Characterization of halobenzoate-degrading, denitrifying *Azoarcus* and *Thauera* isolates and description of *Thauera chlorobenzoica* sp. nov.

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The taxonomic relationships of *Azoarcus* and *Thauera* isolates in the β -subclass of the *Proteobacteria* capable of degrading fluoro-, chloro- or bromobenzoate under denitrifying conditions were analysed. A detailed classification of these strains was performed using a polyphasic approach, which included studies on morphology, phenotypic characterization, fatty acid analysis, 16S rRNA gene sequence analysis, 16S rRNA gene mapping (ribotyping) and DNA–DNA hybridization. The analyses of fatty acids and 16S rRNA gene sequencing differentiated strains 2FB2, 2FB6 and 4FB10 as new members of the genus *Azoarcus* and strains 4FB1, 4FB2, 3CB2, 3CB3 and 3BB1 as new members of the genus *Thauera*. DNA–DNA hybridization experiments established that strains 2FB2, 2FB6 and 4FB10 belong to the species *Azoarcus tolulyticus*. Strains 3CB2 and 3CB3 were assigned to the species *Thauera aromatica* on the basis of DNA–DNA hybridization and ribotyping experiments. Strains 4FB1, 4FB2 and 3BB1 showed close relatedness with strain 3CB-1^T, previously described as *T. aromatica* genomovar *chlorobenzoica*. This group of strains is clearly differentiated from the species *T. aromatica* on the basis of 16S rRNA sequence analysis, DNA homology and ribotyping analysis. Strains 3CB-1^T, 4FB1, 4FB2 and 3BB1 are proposed as members of the new species *Thauera chlorobenzoica* sp. nov., strain 3CB-1^T (= ATCC 700723^T) being the type strain.

Keywords: *Azoarcus*, *Thauera*, halobenzoate degradation

INTRODUCTION

Several bacterial strains capable of growing at the expense of aromatic compounds under denitrifying conditions have been assigned to the genera *Azoarcus* and *Thauera* (Anders *et al.*, 1995; Gallus *et al.*, 1997; Harms *et al.*, 1999; Häggblom & Young, 1999; Rabus & Widdel, 1995; van Schie & Young, 1998; Song *et al.*, 1998, 1999, 2000a; Springer *et al.*, 1998; Zhou *et al.*, 1995). These closely related genera seem to constitute a major group of denitrifying bacteria capable of degrading aromatic compounds. Thus far, the genus

Azoarcus is represented by seven species. On the basis of cell morphology and the ability to fix nitrogen, it was proposed that strains isolated from the rhizosphere microflora of Kallar grass were *Azoarcus indigens* and *Azoarcus communis* (Reinhold-Hurek *et al.*, 1993). Later, a new species capable of degrading aromatic compounds was described under the name *Azoarcus evansii* (Anders *et al.*, 1995). Toluene-degrading denitrifiers were initially assigned to a new species, *Azoarcus tolulyticus* (Zhou *et al.*, 1995), but more detailed taxonomic examination resulted in reclassification and the proposal of two additional species, *Azoarcus toluvorans* and *Azoarcus toluclasticus* (Song *et al.*, 1999). Finally, the species *Azoarcus anaerobius* was assigned to the genus as an obligately denitrifying bacterium with the capacity to degrade α -resorcinol (Springer *et al.*, 1998).

The genus *Thauera* was proposed to accommodate the single species *Thauera selenatis*, represented by one

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA sequences described in this work are AF229857 (strain 2FB2), AF229862 (strain 2FB6), AF229874 (strain 4FB10), AF229867 (strain 4FB1), AF229868 (strain 4FB2), AF229881 (strain 3CB2), AF229882 (strain 3CB3) and AF229887 (strain 3BB1).

strain, which is capable of selenate reduction (Macy *et al.*, 1993). Additional strains of the genus have been assigned to the species *Thauera aromatica*, which has the capacity to degrade aromatic compounds, including toluene, phenol, *m*-xylene and chlorobenzoate, under denitrifying conditions (Anders *et al.*, 1995; Foss & Harder, 1998; Gallus *et al.*, 1997; Häggblom & Young, 1999; Rabus & Widdel, 1995; Song *et al.*, 1998, 2000a). Terpene-degrading denitrifiers were assigned to two new species of the genus, *Thauera linaloolentis* and *Thauera terpenica* (Foss & Harder, 1998). Recently, the species *Thauera mechernichensis* was reported as having an aerobic denitrification property (Scholten *et al.*, 1999). Unfortunately, most of the species are represented by single strains, which conspires against an evaluation of the important question of intraspecies diversity.

In previously described work from our laboratory, strains capable of mineralizing halobenzoates under denitrifying conditions were isolated from samples collected at various geographical and ecological sites (Song *et al.*, 2000b). These strains were assigned to nine genera belonging to either the α -, β - or γ -*Proteobacteria*. Eight strains isolated from estuarine sediments from the Arthur Kill intertidal strait in New York state, Sihwa Lake in Korea and agricultural soil from Wyoming were tentatively assigned to the genera *Azoarcus* and *Thauera* (Song *et al.*, 2000b). In this communication, we provide more detailed classification of the isolates on the basis of morphological and physiological characterization, cellular fatty acid profiles, phylogenetic analysis of the 16S rRNA gene, 16S rRNA gene mapping and the results of DNA–DNA hybridization experiments. On the basis of these results, we propose the new species *Thauera chlorobenzoica* sp. nov. and designate 3CB-1^T (= ATCC 700723^T) as the type strain.

METHODS

Organisms and growth conditions. All of the strains, including halobenzoate-degrading, denitrifying isolates and reference strains of the genera *Azoarcus* and *Thauera*, together with their geographical and ecological origins, are listed in Table 1. All of the strains of *Thauera* (with the exception of *T. selenatis* AX-1^T and *T. mechernichensis* TL1^T) and *Pseudomonas stutzeri* ATCC 17588^T were cultivated anaerobically in a minimal salts medium (Tschech & Fuchs, 1987) with succinate as the carbon source and nitrate as the electron acceptor. *T. selenatis* was grown in a vitamin-amended medium as described previously (Song *et al.* 2000a) and *T. mechernichensis* was cultured aerobically in Luria–Bertani broth. All of the strains of *Azoarcus* were grown on M-R2A medium as described previously (Song *et al.*, 1999).

