***Sphingomonas cloacae* sp. nov., a nonylphenol-degrading bacterium isolated from wastewater of a sewage-treatment plant in Tokyo**

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A nonylphenol-assimilating bacterium isolated at a sewage-treatment plant in Tokyo was studied phenotypically, genotypically and phylogenetically. Analysis of the 16S rDNA sequence, the G+C content of the DNA (63 mol%) and the isoprenoid quinone composition, as well as the presence of sphingoglycolipid and the whole-cell fatty acid profile, revealed that the isolate is a member of the genus *Sphingomonas*. However, the sequence similarity of the 16S rDNA with that of known *Sphingomonas* spp. was found to be at most 96%, implying that the isolate is distinctive. Furthermore, the results of DNA–DNA hybridization experiments and its physiological characteristics clearly indicated that the isolate represents a new *Sphingomonas* species, for which the name *Sphingomonas cloacae* is proposed; strain S-3^T (= JCM 10874^T = IAM 14885^T) is the type strain.

Keywords: *Sphingomonas cloacae* sp. nov., biodegradation, endocrine disrupter chemical, nonylphenol

INTRODUCTION

Nonylphenol (NP) is known as an important intermediate in the production of many kinds of commercial and industrial materials. Above all, NP is used in the production of nonylphenol polyethoxylate, a nonionic surfactant with many industrial applications. However, NP is also known as a ubiquitous pollutant in urban aquatic environments, with concentrations in the p.p.b. ($\mu\text{g l}^{-1}$) order, and is found in both sediment and surface water (Giger *et al.*, 1984; Ahel & Giger, 1985; Brunner *et al.*, 1988; Marcomini *et al.*, 1990; Rudel *et al.*, 1996; Isobe & Takada, 1998; Kojima & Watanabe, 1998). There is growing evidence that NP has a certain oestrogenic activity (Granmo *et al.*, 1989; Ekelund *et al.*, 1990; Soto *et al.*, 1991; Ahel *et al.*, 1993; Jobling & Sumpter, 1993; Gray & Metcalfe, 1997; Ren *et al.*, 1997; Shurin & Dodson, 1997; Ashfield *et al.*, 1998; Coldham *et al.*, 1998; Sonnenschein & Soto, 1998).

Recently, we have found significant NP-degrading activity in wastewater of a sewage-treatment plant in

Tokyo (Fujii *et al.*, 2000) and have isolated a NP-degrading bacterium (K. Fujii and others, unpublished data), which has been designated strain S-3^T. This strain degraded 1000 p.p.m. NP, i.e. a 10000–1000000-fold higher concentration than found in urban environments, almost completely within 10 d. Analysis of 16S rDNA partial sequences strongly suggested that the bacterium is a novel species of the genus *Sphingomonas*.

To precisely identify and phylogenetically place strain S-3^T, phenotypic characterization, analysis of isoprenoid quinone composition, fatty acid composition, polar lipid pattern and DNA G+C content, 16S rDNA sequencing and DNA–DNA hybridization were carried out. On the basis of our results we propose that S-3^T should be placed in a new species of the genus *Sphingomonas*, *Sphingomonas cloacae*.

METHODS

Chemicals. Yeast nitrogen base without amino acids (YNB), Bacto agar and tryptic soy agar were purchased from Difco. NP was obtained from Kanto Chemical. Nutrient broth and nutrient agar were purchased from Eiken Chemical. Other materials and chemicals used were available from commercial sources.

Abbreviation: NP, nonylphenol.

The GenBank/DBJ accession number for the 16S rDNA sequence of strain S-3^T is AB040739.

