Description of *Sphingomonas xenophaga* sp. nov. for strains BN6^T and N,N which degrade xenobiotic aromatic compounds

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The taxonomic position of two bacterial strains, BN6^T and N,N, with the ability to degrade xenobiotic aromatic compounds (naphthalenesulfonates or N,N-dimethylaniline) was investigated. The 16S rRNA gene sequence, the G+C content of the DNA (62–63 mol%) and the detection of ubiquinone Q-10, 2-hydroxymyristic acid and the sphingoglycolipid present clearly placed the two strains into the genus *Sphingomonas*. Both strains are representatives of one species according to the level of DNA relatedness (70.7%). The strains could be separated from all validly described taxa of the genus *Sphingomonas*, according to the 16S rRNA gene sequence (the highest sequence similarity observed was 96% to *Sphingomonas yanoikuyae*), the pattern of the polar lipids and physiological characteristics. Therefore, the new species *Sphingomonas xenophaga* is proposed to accommodate strains BN6^T (= DSM 6383^T) and N,N (= DSM 8566).

Keywords: *Sphingomonas xenophaga* sp. nov., taxonomy, degradation, naphthalenesulfonate, dimethylaniline

INTRODUCTION

The genus *Sphingomonas* is becoming increasingly interesting because it contains various xenobiotic-degrading bacterial isolates. Members of this genus are able to degrade compounds such as biphenyl, naphthalene and pyrene (Balkwill *et al.*, 1997; Khan *et al.*, 1996; Ye *et al.*, 1996), diphenylether and dibenzo-*p*-dioxin (Schmidt *et al.*, 1992; Moore *et al.*, 1993), herbicides and pesticides (Feng *et al.*, 1997; Nagata *et al.*, 1997; Nickel *et al.*, 1997), polyethylene glycols (Takeuchi *et al.*, 1993) and chlorinated phenols (Karlson *et al.*, 1995; Nohynek *et al.*, 1995, 1996). Because of the high physiological versatility within members of the genus *Sphingomonas*, the isolation of new *Sphingomonas* strains with interesting properties

can be expected. Therefore, a reliable taxonomic description of known *Sphingomonas* strains is urgently needed.

The bacterial isolate '*Pseudomonas*' sp. BN6^T has been studied intensively because of its ability to degrade naphthalenesulfonates. The strain was isolated from the River Elbe as a member of a bacterial consortium which completely degraded 6-aminonaphthalene-2-sulfonate. It was found that strain BN6^T oxidized a wide range of (substituted) naphthalenesulfonates to the corresponding (substituted) salicylates (Nörtemann *et al.*, 1986). Different enzymes of the degradative pathway for naphthalenesulfonates have been purified and characterized (Nörtemann *et al.*, 1994; Kuhm *et al.*, 1991, 1993a, b). Different extradiol dioxygenases from this strain have also been studied (Heiss *et al.*, 1995, 1997; Riegert *et al.*, 1998).

Furthermore, strain BN6^T reduced the azo bond of sulfonated azo dyes under anaerobic conditions and

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Abbreviation: DMA, dimethylaniline.

has been used in an anaerobic/aerobic process for the mineralization of sulfonated azo compounds (Haug *et al.*, 1991; Keck *et al.*, 1997; Kudlich *et al.*, 1996, 1997).

During a comparative study with different *Sphingomonas* strains, Nohynek *et al.* (1996) demonstrated that strain BN6^T belongs to the genus *Sphingomonas*, according to its 16S rRNA gene sequence. They suggested that strain BN6^T represents a yet undescribed species within the genus *Sphingomonas*. A taxonomic description of strain BN6^T was hampered by the fact that only this single isolate of the presumed new species was available. Schmidt (1994) isolated another *Sphingomonas* strain (designated N,N), after enrichment with *N,N*-dimethylaniline (*N,N*-DMA) as sole source of carbon and energy, which resembled strain BN6^T in many aspects. Therefore, we compared these strains and suggest that strain BN6^T and strain N,N belong to a new species of the genus *Sphingomonas* for which we propose the name *Sphingomonas xenophaga* sp. nov.

