Sphingomonas jaspsi sp. nov., a novel carotenoid-producing bacterium isolated from Misasa, Tottori, Japan

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A yellow-pigmented, Gram-negative, motile, strictly aerobic, pleomorphic bacterium (strain TDMA-16^T) was isolated from a freshwater sample collected at Misasa (Tottori, Japan). Strain TDMA-16^T was slightly tolerant to gamma-ray irradiation and produced carotenoids, including zeaxanthin, nostoxanthin and an unknown carotenoid, effectively [1.7 mg (g dry cells)⁻¹]. The DNA G+C content of strain TDMA-16^T was 63.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences placed strain TDMA-16^T in a distinct lineage in the family *Sphingomonadaceae*; sequence data showed that strain TDMA-16^T was most closely related to *Sphingomonas mali* IFO 15500^T (95.1%), *Sphingomonas aquatilis* JSS7^T (95.0%), *Sphingomonas pruni* IFO 15498^T (94.9%), *Sphingomonas melonis* DSM 14444^T (94.9%) and *Sphingomonas asaccharolytica* IFO 15499^T (94.5%). The major fatty acids of strain TDMA-16^T were C_{17:1}ω6c (34.5%) and C_{18:1}ω7c (29.3%). The presence of Q-10 as the main ubiquinone, the *Sphingomonadaceae*-specific sphingoglycolipid in the polar lipid profile and 2-hydroxy fatty acids, plus the absence of 3-hydroxy fatty acids, supported identification of this strain as a member of the genus *Sphingomonas sensu stricto*. Phylogenetic distinctiveness and unique phenotypic characteristics differentiated strain TDMA-16^T from closely related *Sphingomonas* species. The results of polyphasic taxonomic analyses suggest that strain TDMA-16^T represents a novel *Sphingomonas* species, for which the name *Sphingomonas jaspsi* sp. nov. is proposed. The type strain is strain TDMA-16^T (=NBRC 102120^T=DSM 18422^T=CCUG 53607^T).

The family *Sphingomonadaceae* belongs to the class *Alpha-proteobacteria* and was first proposed by Kosako *et al.* (2000). Subsequently, the genus description was emended (Takeuchi *et al.*, 2001; Yabuuchi *et al.*, 2002) and included in the second edition of *Bergey's Manual of Systematic Bacteriology* (Yabuuchi & Kosako, 2005). The family comprises the genera *Blastomonas*, *Erythromonas*, *Sandaracino-bacter*, *Sandarakinorhabdus*, *Sphingomonas*, *Sphingosinicella* and *Zymomonas*; it has been proposed recently that the genus *Sphingomonas*, the type genus of this family that was originally described by Yabuuchi *et al.* (1990), should be divided into four genera, *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* (Takeuchi *et al.*, 2001). Sphingomonads are strictly aerobic, chemoheterotrophic, Gram-negative bacteria that are of biotechnological interest due to their ability to degrade various xenobiotic substances (Ederer *et al.*, 1997; Zablotowicz *et al.*, 1999; Zipper *et al.*, 1996) and also

their potential to produce useful exopolysaccharides (White *et al.*, 1996) and carotenoids (Silva *et al.*, 2004).

Zeaxanthin (3,3'-dihydroxy-β-carotene) is a natural, fat-soluble, yellowish carotenoid that is found in some plants, algae and photosynthetic bacteria (Nelis & De Leenheer, 1989), where it serves as an accessory light-gathering pigment, as well as a protectant against the toxic effects of UV radiation and oxygen radicals. Zeaxanthin is currently utilized as an ingredient in feed for fish and poultry to enhance the pigmentation of animal flesh and egg yolk. Additionally, this pigment has a remarkable potential for use in pharmaceuticals as it prevents age-related macular degeneration and tumour formation. At present, the commercial production of zeaxanthin is carried out mostly by extraction from plant tissues or chemical synthesis. However, there is great potential for the development of a large-scale production system for microbial zeaxanthin.

