Sphingomonas oryzae sp. nov. and *Sphingomonas jinjuensis* sp. nov. isolated from rhizosphere soil of rice (*Oryza sativa* L.)

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Two Gram-reaction-negative, yellow–orange-pigmented, rod-shaped bacterial strains, designated YC6722^T and YC6723^T, were isolated from rhizosphere soil samples collected from rice fields in Jinju, Korea. Strains YC6722^T and YC6723^T grew optimally at 25–30 °C and at pH 7.0–8.5. Phylogenetic analyses of 16S rRNA gene sequences showed that strain YC6722^T was most closely related to *Sphingomonas jaspsi* TDMA-16^T (96.6% sequence similarity) and strain YC6723^T was related most closely to *Sphingomonas aquatilis* JSS7^T (96.9%). The two strains contained ubiquinone-10 (Q-10) as the major respiratory quinone system and sym-homospermidine as the major polyamine. The G + C contents of the genomic DNA of strains YC6722^T and YC6723^T were 63.3 and 61.0 mol%, respectively. The major fatty acid was C_{18:1ω7c}. The polar lipids detected in the two strains were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid, phosphatidylmethylethanolamine and other unknown lipids. On the basis of their phylogenetic positions, and their biochemical and phenotypic characteristics, strains YC6722^T and YC6723^T represent two novel species of the genus *Sphingomonas*, for which the names *Sphingomonas oryzae* sp. nov. (=KCTC 22476^T =DSM 21455^T) and *Sphingomonas jinjuensis* sp. nov. (KCTC 22477^T =DSM 21457^T) are proposed.

The genus *Sphingomonas* in the family *Sphingomonadaceae* of the *Gammaproteobacteria* was first proposed by Yabuuchi *et al.* (1990) and has subsequently been reclassified into four genera, namely *Sphingomonas*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* (Takeuchi *et al.*, 2001; Yabuuchi *et al.*, 2002). Since 2009, eight recognized species of the genus *Sphingomonas* have been described, namely *S. aestuarii* (Roh *et al.*, 2009), *S. japonica* (Romanenko *et al.*, 2009), *S. hankookensis* (Yoon *et al.*, 2009), *S. sanxanigenens* (Huang *et al.*, 2009), *S. changbaiensis* (Zhang *et al.*, 2010), *S. histidinilytica* (Nigam *et al.*, 2010), *S. rubra* (Huo *et al.*, 2010) and *S. glacialis* (Zhang *et al.*, 2011).

During analysis of the bacterial community in two rice fields of Gyeongsang National University Farm (Daegok

Valley), north-east Jinju, Korea, managed under conventional and no-tillage practices, soil samples from the rhizosphere were collected from each field in 2007. Samples were sonicated in a Bandelin Sonoplus electronic homogenizer at 260 W cm⁻² for 15 s and then serially diluted extracts, made by adding 50 mM phosphate buffer (pH 7.0) solution, were spread on modified half-strength R2A agar plates (Aslam *et al.*, 2009; Janssen *et al.*, 2002) and incubated at 28 °C for more than 1 month. Single colonies on the plates were purified by transferring them to new plates while keeping other conditions unchanged. Two novel *Sphingomonas*-like strains, designated YC6722^T and YC6723^T, were isolated and characterized in this study.

Cell morphology was observed under a Nikon light microscope at 1000× magnification and the presence of flagella was investigated using a transmission electron microscope (model H-600, Hitachi) with cells grown at 30 °C for 72 h in R2A broth. The Gram reaction was determined by using a bioMérieux Gram stain kit according to the manufacturer's instructions. Catalase

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YC6722^T and YC6723^T are EU707560 and EU707561, respectively.

Three supplementary figures and one supplementary table are available with the online version of this paper.