METHODS

Bacterial strains. Strain BN6^T was isolated from the River Elbe as a member of a 6-aminonaphthalene-2-sulfonate-degrading mixed bacterial culture (Nörtemann *et al.*, 1986). It has been deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, as DSM 6383^T. Strain N,N was obtained from a mixed sample from wastewater treatment plants, contaminated soil, river sediments and rotten wood after a continuous enrichment with a simultaneous supply of 2,4-, 2,5- and 2,6-DMA and *N,N*-DMA (50 mg l⁻¹ each). The strain was isolated from the enrichment culture after plating on agar plates with *N,N*-DMA as sole source of carbon, nitrogen and energy. Repeated transfers to liquid cultures with *N,N*-DMA and solid complex media resulted in a pure culture of strain N,N (Schmidt, 1994). It has been deposited as strain DSM 8566.

Cellular fatty acids. Fatty acid methyl esters were extracted 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).

Polyamines. Polyamines were extracted and analysed according to Busse & Auling (1988).

Polar lipids and quinones. The quinone system and polar lipids were determined by TLC as described previously (Tindall, 1990; Auling *et al.*, 1993).

Extraction and analysis of the pigment. Lyophilized cells (about 0.5 g) were stirred for 3 h at 4 °C in acetone (5 ml). Cells were removed by centrifugation and the dissolved pigment in the supernatant was analysed by UV/visible spectroscopy (IMA Gilford Analysentechnik). Spectra were recorded between 350 and 520 nm against acetone as reference.

Morphology. Gram reaction was tested as described by Gerhardt *et al.* (1994). Cell morphologies were observed under a light microscope (1000×) using cells grown for 3 d at 30 °C on Nutrient Agar.

Physiological and biochemical characterization. The two strains were characterized on the basis of 66 biochemical and physiological characteristics as described previously (Kämpfer *et al.*, 1997).

G + C content. Isolation of genomic DNA and spectrophotometric determination of the G + C content were carried out according to Auling *et al.* (1986).

DNA reassociation experiments. Genomic DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1977). DNA-DNA hybridization studies were carried out by using the thermal renaturation method (Yassin *et al.*, 1993).

RESULTS

Morphological and cultural characteristics

Cells of strains BN6^T and N,N were aerobic Gram-negative, non-spore-forming rods (1.3–4.0 µm in length and 0.7–1.2 µm in diameter). Both strains formed intense yellow colonies on solid medium. No growth was observed at 4 or 42 °C on agar plates. After reaching the stationary growth phase in liquid cultures, both strains rapidly lost the ability to form colonies on solid medium.

DNA reassociation

According to the 16S rDNA gene sequence, strain BN6^T showed the highest degree of similarity to *Sphingomonas yanoikuyae* (96% similarity) (Nohynek *et al.*, 1996). Therefore, DNA reassociation studies were performed with strains BN6^T, N,N and *S. yanoikuyae* DSM 7462^T. The level of DNA relatedness between strains BN6^T and N,N was 70.7%. In contrast, the level of DNA relatedness between strain BN6^T and *S. yanoikuyae* DSM 7462^T was only 40.0%. These results suggested that strains BN6^T and N,N are indeed members of the same species but do not belong to the species *S. yanoikuyae*.

G + C content of DNA

The G + C contents of the genomic DNA from strains BN6^T and N,N were determined to be 62.1 ± 0.2 and 63.3 ± 0.3% mol%, respectively. These values fall within the range observed for members of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990).

Physiological and biochemical characteristics

The results obtained from further tests demonstrated that strains BN6^T and N,N reacted very similarly to each other but showed characteristics different from *S. yanoikuyae*. Strains BN6^T and N,N did not produce acids from various sugars and sugar alcohols tested under aerobic conditions. Only a limited number of carbohydrates as well as simple organic acids were utilized as sources of carbon and energy (Table 1). Distinguishing results between strains BN6^T and N,N were observed in respect to hydrolysis of *p*-nitrophenyl-β-D-xylopyranoside, assimilation of D-

Table 1. Physiological characteristics of different *Sphingomonas* species which are characterized by spermidine as the predominant compound in the polyamine pattern