Morphology and physiological tests. Cell dimensions and morphology were determined by using phase-contrast microscopy. Flagellation was assessed according to Leifson (1951). The presence of catalase and oxidase was tested as described previously (Smibert & Krieg, 1994). The utilization of aromatic compounds (Aldrich; minimum 97% purity) was tested with dense cell suspensions ($OD_{620} = 0.8$)

and substrates at 200 μ M under aerobic and denitrifying conditions. Growth on toluene was determined in an anaerobic toluene atmosphere as described previously (Evans *et al.*, 1991). Growth on various other substrates was determined at 30 °C under both aerobic and denitrifying conditions, using minimal salts medium plates (Tschech & Fuchs, 1987) containing 1 mM final concentrations of acetate, adipate, D-alanine, L-asparagine, betaine, butyrate, caproate, ethanol, fructose, glucose, glutamate, lactose, malate, ornithine, pyruvate, succinate or valerate.

Determination of cellular fatty acids. All of the strains were pre-cultured under denitrifying conditions in minimal salts medium (Tschech & Fuchs, 1987) with 10 mM succinate and 30 mM nitrate. After the OD_{620} of the cultures had reached 0.4, the cells were used as an inoculum (10%) to 50 ml fresh liquid medium containing 10 mM succinate and 30 mM nitrate, in anaerobic serum bottles, and cultivated for 24 h at 30 °C under an argon atmosphere. The cells were collected by centrifugation at room temperature. Fatty acids were saponified, methylated, extracted and analysed by GC, using the SHERLOCK Microbial Identification System (MIDI).

Genomic bacterial DNA isolation. Chromosomal DNA from each strain was isolated as described previously (Song *et al.*, 1998). The extracted DNA was sheared by two passages through a French press at 20000 p.s.i. (138000 kPa) and diluted to 0.4 mg DNA ml⁻¹. The purity of the DNA was determined by measuring the absorbance at 230, 260 and 280 nm. In all cases, the A_{260}/A_{280} and A_{260}/A_{230} ratios were close to 2.

Measurement of G + C content. The G + C content of DNA was measured by HPLC (Mesbah *et al.*, 1989), with some modifications. Phosphate/methanol buffer (90:10) was used at a flow rate of 1.5 ml min⁻¹ as a solvent for the separation of deoxyribonucleosides and ribonucleosides. Phosphate buffer (pH 4.0) was prepared by dissolving KH₂PO₄ (0.049 M) in distilled water and adjusting the pH with 85% H₃PO₄. The nucleosides were analysed by using a model LC-10AS HPLC (Shimadzu), with a reverse-phase C18 column (Spherisorb 4.6 $\times$ 250 mm, 5 μ m particle size; Phenomenex), and the absorbance was monitored at a wavelength of 254 nm.

Determination and analysis of 16S rRNA gene sequences. PCR amplifications of the 16S rRNA genes of strains were performed with a GeneAmp PCR system 2400, as described previously (Song *et al.*, 1998). The complete sequence of the 16S rRNA gene was obtained by using internal 16S rRNA oligonucleotide sequencing primers 27F, 321R, 357F, 685R, 704F, 907R, 1242F and 1522R (Johnson, 1994a) and a model ABI 377A automated sequencer (Perkin Elmer).

The rRNA gene sequences of reference strains and species in the genera *Thauera* and *Azoarcus* were obtained from the GenBank database. All of the sequences were aligned using the PILEUP program of the Genetics Computer Group software package (Devereux *et al.*, 1984). Phylogenetic analysis was carried out according to Kimura's two-parameter method (Kimura, 1980) and a tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987). The SEQBOOT program was used to obtain the confidence level for neighbour-joining analysis using a 100 bootstrapped dataset (Felsenstein, 1985).

The nucleotide sequence accession numbers of the reference strains used in this study are as follows (in parentheses): *T. aromatica* K172^T (X77118), *T. aromatica* T1 (U95176), *T.*

Table 1. Geographical and ecological origins of strains used in this study

Species	Strain	Geographical and ecological source	Reference(s)
Azoarcus			
<i>A. indigenus</i>	VB32 ^T (= LMG 9092 ^T)	Rhizosphere of Kallar grass, Brisbane, Australia	Reinhold-Hurek <i>et al.</i> (1993)
<i>A. tolulyticus</i>	Tol4 ^T (= ATCC 51758 ^T)	Aquifer sediment, Moffett Field, MI, USA	Zhou <i>et al.</i> (1995)
<i>A. toluclasticus</i>	MF63 ^T (= ATCC 700605 ^T)	Aquifer sediment, Moffett Field, MI, USA	Fries <i>et al.</i> (1994), Song <i>et al.</i> (1999)
<i>A. toluvorans</i>	Td21 ^T (= ATCC 700604 ^T)	Muck soil, Clinton, MI, USA	Fries <i>et al.</i> (1994), Song <i>et al.</i> (1999)
<i>A. evansii</i>	KB740 ^T (= DSM 6898 ^T)	Creek sediment, USA	Braun & Gibson (1984), Anders <i>et al.</i> (1995)
<i>Azoarcus</i> sp.	2FB2	Estuarine sediment, Arthur Kill, NJ, USA	Song <i>et al.</i> (2000b)
<i>Azoarcus</i> sp.	2FB6	Estuarine sediment, Arthur Kill, NJ, USA	Song <i>et al.</i> (2000b)
<i>Azoarcus</i> sp.	4FB10	Estuarine sediment, Sihwa, Korea	Song <i>et al.</i> (2000b)
Thauera			
<i>T. selenatis</i>	AX ^T (= ATCC 55363 ^T)	Selenium-contaminated water, CA, USA	Macy <i>et al.</i> (1993)
<i>T. aromatica</i>	K172 ^T (= DSM 6984 ^T)	Anaerobic sludge, Konstanz, Germany	Tschech & Fuchs (1987), Anders <i>et al.</i> (1995)
<i>T. aromatica</i>	T1 (= ATCC 700265)	Petroleum-contaminated soil, CA, USA	Evans <i>et al.</i> (1991), Song <i>et al.</i> (1998)
<i>T. aromatica</i>	AR-1 (= DSM 11528)	Activated sewage sludge, Germany	Gallus <i>et al.</i> (1997)
<i>T. aromatica</i> gv. <i>chlorobenzoica</i>	3CB-1 ^T (= ATCC 700723 ^T)	River sediment, Albany, NY, USA	Häggblom & Young (1999), Song <i>et al.</i> (2000a)
<i>T. linaloolentis</i>	47Lo1 ^T (= DSM 12138 ^T)	Activated sewage sludge, Germany	Foss & Harder (1998)
<i>T. terpenica</i>	58Eu ^T (= DSM 12139 ^T)	Activated sewage sludge, Germany	Foss & Harder (1998)
<i>T. terpenica</i>	21Mo1 (= DSM 12140)	Activated sewage sludge, Germany	Foss & Harder (1998)
<i>T. mechernichensis</i>	TL1 ^T (= DSM 12266 ^T)	Leachate, Mechernich, Germany	Scholten <i>et al.</i> (1999)
<i>Thauera</i> sp.	4FB1	Estuarine sediment, Arthur Kill, NJ, USA	Song <i>et al.</i> (2000b)
<i>Thauera</i> sp.	4FB2	Estuarine sediment, Arthur Kill, NJ, USA	Song <i>et al.</i> (2000b)
<i>Thauera</i> sp.	3CB2	Estuarine sediment, Arthur Kill, NJ, USA	Song <i>et al.</i> (2000b)
<i>Thauera</i> sp.	3CB3	Agricultural soil, WY, USA	Song <i>et al.</i> (2000b)
<i>Thauera</i> sp.	3BB1	Agricultural soil, WY, USA	Song <i>et al.</i> (2000b)