and oxidase tests were performed according to the procedures outlined in Cappuccino & Sherman (2002). Tests for hydrolysis of casein, aesculin, gelatin, starch, L-tyrosine, urea and Tweens 20 and 80 by strains YC6722^T and YC6723^T and the type strains of related species, including *Sphingomonas astaxanthinifaciens* TDMA-17^T, *S. kaistensis* PB56^T, *S. abaci* C42^T, *S. jaspis* TDMA-16^T, *S. faeni* MA-olki^T, *S. aquatilis* JSS7^T, *S. melonis* DAPP-PG224^T, *S. phyllosphaerae* LMG 21958^T, *S. yunnanensis* YIM 003^T and *S. adhaesiva* GIFU 11458^T (Yabuuchi *et al.*, 1990; Lee *et al.*, 2001; Buonauro *et al.*, 2002; Busse *et al.*, 2003, 2005; Rivas *et al.*, 2004; Zhang *et al.*, 2005; Asker *et al.*, 2007a, b; Kim *et al.*, 2007), were performed by using standard protocols (Brown, 2007; Atlas, 1993). Enzyme activities, acid production from different carbohydrates, assimilation of various substrates and growth on carbohydrates were determined by using API ZYM, API 20 E, API 20 NE and API 50 CH kits, at 30 °C, respectively, according to instructions of the manufacturer (bioMérieux). Growth at 4, 10, 15, 20, 26, 30, 35, 40 and 45 °C, and at pH 4.0–12.0 (at intervals of 0.5 pH units) were assayed after 5 days of incubation on R2A agar. Growth on nutrient agar (NA), Luria–Bertani (LB) agar, Czapek–dox agar, MacConkey agar, 1/10-strength trypticase soy agar (TSA) and R2A agar was also evaluated at 30 °C after 5 days of incubation. All culture media were purchased from Difco. Growth under anaerobic conditions was determined on R2A agar plates at 30 °C for 5 days in an anaerobic Gaspak jar containing an atmosphere of CO₂ (Gas-Pack System; Becton Dickinson). Salt tolerance was tested in R2A broth supplemented with 0–6 % (w/v) NaCl (at intervals of 0.5 %) after 5 days of incubation at 30 °C. Duplicate antibiotic-sensitivity tests were performed with filter-paper discs (BD; diameter 8 mm) containing (µg per disc) ampicillin (10), chloramphenicol (30), penicillin (10), gentamicin (10), kanamycin (30), vancomycin (30), streptomycin (10), tetracycline (10) and rifampicin (10). The discs were placed on R2A plates spread with cell suspension and the plates were then incubated at 30 °C for 3 days. Almost all tests performed on strains YC6722^T and YC6723^T were also performed on the reference type strains listed above.

Cells of strains YC6722^T and YC6723^T were Gram-reaction-negative and formed smooth, convex, circular colonies that were 0.5–1.5 mm in diameter after 72 h of incubation at 30 °C. Colonies of strains YC6722^T and YC6723^T were dark-yellow and dark-orange, respectively. Cells of strain YC6722^T were non-motile rods 0.5–0.6 × 0.9–1.0 µm, while cells of strain YC6723^T were non-motile rods 0.6–0.8 × 1.4–1.6 µm (Supplementary Fig. S1, available in IJSEM Online). Cells of strains YC6722^T and YC6723^T occurred singly, in pairs and in clusters. Both strains grew well aerobically on 1/10-strength TSA agar as well as NA and R2A agar but grew slowly on LB agar. The physiological and biochemical characteristics of strains YC6722^T and YC6723^T are summarized in the species descriptions and a comparison of selective characteristics with the type strains of closely related species is given in Table 1.

Genomic DNA of strains YC6722^T and YC6723^T was extracted by using a commercial genomic DNA extraction kit (Core Biosystem). The 16S rRNA gene was PCR-amplified from the purified genomic DNA using primers 27F and 1492R (Lane, 1991). The PCR product was cloned into a TOPO TA vector (Invitrogen) and sequenced by GenoTech. The 16S rRNA gene sequence was compiled by using SeqMan software (DNASTAR) and the sequences of related taxa were obtained from the GenBank database. Multiple sequence alignments were performed using the CLUSTAL X program (Thompson *et al.*, 1997). Gaps were edited by using the BioEdit program (Hall, 1999). Phylogenetic trees were reconstructed using the neighbour-joining method (Saitou & Nei, 1987) in the program MEGA4, with bootstrap values based on 1000 replications (Tamura *et al.*, 2007), and the maximum-likelihood algorithm in the PHYLIP version 3.6 program (Felsenstein, 2002). Pair-wise sequence similarity values between strains YC6722^T and YC6723^T and the type strains of related species were computed by using the EzTaxon server (Chun *et al.*, 2007).

The 16S rRNA gene sequences of strains YC6722^T and YC6723^T were a continuous stretch of 1365 and 1375 nt, respectively, showing 93.9 % sequence similarity to each other. Based on phylogenetic analysis of 16S rRNA gene sequences, the closest relatives of strain YC6722^T were *S. jaspis* TDMA-16^T (96.6 % similarity), *S. kaistensis* PB56^T (96.2 %), *S. astaxanthinifaciens* TDMA-17^T (96.1 %), *S. faeni* MA-olki^T (94.7 %) and *S. abaci* C42^T (94.0 %), whereas the closest relatives of strain YC6723^T were *S. aquatilis* JSS7^T (96.9 %), *S. melonis* DAPP-PG 224^T (96.8 %), *S. phyllosphaerae* LMG 21958^T (96.3 %), *S. yunnanensis* YIM 003^T (96.1 %) and *S. adhaesiva* GIFU 11458^T (95.0 %). In the neighbour-joining phylogenetic tree, strains YC6722^T and YC6723^T grouped in clades with the type strains of their related species with 87 and 60 % bootstrap support, respectively (Fig. 1). The topology of the phylogenetic tree based on the maximum-likelihood algorithm (Supplementary Fig. S2) also supported the suggestion that strains YC6722^T and YC6723^T represent novel species within the genus *Sphingomonas*.

