Sphingopyxis ummariensis sp. nov., isolated from a hexachlorocyclohexane dump site

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A Gram-negative, motile, rod-shaped, yellow-pigmented bacterium, strain UI2^T, was isolated from a hexachlorocyclohexane (HCH) dump site located in Ummari, in northern India. 16S rRNA gene sequence analysis revealed that strain UI2^T belongs to the genus *Sphingopyxis* and showed highest 16S rRNA gene sequence similarity with *Sphingopyxis terrae* IFO 15098^T (98.6%). DNA–DNA relatedness values between strain UI2^T and *Sphingopyxis terrae* IFO 15098^T and other related strains were found to be less than 46%. The major cellular fatty acids of strain UI2^T were C_{18:1}ω7c (28.3%), C_{16:0} (14.4%), 11-methyl C_{18:1}ω7c (10.9%), C_{17:1}ω6c (9.6%) and summed feature 3 (consisting of C_{16:1}ω7c and/or C_{15:0} iso 2-OH; 14.7%). The DNA G + C content of strain UI2^T was 68 mol%. On the basis of DNA–DNA hybridization, phenotypic characteristics and phylogenetic analysis, strain UI2^T is found to represent a novel species of the genus *Sphingopyxis*, for which the name *Sphingopyxis ummariensis* sp. nov. is proposed. The type strain is UI2^T (=CCM 7428^T =MTCC 8591^T).

The genus *Sphingopyxis* was introduced after the subdivision of the genus *Sphingomonas* by Takeuchi *et al.* (2001) and is currently represented by 12 species: *Sphingopyxis terrae* (Takeuchi *et al.*, 1993, 2001), *S. macroglotabida* (Takeuchi *et al.*, 1993, 2001), *S. alaskensis* (Vancanneyt *et al.*, 2001; Godoy *et al.*, 2003), *S. taejonensis* (Lee *et al.*, 2001; Pal *et al.*, 2006), *S. witflariensis* (Kämpfer *et al.*, 2002), *S. chilensis* (Godoy *et al.*, 2003), *S. flavimaris* (Yoon & Oh, 2005), *S. baekryungensis* (Yoon *et al.*, 2005), ‘*S. granuli*’ (Kim *et al.*, 2005; this name has not been validly published), *S. ginsengisoli* (Lee *et al.*, 2008), *S. marina* (Kim *et al.*, 2008) and *S. litoris* (Kim *et al.*, 2008).

A yellow-coloured strain, designated UI2^T, was isolated from a hexachlorocyclohexane (HCH) dump site at Ummari village in northern India. For this purpose, an HCH-contaminated soil sample was serially diluted and plated on nystatin- and streptomycin-amended Luria–Bertani (LB) agar plates (Vanbroekhoven *et al.*, 2004; Dadhwal *et al.*, 2009). A yellow-coloured colony that appeared within 36 h of incubation at 28 °C was picked and purified by repeated streaking on LB agar. Strain UI2^T

was characterized taxonomically by using a polyphasic approach (Prakash *et al.*, 2007b).

Colonies were yellow and circular with entire margins and varied in size from 0.5 to 2 mm within 2 days of incubation. Colony morphology was studied on LB agar, nutrient agar (NA) and tryptic soya agar (TSA). Strain UI2^T grew well under aerobic conditions on LB agar, NA and TSA at 28 °C within 24–48 h of incubation. Gram-staining was performed using a Gram-stain kit (HiMedia) and strain UI2^T was found to stain Gram-negative. After preparing cells as described by Kumar *et al.* (2008), a subpolar (monotrichous) flagellum was observed by transmission electron microscope (Supplementary Fig. S1, available in IJSEM Online). Motility of the organism was also checked on motility agar (Farmer, 1999).

The 16S rRNA gene sequence of the strain UI2^T was amplified by colony PCR using the universal primers 27F and 1492R and sequenced as described by Kumar *et al.* (2008) and Jit *et al.* (2008). A continuous stretch of 1408 bp of the 16S rRNA gene sequence obtained was subjected to similarity searches by using the sequence matching tool of Ribosomal Database Project II (<http://rdp.cme.msu.edu/>) and the NCBI BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The nearly full-length 16S rRNA gene sequences of strains closely related to UI2^T were retrieved for the construction of a phylogenetic tree. The selected sequences were aligned using CLUSTAL X version 1.81b (Thompson *et al.*, 1997). The evolutionary distance matrix was calculated using the distance model of Jukes & Cantor (1969) within TREECON version 1.3b (Van de Peer &

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Abbreviation: HCH, hexachlorocyclohexane.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain UI2^T is EF424391.