Test results were read after 72 h incubation at 30 °C. The following strains were investigated: *S. macrogoltabidus* IFO 15033^T; *S. terrae* IFO 15098^T; *S. chlorophenolica* ATCC 33790^T; *S. yanoikuyae* DSM 7462^T; *S. rosa* IFO 15208^T; *S. capsulata* IFO 12533^T; *S. stygia* SMCC B0712^T; *S. subterranea* SMCC B0478^T; *S. aromaticivorans* SMCC F199^T; *S. herbicidovorans* DSM 11019^T; *S. subarctica* KF1 HAMBI 2110^T (SMCC Subsurface Microbial Culture Collection, Florida State University, FL, USA; IFO Institute of Fermentation, Osaka, Japan). Data for *S. macrogoltabidus*, *S. terrae*, *S. chlorophenolica*, *S. yanoikuyae*, *S. rosa*, and *S. capsulata* were taken from Kämpfer *et al.* (1997). Only those substrates which gave a uniform reaction for strains BN6^T and N,N are reported. All strains hydrolysed L-alanine-*p*-nitroanilide but none of the strains grew with adonitol, i-inositol, D-mannitol, D-sorbitol, putrescine, *trans*-aconitate, 4-aminobutyrate, itaconate, mesaconate, oxoglutarate, β -alanine, L-ornithine, L-serine, 3-hydroxybenzoate or phenylacetate. Abbreviations: *xenop*, *xenophaga*; *macro*, *macrogoltabidus*; *chloro*, *chlorophenolica*; *yanoi*, *yanoikuyae*; *capsu*, *capsulata*; *subter*, *subterranea*; *aroma*, *aromaticivorans*; *herbic*, *herbicidovorans*; *subarc*, *subarctica*; pNP, *p*-nitrophenyl; pNA, *p*-nitroanilide. Symbols: +, Positive; –, negative; (+) weakly positive. Data are in accordance with those published by: a, Yabuuchi *et al.* (1990); b, Takeuchi *et al.* (1993); c, Takeuchi *et al.* (1994); d, Balkwill *et al.* (1997); e, Nohynek *et al.* (1996).