Table 1 List of bacterial species analysed in this study

Species	Strain*	16S rDNA accession no.
<i>Sphingomonas cloacae</i> †	S-3 ^T (= JCM 10874 ^T)	AB040739‡
<i>Sphingomonas yanoikuyae</i>	IFO 15102 ^T (= JCM 7370 ^T)	X85023
<i>Sphingomonas paucimobilis</i>	IFO 13935 ^T (= ATTC 29837 ^T)	X72722
<i>Sphingomonas chlorophenolica</i>	JCM 10275 ^T (= ATTC 33790 ^T)	X87161
<i>Sphingomonas</i> sp. RA2	DSM 6824 (= ATTC 39723)	X87162
<i>Sphingomonas herbicidovorans</i>	DSM 11029 ^T (= IFO 16415 ^T)	AB022428
<i>Sphingomonas</i> sp. HV3	HAMBI 2249	Y12803
<i>Sphingomonas sanguinis</i>	IFO 13937 ^T (= ATCC 51382 ^T)	D13726
<i>Sphingomonas natatoria</i>	DSM 3183 ^T (= ATCC 35951 ^T)	AB024288
<i>Sphingomonas capsulata</i>	IFO 12533 ^T (= ATTC 14666 ^T)	D16147
<i>Sphingomonas asaccharolytica</i>	IFO 15499 ^T (= ATTC 51839 ^T)	Y09639
<i>Sphingomonas trueperi</i>	ATCC 12417 ^T (= DSM 7225 ^T)	X97776
<i>Sphingomonas parapaucimobilis</i>	JCM 7510 ^T (= ATCC 51231 ^T)	D13724
<i>Sphingomonas echinoides</i>	ATCC 14820 ^T (= DSM 1805 ^T)	AB033944
<i>Sphingomonas pruni</i>	IFO 15498 ^T (= ATTC 51838 ^T)	Y09637
<i>Sphingomonas mali</i>	IFO 15500 ^T (= ATTC 51840 ^T)	Y09638
<i>Sphingomonas suberifaciens</i>	DSM 7465 ^T (= ATTC 49355 ^T)	D13737
<i>Sphingomonas macrogoltabidus</i>	IFO 15033 ^T (= ATTC 51380 ^T)	D13723
<i>Sphingomonas terrae</i>	IFO 15098 ^T (= ATCC 51381 ^T)	D13727
<i>Sphingomonas adhaesiva</i>	JCM 15099 ^T (= ATCC 51229 ^T)	X72720
<i>Sphingomonas subarctica</i>	JCM 10398 ^T (= DSM 10700 ^T)	X94102
<i>Sphingomonas subterranea</i>	ATCC 700279 ^T (= DSM 12447 ^T)	AB025014
<i>Sphingomonas aromaticivorans</i>	ATCC 700278 ^T (DSM 12444 ^T)	AB025012
<i>Sphingomonas stygia</i>	ATCC 700280 ^T (DSM 12445 ^T)	AB025013
<i>Sphingomonas xenophaga</i>	DSM 6383 ^T	X94098
<i>Agrobacterium rhizogenes</i>	IFO 13257 ^T (= ATTC 11325 ^T)	D12788

* IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Saitama, Japan; ATTC, American Type Culture Collection, Manassas, VA, USA; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; HAMBI, Culture Collection of the Department of Microbiology, University of Helsinki, Finland.

† Isolated in this study.

‡ Sequenced in this study.

Isolation of the NP-degrading bacterium. Strain S-3^T was isolated from wastewater of a sewage-treatment plant in Tokyo. For isolation of S-3^T, YNB agar (pH 7.0 at 25 °C) containing 0.1 % (w/v), i.e. 1000 p.p.m., NP (NP/YNB agar) was used. YNB consists of (NH₄)₂SO₄ as the nitrogen source, other salts (KH₂PO₄, MgSO₄, NaCl and CaCl₂), trace metals and very small amounts of vitamins, as described in the Difco manual. NP is therefore considered to be nearly the sole carbon source in the NP/YNB medium.

Bacterial strains. The bacterial strains used in this study are listed in Table 1. They were obtained from the Institute for Fermentation (IFO), Osaka, Japan, the Japan Collection of Microorganisms (JCM), Saitama, Japan, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany, and the Culture Collection of the Department of Microbiology (HAMBI), University of Helsinki, Finland. Recommended and known growth media were used to grow these organisms. S-3^T has been deposited in JCM as *Sphingomonas cloacae* JCM 10874^T.

Morphology. Cell morphology was examined using transmission electron microscopy (model H-7000; Hitachi). In preparation for electron microscopy, bacterial cells were

suspended in 500 µl 0.85 % NaCl. Subsequently, the cells were dried on a carbon-coated mesh, stained with 3 % uranyl acetate and examined.