The cellular fatty acids of strains YC6722^T and YC6723^T and related type strains were analysed by using cells harvested from the third quadrant grown on R2A agar at 30 °C for 72 h. Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI). Isoprenoid quinones were extracted and analysed by using reversed-phase HPLC according to the method described by Komagata & Suzuki (1987). Polyamines were analysed as described by Busse & Auling (1988) and Busse *et al.* (1997). For measurement of the G+C content of the chromosomal DNA, genomic DNA was extracted and purified as described by Ausubel *et al.* (1995). This was then degraded enzymically into nucleosides and the G+C content was determined as described by Mesbah *et al.* (1989) via reversed-phase HPLC. Polar lipids were extracted according to a

Table 1. Differential phenotypic characteristics between strains YC6722^T and YC6723^T and the type strains of closely related species of the genus *Sphingomonas*

Strains: 1, YC6722^T; 2, YC6723^T; 3, *S. jaspis* TDMA-16^T; 4, *S. kaistensis* PB56^T; 5, *S. astaxanthinifaciens* TDMA-17^T; 6, *S. faeni* MA-olki^T; 7, *S. abaci* C42^T; 8, *S. aquatilis* JSS7^T; 9, *S. melonis* DAPP-PG 224^T; 10, *S. phyllosphaerae* LMG 21958^T; 11, *S. yunnanensis* YIM 003^T; 12, *S. adhaesiva* GIFU 11458^T. +, Positive; –, negative; (+), weakly positive; ND, not determined; Y, yellow; D-Y, dark-yellow; L-Y, light-yellow; L-O, light-orange; D-O, dark-orange; R-O, red-orange. Data for the reference type strains are from this study unless indicated otherwise.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Colony colour	D-Y	D-O	D-Y	R-O	L-Y	L-O	D-O	Y	L-Y	L-Y	D-Y	D-Y
Motility*	–	–	+	–	+	+	–	+	–	+	+	+
Growth at 40 °C	+	–	+	+	+	–	–	–	–	–	–	+
Growth with 3.0 % NaCl	–	–	–	–	+	–	–	–	–	–	–	+
Hydrolysis of:												
L-Tyrosine	–	+	–	–	–	–	+	+	+	+	–	+
Urea	–	+	+	–	+	–	+	–	+	–	+	+
Enzyme activity												
Cystine arylamidase	–	–	+	–	+	–	+	+	+	–	–	–
Alkaline phosphatase	–	+	+	+	+	+	+	+	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	–	–	+	+	–	+	+	+	+	+	–	+
Valine arylamidase	–	+	+	–	+	+	+	+	+	+	+	–
α-Chymotrypsin	–	+	–	+	+	–	–	–	–	–	–	+
α-Galactosidase	–	+	–	–	–	–	+	–	–	+	+	+
Assimilation of:												
L-Arabinose	–	–	–	–	–	–	–	+	–	(+)	–	–
D-Mannose	–	–	–	–	–	–	–	+	+	–	+	+
Maltose	–	–	–	–	–	–	–	+	+	(+)	(+)	–
Cellobiose	–	+	+	–	–	–	+	+	+	+	+	+
Glycogen	+	–	–	–	–	–	–	–	–	–	–	–
D-Xylose	–	–	–	–	–	–	+	+	+	+	+	–
Aesculin ferric citrate	+	+	–	–	–	+	+	–	+	+	+	+
DNA G + C content (mol%)*	63.3	61.0	63.3	69.1–69.9	67.7	63.1	ND	63.0	65.0	61.0	67.5	67.2

*Data for 3–12 are from Asker *et al.* (2007a), Kim *et al.* (2007), Asker *et al.* (2007b), Busse *et al.* (2003), Busse *et al.* (2005), Lee *et al.* (2001), Buonauro *et al.* (2002), Rivas *et al.* (2004), Zhang *et al.* (2005) and Yabuuchi *et al.* (1990), respectively.