Test	<i>Sphingomonas</i>											
	<i>xenop</i>	<i>macro</i>	<i>terrae</i>	<i>chloro</i>	<i>yanoi</i>	<i>rosa</i>	<i>capsu</i>	<i>stygia</i>	<i>subter</i>	<i>aroma</i>	<i>herbic</i>	<i>subarc</i>
Hydrolysis of:												
Aesculin	+	+ ^b	–	–	+ ^a	(+)	–	+ ^d	+ ^d	+ ^d	+	+ ^e
pNP- β -D-Galactopyranoside	(+)	+	–	–	(+) ^a	+ ^c	+ ^a	–	(+)	+	–	–
pNP- β -D-Glucuronide	–	–	–	–	+	+	+	–	+	+	–	–
pNP- α -D-Glucopyranoside	+	–	+	–	+ ^a	+	+ ^a	+	+	+	+	+
pNP- β -D-Glucopyranoside	+	+	–	–	+ ^a	+	+ ^a	+	+	+	+	+
bis(pNP) Phosphate	+	+	+	+	+	+	+	(+)	(+)	+	–	+
pNP-Phenylphosphonate	+	+	+	+	+	+	+	–	+	+	–	+
pNP-Phosphorylcholine	+	+	+	–	+	+	+	–	–	(+)	–	–
2-Deoxythymidine-5'-pNP-phosphate	+	+	+	+	+	+	+	+	+	+	–	+
L-Glutamate- γ -3-carboxy-pNA	+	+	+	+	+	–	+	–	+	+	+	+
L-Proline-pNA	–	+	+	+	–	–	–	–	–	+	–	–
Assimilation of:												
N-Acetyl-D-glucosamine	–	–	–	–	+ ^a	(+) ^c	+ ^a	–	–	+	–	+
L-Arabinose	+	–	–	–	+ ^a	+ ^c	(+) ^a	– ^d	+ ^d	– ^d	–	+ ^e
D-Fructose	–	– ^b	– ^b	–	–	+ ^c	–	–	–	–	–	– ^e
D-Galactose	–	– ^b	–	–	+ ^a	–	+	–	–	–	–	+ ^e
D-Glucose	+	+	–	+	+ ^c	+ ^c	+	–	+	+	+	+
D-Mannose	–	– ^b	– ^b	–	(–) ^a	+ ^c	(+) ^a	–	+	+	–	–
D-Maltose	+	–	(–)	–	+ ^a	+ ^c	+ ^a	–	+	+	–	+ ^e
α -D-Melibiose	–	–	–	–	+ ^c	+ ^c	–	–	+	–	–	–
L-Rhamnose	–	–	–	–	+ ^c	+ ^c	+	–	+	+	+	+ ^e
D-Ribose	–	– ^b	– ^b	+	– ^a	–	– ^a	–	–	–	–	–
Sucrose	+	–	–	–	+ ^c	+ ^c	+	–	+	+	–	–
Maltitol	–	–	–	–	+	–	–	–	–	–	–	–
Acetate	(+)	+	–	+	+ ^c	–	–	–	–	+	+	+ ^e
Azelate	–	+	–	–	–	–	–	–	–	+	–	–
Citrate	–	–	–	–	+ ^c	– ^c	– ^c	–	–	–	–	– ^e
Fumarate	+	+ ^b	+	+	+ ^c	+	+	–	–	–	+	+
Glutarate	–	–	–	–	–	–	–	–	–	+	–	–
DL-3-Hydroxybutyrate	+	+	+	+	+	–	–	–	+	+	+	+ ^e
DL-Lactate	–	–	–	–	+ ^c	– ^c	–	–	–	–	–	+ ^e
Pyruvate	–	–	–	+	+	–	–	–	+	+	+	+
Suberate	–	+	–	+	–	+	–	–	(+)	+	–	–
L-Aspartate	–	+	–	–	+	–	+	–	–	+	+	+ ^e
L-Histidine	–	+	–	–	+	+	+	–	–	–	–	+
L-Leucine	–	+	–	+	+	–	+	–	–	+	–	+ ^e
L-Phenylalanine	–	+	+	–	+	–	–	–	–	–	–	–
L-Tryptophan	–	+	–	–	–	–	–	–	–	–	–	–

Table 2. Major fatty acid composition of different *Sphingomonas* species showing spermidine as the major compound in the polyamine pattern

The following strains were investigated: *S. xenophaga* BN6^T (DSM 6383^T) and N,N (DSM 8566); *S. macroglabridus* IFO 15033^T; *S. terrae* IFO 15098^T; *S. chlorophenolica* ATCC 33790^T; *S. yanoikuyae* DSM 7462^T; *S. rosa* IFO 15208^T; *S. capsulata* IFO 12533^T; *S. stygia* SMCC B0712^T; *S. subterranea* SMCC B0478^T; *S. aromaticivorans* SMCC F199^T; *S. herbicidovorans* DSM 11019^T; *S. subarctica* KFI HAMB1 2110^T. Data for *S. macroglabridus*, *S. terrae*, *S. chlorophenolica*, *S. yanoikuyae*, *S. rosa* and *S. capsulata* are taken from Kämpfer *et al.* (1997). Abbreviations are defined in the legend to Table 1.