Physiological and biochemical characterization. Oxygen requirement for growth was tested with nutrient agar and the BBL GasPak Anaerobic System (Becton Dickinson). Oxidase and catalase tests were carried out with the Poremedia Oxidase Test Indicator (Eiken) and 3.0 % hydrogen peroxide, respectively. API50 carbohydrate substrate strips (BioMérieux) were used to determine the assimilation pattern of test organisms. Assimilation of carbohydrates was also examined by aerobic cultivation with YNB (pH 7.0 at 25 °C) containing 1.0 % carbohydrate to confirm the data obtained with API50. APIZYM (BioMérieux) was used to determine biochemical characteristics.

DNA preparation. Chromosomal DNA was purified by standard procedures (Sambrook *et al.*, 1989) from bacterial cells harvested from nutrient broth after growth for 48 h at 25 °C. Subsequently, the bacterial cells were suspended in Tris/EDTA buffer (pH 8.0) and lysed with lysozyme (final concentration 2 mg ml⁻¹) and SDS (final concentration 0.5 %). Chromosomal DNA was then recovered from the lysate by phenol/chloroform extraction, followed by RNase treatment, cetyltrimethylammonium bromide (CTAB) treat-

ment and ethanol precipitation. We performed each procedure twice to obtain highly pure DNA.

DNA base composition and DNA–DNA hybridization. The DNA base composition, i.e. the G+C content, was determined by a HPLC method, as described by Tamaoka & Komagata (1984). *Sphingomonas chlorophenolica* was used as control.

DNA–DNA hybridization experiments were carried out by microplate hybridization methods (Ezaki *et al.*, 1989) using photobiotin labelling and colorimetric detection (Satomi *et al.*, 1997), with 1,2-phenylenediamine (Sigma) as the substrate and streptavidin–peroxidase conjugate (Boehringer Mannheim) as the colorimetric enzyme. The experiment was independently done three times.

16S rDNA sequencing and phylogenetic analysis. Nearly complete 16S rDNA (1417 bases) was amplified by PCR, using a universal primer set corresponding to positions 8–27 (forward primer) and 1492–1510 (reverse primer) of the *Escherichia coli* numbering system (Weisburg *et al.*, 1991). The PCR operating conditions were similar to those described by Suzuki & Yamasato (1994). Direct sequencing of the amplified DNA fragments was carried out as described by Satomi *et al.* (1997). Similarity of 16S rDNA sequences between strain S-3^T and other species was compared with all known sequence data in the GenBank, EMBL and DDBJ databases using the BLAST algorithm (Altschul *et al.*, 1990). Data analysis was performed with CLUSTAL W software (Thompson *et al.*, 1994) and the PHYLIP program (Felsenstein, 1995). Nucleotide substitution rates (K_{nuc} values) were calculated by the methods of Kimura (1980), and a phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987). The sequence accession numbers of bacteria used in the phylogenetic analysis are shown in Table 1.

Fatty acid composition. Whole-cell lipids were extracted according to the methods of Bligh & Dyer (1959). The extracted lipids were converted to fatty acid methyl esters following the methodology of the American Oil Chemists' Society (1990). Hydroxylated fatty acids were trimethylsilylated prior to GC/MS analysis, for which a GC-17A gas chromatograph interfaced with a QP-5000 mass spectrometer (Shimadzu) was used. The experimental conditions used were: OMEGA WAX column (30 m × 0.25 mm; Supelco); injection volume, 0.8 µl; carrier gas, helium (1 ml min⁻¹); temperature gradient, 150 °C for 4 min, 150 to 180 °C at 5 °C min⁻¹, 180 to 240 °C at 2 °C min⁻¹, 240 °C for 10 min, resulting in a total run time of 50 min. The injection port temperature was 250 °C. The ionization energy and temperature for electron impact ionization were 70 eV and 280 °C, respectively. *S. chlorophenolica* was used as control.

TLC of polar lipids. Polar lipids were extracted from cell membranes by the methods of Bligh & Dyer (1959), and subsequently analysed by two-dimensional TLC. The crude lipids were separated twice on Silica gel 60 TLC plates (20 × 20 cm; Merck) with two solvent systems, one being chloroform/methanol/water (65:25:4, by vol.) and the other chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). A 50% sulphuric acid solution was used to detect spots of all kinds of lipids. α -Naphthol/sulphuric acid, periodate-Schiff reagent, Zinzadze (Dittmer) reagent and ninhydrin were used for the detection of sugars, α -glycols, phosphate and free amino groups, respectively. Crude lipids of *S. chlorophenolica* were used as control.

