Sphingomonas kaistensis sp. nov., a novel alphaproteobacterium containing *pufLM* genes

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Three Gram-negative, non-motile, non-spore-forming short rods (strains PB56^T, PB180, PB229) were isolated from soil in South Korea. Cells were orange–red in colour. Strains PB180 and PB229 contained small amounts of bacteriochlorophyll *a*, which was not detected in strain PB56^T. However, all three isolates contained the genes for the photosynthetic type II reaction centre, *pufLM*. They contained Q-10 as the dominant quinone and C_{18:1} as the dominant fatty acid. The highest 16S rRNA gene sequence similarities were found to *Sphingomonas oligophenolica* JCM 12082^T (95.8%), *Sphingomonas koreensis* KCTC 2882^T (95.1%), *Sphingomonas mali* IFO 15500^T (95.1%), *Sphingomonas faeni* DSM 14747^T (94.8%), *Sphingomonas pruni* IFO 15498^T (94.7%) and *Sphingomonas aquatilis* KCTC 2881^T (94.6%), as well as to *Sphingosinicella microcystinivorans* Y2^T and *Sphingosinicella xenopeptidilytica* 3-2W4^T (95.0–95.2%). Phylogenetic analyses supported the assignment of strains PB56^T, PB180, PB229 to the genus *Sphingomonas*. The novel isolates differ from all established species of the genus *Sphingomonas* by their higher G+C content and the absence of straight-chain 2-hydroxy fatty acids. Based on the phylogenetic distances from species with validly published names and their phenotypic properties, the strains constitute a separate species, for which the name *Sphingomonas kaistensis* sp. nov. is proposed. The type strain is PB56^T (=KCTC 12334^T = DSM 16846^T).

Based on the current classification, the *Sphingomonadales* represents one of the six orders of the *Alphaproteobacteria* and includes the single family *Sphingomonadaceae* (Yabuuchi & Kosako, 2005). Bacteria of the order *Sphingomonadales* are characterized by glucuronosyl ceramide and 2-OH myristic acid or non-hydroxy myristic acid, which replace the lipopolysaccharides of the Gram-negative cell wall. They contain octadecenoic acid (C_{18:1}ω7c, C_{18:1}ω9t) as the major non-polar fatty acid and ubiquinone Q-10 as the major respiratory quinone.

Members of the *Sphingomonadaceae* have been repeatedly reclassified and presently comprise the six chemo-organotrophic genera *Sphingomonas*, *Sphingobium*, *Sphingopyxis*,

Sphingosinicella, *Novosphingobium* and *Zymomonas* and six genera of aerobic anoxygenic phototrophic bacteria, *Blastomonas*, *Erythrobacter*, *Erythromicrobium*, *Porphyrobacter*, *Sandaracinobacter* and *Sandarakinorhabdus*. ‘*Lutibacterium*’ and ‘*Citromicrobium*’ represent two additional lineages (Takeuchi *et al.*, 2001; Yabuuchi & Kosako, 2005; Gich & Overmann, 2006).

In an attempt to assess the diversity of aerobic phototrophic bacteria systematically, we isolated a large number of red-, orange- and yellow-pigmented bacteria from surface soil collected on the campus of the Korea Advanced Institute of Science and Technology (KAIST) in Daejeon city, South Korea. After collection, 1 g soil was suspended in distilled water and serially diluted (1:10) three times. Aliquots (100 µl) of each dilution were spotted on 10-fold-diluted R2A agar (Difco) plates and the plates were incubated at 25 °C. Isolated strains were also subcultured on undiluted R2A medium. For screening of the isolates, the *pufL* and *pufM* genes, which encode the typical type II photosynthetic reaction centre of proteobacteria, were amplified and sequenced as described by Nagashima *et al.* (1997). Of over 300 isolates, about 100 were found to contain the *pufL* and

Abbreviations: BChl *a*, bacteriochlorophyll *a*; FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains PB56^T, PB180 and PB229 are respectively AY769083, AY769084 and AY785128.