Fatty acid*	<i>xenop</i>		<i>macro</i>	<i>terrae</i>	<i>chloro</i>	<i>yanoi</i>	<i>rosa</i>	<i>capsu</i>	<i>stygia</i>	<i>subter</i>	<i>aroma</i>	<i>herbic</i>	<i>subarc</i>
	BN6 ^T	N,N											
14:0	—	—	—	—	—	0.5	1.5	—	—	0.9	0.5	—	—
12:0 2-OH	—	—	—	—	—	—	—	—	0.6	—	1.0	—	—
13:0 2-OH	—	—	—	—	—	—	—	—	0.6	—	2.6	—	—
14:0 2-OH	6.7	10.1	3.0	0.8	9.4	7.1	12.8	14.7	14.9	13.0	16.8	5.5	7.6
Summed feature 4	23.9	27.4	31.8	8.0	9.6	16.3	22.8	6.7	23.1	20.1	20.0	13.4	12.6
16:0	8.0	9.3	12.8	4.5	9.5	11.2	10.9	8.1	2.5	4.7	2.4	7.4	10.2
Summed feature 7	55.2	47.2	41.2	13.4	60.1	56.0	52.0	64.8	49.3	48.2	37.7	68.3	61.1
17:1 ω 6 <i>c</i>	2.5	3.0	4.5	48.1	6.4	3.2	—	—	4.1	6.4	10.2	1.7	3.3
18:1 ω 5 <i>c</i>	1.6	1.5	0.9	0.6	2.5	2.1	—	—	0.8	—	—	2.0	—
17:0	—	—	—	3.8	—	—	—	—	—	—	—	—	—
16:1 ω 5 <i>c</i>	2.0	2.0	2.9	2.1	2.4	2.7	—	1.7	0.6	—	0.7	1.6	2.9
16:0 2-OH	—	1.0	2.9	—	—	0.9	—	—	—	—	—	—	2.1
15:0	—	—	—	5.9	—	—	—	—	—	—	0.9	—	—
17:1 ω 8 <i>c</i>	—	—	—	7.9	—	—	—	—	0.8	1.0	2.6	—	—
15:0 2-OH	—	—	—	4.9	—	—	—	—	2.5	3.3	4.5	—	—
18:0	—	—	—	—	—	—	—	—	—	3.2	—	—	—

* The position of the double bond in unsaturated fatty acids can be located by counting from the methyl (ω) end of the carbon chain. Summed features represent groups of two or three fatty acids that could not 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 15:0 iso 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).

cellobiose, D-mannose, 4-aminobutyrate, L-alanine, L-aspartate, L-leucine and 4-hydroxybenzoate.

Where possible, a detailed comparison was made with all previously published data (Takeuchi *et al.*, 1993, 1994; Yabuuchi *et al.*, 1990; Balkwill *et al.*, 1997; Nohynek *et al.*, 1996). Table 1 shows that the majority of test results which could be compared directly with data from previously published papers were confirmed. Table 1 shows also that the new species proposed in this paper, *Sphingomonas xenophaga* sp. nov., can be differentiated from all other *Sphingomonas* species on the basis of several tests. Additional physiological and biochemical characteristics of *S. xenophaga* are given below.

Characterization of the yellow pigment

Strains BN6^T and N,N formed bright yellow colonies on agar plates. The yellow pigment of '*Pseudomonas paucimobilis*' has been identified as the carotenoid nostoxanthin [(2R, 3R, 2'R, 3'R)- β,β -carotene-2,3,2',3'-tetrol] (Jenkins *et al.*, 1979). The extracted pigments of strains BN6^T and N,N showed the same spectral characteristics in acetone ($\lambda_{\max}$ = 453 and 480 nm) as nostoxanthin.

Quinones and polyamines

The extracted isoprenoid quinones from both strains gave one characteristic spot by HPLC analysis which corresponded to ubiquinone Q-10 upon comparison with authentic standards. In both strains spermidine was the major polyamine [31.7–38.3 μ mol (g dry wt)⁻¹]. In strain BN6^T small amounts of spermine [3.5 μ mol (g dry wt)⁻¹] were detected. The combination of these two features is in accordance with the characteristics of other species of the genus *Sphingomonas* such as *Sphingomonas capsulata* and *S. yanoikuyae* (Segers *et al.*, 1994).

Whole-cell fatty acid composition and polar lipid pattern from strains BN6^T and N,N

Strains BN6^T and N,N contained the fatty acids 16:0, summed feature 7 (18:1 ω 7*c*, 18:1 ω 9*t* and/or 18:1 ω 12*t*), 14:0 2-OH and summed feature 4 (16:1 ω 7*c* and/or 15:0 iso 2-OH) (Table 2), as found in other *Sphingomonas* species (Takeuchi *et al.*, 1993, 1994; Yabuuchi *et al.*, 1990; Kämpfer *et al.*, 1997). 3-Hydroxy acids were not detected. Table 2 shows that the type strains of *Sphingomonas* species containing spermidine as the major polyamine exhibit hetero-