Recently, some carotenoid-producing bacteria were isolated from freshwater samples that were collected at Misasa (Tottori, Japan), a region known for its high natural radioactivity (Kametani & Matsumura, 1983). A comprehensive taxonomic characterization based on 16S rRNA gene

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TDMA-16^T is AB264131.

SEMs of cells of strain TDMA-16^T are available as supplementary material with the online version of this paper.

sequences has revealed a unique diversity of carotenoid producers that exist in this radioactive region (D. Asker, T. Beppu & K. Ueda, unpublished results). Among the isolates, a unique heterotrophic, aerobic, yellow-pigmented bacterium (strain TDMA-16^T) was found that belongs to the *Sphingomonadaceae*. This paper deals with the taxonomic characteristics of this organism.

Strain TDMA-16^T was isolated from a water sample collected at Misasa on 1 September, 2005 by the conventional dilution-plating method using nutrient agar (NA; Difco). Unless otherwise specified, all the characteristics described hereafter are those of cells of strain TDMA-16^T grown on NA for 48 h at 37 °C. The phylogenetic position of the isolate was studied by using standard analysis based on the 16S rRNA gene sequence. The genomic DNA of strain TDMA-16^T was extracted using a bacterial genomic DNA purification kit (Edge BioSystems). The DNA fragment that contained the nearly complete 16S rRNA gene of strain TDMA-16^T (1431 bp) was amplified using the bacterial universal primers B8F (5'-AGAGTTTGATCCTGGCTCAG; nt 8–27 based on *Escherichia coli* numbering) and B1492R (5'-GGTTACCTTGTTACGACTT; nt 1510–1492 based on *E. coli* numbering). The 16S rRNA gene was sequenced directly by using a BigDye Terminator v3.1 cycle sequencing kit on an ABI 3100 automated DNA sequencer (Applied Biosystems).

A sequence similarity search in the GenBank/EMBL/DBJ nucleotide sequence database performed using the program BLASTN (<http://www.ncbi.nlm.nih.gov/blast/>) revealed that strain TDMA-16^T belongs to the family *Sphingomonadaceae*. Highest similarity was found with sequences of established species of the genus *Sphingomonas sensu stricto* including *Sphingomonas mali* IFO 15500^T (95.1 %), *Sphingomonas aquatilis* JSS7^T (95.0 %), *Sphingomonas pruni* IFO 15498^T (94.9 %), *Sphingomonas melonis* DSM 14444^T (94.9 %), *Sphingomonas asaccharolytica* IFO 15499^T (94.5 %), *Sphingomonas faeni* MA-olki^T (94.3 %), *Sphingomonas aerolata* NW12^T (94.2 %), *Sphingomonas abaci* C42^T (94.2 %), *Sphingomonas echinoides* ATCC 14820^T (94.2 %), *Sphingomonas panni* C52^T (94.0 %) and *Sphingomonas aurantiaca* MA101b^T (93.9 %). These results clearly demonstrated that strain TDMA-16^T represents a novel species within the genus *Sphingomonas sensu stricto*.

A neighbour-joining phylogenetic tree was constructed using the programs CLUSTAL W (Thompson *et al.*, 1994) and NJ plot (Perrière & Gouy, 1996). The tree topology was estimated by bootstrap analysis (Felsenstein, 1993) with 1000 resamplings of the dataset. Phylogenetic analysis of a nearly full-length 16S rRNA gene sequence of strain TDMA-16^T indicated that it is a member of the genus *Sphingomonas sensu stricto* (Takeuchi *et al.*, 2001), forming a distinct phylogenetic lineage (Fig. 1). The 16S rRNA gene

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences of strain TDMA-16^T and representative members of related species of the genus *Sphingomonas sensu stricto*. The tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). Bar, 1 substitution per 100 nt positions. Bootstrap values, expressed as a percentage of 1000 replicates, are given at the branching points. Values above 50 % are shown. GenBank accession numbers are shown in parentheses.

sequence of strain TDMA-16^T retained all the signature nucleotides, namely C:G at position 52:359, G at position 134, G at position 593, G:C at position 987:1218 and U:G at position 990:1215 (based on *E. coli* numbering) (Brosius *et al.*, 1978), that are conserved in the genus *Sphingomonas sensu stricto* (Takeuchi *et al.*, 2001).