gv., Genomovar.

aromatica mXyN1 (X83533), *T. aromatica* genomovar *chlorobenzoica* 3CB-1^T (AF123264), *T. selenatis* AX^T (X68491), *T. mechernichensis* TL1^T (Y17590), *T. linaloolentis* 47Lo1^T (AJ005817), *T. terpenica* 58Eu^T (AJ005817), *A. evansii* KB740^T (X77679), *A. tolulyticus* Tol4^T (L33687), *A. tolulyticus* Td1 (L33687), *A. tolulyticus* Td2 (L33691), *A. toluvorans* Td21^T (L33692), *A. toluclasticus* MF63^T (AF123077), *A. anaerobius* LuFRes1^T (Y14701), *A. indigenus* VB32^T (AF011345) and *A. communis* Swub3^T (AF011343).

DNA–DNA hybridization. The DNA preparations were labelled using the BRL nick translation kit with ³H-radiolabelled dTTP. DNA–DNA hybridization was performed using the S1 nuclease method (Johnson, 1994b), with the modification of Song *et al.* (1998). The reannealing temperature was 68.3 °C.

DNA homology value transformation. The construction of a three-dimensional model for the DNA homology values was performed with the program 3D; this calculated three-dimensional co-ordinates by using the DNA homology value (Hildebrand *et al.*, 1982). The accuracy, limitations and predictability of the program have been reported elsewhere (Hildebrand *et al.*, 1984).

Restriction mapping of small-subunit rRNA (ribotyping). Strains belonging to the genus *Thauera* were tested for differences in genomic structures by mapping small-subunit rRNA genes. *T. aromatica* strains K172^T and T1 and strain 3CB-1^T were used for comparison. The genomic DNAs of the strains were digested with restriction endonucleases *Bgl*I, *Eco*RI and *Eco*RV. These enzymes were selected because sequence analysis indicated internal cut sites in the small-subunit genes. The digested samples were run on a 1% agarose gel and transferred to nylon membranes (Boehringer Mannheim).

Samples were probed with a digoxigenin-labelled, 500 bp fragment from the 5'-end of the *Escherichia coli* 16S rRNA gene, as described previously (Kerkhof & Speck, 1997).

Genomic Southern blots were performed according to standard methods (Maniatis *et al.*, 1982) with 65 °C as the hybridization temperature and using the colorimetric substrates NBT and X-phosphate for visualization.

RESULTS AND DISCUSSION

16S rRNA gene sequence analysis

A comparative analysis of the almost complete sequences of the 16S rRNA genes differentiated the halobenzoate-degrading strains into three groups located in the genera *Azoarcus* and *Thauera* (Fig. 1). Isolates 2FB2, 2FB6 and 4FB10 belong to the genus *Azoarcus* (Fig. 1) and are closely related to *A. tolulyticus* Tol4^T, having sequence similarities of 98.2, 99.2 and 98.6%, respectively. Isolates 3CB2, 3CB3, 3BB1, 4FB1 and 4FB2 are located in the genus *Thauera* (Fig. 1). Isolates 3CB2 and 3CB3 are near to *T. aromatica* K172^T, having sequence similarities of 99.4 and 99.2%, and isolates 4FB1, 4FB2 and 3BB1 are located close to *T. aromatica* genomovar *chlorobenzoica* 3CB-1^T and *T. mechernichensis*, having sequence similarities of more than 98%.

Determination of cellular fatty acids

Cluster analysis of cellular fatty acid profiles (Fig. 2) showed agreement with the phylogenetic analysis of 16S rDNA for the grouping of the halobenzoate-degrading strains into the genera *Azoarcus* and *Thauera*. Strain 4FB10 was located near to *A. tolulyticus*, strains 2FB2 and 2FB6 were close to *A. toluclasticus* and the strains belonging to the genus *Thauera* were located in the cluster of *T. aromatica*.

Fig. 1. Phylogenetic analysis of 16S rRNA genes from halobenzoate-degrading isolates with the species of the genera *Azoarcus* and *Thauera* of the β -subclass of the *Proteobacteria*; 1500 bases were used to build the tree. Neighbour-joining topology was used for tree construction. Bootstrap percentages (> 50%) are shown at nodes. Bar, 1 nucleotide substitution in 100 bases.