An EM of a cell of strain UI2^T, results of TLC analysis of its polar lipids and tables of DNA–DNA relatedness values and fatty acid compositions are available as supplementary material with the online version of this paper.

De Wachter, 1994) using a 1330 bp alignment and the resultant tree topology was evaluated using bootstrap analysis of 1000 resamplings. A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987). Evaluation of the tree topology revealed that strain UI2^T clustered with members of the genus *Sphingopyxis* and formed a monophyletic clade with *S. terrae* IFO 15098^T (Fig. 1). A similar tree topology was also observed using neighbour-joining and maximum-parsimony methods with PHYLIP version 3.5 (Felsenstein, 1993).

16S rRNA gene sequence similarity values between strain UI2^T and *Sphingopyxis* strains ranged from 93.6 to 98.6 %, with the highest similarity to *S. terrae* IFO 15098^T (98.6 %), followed by *S. witflariensis* W-50^T (97.9 %), '*S. granul*' Kw07 (97.9 %), *S. ginsengisoli* Gsoil 250^T (97.9 %), *S. taejonensis* JSS54^T (97.4 %), *S. alaskensis* RB2256^T (97.3 %), *S. chilensis* S37^T (97.2 %), *S. macrogoltabida* IFO 15033^T (97.1 %), *S. flavimaris* SW-151^T (95 %), *S. baekryungensis* SW-150^T (94.4 %), *S. marina* FR1087^T (94.4 %) and *S. litoris* FR1093^T (93.6 %).

DNA–DNA hybridizations between strain UI2^T and strains of *Sphingopyxis* species with 16S rRNA gene sequence similarity values greater than 97 % were carried out by the membrane-filter method (Tourova & Antonov, 1988; Prakash *et al.*, 2007a; Kumar *et al.*, 2008). *S. terrae* DSM 8831^T, *S. witflariensis* DSM 14551^T, *S. taejonensis* DSM 15583^T, *S. alaskensis* DSM 13593^T, *S. chilensis* DSM 14889^T and *S. macrogoltabida* DSM 8826^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. '*S. granul*' KCTC 12209 and *S. ginsengisoli* KCTC 12582^T were obtained from Korean Collection for Type Cultures, Seoul, Korea. Strain

UI2^T was most closely related to *S. terrae* DSM 8831^T (DNA–DNA relatedness value 42.2 %; Supplementary Table S1). Hybridization values of strain UI2^T with other strains of the genus *Sphingopyxis* were less than 46 %. Since all hybridization values were below 70 %, as is recommended for the delineation of species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994), this confirms that strain UI2^T represents a novel species of the genus *Sphingopyxis*.

Fatty acid methyl ester analysis was performed as described by Prakash *et al.* (2007a). The polar lipid profile of strain UI2^T was generated by two-dimensional TLC as described by Gupta *et al.* (2009). Aqueous premulin (1 %) solution was used for detection of lipids under UV light. Polyamines were extracted as described by Busse & Auling (1988) and analysed by TLC (silica gel 60 F₂₅₄ 20 × 20 cm, 1.05554.0007; Merck) at IMTECH (Chandigarh, India). Quinones were extracted from 200 mg dry cell mass with a 10 % aqueous solution of 0.3 % (w/v) NaCl in methanol and petroleum ether (boiling point 60–80 °C) at a ratio of 1:1 (v/v). The upper phase was collected and dried in a Buchi Rotavapor and the residue was dissolved in 100 µl acetone. The extract was analysed by TLC, as above using petroleum ether and diethyl ether at a ratio of 85:15 (v/v). Purified ubiquinones were dissolved in 2-propanol and analysed by reversed-phase TLC according to Collins & Jones (1980).