Gram staining was performed according to the method described by Smibert & Krieg (1994) and bacterial cells were observed under a Zeiss Axioskop 2 microscope (Carl Zeiss Microimaging). SEM observation was performed using a model VE-8800 (Keyence). Anaerobic growth was assessed on NA incubated in a GasPak anaerobic system (BBL). The following tests were performed as described in each reference: casein hydrolysis (Norris *et al.*, 1985); catalase activity, spore formation and hydrolysis of Tween 80, DNA and starch (Smibert & Krieg, 1994); and hydrolysis of chitin, carboxymethylcellulose (high viscosity; Sigma) and agar (Barrow & Feltham, 1993). Oxidase activity was tested by using commercial cytochrome oxidase test strips (BioChemika). Other enzyme activities, growth on carbohydrates, acid production from carbohydrates, nitrate reduction, and the production of H₂S, indole and acetoin were examined using the commercial systems API 20E, API 20NE and API 50 CH (bioMérieux) according to the manufacturer's instructions. To determine optimal growth temperature, the strain was cultivated on NA at 4, 10, 20, 30, 37, 40 and 45 °C. NaCl tolerance was studied using nutrient broth (NB) supplemented with different concentrations of NaCl. Growth at pH 3.0–10.0 was evaluated in NB adjusted with HCl or NaOH. To determine the survival rate after exposure to gamma radiation, cells of strain TDMA-16^T were grown on NA plates and suspended in NB (5 ml). Aliquots of the cell suspension (1 ml) were irradiated at 2.35 kGy (13 Gy min⁻¹, ¹³⁷Cs source), serially diluted and plated onto NA. The plates were incubated aerobically at 37 °C. *E. coli* C600 was treated similarly. Percentage survival was determined by comparison with unirradiated cultures.

Cells of strain TDMA-16^T were Gram-negative, pleomorphic, motile, short rods (0.4–0.5 × 0.7–1.0 µm). Spores were not observed. Buds formed on the mature cells (see SEMs available as Supplementary Fig. S1 in IJSEM Online). Strain TDMA-16^T formed yellow-pigmented, convex, circular colonies (1–2 mm in diameter) after 48 h incubation at 37 °C. The temperature range for growth of strain TDMA-16^T was 20–40 °C (optimum, 35–37 °C). No growth occurred at 45 °C. The pH range for growth was pH 6.0–9.0 (optimum, pH 6.0–7.0). Strain TDMA-16^T was strictly aerobic and chemo-organotrophic. The strain was positive for oxidase and catalase, but negative in the oxidation/fermentation test and gas/acid production test with glucose. Strain TDMA-16^T was slightly tolerant to gamma-ray irradiation, showing 0.45 % survival rate after exposure to a 2.3 kGy dose (13 Gy min⁻¹, ¹³⁷Cs source). At this dose, the survival rate of *E. coli* C600 was 0 %.

Other phenotypic properties of strain TDMA-16^T are given in the species description and those characteristics

that differentiate strain TDMA-16^T from related members of the genus *Sphingomonas sensu stricto* are listed in Table 1.

Unlike *Sphingomonas mali* IFO 15500^T, *Sphingomonas pruni* IFO 15498^T, *Sphingomonas asaccharolytica* IFO 15499^T, *Sphingomonas melonis* DSM 14444^T and *Sphingomonas dokdonensis* DS-4^T, strain TDMA-16^T showed severe growth arrest in the presence of >0.25 % NaCl. Strain TDMA-16^T differed from *Sphingomonas mali* IFO 15500^T, *Sphingomonas aerolata* NW12^T, *Sphingomonas faeni* MA-olki^T and *Sphingomonas aurantiaca* MA101b^T by: (i) being unable to assimilate L-arabinose, D-mannose, N-acetyl-D-glucosamine, gluconate or adipate; (ii) being able to hydrolyse gelatin and starch; and (iii) being unable to hydrolyse aesculin or DNA. Strain TDMA-16^T differed from *Sphingomonas pruni* IFO 15498^T and *Sphingomonas asaccharolytica* IFO 15499^T by: (i) being unable to assimilate L-arabinose, D-mannose or N-acetyl-D-glucosamine; (ii) being able to hydrolyse gelatin; and (iii) being unable to hydrolyse aesculin. Strain TDMA-16^T differed from *Sphingomonas melonis* DSM 14444^T by: (i) being motile; (ii) being unable to assimilate L-arabinose, D-mannose, N-acetyl-D-glucosamine or DL-malic acid; (iii) being able to hydrolyse gelatin and starch; and (iv) being unable to hydrolyse aesculin. Strain TDMA-16^T differed from *Sphingomonas aquatilis* JSS7^T by: (i) being unable to assimilate L-arabinose; (ii) being able to assimilate glucose; and (iii) being able to hydrolyse gelatin. Additionally, strain TDMA-16^T could be distinguished from other closely related species by the traits detailed in Table 1.