Fig. 2. Dendrogram analysis of strains of the genera *Azoarcus* and *Thauera* on the basis of cellular fatty acid analysis, with *Pseudomonas stutzeri* as the outgroup. Strains were grown with 10 mM succinate and 30 mM nitrate for 24 h under denitrifying conditions.

Table 2. Fatty acid composition of strains in the genera *Thauera* and *Azoarcus*

Values are percentages of total fatty acid content. Strains are identified as: 1, 3CB-1^T; 2, CB2; 3, CB3; 4, 3BB1; 5, 4FB1; 6, 4FB2; 7, *T. aromatica* K172^T; 8, *T. aromatica* T1; 9, *T. aromatica* AR-1; 10, *T. linaloolentis* 47Lol^T; 11, *T. terpenica* 58Eu^T; 12, *T. terpenica* 21Mol; 13, *T. selenatis* AX^T; 14, 2FB2; 15, 2FB6; 16, 4FB10; 17, *A. tolulyticus* Tol4^T; 18, *A. tolulyticus* Td1; 19, *A. tolulyticus* Td2; 20, *A. tolulyticus* MF66; 21, *A. toluvorans* Td21^T; 22, *A. toluclasticus* MF63^T; and 23, *A. evansii* KB740^T. Cells were cultivated with 10 mM succinate and 30 mM nitrate under anaerobic conditions for 24 h at 30 °C. *T. selenatis* was cultivated in the same denitrifying medium but with the addition of 5 × vitamin solution.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
10:0	0.0	0.5	0.4	0.0	0.4	0.4	0.0	0.4	0.5	0.3	0.2	0.3	0.0	0.6	0.4	0.4	0.6	0.5	0.7	0.6	0.5	0.5	0.8
10:0 3-OH	5.5	5.2	4.9	4.9	4.8	5.0	5.5	6.3	6.2	4.5	3.8	4.2	5.0	5.8	4.2	4.8	6.6	6.0	6.2	6.6	3.1	5.5	8.0
12:0	5.5	6.3	5.1	6.4	5.1	5.3	5.7	6.0	6.4	5.2	5.0	4.7	7.8	6.0	6.2	6.5	7.1	7.0	7.0	7.1	4.7	5.6	6.9
12:0 3-OH	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.6	0.7	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14:0	0.0	0.6	0.7	0.9	0.6	0.6	0.0	0.8	0.6	0.2	0.4	0.5	0.0	0.0	0.0	0.0	0.8	1.0	1.0	1.3	0.8	0.4	0.8
16:1 ω 7c	45.7	46.7	45.6	49.3	45.6	46.4	46.2	42.5	42.6	45.6	49.5	48.5	40.9	49.6	49.9	50.9	52.7	52.8	51.2	52.5	51.0	51.8	46.9
16:1 ω 5c	0.5	1.0	0.9	1.1	0.9	0.9	1.3	0.6	0.7	0.6	0.8	0.6	0.0	0.7	0.7	0.6	0.5	0.4	0.0	0.0	0.0	0.8	0.5
16:0	28.7	25.3	28.5	24.9	28.6	28.3	25.1	31.5	26.2	28.5	23.8	25.2	24.6	26.7	26.7	28.1	25.4	27.5	26.8	26.7	29.7	25.6	27.4
17:0 cyclo	0.7	2.1	0.9	0.9	0.9	0.9	1.7	1.5	0.0	0.0	0.0	0.0	0.0	0.7	0.9	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:1 ω 7c/ ω 9t/ ω 12t	13.4	12.3	13.0	10.8	12.8	12.4	14.5	10.3	17.1	14.6	15.7	14.9	17.6	9.5	9.1	6.2	6.8	4.8	6.7	5.3	10.3	9.3	8.2
18:1 ω 5c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:0	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	0.0	0.2	0.2	0.4	1.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 3. Comparison of the characteristics of halobenzoate-degrading isolates and the species in the genus *Azoarcus*

Data were obtained from this study and the following sources: Zhou *et al.* (1995) and Song *et al.* (1999) (*A. toluolyticus* Tol4^T, *A. toluovorans* Td21^T and *A. toluclasticus* MF63^T); Anders *et al.* (1995) (*A. evansii* KB740^T); Springer *et al.* (1998) (*A. anaerobius* LuFRes1^T); and Reinhold-Hurek *et al.* (1993) (*A. indigenus* VB32^T). Growth is scored as + (growth) or – (no growth). ND, Not determined. Cells of all strains listed are motile rods.

Characteristic	<i>A. toluolyticus</i>				<i>A. toluovorans</i> Td21 ^T	<i>A. toluclasticus</i> MF63 ^T	<i>A. evansii</i> KB740 ^T	<i>A. anaerobius</i> LuFRes1 ^T	<i>A. indigenus</i> VB32 ^T
	2FB2	2FB6	4FB10	Tol4 ^T					
Cell size (µm)	1.6–3.3 × 0.8	1.4 × 0.93	1.5–4 × 0.85	1.4–2.1 × 0.78	1.3–2.1 × 0.77	1.5–2.0 × 0.75	1.5–3.0 × 0.7	2.7–3.3 × 1.5	2.0–4.0 × 0.6
Flagellation	Monotrichous	Monotrichous	Monotrichous	Monotrichous	Monotrichous	Monotrichous	Monotrichous	ND	Monotrichous
Catalase/oxidase	+/+	+/+	+/+	+/+	+/+	+/+	+/+	–/+	+/+
G+C content (mol%)	66.1	64.0	66.0	66.9	67.8	67.3	67.5	66.5	66.6
Optimum pH for growth	8.0	7.5	7.0	ND	ND	ND	7.8	7.2	7.0–7.5
Optimum temperature for growth (°C)	37	30	30	ND	ND	ND	28–37	20–32	40
Electron acceptors	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–]	O ₂
Use of sole carbon substrate for growth under aerobic (O ₂)/denitrifying (NO ₃ [–]) conditions:	O ₂ /NO ₃ [–]	O ₂ /NO ₃ [–]	O ₂ /NO ₃ [–]	O ₂ /NO ₃ [–]	O ₂ /NO ₃ [–]	O ₂ /NO ₃ [–]	O ₂ /NO ₃ [–]	NO ₃ [–]	O ₂
Acetate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+	+
Adipate	+/+	+/+	+/+	ND	ND	ND	–/ND	–	–
D-Alanine	+/+	+/+	+/+	ND	ND	ND	ND	ND	–
L-Asparagine	+/+	+/+	+/+	ND	ND	ND	+/ND	ND	–
Betaine	+/+	+/+	+/+	ND	ND	ND	ND	ND	–
Butyrate	+/+	+/+	+/+	+/+	+/+	+/+	+/ND	+	+
Caproate	+/+	+/+	+/+	+/+	+/+	–/–	ND	ND	–
D-Fructose	+/+	+/+	+/+	+/+	+/+	+/+	+/ND	–	–
D-Glucose	+/+	+/+	+/+	+/+	–/–	+/+	ND/+	–	–
Glutamate	+/+	+/+	+/+	ND	ND	ND	+/ND	ND	+
Lactose	+/+	+/+	+/+	ND	ND	ND	ND	ND	–
Malate	+/+	+/+	+/+	ND	ND	ND	+/ND	–	+
Ornithine	+/+	+/+	+/+	ND	ND	ND	+/ND	ND	–
Pyruvate	+/+	+/+	+/+	+/+	+/+	+/+	+/ND	+	+
Succinate	+/+	+/+	+/+	+/+	+/+	+/+	+/ND	+	+
Valerate	+/+	+/+	+/–	–/–	+/–	–/–	ND	+	ND

