similarities of greater than 99.6% were found between the 16S rDNA sequences from all three strains of *Aminobacter*, that from *C. heintzii* and from that of the methylo-trophic proteobacterium strain IMB-1 (accession number AF034798) (Table 4). FASTA analysis of these sequences showed highest similarities to those from another methylo-trophic isolate, strain ER2 and to members of the species *Mesorhizobium loti* and *Mesorhizobium ciceri*.

An alignment of the 16S rDNA sequences from the strains BN12^T, THI 051^T and ER2, members of the genus *Aminobacter*, the type strains of the six species of the genus *Mesorhizobium* (Jarvis *et al.*, 1997), and more distant members of the family *Rhizobiaceae* was made, and a neighbour-joining tree was constructed following pairwise comparison of these aligned sequences (1376 positions, after exclusion of any position in which a gap was found). The strains BN12^T and THI 051^T form a distinct group, with overwhelming bootstrap support, which is clearly separate from

Table 4. Percentage similarities between 16S rDNA sequences from members of the genera *Aminobacter*, *Chelatobacter*, *Pseudaminobacter*, *Mesorhizobium* and related members of the *Rhizobiaceae* family and the related isolate ER2

Strain: 1, *Aminobacter aganoensis* DSM 7051^T; 2, *Aminobacter aminovorans* DSM 7048^T; 3, *Chelatobacter heintzii* DSM 6450^T; 4, *Aminobacter niigataensis* DSM 7050^T; 5, IMB-1; 6, ER2; 7, *Pseudaminobacter salicylatoxidans* BN12^T; 8, *Pseudaminobacter defluvii* THI 051^T; 9, *Mesorhizobium loti* LMG 6125^T; 10, *Mesorhizobium ciceri* UPM-Ca7^T; 11, *Mesorhizobium mediterraneum* UPM-Ca36^T; 12, *Mesorhizobium huakuii* IFO 15243^T; 13, *Mesorhizobium plurifarum* LMG 11892^T; 14, *Mesorhizobium tianshanense* A-1BS^T; 15, *Phyllobacterium myrsinacearum* IAM 13584^T; 16, *Ochrobactrum anthropi* IAM 14119^T; 17, *Agrobacterium tumefaciens* DSM 30105^T.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	100	99.8	99.9	100	99.6	97.9	96.8	96.7	97.8	97.8	96.8	96.6	96.7	96.0	96.2	93.8	93.6
2		100	99.8	99.8	99.6	97.7	96.6	96.5	98.1	98.0	97.0	96.8	96.9	96.1	96.2	93.8	93.5
3			100	99.9	99.8	97.9	96.8	96.7	98.0	97.8	96.6	96.7	96.9	96.1	96.4	93.6	93.9
4				100	99.6	97.9	96.8	96.7	97.8	97.8	96.8	96.6	96.7	96.0	96.2	93.8	93.6
5					100	97.8	96.9	96.5	97.7	97.7	96.7	96.4	96.6	95.9	96.1	93.6	93.2
6						100	95.4	94.9	95.9	95.8	95.0	95.0	94.9	94.7	94.4	92.3	91.9
7							100	98.0	95.7	95.9	95.9	95.3	95.8	94.7	95.1	92.8	92.6
8								100	96.7	96.6	96.6	96.3	96.8	95.5	96.0	93.2	92.3
9									100	99.5	98.4	98.0	98.0	97.3	96.4	94.0	93.4
10										100	98.5	97.8	97.9	97.3	96.3	94.0	93.3
11											100	98.8	98.8	97.7	96.1	94.6	93.7
12												100	99.2	97.7	96.0	94.7	93.7
13													100	97.4	96.4	94.8	93.8
14														100	94.8	94.3	94.6
15															100	94.6	92.9
16																100	93.3
17																	100

both the genera *Aminobacter* and *Mesorhizobium*. Moreover, parsimony analysis of these sequences provided additional confirmation of BN12^T and THI 051^T forming such a distinct phylogenetic group (data not shown). On the basis of both their phylogenetic and physiological distinction, we propose the description of a new genus *Pseudaminobacter* gen. nov. containing the species *Pseudaminobacter salicylatoxidans* sp. nov. and *Pseudaminobacter defluvii* sp. nov. The study of the generic relationships of members of the genera *Aminobacter* and *Chelatobacter* based on 16S rRNA sequence comparisons showed a close relationship between *C. heintzii* and the three *Aminobacter* species (Fig. 4, Table 4). Results of chemotaxonomic and phenotypic investigations support this (Tables 1 and 2). DNA–DNA similarity studies, however, clearly indicated its species status, but it is obvious that the taxonomic status of the genus *Chelatobacter* needs revision.

Description of *Pseudaminobacter* gen. nov.

Pseudaminobacter (Pseud.ami.no.bac.ter. Gr. adj. *pseudos* false; M.L. *Aminobacter* generic name of a bacterium, false aminobacters).