The predominant fatty acids of strain UI2^T were C_{18:1}ω7c (28.3 %), C_{16:0} (14.4 %), 11-methyl C_{18:1}ω7c (10.9 %), C_{17:1}ω6c (9.6 %) and summed feature 3 (consisting of C_{16:1}ω7c and/or C_{15:0} iso 2-OH; 14.7 %) (Supplementary Table S2). C_{16:1}ω5c (3.3 %) and C_{18:0} (2.4 %) were also detected, along with three characteristic hydroxylated fatty acids, C_{14:0} 2-OH (6.1 %), C_{15:0} 2-OH (4.1 %) and C_{16:0}

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationship of strain UI2^T and members of related genera. The sequence of *Erythrobacter litoralis* DSM 8509^T was used as an outgroup. Bootstrap values (>500) based on 1000 resamplings are shown at branch nodes. Bar, 0.02 substitutions per nucleotide position.

2-OH (3.2 %). In addition, C_{19:0} cyclo ω 8c (3.2 %), which has not been reported earlier in the genus *Sphingopyxis*, was also detected in strain UI2^T. Fatty acid analysis confirmed the inclusion of strain UI2^T in the genus *Sphingopyxis*. The polar lipid analysis of strain UI2^T exhibited the presence of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylmonomethylethanolamine, sphingoglycolipids and traces of unidentified glycolipids and lipids (Supplementary Fig. S2). These are the common polar lipids reported for sphingomonads (Busse *et al.*, 1997). Strain UI2^T contained both spermidine and spermine, and spermidine was identified as the major polyamine. Strain UI2^T contained ubiquinone Q-10 as the major respiratory quinone. The G+C content of strain UI2^T, calculated by the method described by Gonzalez & Saiz-Jimenez (2002) and the Applied Biosystems 7500 Real-Time PCR system, was found to be 68 mol%.

The results of the chemotaxonomic analyses are given in the species description and Table 1. Growth at 4, 10, 20, 28, 33, 37 and 43 °C, at pH 4–9 (in increments of one pH unit) and with 0, 1, 3, 5 and 7 % (w/v) NaCl was tested in LB as described by Arden Jones *et al.* (1979). All biochemical tests were carried out at 28 °C. Acid production from carbohydrates and assimilation of substrates on mineral medium were determined as described by Gordon *et al.* (1974). Production of indole and hydrolysis of casein, gelatin, starch and aesculin were tested as described by Cowan & Steel (1965). Degradation of α -, β -, γ - and δ -isomers of HCH was investigated as described by Kumari *et al.* (2002). Nitrate reduction was tested as described by Prakash *et al.* (2007a). Catalase production was tested with 3 % (v/v) hydrogen peroxide solution as described by McCarthy & Cross (1984). Oxidase production was tested using bacteriological differentiation discs. Urease activity was tested according to Christensen (1946). Hydrolysis of Tween 20 was tested according to Arden Jones *et al.* (1979). Degradation of hypoxanthine and xanthine was tested according to Gordon *et al.* (1974). β -Galactosidase activity was observed using ONPG discs. Susceptibility towards antibiotics was determined on Mueller–Hinton agar using ready-made discs as follows (μ g per disc): ampicillin (10), chloramphenicol (30), chlortetracycline (30), erythromycin (15), gentamicin (10), kanamycin (30), neomycin (30), novobiocin (30), oxytetracycline (30), penicillin G (10), rifampicin (5), tetracycline (30) and vancomycin (30).

On the basis of the phenotypic, chemotaxonomic and phylogenetic analysis, it is proposed that strain UI2^T represents a novel species, for which the name *Sphingopyxis ummariensis* sp. nov. is proposed.

Description of *Sphingopyxis ummariensis* sp. nov.

Sphingopyxis ummariensis (um.ma.ri.en'sis. N.L. fem. n. *ummariensis* pertaining to Ummari village, the place of isolation of the type strain).