Fig. 3. *Azoarcus* sp. strain 2FB6. Cells were grown in Bacto Tryptic Soy broth with 1% Difco yeast extract for 24 h at 28 °C. Insets, cells with single polar flagella (stained using Leifson's method). Bar, 5 µm.

The profiles of cellular fatty acids of each strain and the species of the genera *Azoarcus* and *Thauera* are compared in Table 2. The predominant fatty acids of the genus *Thauera* are 10:0 3-OH (3.8–6.3%), 12:0 (4.7–7.8%), 16:1 ω 7c (40.9–49.5%), 16:0 (23.8–31.5%) and 18:1 (10.3–17.6%), and members of the genus *Azoarcus* have 10:0 3-OH (3.1–8.0%), 12:0 (4.7–7.1%), 16:1 ω 7c (46.9–52.8%), 16:0 (25.4–28.1%) and 18:1 (4.8–10.3%) fatty acids after growth under denitrifying conditions. The strains and species in the genus *Thauera* had a higher proportion of 18:1 fatty acid and a lower proportion of 16:1 ω 7c than those of the genus *Azoarcus*. Halobenzoate-degrading strains and the strains of *T. aromatica* had 17:0 cyclo (0.7–2.1%) fatty acids in their cellular profiles, except in the case of strain AR-1, which did not have a detectable amount of this fatty acid.

Morphological and physiological characterization

The cells of strains 2FB2, 2FB6 and 4FB10 in the genus *Azoarcus* are rods with dimensions of 1.4 × 0.8–0.93 µm and are motile, each cell having a monotrichous flagellum; this is in agreement with the description of the species *A. tolulyticus* (Table 3). The

general morphology and flagellation of strain 2FB6 are shown in Fig. 3. The mean length of the cells is 1.13 µm and the mean width is 0.67 µm. The physiological characteristics of strains and species in the genus *Azoarcus* are listed in Table 3. The optimum pH and temperatures for growth were 7.0–8.0 and 30–37 °C. Strains 2FB2, 2FB6 and 4FB10 could use O₂, NO₃[−] or NO₂[−] as the electron acceptor for growth. Strains in the genus *Azoarcus* demonstrated versatility of growth on different sugars and with short-chain fatty acids as carbon sources (Table 3).

Strains 3CB2, 3CB3, 3BB1, 4FB1 and 4FB2 in the genus *Thauera* are short rods, with dimensions of 1.2–2.7 × 0.85–1 µm, and are motile with peritrichous flagella, which are characteristic of the species *T. aromatica* (Table 4) but not of the rest of the genus. The typical morphology and flagellation of strain 4FB2 are shown in Fig. 4. The mean length of the cells is 1.66 µm and the mean width is 0.72 µm; in some complex media, they tended to form long chains. In some of the cells, inclusions were visible, using phase-contrast optics. The physiological characteristics of strains in the genus *Thauera* are compared in Table 4. The optimum pH and temperature for growth were 7.5–8.0 and 30–37 °C. The halobenzoate-degrading isolates were able to grow with O₂, NO₃[−] or NO₂[−] as the electron acceptors in the presence of succinate, but not with SO₄^{2−}, SO₃^{2−}, S₂O₃^{2−}, SeO₄^{2−} or SeO₃^{2−}. Growth tests on various substrates showed that the strains were unable to utilize sugars but that they could grow well on short-chain fatty acids (Table 4).

The utilization of aromatic compounds under aerobic and denitrifying conditions was tested with all of the halobenzoate-degrading isolates and some strains in the genera *Azoarcus* and *Thauera*. The data on utilization of aromatic compounds under either denitrifying or aerobic conditions are listed in Table 5. All of the strains were able to utilize benzoate, phenylacetate, vanillate, 3-hydroxybenzoate, 4-hydroxybenzoate, 2-fluorobenzoate and 4-fluorobenzoate. None of them could degrade phthalate, 4-aminobenzoate, 2-chlorobenzoate, 4-chlorobenzoate, 2-bromobenzoate, 4-bromobenzoate, 2-methylbenzoate or 4-methylbenzoate under either aerobic or denitrifying conditions. Only those strains isolated on 3-chlorobenzoate or 3-bromobenzoate were able to use 3-aminobenzoate, 3-methylbenzoate, 3-chlorobenzoate and 3-bromobenzoate under denitrifying conditions. Interestingly, *T. aromatica* strains K172^T, T1 and AR-1 can degrade 2-fluorobenzoate and 4-fluorobenzoate and strain T1 can also utilize 3-chlorobenzoate and 3-bromobenzoate under denitrifying conditions. *A. tolulyticus* strain Tol4^T can use 2-fluorobenzoate under denitrifying conditions.