Fig. 1. Polar lipids of *Sphingomonas* sp. BN6^T after separation by two-dimensional TLC. PE, phosphatidylethanolamine; PME, phosphatidylmonomethylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PDE, phosphatidyl dimethylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipid; PL3 and PL4, unidentified phospholipids; L, unidentified polar lipid; P, pigment.

geneity in their fatty acid profiles, which correlates with the heterogeneity found in the polar lipid profiles. However, the presence of 2-hydroxymyristic acid (14:0 2-OH) as the dominant hydroxylated acid and the absence of 3-hydroxylated fatty acids are consistent with the description of the genus *Sphingomonas* and confirm the results of other studies (Takeuchi *et al.*, 1993, 1994; Yabuuchi *et al.*, 1990; Kämpfer *et al.*, 1997).

The examination of total polar lipids revealed the presence of identical patterns with eight compounds in strains BN6^T and N,N (Fig. 1). Using authentic standards or in comparison with a lipid extract from *Sphingomonas paucimobilis*, phosphatidylethanolamine (PE), phosphatidyl dimethylethanolamine (PDE), phosphatidylglycerol (PG), sphingoglycolipid (SGL), diphosphatidylglycerol (DPG) and an unidentified phospholipid (PL4) were identified as the major lipids. Phosphatidylmonomethylethanolamine (PME), phosphatidylcholine (PC), an unidentified phospholipid (PL3) and an unidentified polar lipid (L) were detected in minor amounts. This polar lipid pattern clearly distinguishes strains BN6^T and N,N from *S. yanoikuyae* DSM 7462^T (Kämpfer *et al.*, 1997).

DISCUSSION

The results from DNA–DNA hybridization and all other experiments demonstrated that strains BN6^T

and N,N are closely related to each other and should be considered as different strains of the same species. Nevertheless, strain BN6^T is not able to grow with *N,N*-DMA and strain N,N could not oxidize naphthalenesulfonates. It was recently shown that the ability to degrade naphthalenesulfonates is a plasmid-encoded trait in strain BN6^T (J. Klein, personal communication). Therefore, these degradative abilities presumably do not have importance for the taxonomic classification of these strains.

The 16S rDNA gene sequence, the G + C content of the DNA, the detection of ubiquinone Q-10, 2-hydroxymyristic acid in the fatty acid profile and the sphingoglycolipid in the polar lipid pattern clearly place strains BN6^T and N,N into the genus *Sphingomonas* as it is currently defined (Yabuuchi *et al.*, 1990). The 16S rDNA gene sequence placed strain BN6^T in the neighbourhood of *S. yanoikuyae*, *Sphingomonas chlorophenolica* and '*S. paucimobilis* EPA505'. According to the 16S rRNA gene sequence comparison, a previously described strain (C7) with the ability to decolorize sulfonated azo compounds under aerobic conditions shared the highest sequence similarity (99%) with strain BN6^T (Govindaswami *et al.*, 1993). Unfortunately, we could not obtain strain C7 and therefore it was not included in this study. The allocation of strain BN6^T in close proximity to *S. yanoikuyae* was also indicated by the presence of spermidine as the major polyamine. The members of the genus *Sphingomonas* can be divided into two major groups on the basis of their polyamine pattern: spermidine is the main compound in *Sphingomonas aromaticivorans*, *S. capsulata*, *S. chlorophenolica*, *Sphingomonas herbicidovorans*, *Sphingomonas macrogoltabidus*, *Sphingomonas rosa*, *Sphingomonas stygia*, *Sphingomonas subterranea*, *Sphingomonas terrae*, *S. yanoikuyae*, '*S. paucimobilis* EPA505' and three so-called *Sphingomonas*–*Rhizomonas*-like strains. Other member species which can be considered as representative of the genus, *Sphingomonas adhaesiva*, *Sphingomonas asaccharolytica*, *Sphingomonas mali*, *Sphingomonas parapaucimobilis*, *S. paucimobilis*, *Sphingomonas pruni*, *Sphingomonas sanguinis* and *Sphingomonas trueperi*, are characterized by the presence of *sym*-homospermidine as the major compound in their polyamine pattern (Busse & Auling, 1988; Hamana & Matsuzaki, 1992; Segers *et al.*, 1994; Kämpfer *et al.*, 1997; Takeuchi *et al.*, 1994; H. J. Busse, unpublished data).