Details of the analysis of the carotenoids of the novel strains are available in a supplementary figure and table with the online version of this paper.

pufM genes. The three orange-red-pigmented strains PB56^T, PB180 and PB229 were chosen for detailed characterization and were found to represent a novel lineage among the *Sphingomonadaceae*.

For an initial phylogenetic characterization, chromosomal DNA was extracted and purified using the DNeasy Tissue kit (Qiagen). PCR amplification of 16S rRNA genes and direct sequencing of the purified PCR products were performed as described previously (Yoon *et al.*, 1997, 1998), thereby obtaining the almost-complete (i.e. 1393–1421 nt) 16S rRNA gene sequences of the strains. The 16S rRNA gene sequences of the closest relatives were retrieved by a BLAST search (Altschul *et al.*, 1997) and the sequences were aligned with the CLUSTAL_X program (Thompson *et al.*, 1997). Evolutionary distances were then calculated using the Kimura two-parameter model (Kimura, 1983) and a phylogenetic tree was constructed by neighbour joining (Saitou & Nei, 1987) in MEGA version 2.1 (Kumar *et al.*, 2001) with bootstrap values based on 1000 replications (Felsenstein, 1985).

Based on the phylogenetic analysis (Fig. 1), strains PB56^T, PB180 and PB229 are most closely related to species of the genus *Sphingomonas*, but form a distinct lineage which is supported by a bootstrap value of 100 %. The closest relatives of strain PB56^T are *Sphingomonas oligophenolica* JCM 12082^T (95.8 % 16S rRNA gene sequence similarity), *Sphingomonas koreensis* KCTC 2882^T (95.1 %), *Sphingomonas mali* IFO 15500^T (95.1 %), *Sphingomonas faeni* DSM 14747^T (94.8 %), *Sphingomonas pruni* IFO 15498^T (94.7 %), and *Sphingomonas aquatilis* KCTC 2881^T (94.6 %). Although the 16S rRNA gene sequences of *Sphingosinicella microcystinivorans* Y2^T and *Sphingosinicella xenopeptidilytica* 3-2W4^T also showed rather high similarity (95.0–95.2 %), the three new isolates clustered with members of the genus *Sphingomonas*. This branching pattern was clearly supported by our bootstrap analysis (Fig. 1). Lower sequence similarities were found with the other described *Sphingomonas* species (<94.6 %) and with *Sandaracinobacter sibiricus* (92.3 %). The values of 16S rRNA gene sequence similarity suggest that the isolated strains represent a novel bacterial species within the genus *Sphingomonas*. The closest phylogenetic relatives listed above were selected for subsequent comparisons of physiological and biochemical features (Tables 1 and 2).

The G + C content of the genomic DNA was determined using HPLC after enzymic degradation of the DNA to nucleosides (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984). Genomic DNA of strains PB56^T, PB180 and PB229 had a G + C content between 69.1 and 69.9 mol% (Table 1), whereas the G + C content of all *Sphingomonas* species described to date ranges between 59 and 67 mol% (Yabuuchi *et al.*, 2002).

The 16S rRNA gene sequence similarities between the three strains PB56^T, PB180 and PB229 were 98.6–98.8 %. DNA–DNA hybridization was performed in five replicates by the

fluorometric method of Ezaki *et al.* (1989). The DNA–DNA hybridization values between strains PB56^T and PB180 were 67.7 ± 11.7 % and those between strains PB56^T and PB229 were 73.1 ± 5.0 %. Based on the commonly accepted criteria for the delineation of a bacterial species (≥ 70 % DNA–DNA hybridization and ≥ 97 % 16S rRNA gene sequence similarity; Rosselló-Mora & Amann, 2001), and considering the values of the standard deviations, the three isolated strains represent members of a single species.