Table 1. Differential morphological and physiological characteristics of strain UI2^T and other members of genus *Sphingopyxis*

Strains: 1, *Sphingopyxis ummariensis* sp. nov. UI2^T; 2, *S. terrae* DSM 8831^T; 3, *S. witflariensis* DSM 14551^T; 4, *S. taejonensis* DSM 15583^T; 5, *S. alaskensis* DSM 13593^T; 6, *S. chilensis* DSM 14889^T; 7, *S. macrogoltabida* DSM 8826^T; 8, *S. ginsengisoli* KCTC 12582^T; 9, '*S. granuli*' KCTC 12209. Data were taken from this study. All strains were Gram-negative, rod-shaped, motile, positive for catalase, oxidase and Tween 20 hydrolysis and negative for utilization of citrate and adonitol, hydrolysis of casein, hypoxanthine, xanthine and starch, indole production and nitrate reduction. +, Positive; (+), weakly positive; v, variable reaction in triplicate samples; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9
Colony colour*	Y	LY/DY	Y	PY	Y–BG	Y	WHB/Y	Y–BG	WH
Assimilation of:									
L-Arabinose	+	–	–	–	–	–	+	–	–
Fructose	+	–	–	+	–	+	–	–	–
Lactose	(+)	–	(+)	–	–	–	+	–	–
D-Mannitol	–	+	–	–	–	–	(+)	–	–
D-Ribose	+	+	–	–	–	–	ND	–	–
Sorbitol	(+)	+	–	–	–	+	(+)	–	–
Xylose	+	–	–	+	–	(+)	–	–	–
Hydrolysis of:									
Aesculin	+	v	–	–	+	+	(+)	+	+
Gelatin	–	–	–	–	–	–	v	–	–
Urea	–	–	–	–	v	–	–	–	–
β -Galactosidase	–	–	–	+	+	(+)	+	–	–
Citrate utilization	–	–	–	–	–	–	–	–	+

*BG, Beige; DY, deep yellow; LY, light yellow; PY, pale yellow; WH, white; WHB, whitish brown; Y, yellow.

Gram-negative, motile, aerobic, rod-shaped bacterium. Subpolar flagellum present. Cell size 0.8 × 0.1 μ m. Colonies are yellow and circular with entire margins, 1.2–2 mm in diameter after 72 h of incubation on LB agar. Optimum conditions for growth are 28 °C, pH 7.5 and 1 % NaCl. Does not degrade α -, β -, γ - or δ -isomers of HCH (5 μ g ml^{–1}). Nitrate is not reduced to nitrite or nitrogen. Catalase- and oxidase-positive. Indole production is negative. No β -galactosidase activity detected. Hydrolyses aesculin and Tween 20, but does not hydrolyse gelatin, starch, casein, urea, hypoxanthine or xanthine. Acid is produced from trehalose, D-glucose and D-ribose. Assimilates cellobiose, maltose, D-mannose, D-ribose, fructose, D-glucose, L-arabinose, xylose and trehalose; assimilates galactose, lactose and sorbitol weakly. Sensitive to chloramphenicol, chlortetracycline, erythromycin, gentamicin, kanamycin, neomycin, novobiocin, oxytetracycline, penicillin G, rifampicin, tetracycline and vancomycin; less sensitive to

ampicillin. Major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and sphingoglycolipid. The fatty acids C_{18:1}ω7c, C_{16:0}, 11-methyl C_{18:1}ω7c, C_{17:1}ω6c, C_{19:0} cyclo ω8c and summed feature 3 (C_{16:1}ω7c 2-OH and/or C_{15:0} iso 2-OH) are detected in addition to C_{14:0} 2-OH, C_{15:0} 2-OH, C_{16:0} 2-OH, C_{16:1}ω5c and C_{18:0}. Ubiquinone Q-10 is the major respiratory quinone. The polyamines spermidine and spermine are present. The DNA G+C content of the type strain is 68 mol%.

The type strain, UI2^T (=CCM 7428^T =MTCC 8591^T), was isolated from HCH-contaminated soil at Ummari village, Lucknow, India.

Acknowledgements

This research was supported by funds from Department of Biotechnology (DBT) and National Bureau of Agriculturally Important Microorganisms (NBAIM), Government of India. P. S., M. V., K. B. and A. N. gratefully acknowledge the Council of Scientific and Industrial Research (CSIR) and DBT for providing research fellowships and SAIF-DST, Department of Anatomy, AIIMS for providing the TEM facility. The authors also thank M. Dadhwal and S. K. Gupta for work on the HCH dump site and polar lipid profiling, respectively.

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