The yellow pigments were extracted by agitating bacterial cells in methanol on a rotary shaker (100 r.p.m.) at 50 °C in the dark until the cells were bleached followed by centrifugation at 5000 g for 5 min. The resultant supernatant was scanned for absorbance between 260 and 700 nm at room temperature on a Hitachi U2000 spectrophotometer. Carotenoid composition was characterized by HPLC-MS (LCMS-2010EV; Shimadzu) using a Shim-Pack FC-ODS (150 × 4.6 mm, 5 µm particle size; column temperature 35 °C) and acetonitrile:methanol:tetrahydrofuran (5.8:3.5:0.7, by vol.) as the mobile phase at a flow rate of 0.8 ml min⁻¹. Zeaxanthin was identified by its retention time, absorption spectrum and molecular mass. Commercial zeaxanthin (DHI; Water & Environment) was used as a standard. The menaquinone content was determined by an HPLC method (Collins, 1994) using an extract of *Sphingopyxis alaskensis* DSM 13593^T and *Sphingomonas asaccharolytica* NBRC 15499^T as a reference for Q-10. The fatty acid methyl esters of strain TDMA-16^T were extracted and analysed according to the standard protocol of the Sherlock Microbial Identification System (version 5.0; MIDI). Glycosphingolipids were analysed by TLC as described by Kawahara *et al.* (1991) and Takeuchi *et al.* (2001). Polar lipids were determined according to Tindall (1990). The DNA G+C content was determined by using an HPLC method (Mesbah & Whitman, 1989).

Table 1. Differential characteristics of strain TDMA-16^T (*Sphingomonas jaspsi* sp. nov.) and related type strains