All three strains formed orange-red, circular, convex and smooth colonies. As observed by phase-contrast microscopy (Zeiss Axiostar Plus), cells grown for 4 days at 25 °C in R2A broth were non-motile rods, 0.7 µm wide and 1.1 µm long. Cells were Gram-negative as determined by the KOH method (Buck, 1982), oxidase-positive (determined by oxidation of 1 % *p*-aminodimethylaniline oxalate) and catalase-positive (determined by gas production upon addition of 3 % H₂O₂). Growth was obligately aerobic. Reduction of nitrate and production of indole was not observed. The optimum temperature for growth on R2A agar was 25–30 °C. The strains did not require any vitamins. They did not grow on MacConkey agar (Difco) and only strain PB180 grew readily on nutrient agar at 25 °C (Table 1).

Substrate utilization was assessed with the API 20NE and API 32GN systems and enzyme activities were assessed with the API ZYM microtest system according to the recommendations of the manufacturer (bioMérieux). For reference, we chose the type strains of the five most closely related *Sphingomonas* species (see above) and *Sphingosinicella microcystinivorans* Y2^T. In addition, the type strains of the four orange-pigmented species *Sphingomonas abaci*, *Sphingomonas aerolata*, *Sphingomonas aurantiaca* and *Sphingomonas roseiflava* were included in this comparison. The three newly isolated strains utilized D-glucose, L-rhamnose, N-acetylglucosamine, D-mannitol, 4-hydroxybenzoate, L-proline, L-serine and glycogen. In addition, L-arabinose, maltose, mannose, malate, malonate, adipate, caprate and salicin were utilized by individual strains. Of the enzymes tested, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and trypsin were present (Table 1). The three novel isolates differ from most of their closest phylogenetic relatives by the capacity to use D-mannitol, L-serine and 4-hydroxybenzoate, the poor utilization of gluconate and by the lack of β-glucosidase (aesculin hydrolysis) and β-galactosidase and the lack of growth on tryptic soy agar (Table 1).

For the analysis of fatty acids, the three strains were cultivated for 4 days at 25 °C on R2A agar. The cell mass was saponified and fatty acid methyl esters (FAMES) were methylated and then extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, Inc.). Fatty acids were analysed by gas chromatography (Hewlett Packard 6890) and identified by the Microbial Identification software package (Sasser, 1990).

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strains PB56^T, PB180 and PB229 and other related taxa based on 16S rRNA gene sequences. Bar, 0.01 substitutions per nucleotide position. Numbers at nodes give bootstrap values for each node out of 100 bootstrap resamplings (only values >50 are shown).

Table 1. Biochemical and chemotaxonomic characteristics of strains PB56^T, PB180 and PB229 and related taxa