Isoprenoid quinone composition. The isoprenoid quinone type and isoprenoid length were analysed according to the

methods described by Yamada & Kuraishi (1982). Total acetone-soluble extracts of whole cells were separated by one-dimensional TLC with benzene as the eluent. Isoprenoid length was analysed by HPLC using a reverse-phase column (Cosmosil C-18 Econopak column; Nakarai Tesque). *S. chlorophenolica* was used as control.

Nucleotide sequence accession number of the new isolate. The 16S rDNA sequence of strain S-3^T has been deposited in the DDBJ database (DNA Database of Japan, Shizuoka, Japan) under accession number AB040739.

RESULTS AND DISCUSSION

Morphological characteristics

Strain S-3^T was found to be aerobic, Gram-negative and rod-shaped (2.0–3.1 µm in length and 1.1–1.4 µm in diameter) (Fig. 1). The strain formed creamy white colonies on nutrient agar and tryptic soy agar in 3–4 d at 25 °C. It required about 1 week to form visible colonies on NP/YNB agar at this temperature. This may be explained by the fact that NP/YNB is a minimal salt medium, as described in Methods. Colony growth was not observed at temperatures of 4 or 42 °C.

Physiological and biochemical characteristics

The characteristics of S-3^T are summarized in Table 2. S-3^T did not assimilate any carbohydrates tested in this study. Since other *Sphingomonas* spp. tested assimilated many different kinds of carbohydrates under the same experimental conditions, the experimental system seemed to work properly. S-3^T was also cultured aerobically in YNB (pH 7.0 at 25 °C) containing 1.0% carbohydrate or NP using a rotary shaker. Again, no carbohydrates were assimilated, whereas NP was utilized for bacterial growth, suggesting that the results obtained from our experiments were reliable. We suspected that the negative results of S-3^T might be due

Fig. 1. Transmission electron micrograph of S-3^T cells. Bar, 1 µm.

Table 2 Characteristics which differentiate S-3^T from other *Sphingomonas* species

Abbreviations: *pau*, *S. paucimobilis*; *yan*, *S. yanoikuyae*; *chl*, *S. chlorophenolica*; *hrb*, *S. herbicidovorans*; RA2, *Sphingomonas* sp. RA2; HV3, *Sphingomonas* sp. HV3; *sng*, *S. sanguinis*; *cps*, *S. capsulata*; *nat*, *S. natatoria*; *asc*, *S. asaccharolytica*. +, Positive result; –, negative result.