Determination of DNA base composition

The DNAs of the halobenzoate-degrading strains belonging to the genus *Thauera* had G + C contents of 67.1–69.4 mol% (Table 4) and the DNAs of strains

Table 4. Comparison of the characteristics of halobenzoate-degrading isolates and the species in the genus *Thauera*

Data were obtained from this study and the following sources: Anders *et al.* (1995) and Song *et al.* (1998) (*T. aromatica* K172^T); Foss & Harder (1998) (*T. linaloolentis* 47Lol^T and *T. terpenica* 58Eu^T) (with the exception of the determination of flagellation); Scholten *et al.* (1999) (*T. mechernichensis* TL1^T); and Macy *et al.* (1993) (*T. selenatis* AX^T). Growth is scored as + (growth) or – (no growth). ND, Not determined. All strains listed are catalase- and oxidase-positive.

Characteristic	<i>T. chlorobenzoica</i>				<i>T. aromatica</i>			<i>T. linaloolentis</i>	<i>T. terpenica</i>	<i>T. mechernichensis</i>	<i>T. selenatis</i>
	3CB-1 ^T	4FB1	4FB2	3BB1	3CB2	3CB3	K172 ^T	47Lol ^T	58Eu ^T	TL1 ^T	AX ^T
Cell morphology	Rods	Rods	Rods	Rods	Rods	Rods	Coccoid rods	Rods	Rods	Short rods	Rods
Cell size (µm)	3.0–3.7	1.5–2.7 × 0.85	1.5–2.5 × 0.87	1.2–1.7 × 0.97	1.6–2.7 × 1	1.6–2.7 × 0.88	1.0–2.5 × 1.0	1.4–2.7 × 0.7	1.6–2.2 × 0.9	1.5–2.0 × 0.75	1.4 × 0.56
Motility	+	+	+	+	+	+	+	–	+	+	+
Flagellation	Peritrichous	Peritrichous	Peritrichous	Peritrichous	Peritrichous	Peritrichous	Peritrichous	None	Monotrichous	Monotrichous	Monotrichous
G + C content (mol%)	68.6	69.3	69.4	ND	67.1	67.1	67.0	65.6	64.2	65.0	66.0
Optimum pH for growth	7.5–8.0	8.0	8.0	7.5	8.0	8.0	7.0–7.4	7.0–7.3	7.9–8.8	ND	8.0
Optimum temperature for growth (°C)	30	37	37	30	37	37	28	32	32	40	25–30
Electron acceptors	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂	NO ₃ [–] , O ₂ , Se(VI), Se(IV)
Use of sole carbon substrates for growth under aerobic (O ₂)/denitrifying (NO ₃ [–]) conditions:	O ₂ , NO ₃ [–]	O ₂ , NO ₃ [–]	O ₂ , NO ₃ [–]	O ₂ , NO ₃ [–]	O ₂ , NO ₃ [–]	O ₂ , NO ₃ [–]	O ₂ , NO ₃ [–]	NO ₃ [–]	NO ₃ [–]	O ₂ , NO ₃ [–]	O ₂
Acetate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+	+	ND	+
Adipate	–/–	+/+	+/+	+/+	–/+	–/–	+/+	ND	ND	ND	ND
D-Alanine	–/–	–/–	–/–	–/–	–/+	+/–	–/–	ND	ND	ND	+
L-Asparagine	–/–	–/–	–/–	+/+	–/+	+/+	–/–	ND	ND	ND	+
Betaine	–/–	–/–	–/–	–/–	–/–	–/–	–/–	ND	ND	ND	ND
Butyrate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+	+	ND	ND
Caproate	–/+	+/+	+/+	+/+	–/+	+/+	–/–	ND	ND	ND	ND
Ethanol	–/+	–/+	–/+	–/–	–/+	–/+	+/+	+	–	ND	ND
D-Fructose	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–	–	ND	+
D-Glucose	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–	–	ND	+
Glutamate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+	+	ND	+
Lactose	–/–	–/–	–/–	–/–	–/–	–/–	–/–	ND	ND	ND	+
Malate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+	+	ND	ND
Ornithine	–/+	+/+	+/+	+/+	+/+	+/+	–/+	ND	ND	ND	ND
Pyruvate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+	+	ND	+
Succinate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+	+	ND	+
Valerate	–/–	+/–	+/+	+/–	–/+	+/+	+/+	ND	ND	ND	ND

Fig. 4. *Thauera* sp. strain 4FB2. Cells were grown in Bacto brain–heart infusion for 24 h at 28 °C. Inclusions can be seen in some of the cells. Bottom, cells with ‘degenerately peritrichous’ flagella (stained using Leifson’s method). Bar, 5 µm.

from the genus *Azoarcus* had G+C contents of 64.0–66.1 mol % (Table 3).

DNA–DNA hybridization

Strains 2FB2, 2FB6 and 4FB10 belonging to the genus *Azoarcus* shared 80–91 % DNA similarity with *A. tolulyticus* strain Tol4^T, but only 18–48 % DNA similarity with the type strains of *A. toluvorans* and *A. toluclasticus* (Table 6). The polyphasic classification is in agreement with the placement of these strains into the species *A. tolulyticus*. Thus, strains 2FB2, 2FB6 and 4FB10 are new strains of *A. tolulyticus* (based on the definition specifying that there must be DNA similarity of more than 70 % between strains of the same species; Wayne *et al.*, 1987) with the capacity to degrade fluorinated benzoates under denitrifying conditions.

In contrast, strains 3CB2, 3CB3, 4FB1 and 4FB2 had less than 70 % DNA similarity to strains K172^T and T1 of *T. aromatica*, but they shared more than 70 % DNA similarity with *T. aromatica* strain AR-1 (Table 6). *T. aromatica* genomovar *chlorobenzoica* strain 3CB-1^T showed more than 70 % DNA similarity to strains 4FB1 and 4FB2 and about 50 % similarity to strains 3CB2 and 3CB3 (Table 6). In addition, the species *T. linaloolentis* and *T. terpenica* shared only about 30 % DNA similarity with strain 4FB1 and the species *T. selenatis* had 20 % DNA similarity to strains 3CB2, 3CB3, 4FB1 and 4FB2 (Table 6). Interestingly, *T. mechernichensis* shared less than 20 % DNA similarity with strains 3CB-1^T, 3CB2 and 4FB1, although they showed 16S rRNA sequence similarity of more than 98 %.