Strains: 1, PB56^T; 2, PB180; 3, PB229; 4, *Sphingosinicella microcystinivorans* (three strains) (data from Maruyama *et al.*, 2006); 5, *Sphingomonas abaci* C42^T (Busse *et al.*, 2005); 6, *Sphingomonas aerolata* (three strains) (Busse *et al.*, 2003); 7, *Sphingomonas aurantiaca* (three strains) (Busse *et al.*, 2003); 8, *Sphingomonas faeni* DSM 14747^T (Busse *et al.*, 2003); 9, *Sphingomonas aquatilis* KCTC 2881^T (Lee *et al.*, 2001); 10, *Sphingomonas koreensis* (two strains) (Lee *et al.*, 2001); 11, *Sphingomonas mali* (three strains) (Takeuchi *et al.*, 1995); 12, *Sphingomonas pruni* (three strains) (Takeuchi *et al.*, 1995); 13, *Sphingomonas oligophenolica* S213^T (Ohta *et al.*, 2004); 14, *Sphingomonas roseiflava* IAM 14823^T (Yun *et al.*, 2000). All strains tested, including PB56^T, PB180 and PB229, were positive for catalase and the presence of sphingolipids and negative for indole production, urease and assimilation of 5-ketogluconate and phenylacetate. Positive results were obtained for strains PB56^T, PB180 and PB229 for the following tests, but no data are available for the other strains: utilization of L-proline and glycogen, growth on R2A and presence of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and trypsin. Negative results were obtained for strains PB56^T, PB180 and PB229 for the following tests, with no data available for the other strains: utilization of 2-ketogluconate, L-alanine, propionate and valerate, growth on MacConkey agar and activity of lipase (C14), *N*-acetyl- β -glucosaminidase, α -fucosidase, α -galactosidase, α -mannosidase and β -glucuronidase. ++, Strongly positive; +, all strains positive; –, all strains negative; v, variable among strains of one species; w, weakly positive; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Motility	–	–	–	+	–	+	+	+	+	+	+	+	–	ND
Cell length (μ m)	1.5	0.7	1.5	0.6–1.0	1.5–3.0	1.5–2.6	1.0–2.7	2.0–2.6	ND	ND	ND	ND	1.0–1.5	ND
Cell width (μ m)	1.0	0.5	1.0	0.3–0.7	0.4–0.5	0.6–0.8	0.6–0.8	0.6–0.8	ND	ND	ND	ND	0.4–0.6	ND
Colony colour*	PR	PR	PR	Y	O	O	O	O	Y	Y	Y	Y	Y	PY
Oxidase	+	+	+	+	–	+	+	+	+	+	ND	ND	+	+
Reduction of nitrate	–	–	–	–	ND	–	–	–	–	–	–	–	+	+
Production of acid from glucose	W	–	W	–	–	ND	ND	ND	–	–	–	W	–	–
Enzyme activities														
Protease (gelatin hydrolysis)	–	–	–	–	ND	–	–	–	–	–	–	–	+	+
β -Galactosidase (PNPG hydrolysis)	–	–	–	–	+	+	W	W	+	+	+	+	ND	ND
α -Glucosidase (aesculin hydrolysis)	–	–	–	–	+	+	+	+	–	–	+	+	ND	+
Assimilation of:														
Acetate	–	–	–	ND	ND	+	+	+	ND	ND	–	W	+	ND
Adipate	+	–	–	–	ND	W	W	W	–	–	+	–	ND	W
Caproate	–	–	+	–	ND	ND	ND	ND	+	–	–	–	ND	ND
Citrate	–	–	–	+	–	ND	ND	+	–	–	–	–	–	+
Gluconate	W	–	W	–	W	+	+	+	–	–	+	+	+	+
DL-Lactate	–	–	–	ND	–	V	+	+	ND	ND	–	–	+	ND
Malate	+	–	–	–	ND	V	+	–	–	+	–	W	–	+
Malonate	–	–	+	ND	ND	ND	ND	–	–	–	–	–	–	ND
4-Hydroxybenzoate	+	+	+	ND	ND	–	V	–	ND	–	–	–	+	ND
Propionate	–	–	–	ND	ND	–	V	+	ND	ND	–	–	ND	–
L-Arabinose	–	–	+	–	–	+	+	+	+	–	W	+	+	+
D-Glucose	+	+	+	–	–	+	+	+	+	+	+	+	+	+
Maltose	+	–	+	–	–	+	+	+	+	+	+	+	+	ND
D-Mannose	+	–	W	–	–	+	+	+	–	–	+	+	+	W
D-Melibiose	–	–	+	ND	ND	V	+	+	–	+	+	W	ND	ND
L-Rhamnose	++	+	++	ND	–	–	–	+	+	+	+	+	ND	–
Sucrose	–	–	+	ND	–	+	+	+	+	+	+	+	ND	ND
D-Mannitol	+	+	+	–	ND	–	–	–	ND	ND	–	–	ND	ND
D-Sorbitol	–	–	+	ND	ND	–	–	–	–	–	–	–	ND	–
<i>N</i> -Acetylglucosamine	+	+	++	–	–	+	+	+	+	+	+	+	ND	–
Salicin	–	+	+	ND	ND	+	+	+	+	+	+	+	ND	ND
L-Serine	+	+	+	ND	ND	–	–	–	ND	+	–	–	ND	ND
L-Histidine	–	–	–	ND	–	–	–	–	–	+	–	–	–	ND
DNA G + C content (mol%)	69.9	69.9	69.1	63.6–63.7	ND	65.4	64.7	63.1	63.0	66.0	65.4–65.9	65.4	64.2	67.2