Strains: 1, strain TDMA-16^T; 2, *Sphingomonas mali* IFO 15500^T (data from Takeuchi *et al.*, 2001); 3, *Sphingomonas pruni* IFO 15498^T (Takeuchi *et al.*, 2001); 4, *Sphingomonas asaccharolytica* IFO 15499^T (Takeuchi *et al.*, 2001); 5, *Sphingomonas aquatilis* JSS7^T (Lee *et al.*, 2001); 6, *Sphingomonas melonis* DSM 14444^T (Buonaurio *et al.*, 2002; Busse *et al.*, 2005); 7, *Sphingomonas abaci* C42^T (Busse *et al.*, 2005); 8, *Sphingomonas aerolata* NW12^T (Busse *et al.*, 2003); 9, *Sphingomonas faeni* MA-olki^T (Busse *et al.*, 2003); 10, *Sphingomonas aurantiaca* MA101b^T (Busse *et al.*, 2003); 11, *Sphingomonas echinoides* ATCC 14820^T (Denner *et al.*, 1999); 12, *Sphingomonas soli* T5-04^T (Yang *et al.*, 2006); 13, *Sphingomonas koreensis* JSS26^T (Lee *et al.*, 2001); 14, *Sphingomonas panni* C52^T (Busse *et al.*, 2005); 15, *Sphingomonas dokdonensis* DS-4^T (Yoon *et al.*, 2006). +, Positive; (+), weakly positive; –, negative; ND, not determined; NG, no growth; V, variable. All strains are negative for urease activity, indole production, glucose fermentation, arginine dihydrolase, and assimilation of D-mannitol, caprate and acetate.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Colony colour*	DY	LY	LY	LY	Y	DY	O	O	O	O	Y	Y	Y	Y	Y
Cell shape†	PSR	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	+	+	+	+	+	–	–	+	+	+	+	–	+	–	+
Budding formation	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Growth in 3 % NaCl	–	+	+	+	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	+
Assimilation of:															
Glucose	(+)	+	+	–	–	+	–	+	+	+	(+)	+	(+)	–	–
L-Arabinose	–	(+)	+	(+)	+	+	–	+	+	+	+	+	–	–	–
D-Mannose	–	+	+	(+)	–	+	–	+	+	+	–	–	–	(+)	+
N-Acetyl-D-glucosamine	–	+	+	+	–	+	–	+	+	+	+	–	+	–	–
Maltose	(+)	+	+	+	+	+	–	+	+	+	(+)	+	+	(+)	–
Gluconate	–	+	+	–	–	–	(+)	+	+	+	–	–	–	–	–
Adipate	–	+	–	–	–	–	–	+	+	+	–	–	–	–	+
DL-Malic acid	–	–	(+)	–	–	+	+	V	–	+	+	–	+	+	–
Citrate	–	–	–	–	–	–	–	V	+	+	–	–	–	+	–
Nitrate reduction	–	–	–	–	–	–	ND	–	–	–	+	–	–	ND	–
β-Galactosidase	+	+	+	+	+	+	ND	+	(+)	(+)	+	–	+	ND	–
Oxidase activity	+	+	+	+	+	+	–	+	+	+	+	+	+	–	+
Hydrolysis of:															
Aesculin	–	+	+	+	–	+	+	+	+	+	+	+	–	+	+
Gelatin	(+)	–	–	–	–	–	ND	–	–	–	–	+	–	ND	+
Starch	+	–	+	+	ND	–	ND	–	–	–	ND	ND	ND	ND	+
Tween 80	+	+	+	+	ND	–	ND	ND	ND	ND	ND	ND	ND	ND	+
DNA	NG	+	NG	NG	ND	NG	ND	(+)	(+)	V	ND	ND	ND	ND	ND
DNA G + C content (mol%)	63.3	65.4–65.9	65.4	64.8	63.0	65.0	ND	65.4	63.1	64.7	65.8	63.9	66.0	ND	66.9

*Y, Yellow; LY, light yellow; DY, deep yellow; O, orange.

†PSR, Pleomorphic short rods; R, rods.

The yellow pigments of strain TDMA-16^T were identified as carotenoids, including zeaxanthin [UV-vis $\lambda_{\max}$ 451 and 476 nm; molecular mass ($M^+ + 1$), 569], nostoxanthin [UV-vis $\lambda_{\max}$ 449 and 475 nm; molecular mass ($M^+ + 1$), 601] and an unidentified polar carotenoid. Nostoxanthin is also found in *Sphingomonas paucimobilis* and *Sphingomonas echinoides* (Jenkins *et al.*, 1979; Rowe *et al.*, 2000). The quinone system of strain TDMA-16^T consisted mainly of ubiquinone Q-10. This quinone profile is a characteristic of the majority of species within the class *Alphaproteobacteria* (Collins & Jones, 1981), including the genus *Sphingomonas sensu stricto* (Busse *et al.*, 1999; Takeuchi *et al.*, 2001). The polar lipid profile of strain TDMA-16^T was characterized by the presence of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid and an

unidentified glycolipid as the predominant lipids and moderate amounts of two unidentified glycolipids. Additionally, strain TDMA-16^T contained minor amounts of various unidentified lipids, including an aminophospholipid and two glycolipids. Phosphatidylmonomethylethanolamine and phosphatidyltrimethylethanolamine were not detected in strain TDMA-16^T. The polar lipid profile of strain TDMA-16^T was distinguishable from that of *Sphingomonas mali*, *Sphingomonas pruni* and *Sphingomonas asaccharolytica* in that phosphatidylmonomethylethanolamine and phosphatidyltrimethylethanolamine were absent (Busse *et al.*, 1999; Kämpfer *et al.*, 1997). The major fatty acids were C_{17:1}ω6c (34.5 %) and C_{18:1}ω7c (29.3 %). Table 2 shows the detailed fatty acid composition of strain TDMA-16^T. This profile is in excellent agreement with