Three-dimensional representation of the strains in the

Table 5. Utilization of aromatic compounds by strains in the genera *Azoarcus* and *Thauera*

A dense cell suspension was incubated at 30 °C for 7 d after feeding with 200 µM substrate. +, Complete loss of substrate; –, less than 10 % consumption of substrate; ND, not determined. Utilization under aerobic/denitrifying conditions is indicated (i.e. +/– indicates that a compound is used under aerobic conditions but not under denitrifying conditions). Positive results were obtained with all strains for the utilization of benzoate and phenylacetate under both aerobic and denitrifying conditions. Negative results were obtained with all strains for the utilization of the following compounds under both aerobic and denitrifying conditions: phthalate, 4-aminobenzoate, 2-chlorobenzoate, 4-chlorobenzoate, 2-bromobenzoate, 4-bromobenzoate, 2-methylbenzoate, 4-methylbenzoate.

Compound	3CB-1 ^T	3CB2	3CB3	3BB1	4FB1	4FB2	K172 ^T	T1	AR-1	2FB2	2FB6	4FB10	Tol4 ^T
Phenol	–/–	–/–	–/–	–/–	–/–	–/–	–/+	–/–	–/–	–/+	–/–	–/–	–/–
Toluene	–/–	–/–	–/+	–/–	–/–	–/–	–/+	–/+	ND/ND	ND/ND	ND/ND	ND/ND	+/+
Vanillate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/–	–/–	–/–	–/+	–/–
3-Aminobenzoate	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/+	–/–	–/–	–/–	–/–	–/–
2-Hydroxybenzoate	–/–	–/–	+/–	+/–	+/–	+/–	–/–	–/–	ND/ND	+/+	+/–	–/–	–/–
3-Hydroxybenzoate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	ND/ND	+/+	+/+	+/+	+/+
4-Hydroxybenzoate	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	ND/ND	+/+	+/+	+/+	+/+
2-Fluorobenzoate	+/+	+/+	+/+	+/+	–/+	–/+	+/+	+/+	–/+	+/+	+/+	–/+	–/+
3-Fluorobenzoate	+/–	–/–	+/–	–/–	+/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
4-Fluorobenzoate	+/+	–/+	–/+	–/+	+/+	+/+	–/+	–/+	–/+	–/–	–/–	–/+	–/–
3-Chlorobenzoate	–/+	–/+	–/+	–/+	–/–	–/–	–/–	–/+	–/–	–/–	–/–	–/–	–/–
3-Bromobenzoate	–/+	–/+	–/+	–/+	–/–	–/–	–/–	–/+	–/–	–/–	–/–	–/–	–/–
3-Methylbenzoate	–/+	–/+	–/+	–/+	–/–	–/–	–/+	–/+	–/–	–/–	–/–	–/–	–/–

Table 6. DNA–DNA hybridization between strains of *Azoarcus* and *Thauera*

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. 3CB-1 ^T																							
2. 3CB2	56.7																						
3. 3CB3	50.5	93.1																					
4. 4FB1	90.7	78.9	47.4																				
5. 4FB2	74.6	96.5	52.6	87.0																			
6. <i>T. aromatica</i> K172 ^T	48.6	59.2	70.7	52.6	–																		
7. <i>T. aromatica</i> T1	47.5	66.0	57.9	65.1	46.8	76.5																	
8. <i>T. aromatica</i> AR-1	73.1	89.4	86.5	80.3	78.5	–	80.2																
9. <i>T. linaloolentis</i> 47Lol ^T	13.2	–	–	31.0	–	–	5.9	–															
10. <i>T. terpenica</i> 21Mol	22.4	–	–	30.3	–	–	22.7	–	25.4														
11. <i>T. terpenica</i> 58Eu ^T	32.0	–	–	–	–	27.6	–	–	24.3	41.2													
12. <i>T. mechernichensis</i> TL1 ^T	13.4	9.3	–	23.2	–	20.3	–	–	0.4	5.7	–												
13. <i>T. selenatis</i> AX ^T	21.9	24.5	22.7	24.8	23.4	24.3	23	13.8	17.1	17	13.2	19.1											
14. 2FB2	3.2	–	–	–	–	–	–	–	–	–	–	–	–										
15. 2FB6	–	–	–	–	–	–	–	–	–	–	–	–	–	–									
16. 4FB10	8.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–								
17. <i>A. tolulyticus</i> Tol4 ^T	3.1	–	–	–	–	0.1	1.6	–	–	–	–	20.3	–	82.5	79.9	90.7							
18. <i>A. tolulyticus</i> Td1	–	–	–	–	–	11.0	–	–	–	–	–	–	–	–	–	–	89.0						
19. <i>A. tolulyticus</i> Td2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	99.0					
20. <i>A. tolulyticus</i> MF66	7.7	–	–	–	–	–	9.7	–	–	–	–	–	–	–	–	–	96.0	91.8	75.0				
21. <i>A. toluvorans</i> Td21 ^T	7.5	–	–	–	–	3.9	3.5	–	–	–	–	–	–	20.2	–	23.4	36.0	28.0	32.6	29.0			
22. <i>A. toluclasticus</i> MF63 ^T	4.8	–	–	–	–	–	13.5	–	–	–	–	–	–	18.2	–	48.1	38.0	39.0	–	45.0	29.0		
23. <i>A. evansii</i> KB740 ^T	2.9	–	–	–	–	–	0.1	–	13.5	–	–	10.3	–	14.5	–	30.5	–	57.2	–	–	32.9	30.2	

–, Not determined.

Fig. 5. Three-dimensional representation of the strains of the genera *Azoarcus* and *Thauera*. Three-dimensional co-ordinates were calculated from the data from DNA–DNA hybridization. Strains in the *T. aromatica* (●) and *A. tolulyticus* (■) groups are not individually labelled.