Characteristic	S-3 ^T	<i>pau</i>	<i>yan</i>	<i>chl</i>	<i>hrb</i>	RA2	HV3	<i>sng</i>	<i>cps</i>	<i>nat</i>	<i>asc</i>
Assimilation of:*											
L-Arabinose	–	+	+	–	–	–	+	+	–	–	–
D-Xylose	–	+	+	–	–	–	–	+	+	+	–
Galactose	–	+	–	–	+	–	+	+	–	–	–
D-Glucose	–	+	+	+	+	+	+	+	+	+	+
D-Fructose	–	+	–	–	–	–	+	+	+	–	–
D-Mannose	–	+	–	–	–	–	–	+	–	–	+
Rhamnose	–	–	+	–	+	–	–	–	–	–	–
N-Acetylglucosamine	–	+	–	–	–	–	–	+	–	–	+
Arbutin	–	+	–	–	–	–	–	+	–	–	–
Salicin	–	+	–	–	–	–	–	+	–	+	–
Cellobiose	–	+	+	+	–	–	–	+	+	–	–
β-Gentiobiose	–	+	–	+	–	–	–	+	–	–	–
Amidon	–	+	+	–	–	–	–	+	–	–	–
Amygdalin	–	+	–	–	–	–	–	+	+	–	–
D-Raffinose	–	+	+	–	–	–	–	+	–	–	–
Melezitose	–	+	–	–	–	–	–	+	+	–	–
Lactose	–	+	+	–	–	–	–	+	+	–	+
Maltose	–	+	+	–	–	–	+	+	+	+	+
Melibiose	–	+	+	–	–	–	–	+	–	–	+
Sucrose	–	+	+	–	–	–	–	+	+	+	–
Trehalose	–	+	–	–	–	–	–	+	+	–	–
D-Fucose	–	+	+	–	–	–	+	–	+	–	–
L-Fucose	–	–	–	–	+	–	–	+	–	–	–
D-Turanose	–	+	–	–	–	–	–	+	–	–	+
α-Methyl-D-glucoside	–	–	–	–	–	–	–	+	–	–	–
Glycerol	–	–	–	–	–	–	–	+	–	–	–
Activity of:†											
Esterase (C-4)	+	+	+	+	–	+	+	+	+	+	+
Esterase lipase (C-8)	–	+	+	+	+	+	+	+	+	+	+
Lipase (C-4)	–	+	+	–	–	–	–	–	+	+	–
Valine arylamidase	+	+	+	+	+	+	+	+	+	–	+
Cystine arylamidase	–	+	+	–	–	–	–	+	+	+	+
Trypsin	–	–	+	+	–	–	+	–	+	+	+
Chymotrypsin	–	+	+	+	–	+	–	–	+	+	–
α-Galactosidase	–	–	–	–	–	–	–	–	–	–	+
β-Galactosidase	–	+	+	–	–	–	–	+	+	+	+
β-Glucuronidase	–	–	–	–	–	–	–	–	+	+	+
α-Glucosidase	–	+	+	+	+	+	+	+	+	+	+
β-Glucosidase	–	+	+	+	+	+	–	+	+	+	+
N-Acetyl-β-glucosaminidase	–	–	–	–	–	–	–	+	–	–	–

* Assimilation tests for which all strains gave negative results were: D-arabinose, L-arabinose, L-xylose, L-sorbose, D-lyxose, D-tagatose, D-arabitol, β-methylxylose, ribose, inositol, inulin, erythritol, adonitol, dulcitol, xylitol, mannitol, sorbitol, glycogen, α-methyl-D-mannose, gluconate, 2-keto-gluconate and 5-keto-gluconate.

† Enzyme activity tests for which all strains gave positive results were: alkaline phosphatase, acid phosphatase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase; tests for which all strains gave negative results were: α-mannosidase and α-fucosidase.

to adaptation to nonylphenol. It is known that highly adapted strains must be subcultured several times in a full medium before they again show their typical physiological behaviour. Thus we subcultured S-3^T

repeatedly (five times) with nutrient broth and nutrient agar before the assimilation pattern tests. However, S-3^T again did not show any positive results in the tests, while NP-degrading activity was stable.

Table 3 Levels of DNA–DNA reassociation of S-3^T with other *Sphingomonas* strains

Strain	G + C content (mol %)*	Reassociation (%) with labelled DNA from:										
		S-3 ^T	yan	pau	chl	hrb	HV3	RA2	sng	cps	nat	asc
S-3 ^T	63	100	17	10	20	16	16	18	5	3	1	0
<i>S. yanoikuyae</i> (yan)	62 ^a	19	100	8	19	17	15	16	6	5	4	3
<i>S. paucimobilis</i> (pau)	64 ^a	13	10	100	9	7	5	8	40	6	5	7
<i>S. chlorophenolica</i> (chl)	65	27	23	11	100	23	31	66	9	6	4	2
<i>S. herbicidovorans</i> (hrb)	NA	16	15	7	17	100	18	15	5	5	4	2
<i>Sphingomonas</i> sp. HV3 (HV3)	64 ^b	18	19	7	31	23	100	35	7	5	5	4
<i>Sphingomonas</i> sp. RA2 (RA2)	NA	23	21	9	69	23	36	100	8	6	6	5
<i>S. sanguinis</i> (sng)	62 ^a	9	9	42	9	6	5	5	100	4	3	5
<i>S. capsulata</i> (cps)	64 ^a	9	9	6	8	6	5	4	4	100	3	0
<i>S. natatoria</i> (nat)	NA	5	7	6	6	6	6	6	5	5	100	5
<i>S. asaccharolytica</i> (asc)	65 ^c	3	4	7	4	3	3	3	6	1	3	100

NA, Not available.