those of representatives of the genus (Table 2) (Busse *et al.*, 1999, 2005, 2003; Yang *et al.*, 2006) and consistent with the assignment of strain TDMA-16^T to the genus *Sphingomonas sensu stricto*. The presence of 2-hydroxy fatty acids and the lack of 3-hydroxy fatty acids are important characteristics of members of the family *Sphingomonadaceae* (Busse *et al.*, 1999; Takeuchi & Hiraishi, 2001; Takeuchi *et al.*, 2001). However, strain TDMA-16^T differed from members of the genus *Sphingomonas sensu stricto* both qualitatively and quantitatively with regard to certain fatty acids and by the presence of 2-OH C_{18:1} as the major hydroxy fatty acid (Table 2).

The DNA G+C content of strain TDMA-16^T was 63.3 mol% (Table 1), which is in agreement with the values of 62–68 mol% given for the genus (Takeuchi *et al.*, 2001).

The results of the analysis of the 16S rRNA gene sequence, quinone system, fatty acids, polar lipids and DNA G+C content thus proved that strain TDMA-16^T is a member of the genus *Sphingomonas sensu stricto*. The strain can be distinguished from its close relatives by its 16S rRNA gene sequence, fatty acid composition and numerous physiological characteristics. On the basis of these results, it is

Table 2. Comparison of whole-cell fatty acid profiles of strain TDMA-16^T (*Sphingomonas jaspsi* sp. nov.) and related species

Taxa: 1, strain TDMA-16^T; 2, *Sphingomonas mali* IFO 15500^T (data from Yang *et al.*, 2006); 3, *Sphingomonas pruni* IFO 15498^T (Yang *et al.*, 2006); 4, *Sphingomonas asaccharolytica* IFO 15499^T (Yang *et al.*, 2006); 5, *Sphingomonas aquatilis* JSS7^T (Busse *et al.*, 2005); 6, *Sphingomonas melonis* DSM 14444^T (Busse *et al.*, 2005); 7, *Sphingomonas abaci* C42^T (Busse *et al.*, 2005); 8, *Sphingomonas aerolata* (three strains; Busse *et al.*, 2003); 9, *Sphingomonas faeni* MA-olki^T (Busse *et al.*, 2003); 10, *Sphingomonas aurantiaca* (three strains; Busse *et al.*, 2003); 11, *Sphingomonas echinoides* ATCC 14820^T (Denner *et al.*, 1999); 12, *Sphingomonas soli* T5-04^T (Yang *et al.*, 2006); 13, *Sphingomonas koreensis* JSS26^T (Busse *et al.*, 2005); 14, *Sphingomonas panni* C52^T (Busse *et al.*, 2005); 15, *Sphingomonas dokdonensis* DS-4^T (Yoon *et al.*, 2006). Values are percentages of total fatty acids.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Saturated															
C _{14:0}	0.4	1.4	16.8		0.7	1.0		1.6–1.9	2.0	2.1–3.0	0.9		2.6	1.1	0.8
C _{15:0}	2.1	0.2		3.8		0.5		0.0–0.5		0.0–0.8		0.8		1.1	
C _{16:0}	4.2	10.9	16.9	7.5	18.4	10.9	12.4	12.0–15.8	11.2	12.2–15.6	10.0	14.3	13.0	17.8	19.0
C _{17:0}	3.8			8.9						0.0–0.2					
C _{18:0}	0.2	0.5		0.4	1.2	0.9				0.0–0.3		0.5	0.8		
C _{17:0} cyclo					0.4			0.0–0.4							
C _{19:0} cyclo ω8c					0.4	0.5			0.8	0.4–0.7					
Unsaturated															
C _{15:1} ω6c	0.2														
C _{16:1} ω5c	2.0				1.0	1.1		1.2–2.0	1.6	1.6–1.8	0.8		1.6	2.1	1.3
C _{16:1} ω7c											2.9				
C _{16:1}		1.4													
C _{17:1} ω6c	34.5				0.4	2.6	1.6	0.0–1.7	2.6	0.8–3.0			0.8	3.6	
C _{17:1}		1.7	4.9	27.5								9.6			
C _{18:1} ω5c	2.1	1.8			1.5	2.0		0.6–0.8	1.0	0.6–1.0	2.7		1.4	0.7	
C _{18:1} ω7c	29.3				60.3	63.0	64.8						62.5	51.3	45.7
11-Methyl C _{18:1} ω7c					5.4	6.9				0.0–3.5			4.1		6.3
Hydroxy															
2-OH C _{12:0}								0.3–0.6	0.6	0.4					
2-OH C _{13:0}	0.1														
2-OH C _{14:0}	0.7	9.8		0.4	5.5	7.6	12.9	8.7–14.1	9.7	10.0–10.6	7.2	3.4	7.5	5.3	12.1
2-OH C _{15:0}	0.5	0.3		1.0		0.5						0.9			
2-OH C _{16:0}	0.4	0.8				0.8									
2-OH C _{16:1}								0.0–0.5							
2-OH C _{18:1}	6.1				0.6								0.8		
Summed features*															
4	8.1	2.1	2.5		4.3	1.4	8.3	24.6–26.8	29.2	25.7–28.0	2.45	3.9	5.0	17.1	14.8
7	0.5	69.2	58.4	50.2				38.5–46.0	41.4	40.0–40.5	73.1	65.5			