Various traits demonstrated that strains BN6^T and N,N are different from all other previously described *Sphingomonas* species. This was already evident from the dendrograms obtained by Nohynek *et al.* (1996) for the comparison of 16S rRNA gene sequences and the whole-cell fatty acid composition of different *Sphingomonas* strains, including BN6^T. The 16S rRNA gene of strain BN6^T showed only 96% similarity to the most related validly described species (*S. yanoikuyae*), which suggested that this strain is a representative of a new *Sphingomonas* species. A comparison of the

phenotypical characteristics obtained in the present study for strains BN6^T and N,N with previously published data for other *Sphingomonas* species (Kämpfer *et al.*, 1997) also demonstrated significant differences. Also, the pattern in the polar lipids was different from other sphingomonads. While strains BN6^T and N,N displayed identical polar lipid patterns, they were clearly distinguishable from their nearest phylogenetic neighbour, *S. yanoikuyae*, because of the lack of an unknown glycolipid (GL1) described for *S. yanoikuyae* (Kämpfer *et al.*, 1997). Taking these results into consideration, we propose strains BN6^T and N,N as representatives of a new species of the genus *Sphingomonas*, *Sphingomonas xenophaga* sp. nov.

Description of *Sphingomonas xenophaga* sp. nov.

Sphingomonas xenophaga [xe.no'pha.ga. Gr. adj. *xenos* foreign; Gr. n. *phagos* eater; M.L. fem. adj. *xenophaga* eating foreign (xenobiotic) compounds].

Gram-negative, rod-shaped, non-spore-forming bacteria (1.3–4.0 µm long; 0.7–1.2 µm diam.) which may be motile by the formation of a polar flagellum. Colonies are yellow, circular, low convex and smooth. Oxidase-positive. Catalase-positive (Nörtemann *et al.*, 1986). Aesculin is hydrolysed. The following compounds are hydrolysed: *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, bis(*p*-nitrophenyl) phosphate, bis(*p*-nitrophenyl) phenylphosphonate, bis(*p*-nitrophenyl) phosphorylcholine, 2-deoxythymidine-5-*p*-nitrophenylphosphate, *L*-aniline-*p*-nitroanilide and γ-*L*-glutamate-*p*-nitroanilide. The following compounds are not hydrolysed: *p*-nitrophenyl-β-D-glucuronide and *L*-proline-*p*-nitroanilide. The following compounds are assimilated: *L*-arabinose, D-glucose, D-maltose, D-sucrose, acetate, fumarate, DL-3-hydroxybutyrate and *L*-malate. The following compounds are not assimilated: *N*-acetylglucosamine, D-fructose, D-galactose, D-maltose, D-mannose, α-D-melibiose, *L*-rhamnose, D-ribose, adonitol, *i*-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, glutarate, itaconate, DL-lactate, mesaconate, 2-oxoglutarate, pyruvate, suberate, β-alanine, *L*-aspartate, *L*-histidine, *L*-leucine, *L*-ornithine, *L*-phenylalanine, *L*-serine, *L*-tryptophan, 3-hydroxybenzoate and phenylacetate. No acids are produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, *L*-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl-D-glucoside, erythritol, melibiose, D-arabitol or D-mannose. The G+C content of the DNA is 62.1–63.3 mol%. The major isoprenoid quinone is ubiquinone Q-10. The main component in the polyamine pattern is spermidine. The major non-polar fatty acids are 18:1 and 16:1, and the major 2-hydroxy fatty acid is 14:0 2-OH. No 3-hydroxy acids were found. Sphingoglycolipid present. Phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidyl dimethyl-

ethanolamine, phosphatidylglycerol, phosphatidylcholine and an unknown phospholipid are detected in the polar lipid pattern. Type strain is BN6^T (= DSM 6383^T). Strain BN6^T was isolated from river water (River Elbe, Germany). The 16S rRNA gene sequence is deposited at the EMBL database under the accession number X94098 (Nohynek *et al.*, 1996).

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