*O, Orange; PR, pink-red; PY, pink-yellow; Y, yellow.

Table 2. Cellular fatty acid profiles of the novel isolates in comparison with closely related species of the order *Sphingomonadales*

Strains: 1, PB56^T; 2, PB180; 3, PB229; 4, *Sphingosinicella microcystinivorans* Y2^T (data from Maruyama *et al.*, 2006); 5, *Sphingomonas oligophenolica* S213^T (Ohta *et al.*, 2004); 6, *Sphingomonas koreensis* (mean values for two strains) (Lee *et al.*, 2001); 7, *Sphingomonas faeni* DSM 14747^T (Busse *et al.*, 2003); 8, *Sphingomonas aquatilis* KCTC 2881^T (Lee *et al.*, 2001). –, Below detection limit; tr, trace amount (<0.5%). For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. C_{17:0}, C_{17:0} iso and C_{17:1} ω 5c were below the detection limit in all strains.

Fatty acid	1	2	3	4	5	6	7	8
Saturated fatty acids								
C _{14:0}	–	0.6	0.7	1.0	3.0	1.4	2.0	0.7
C _{15:0}	–	0.9	0.6	–	–	0.2	–	–
C _{16:0}	10.0	15.7	12.4	8.0	10.0	10.9	11.2	20.6
C _{18:0}	–	–	–	tr	–	0.5	–	1.0
C _{19:0} cyclo ω 8c	–	–	–	–	–	–	0.8	–
Unsaturated fatty acids								
C _{16:1}	–	–	–	–	6.0	1.4	1.6	0.8
C _{16:1} ω 5c	4.0	2.4	3.8	3.0	–	–	–	–
C _{17:1} *	–	–	–	–	1.0	1.7	2.6	–
C _{17:1} ω 6c	9.6	5.3	6.1	tr	–	–	–	–
C _{17:1} ω 8c	–	0.6	0.8	–	–	–	–	–
C _{18:1} ω 5c	–	–	–	1.0	–	1.8	1.0	1.5
Hydroxy fatty acids								
C _{12:0} 2-OH	–	–	–	tr	–	–	0.6	–
C _{14:0} 2-OH	–	–	–	12.0	19.0	9.8	9.7	10.9
C _{15:0} 2-OH	–	–	–	tr	–	0.3	–	–
C _{16:0} 2-OH	–	–	–	1.0	–	0.8	–	–
C _{16:0} iso 3-OH	–	–	–	tr	–	–	–	–
C _{16:1} 2-OH	–	–	–	tr	–	–	–	–
C _{18:1} 2-OH	–	0.9	–	–	–	–	–	–
Summed features†								
Summed feature 4	36.3	31.6	35.9	34.0	–	2.1	29.2	4.6
Summed feature 7	40.0	41.9	39.7	38.0	56.0	69.2	41.4	59.9

*The position or stereochemistry of the double bond was not specified in these studies.

†Summed features are groups of two or three fatty acids that cannot be separated reliably by GLC with the MIDI system. Summed feature 4 contained C_{16:1} ω 7c and/or C_{15:0} iso 2-OH; summed feature 7 contained C_{18:1} ω 7c/ ω 9t/ ω 12t.