*Summed feature 4 contains 2-OH iso-C_{15:0} and/or C_{16:1}ω7c. Summed feature 7 contains C_{18:1}ω7c, C_{18:1}ω9t and/or C_{18:1}ω12t.

concluded that strain TDMA-16^T represents a novel carotenoid-producing species of the genus *Sphingomonas sensu stricto*, for which the name *Sphingomonas jaspsi* sp. nov. is proposed.

Description of *Sphingomonas jaspsi* sp. nov.

Sphingomonas jaspsi [ja.sp'.si. N.L. n. *jaspsum* arbitrary name derived from the acronym JSPS (Japan Society for the Promotion of Science); N.L. gen. n. *jaspsi* of JSPS, the organization that supported this study].

Cells are Gram-negative-staining pleomorphic short rods that are strictly aerobic, motile and non-spore-forming. Cells are 0.4–0.5 µm in width and 0.7–1.0 µm in length. Budding morphology may be observed. On NA, forms yellow, circular, dry colonies (1–2 mm in diameter) within 48 h. Carotenoids such as zeaxanthin and nostoxanthin are produced. Grows at 20.0–40.0 °C (optimum, 35.0–37.0 °C) and pH 6.0–9.0 (optimum, pH 6.0–7.0). Optimal growth occurs in the absence of NaCl; growth is inhibited by the presence of >0.25 % (w/v) NaCl. Anaerobic growth does not occur. Tween 80, gelatin and starch are hydrolysed, whereas aesculin, agar, DNA, casein, cellulose and chitin are not. Negative reactions are observed for glucose fermentation and acid production from all tested sugars. Indole and H₂S are not produced. Nitrate is not reduced. Catalase- and oxidase-positive. β-Galactosidase-positive. Negative for tryptophan deaminase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease activities. Glucose and maltose are assimilated, but arabinose, mannose, mannitol, N-acetylglucosamine, gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid are not. The major fatty acids are C_{17:1}ω6c (34.5 %), C_{18:1}ω7c (29.3 %) and summed feature 4 (containing C_{16:1}ω7c and/or 2-OH iso-C_{15:0}) (8.1 %). The major hydroxy fatty acid is 2-OH C_{18:1} (6.1 %). The predominant ubiquinone is Q-10. Glycosphingolipids are present.

The type strain is TDMA-16^T (=NBRC 102120^T=DSM 18422^T=CCUG 53607^T), which was isolated from a freshwater sample collected at Misasa (Tottori, Japan). The DNA G+C content of the type strain is 63.3 mol%.

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