Fig. 6. Three-dimensional representation of strains in the species *Thauera aromatica* and *Thauera chlorobenzoica*.

genera *Azoarcus* and *Thauera* (Fig. 5) demonstrates that *A. tolulyticus* strains and strains 2FB2, 2FB6 and 4FB10 are tightly grouped. The strains of the species *T. aromatica* and strains 3CB2, 3CB3, 4FB1 and 4FB2 also formed a cluster. However, detailed differentiation of the *T. aromatica* strains (Fig. 6) showed the complexity of this species. Strains K172^T, T1 and AR-

1 of the species *T. aromatica* and strains 3CB2 and 3CB3 grouped together and were located separately from the other strains. The fluorobenzoate-degrading strains 4FB1 and 4FB2, sharing 87% DNA similarity (Table 6), were grouped together with strain 3CB-1^T, which was previously proposed as *T. aromatica* genomovar *chlorobenzoica* (Song *et al.*, 2000a).

Fig. 7. Ribotype patterns of strains 3CB3, 4FB1, 4FB2 and 3CB-1^T and *T. aromatica* strains K172^T, T1 and AR-1. DNA samples were digested with *Eco*RI and a 500 bp 16S rRNA gene probe from *E. coli* was used for hybridization.

16S rRNA gene mapping (ribotyping)

Halobenzoate-degrading strains assigned to the genus *Thauera* and strains in the species *T. aromatica* were examined. Ribotyping of the DNA samples digested with *Eco*RI provided clear banding patterns and allowed the detection of four different sizes of fragments from each sample (Fig. 7), indicating that the strains had four copies of 16S rRNA gene in their genomes. The copy numbers of 16S rRNA genes in marine bacteria were determined in the same way (Kerkhof & Speck, 1997). On the basis of restriction fragment polymorphism, two distinct groups are apparent. *T. aromatica* strains K172^T, T1 and AR-1 and strain 3CB2 formed one group, and *T. aromatica* genomovar *chlorobenzoica* 3CB-1^T and strains 4FB1 and 4FB2 formed another (Fig. 7). In agreement with the analysis of DNA–DNA hybridization, strains 3CB2 and 3CB3 are assigned to the species *T. aromatica*, having the capacity to degrade halobenzoate under denitrifying conditions. However, strains 3CB-1^T, 4FB1 and 4FB2 should be separated as a new species.

Complexity of the genus *Thauera*

The definition of the genus *Thauera* is quite complex, since it includes a variety of morphological and physiological attributes, some of which could justify subdivision into at least two genera. Thus, whilst *T. selenatis* (the type species of the genus), *T. terpenica*

and *T. mechernichensis* have cells that are each motile by means of a single polar flagellum, *T. aromatica* and *T. chlorobenzoica* sp. nov. have a type of flagellation that has been designated as ‘degenerately peritrichous’. The species *T. linaloolentis* cannot be included in the discussion in view of the fact that we could never detect either motility or the presence of flagella in the cells.

As a result of the discovery of the peritrichous flagellation of *T. aromatica*, and in view of the weight assigned to morphological attributes in classical bacterial taxonomy, we have already mentioned the evident heterogeneity that perhaps points to the existence of a separate phylogenetic category. In support of this opinion, the phylogenetic analysis of 16S rRNA sequences (Fig. 1) and the three-dimensional representation of DNA similarities among the strains (Fig. 5) all point to a separation of *T. aromatica* and *T. chlorobenzoica* from the remaining species of *Thauera*. This argument is also supported by physiological differences between the two groups. Future studies including many more strains might provide a more solid basis for a proposal of differentiation at the generic level.

Description of *Thauera chlorobenzoica* sp. nov.

Thauera chlorobenzoica (chlor.o.ben.zo'i.ca. M. L. adj. *chloro* pertaining to chlorine, from Gr. adj. *chloros* pale green; M. L. adj. *benzoicus* pertaining to benzoic acid; M. L. fem. adj. *chlorobenzoica* indicating the ability to utilize chlorobenzoic acid).

Cells are short, fat rods with lengths of 1.2–2.7 µm and widths of 0.85–0.97 µm and with ‘degenerately peritrichous’ flagellation. Fig. 4 shows the cell morphology and flagellation of strain 4FB2. All of the strains are oxidase- and catalase-positive. The predominant fatty acids are 10:0 3-OH, 12:0, 16:1ω7c, 16:0 and 18:1ω7c, and a small amount of 17:0 cyclo fatty acid is also found. They can grow under aerobic and denitrifying conditions with acetate, adipate, butyrate, glutamate, malate, ornithine, pyruvate, succinate, benzoate, phenylacetate, vanillate, 3-hydroxybenzoate and 4-hydroxybenzoate. Under denitrifying conditions, 3-aminobenzoate, 2-fluorobenzoate and 4-fluorobenzoate are used. In addition, strains 3CB-1^T and 3BB1 can use 3-chlorobenzoate, 3-bromobenzoate and 3-methylbenzoate under denitrifying conditions. The analysis of the 16S rRNA genes shows sequence similarity to *T. aromatica* K172^T of more than 98%. Strains 3CB-1^T, 4FB1 and 4FB2 showed very similar ribotyping patterns, distinct from that of *T. aromatica* K172^T. The genomic DNA similarity measured using DNA–DNA hybridization is more than 70% within the species and in the range 45–65% with respect to *T. aromatica* strains K172^T and T1.

Strain 3CB-1^T was initially proposed as *Thauera aromatica* genomovar *chlorobenzoica* (Song *et al.*, 2000a) because insufficient information was available

to differentiate it as a new species. However, additional isolates capable of halobenzoate degradation under denitrifying conditions were found to belong to this genomovar group (4FB1, 4FB2 and 3BB1) and showed clear genetic differences from the type strain of *T. aromatica*, K172^T, and closely grouped strains.

Strains 3CB-1^T, 4FB1, 4FB2 and 3BB1 are proposed as members of the new species *Thauera chlorobenzoica* sp. nov. The type strain is strain 3CB-1^T (= ATCC 700723^T).

ACKNOWLEDGEMENTS

This work was supported by grants from the United States Environmental Protection Agency (R822457) and the Office of Naval Research (N00014-94-0434 and N0014-99-1-0761).

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