Strains PB56^T, PB180 and PB229 showed cellular fatty acid profiles with large amounts of the saturated and unsaturated fatty acids C_{16:0} (12.8%), C_{16:1} (3.2%), C_{17:1} (7.5%), summed feature 7 (C_{18:1} ω 7c/ ω 9t/ ω 12t) (41.0%) and summed feature 4 (C_{16:1} ω 7c/C_{15:0} iso 2-OH) (33.9%) (Table 2). No significant differences in the fatty acid profiles were found between the three isolates. There were some

differences in FAME composition between these strains and their nearest phylogenetic neighbours in the genera *Sphingomonas*, *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter*. The common dominant fatty acids for all strains are C_{16:0} and summed feature 7. Strains PB56^T, PB180 and PB229 differ from the related *Sphingomonas* species with respect to the absence of 2-OH myristic acid (C_{14:0} 2-OH), which dominates the 2-hydroxy fatty acids in all other groups. 2-Hydroxy fatty acids are not completely absent in the novel isolates, however, since C_{18:1} 2-OH occurs in strain PB180 (Table 2). Furthermore, C_{15:0} iso 2-OH (contained in summed feature 4) is potentially present in all three strains. Another conspicuous feature of strains PB56^T, PB180 and PB229 is the large amount of fatty acids of summed feature 4 (C_{16:1} ω 7c/C_{15:0} iso 2-OH), which has so far been found only in *Sphingomonas faeni* (Busse *et al.*, 2003; Yabuuchi & Kosako, 2005) and *Sphingosinicella microcystinivorans* Y2^T (Maruyama *et al.*, 2006).

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by TLC on Merck silica gel 60F₂₅₄ plates (20 × 20 cm, 0.5 cm thick) using a mixture of petroleum benzene and diethyl ether (85:15, v/v) as the solvent. Spots were scraped off and then analysed by HPLC as described earlier (Collins & Jones, 1981; Shin *et al.*, 1996). The major isoprenoid quinone of isolates PB56^T, PB180 and PB229 was ubiquinone Q-10, as in all known members of the *Sphingomonadaceae* (Rainey *et al.*, 2003).

Within the genus *Sphingomonas sensu stricto*, only *Sphingomonas* (*Blastomonas*, *Erythromonas*) *ursincola* DSM 9006^T and *Sphingomonas* (*Blastomonas*) *natatoria* DSM 3183^T are currently known to be capable of synthesizing bacteriochlorophyll (BChl) *a* (Yabuuchi & Kosako, 2005). The other BChl *a*-containing members of the family *Sphingomonadaceae* are found within the genera *Erythrobacter* (*Erythrobacter longus* DSM 6997^T, *Erythrobacter litoralis* DSM 8509^T), *Erythromicrobium* [*Erythromicrobium ramosum* DSM 8510^T, ‘*Erythromicrobium ezovicum*’ E-1, ‘*Erythromicrobium hydrolyticum*’ E4(1)] and *Porphyrobacter* (*Porphyrobacter cryptus* DSM 12079^T, *P. dokdonensis* DSM 17193^T, *P. donghaensis* DSM 16220^T, *P. neustonensis* DSM 9434^T, *P. sanguineus* ATCC 25659^T, *P. tepidarius* DSM 10594^T) and also include ‘*Citromicrobium bathyomarinum*’ JF-1, *Sandaracinobacter sibiricus* RB16-17^T and *Sandarakinorhabdus limnophila* so42^T (Hiraishi *et al.*, 2002; Rainey *et al.*, 2003; Yabuuchi & Kosako, 2005; Gich & Overmann, 2006; Yoon *et al.*, 2004, 2006).