Gram-negative, rod-shaped cells, 1–2 µm long by 0.5–0.8 µm wide, with rounded ends. Cells are motile. Oxidative metabolism. Oxidase-positive. Catalase-

poistive. Contains ubiquinone Q-10. The major polyamines are spermidine, *sym*-homospermidine and putrescine. The overall polar lipid pattern was characterized by nearly the same amounts of the polar lipids PC, PG, PDE, PME, PE and DPG. The cellular fatty acids include a large amount of straight-chain 18:1 acid. The hydroxy acids include the hydroxylated fatty acid 15:0iso 3-OH. Colonies are circular, entire, slightly convex and smooth, glistening and pale beige on nutrient agar at 25 °C. Growth occurs on nutrient agar (Oxoid), Caso agar, R2A agar (Oxoid) and TSB agar (BBL). Several organic compounds can be used as sole source of carbon (Table 1). The G + C contents of the DNAs are 62.9–63.9 mol% (as determined by HPLC). Phylogenetically, the genus is a member of the α -subclass of the *Proteobacteria*. The type species is *Pseudaminobacter salicylatoxidans*. At the molecular level, the genus can be recognized by 16S rRNA sequences and fatty acid profiles (Table 3).

Description of *Pseudaminobacter salicylatoxidans* sp. nov.

Pseudaminobacter salicylatoxidans (sa.li.cy.lat. oxi.dans. N.L. *salicylatoxidans* oxidizing salicylate, because the organism oxidizes salicylate in an unusual manner).

Gram-negative, rod-shaped cells are 1–1.5 µm long by

Fig. 4. Neighbour-joining distance tree showing the estimated relationship between the strains BN12^T and THI 051^T, ER2, species of the genus *Aminobacter* and species of related genera from the *Rhizobiaceae*. Accession numbers of these related sequences are as follows: methylotrophic sp. strain ER2, L20802; methylotrophic proteobacterium IMB-1, AF034798; *A. aminovorans*, AJ011759; *A. aganoensis*, AJ011760; *A. niigataensis*, AJ011761; *C. heintzii*, AJ011762; BN12^T, AF072542; THI 051^T, D32248; *Mesorhizobium loti*, X67229; *Mesorhizobium ciceri*, U07934; *Mesorhizobium mediterraneum*, L38825; *Mesorhizobium huakuii*, D13431; *M. plurifarum*, Y14158; *Mesorhizobium tianshanense*, U71079; *Phyllobacterium myrsinacearum*, D12789; *Ochrobactrum anthropi*, D12794; and *Agrobacterium tumefaciens*, M11223. Evolutionary distances are shown as nucleotide substitutions per site. Bootstrap values (1000 replicates) are shown to the left of the node being considered.

0.5–0.8 µm wide, with rounded ends. Cells are motile. Forms circular, entire, slightly convex and smooth, glistening beige colonies with a diameter of 1–3 mm on nutrient agar (Oxoid). Oxidative metabolism. Oxidase-positive. Able to grow on nutrient agar (Oxoid), R2A agar (Oxoid) and TSB agar (BBL) at temperatures of 20–40 °C. Contains ubiquinone Q-10. The major polyamines are putrescine, spermidine and sym-homospermidine. The overall polar lipid pattern was characterized by nearly the same amounts of the polar lipids PC, PG, PDE, PME, PE and DPG. The cellular fatty acids include a large amount of straight-chain 18:1 acid. The hydroxy acids include the hydroxylated fatty acid 15:0iso 3-OH. The utilization of L-arabinose, D-cellobiose, D-galactose, D-mannose, sucrose, L-ornithine and L-serine (all negative for BN12^T) and adonitol and *cis*-aconitate (positive for BN12^T) allows differentiation from all *Aminobacter* species. Hydrolysis of *p*-nitrophenyl α-D-glucopyranoside and *p*-nitrophenyl β-D-glucopyranoside is negative in contrast to all *Aminobacter* and *Chelatobacter* strains. The utilization of L-ornithine and L-serine (all negative for BN12^T) and D-maltose, D-trehalose, *cis*-aconitate, glutarate and L-malate (positive for BN12^T) allows differentiation from THI 051^T. Other characteristics

are shown in Tables 1 and 2. The type strain is strain BN12^T (= DSM 6986^T). The G+C content is 63.9 mol %.

Description of *Pseudaminobacter defluvii* sp. nov.

Pseudaminobacter defluvii (de.flu.vi.i. L. neut. gen. n. *fluvius* wastewater, because the organism was isolated from activated sludge).

Gram-negative, coccoid to rod-shaped cells are 0.8–1.2 µm long by 0.5–0.8 µm wide. Cells are motile. Forms circular, entire, slightly convex and smooth, glistening beige colonies with a diameter of 1–3 mm on nutrient agar (Oxoid). Oxidative metabolism. Oxidase-positive. Contains ubiquinone Q-10. The major polyamines are putrescine, spermidine and sym-homospermidine. The overall polar lipid pattern is characterized by nearly the same amounts of the polar lipids PC, PG, PDE, PME, PE and DPG. The cellular fatty acids include a large amount of straight-chain 18:1 acid. The hydroxy acids include the hydroxylated fatty acid 15:0iso 3-OH. In addition to the characteristics given in the genus description, *P. defluvii* has the following characteristics. Growth occurs between

10 and 40 °C and on nutrient agar (Oxoid), Caso agar, R2A agar (Oxoid) and TSB agar (BBL). No growth was visible after incubation at 5 and 45 °C. Several organic compounds can be used as sole source of carbon (Table 1). Other characteristics are shown in Tables 1 and 2. The type strain is strain THI 051^T (= IFO 14570^T). The G+C content is 62.9 mol %

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