* Data from: *a*, Takeuchi *et al.* (1993); *b*, Yrjala *et al.* (1998); *c*, Takeuchi *et al.* (1995).

On the other hand, we found that S-3^T could grow aerobically with YNB containing 0.1% tryptone or proline as sole carbon source. Therefore, it is less likely that the negative results of S-3^T are due to adaptation to NP. We think that S-3^T can utilize some uncommon carbon sources, including at least NP and proline, but cannot assimilate the carbohydrates tested in our study.

Tests of oxidase and catalase activities were found to be positive, and several other enzyme activities were also detected. However, tests of the carbohydrate-degrading enzymes of S-3^T were all negative, which is consistent with its carbohydrate assimilation pattern (Table 2).

DNA analysis

The G + C content of the genomic DNA of S-3^T was determined as 63 mol% (Table 3). This value falls within the range observed for other members of the genus *Sphingomonas*, namely 61.6–67.8 mol% (Takeuchi *et al.*, 1993; Yabuuchi *et al.*, 1990).

From the analysis of 16S rDNA sequences, S-3^T showed similarity to several *Sphingomonas* spp., including *Sphingomonas yanoikuyae*, *Sphingomonas chlorophenolica*, *Sphingomonas paucimobilis*, *Sphingomonas herbicidovorans*, *Sphingomonas* sp. HV3 and *Sphingomonas* sp. RA2 (Fig. 2). We have confirmed that other clustering methods (maximum-likelihood methods) using the PHYLIP program (Felsenstein, 1995) lead to the same grouping.

However, the sequence similarity of the 16S rDNA of S-3^T with that of these species was 96% at most, implying that S-3^T should be regarded as distinctive. Therefore, DNA–DNA hybridization experiments were carried out to obtain more conclusive infor-

Fig. 2. Phylogenetic tree constructed by the neighbour-joining method based on 16S rDNA sequences of S-3^T and related bacteria. Scale bar represents an evolutionary distance (K_{nucl}) of 0.01.

mation on the relationship between S-3^T and known *Sphingomonas* species. Six species forming a cluster with S-3^T, and four random species forming a distinct grouping in the phylogenetic tree of 16S rDNA (Fig. 2) were studied. Table 3 shows levels of DNA–DNA reassociation values among these species, indicating

Table 4 Whole-cell fatty acid profile of S-3^T

S. chlorophenolica (*chl*) was used as control.

Fatty acid	Percentage in:	
	S-3 ^T	<i>chl</i>
Saturated fatty acids		
14:0	0.12	0.11
14:0 2-OH	2.8	2.7
16:0	7.2	8.7
16:0 2-OH	0.20	0.38
17:0	0.33	0.22
18:0	0.66	0.86
19:0	1.7	0.21
22:0	tr	tr
Unsaturated fatty acids		
16:1(9)	3.3	8.8
16:1(7)	0.73	1.6
16:1 2-OH	1.3	1.3
18:1(11)	1.3	ND
Summed feature*	80	75
3-OH fatty acids	ND	ND

ND, Not detected.

tr, Trace (less than 0.1 %).

* Summed feature consists mainly of 18:1 fatty acid and could not be separated completely under our experimental conditions.

relatively low levels of hybridization (maximum 27 %). It has been recommended that phylogenetically defined species should consist of strains that exhibit approximately 70 % or higher DNA–DNA hybridization values (Wayne *et al.*, 1987). Therefore, our findings strongly suggest that S-3^T is distinct from other known *Sphingomonas* species.

Cellular fatty acid composition and polar lipid pattern of S-3^T

The whole-cell fatty acid profile of S-3^T is shown in Table 4. The major non-polar fatty acids that were found were 18:1 and 16:0, while 2-hydroxymyristic acid (14:0 2-OH) was present as the dominant hydroxylated fatty acid. However, 3-hydroxylated fatty acids were not found. These results are consistent with the description of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1993, 1994; Kampfer *et al.*, 1997; Stolz *et al.*, 2000).

The total polar lipid profile was examined by two-dimensional TLC (data not shown). Several major spots of phospholipids and minor spots of unidentified lipids emerged on the TLC plate. Furthermore, staining of the plate with α -naphthol/sulphuric acid, periodate-Schiff reagent, Zinzadze (Dittmer) reagent and ninhydrin revealed a spot of sphingoglycolipid, the unique lipid found in the genus *Sphingomonas* (Kampfer *et al.*, 1997), suggesting that S-3^T is a *Sphingomonas* species.