Using the 1.5 kb amplification product obtained for the *pufLM* genes (see above), the respective nucleotide sequences were determined and a phylogenetic tree was constructed as described for the 16S rRNA gene sequences. The *pufLM* gene sequences of strains PB56^T, PB180 and PB229 were found to be most closely related to those of *Sphingomonas* (*Blastomonas*, *Erythromonas*) *ursincola* DSM 9006^T and *Sphingomonas* (*Blastomonas*) *natatoria* DSM 3183^T and to form a distinct lineage separate from all other species of the family *Sphingomonadaceae*, which is

Fig. 2. Neighbour-joining tree showing the phylogenetic positions of strains PB56^T, PB180 and PB229 and other related taxa based on *pufL* and *pufM* gene sequences. Bar, 0.1 substitutions per nucleotide position.

supported by high bootstrap resampling values (Fig. 2). The closer affiliation with the only BChl *a*-containing members of genus *Sphingomonas sensu stricto* rather than the other aerobic anoxygenic phototrophic genera is commensurate with the 16S rRNA gene phylogeny.

In order to test for the presence of BChl *a* and to determine the carotenoid composition, pigments were extracted from cell pellets with methanol/acetone (2:7, v/v) in the dark and consecutively analysed on an HPLC system equipped with a reversed-phase ET 250/8/4 Nucleosil 5 C18 PPN column (Macherey-Nagel) as described by Koblížek *et al.* (2003). Spectra were recorded by an absorption UV/Vis diode array detector and recorded at 450 and 770 nm. At these wavelengths, absorbance coefficients used for quantification were 128.0 mol l⁻¹ cm⁻¹ (Yurkov *et al.*, 1994) for carotenoids and 54.8 mol l⁻¹ cm⁻¹ for BChl *a* (Permentier *et al.*, 2000), respectively. Excreted sulfated carotenoids were extracted directly from lyophilized culture supernatants. To remove the sulfate group, they were hydrolysed with 0.6 M HCl in tetrahydrofuran for 10 min at room temperature (<http://lipidbank.jp/>) and then subjected to HPLC analysis.

All strains produced numerous carotenoids. With the exception of spirilloxanthin and β -carotene, which constituted only minor amounts (peaks 12 and 14; see Supplementary Fig. S1 available in IJSEM Online), all other compounds differed from known carotenoids with respect to retention time and absorption spectrum (Supplementary Table S1). However, most carotenoids were ketocarotenoids, as indicated by their absorption spectra, which exhibited only one broad maximum (data not shown). The main carotenoid was a very polar xanthophyll sulfate (compound 2 in

Supplementary Fig. S1), but differed from erythroxanthin sulfate or caloxanthin sulfate produced by *Erythrobacter longus* and *Erythromicrobium ramosum* (Supplementary Table S1). The spectra of PB180 and PB229 were almost identical (Supplementary Fig. S1b), but differed from the spectrum of PB56^T (Supplementary Fig. S1a and Supplementary Table S1). Strains PB180 and PB229 released part of the sulfated carotenoid into the medium during growth. The excreted sulfated carotenoid corresponded to peak 2 of the cellular extract.

No BChl *a* was detected in cells of strain PB56^T, whereas cells of strains PB180 and PB229 contained small amounts of BChl *a*. Aerobic anoxygenic photosynthetic bacteria analysed so far contain BChl *a* esterified with phytol (BChl *a*_P; Takaichi, 1999). Strain PB229 also had BChl *a*_P (Supplementary Fig. S1b, peak c). In addition, PB180 contained two more types of BChl *a*, tentatively identified as BChl *a* esterified with farnesol (BChl *a*_F; Supplementary Fig. S1b, peak a) and BChl *a* esterified with geranylgeraniol (BChl *a*_{GG}; Supplementary Fig. S1b, peak b) based on a comparison with pigment extracts from *Rhodobacter capsulatus* DSM 155 and from *Rhodospirillum rubrum* DSM 107. The ratio of BChl *a* to cell dry weight ranged from 0.5 (strain PB229) to 12.2 (PB180) $\mu\text{g g}^{-1}$, depending on the medium composition and the illumination. The maximum cellular BChl *a* content in strain PB180 thus reached 6.4×10^{-22} mol per cell, which is about 200 times lower than the average BChl *a* content of aerobic anoxygenic photosynthetic bacteria (1.2×10^{-19} mol per cell; Jiao *et al.*, 2003; Kolber *et al.*, 2001). The ratio of BChl *a* to carotenoids in the cells of strains PB180 and PB229 ranged from 1:60 to 1:600.

Strains PB56^T, PB180 and PB229 thus differ clearly from *Sphingomonas* (*Blastomonas*) *ursincola* DSM 9006^T and *Sphingomonas natatoria* DSM 3183^T and from the type species of the aerobic anoxygenic phototrophic genera *Sandaracinobacter*, *Erythrobacter*, *Erythromicrobium* and *Porphyrobacter* in that they were non-motile, differed in colour, did not contain nostoxanthin and zeaxanthin and had no (PB56^T) or only trace amounts (PB180, PB229) of BChl *a*. These morphological and physiological differences are in accordance with the sequence divergence of the respective 16S rRNA and *pufLM* genes.

Based on the results of the present characterization, strains PB56^T, PB180 and PB229 are classified as members of the genus *Sphingomonas sensu stricto* (Takeuchi *et al.*, 2001). However, the three strains represent a separate phylogenetic lineage and exhibit considerable physiological differences from the recognized *Sphingomonas* species such as non-motile cells, orange-red colour, lack of β -glucosidase and β -galactosidase, utilization of D-mannitol, L-serine and 4-hydroxybenzoate and lack of 2-OH myristic acid. Therefore, strains PB56^T, PB180 and PB229 are assigned to a novel species, for which the name *Sphingomonas kaistensis* sp. nov. is proposed.

Description of *Sphingomonas kaistensis* sp. nov.

Sphingomonas kaistensis [ka.is.ten'sis. N.L. fem. adj. *kaistensis* of or pertaining to the Korea Advanced Institute of Science and Technology (KAIST)].

Cells are 0.7 µm wide and 1.1 µm long. Gram-negative, non-motile, non-spore-forming rods that grow aerobically. Colonies are circular, entire, low-convex, smooth, opaque and orange-red-coloured and grow chemo-organotrophically on R2A agar (Difco) without requirement for vitamins. All known strains contain two structural genes of the type II photosynthetic reaction centre (*pufL* and *pufM*), but BChl *a* synthesis is detected in strains PB180 and PB229 only in very small amounts. Oxidase- and catalase-positive. Optimum temperature is 25–30 °C. Nitrate is not reduced to nitrite or N₂. Indole is not produced. Strains are positive for enzyme activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and trypsin and negative for enzyme activities of arginine dihydrolase, lipase (C14), *N*-acetyl-β-glucosaminidase, protease (gelatin hydrolysis), urease, β-glucosidase (aesculin hydrolysis), α-galactosidase, β-galactosidase (PNPG hydrolysis), β-glucuronidase, α-fucosidase and α-mannosidase. Strains do not grow on MacConkey agar. All strains utilize D-glucose, L-rhamnose, D-mannitol, *N*-acetylglucosamine, 4-hydroxybenzoate, L-proline, L-serine and glycogen. 2-Ketogluconate, 5-ketogluconate, acetate, propionate, valerate, citrate, lactate, phenylacetate, L-alanine and L-histidine are not utilized. The DNA base composition is 69.1–69.9 mol% G + C. The major isoprenoid quinone is ubiquinone Q-10 and major cellular fatty acids are C_{18:1}, summed feature 4 (C_{16:1ω7c}/C_{15:0} iso 2-OH) and C_{16:0}, but cells lack any 2-hydroxy fatty acid.

The type strain is PB56^T (=KCTC 12334^T=DSM 16846^T), which was isolated from soil on the campus of KAIST in Daejeon city in South Korea.

Acknowledgements

This work was supported by the International Research Internship Program of the Korea Science and Engineering Foundation (KOSEF) to M. K. K. and a grant of the BMBF (Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie) to J.O. (Biolog/01LC0021).

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