Isoprenoid quinone analysis

The isoprenoid quinone composition of S-3^T was determined. It contained ubiquinone, which consisted mainly of Q-10. The presence of ubiquinone Q-10 as the predominant isoprenoid quinone is typical for members of the α -subclass of the *Proteobacteria*, which includes the genus *Sphingomonas* (Yabuuchi *et al.*, 1990; Yrjala *et al.*, 1998; Stolz *et al.*, 2000).

Conclusions

Recently, study of the genus *Sphingomonas* has become one of the most competitive research fields since various xenobiotic-degrading species have been reported. For example, *S. paucimobilis* and *S. yanai-kuyae* are known to assimilate naphthalene and biphenyl (Gibson *et al.*, 1973; Furukawa *et al.*, 1983; Yabuuchi *et al.*, 1990; Kuhm *et al.*, 1991; Khan *et al.*, 1996). *Sphingomonas chlorophenolica*, *Sphingomonas subarctica* and *Sphingomonas* sp. RA2 are able to assimilate chlorinated phenols (Radehaus & Schmidt, 1992; Karlson *et al.*, 1995; Nohynek *et al.*, 1996; McCarthy *et al.*, 1997; Ohtsubo *et al.*, 1999). *S. herbicidovorans* has been found to utilize herbicides as carbon sources (Horvath *et al.*, 1990; Zipper *et al.*, 1996, 1998). *Sphingomonas xenophaga* and *Sphingomonas* sp. HV3 have been reported to assimilate several aromatic and chloroaromatic compounds (Kilpi *et al.*, 1980; Yrjala *et al.*, 1998; Stolz *et al.*, 2000).

The 16S rDNA sequence (Fig. 2), the G + C content of the genomic DNA (Table 3), the isoprenoid quinone composition, the whole-cell fatty acid profile (Table 4) and the presence of sphingoglycolipid suggested that S-3^T belongs to the genus *Sphingomonas*.

However, the level of similarity of the 16S rDNA between S-3^T and other known *Sphingomonas* spp. suggested that S-3^T is a novel species within this genus. Comparison of the phenotypic characteristics of S-3^T (Table 2) with those of other members of *Sphingomonas* (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1995; Balkwill *et al.*, 1997; Kampfer *et al.*, 1997; Stolz *et al.*, 2000) also demonstrated significant differences, supporting our conclusion that S-3^T is distinct from known members of the genus. Finally, the DNA–DNA hybridization experiments (Table 3) clearly showed that S-3^T is a novel species of the genus *Sphingomonas*.

Based on the phenotypic, genotypic and phylogenetic data obtained in our study, it was concluded that the strain S-3^T should be classified as a new species of the genus *Sphingomonas*. We therefore propose the name *Sphingomonas cloacae* for this novel organism.

Description of *Sphingomonas cloacae* sp. nov.

Sphingomonas (clo.a'cae. L. n. *cloaca* sewer, the source of the organism).

Gram-negative, aerobic and rod-shaped bacterium (2.0–3.1 μ m long, 1.1–1.4 μ m diameter). Colonies are circular, entire, convex, dry, opaque and creamy white.

Cells are able to grow in nutrient broth at 25 °C, but not at 4 or 42 °C. NP, an endocrine disrupter chemical, and proline are assimilated, but the carbohydrates tested in our study, including glucose, galactose, xylose, arabinose, trehalose, fructose, sucrose and maltose, are not. Oxidase, catalase, alkaline phosphatase, acid phosphatase, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrazidase and esterase activities are positive. The G+C content of the DNA is 63 mol%. The major non-polar fatty acids are 18:1 and 16:0, and the major 2-hydroxy fatty acid is 14:0 2-OH. 3-Hydroxy fatty acids were not detected. Sphingoglycolipid is present. The major isoprenoid quinone is ubiquinone Q-10. Isolated from wastewater of a sewage-treatment plant in Tokyo. The type strain is strain S-3^T (= JCM 10874^T = IAM 14885^T). The 16S rDNA sequence has been deposited at the DDJB database under accession number AB040739.

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