modification of the method of Minnikin *et al.* (1977) and were separated by TLC on a Merck Kieselgel 60-HPTLC. For two-dimensional TLC development, a chloroform, methanol and water (65:25:4, v/v/v) mixture followed by a chloroform, methanol, acetic acid and water (80:12:15:4, v/v/v/v) mixture were used as mobile phase. Aminolipids were detected by spraying the plate with a 0.2 % (w/v) solution of ninhydrin in butanol saturated with water followed by heating at 105 °C for 10 min (Ross *et al.*, 1985). Phospholipids were detected by spraying the plate with Zinzadze reagent (Dittmer & Lester, 1964). Glycolipids were detected with 0.5 % 1-naphthol in methanol/water (1:1, v/v) and sulfuric acid/ethanol (1:1, v/v), followed by heating at 120 °C for 5–10 min (Xin *et al.*, 2000). The presence of phosphatidylcholine was detected with Dragendorff reagent (Sigma–Aldrich). Total lipid profiles were detected by spraying with phosphomolybdic acid solution (Sigma–Aldrich) followed by heating at 150 °C.

Cellular fatty acid profiles of strains YC6722^T and YC6723^T and of the type strains of related species are shown in

Supplementary Table S1. The major fatty acids of strain YC6722^T were C_{18:1}ω7c (26.3 %), summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) (23.0 %) and C_{16:0} (21.3 %), which are shared with the majority of members of the genus *Sphingomonas*. Similarly, the predominant fatty acids of strain YC6723^T were C_{18:1}ω7c (49.5 %), C_{14:0} 2-OH (17.4 %) and summed feature 3 (12.8 %). The total fatty acid profiles were consistent with those of members of the family *Sphingomonadaceae*, displaying the presence of 2-hydroxy fatty acids and a lack of 3-hydroxy fatty acids. However, the total fatty acid profiles of strains YC6722^T and YC6723^T differed from those of representatives of the genus *Sphingomonas* both qualitatively and quantitatively (Busse *et al.*, 1999; Takeuchi & Hiraishi, 2001; Takeuchi *et al.*, 2001). The major isoprenoid quinone of strains YC6722^T and YC6723^T was ubiquinone Q-10, consistent with data for the type strains of recognized species of the genus *Sphingomonas* (Busse *et al.*, 1999; Yabuuchi *et al.*, 1990; Yoon *et al.*, 2006). The DNA G + C content of strains YC6722^T and YC6723^T was 63.3 and 61.0 mol%, respectively; these values closely resembled those of recognized

Fig. 1. Phylogenetic tree reconstructed from comparative analysis of 16S rRNA gene sequences showing the relationship of strains YC6722^T and YC6723^T with the type strains of related species. The phylogenetic tree was reconstructed by using the neighbour-joining method and Jukes–Cantor evolutionary distance matrix data obtained from aligned nucleotides. Bootstrap values >50% (based on 1000 replications) are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

species of the genus *Sphingomonas* (Asker *et al.*, 2007a; Rivas *et al.*, 2004; Yabuuchi *et al.*, 2002). The polyamine patterns of the two strains comprised *sym*-homospermidine and minor amounts of spermidine, which was in agreement with those of members of the genus *Sphingomonas* (Huang *et al.*, 2009; Roh *et al.*, 2009; Busse *et al.*, 2005). The polar lipid profiles of strains YC6722^T and YC6723^T were characterized by the presence of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid and phosphatidyltrimethylethanolamine, which is in accordance with data for the genus *Sphingomonas* (Busse *et al.*, 1999). Although the polar lipid profiles of strains YC6722^T and YC6723^T were similar, they differed in terms of their phosphoglycolipid content. Three unknown phosphoglycolipids (PGL1, PGL2 and PGL5) and three unknown lipids (L1, L2 and L3) were detected in strain YC6722^T while three other unknown phosphoglycolipids (PGL3, PGL4 and PGL6) were detected in strain YC6723^T (Supplementary Fig. S3).

On the basis of phylogenetic analyses (Fig. 1 and Supplementary Fig. S2), physiological and biochemical characteristics (Table 1), polyamine patterns, polar lipid analyses (Supplementary Fig. S3) and fatty acid profiles (Supplementary Table S1), strains YC6722^T and YC6723^T represent two novel species of the genus *Sphingomonas*, for which the names *Sphingomonas oryzae* sp. nov. and *Sphingomonas jinjuensis* sp. nov. are proposed, respectively.

Description of *Sphingomonas oryzae* sp. nov.

Sphingomonas oryzae (o.ry.zi.ter'ra.e. L. n. *oryza* rice; L. n. *terra* earth, soil; N.L. gen. n. *oryzae* of rice soil or field, referring to the source of isolation of the type strain).

Cells are Gram-reaction-negative, aerobic, non-motile short rods, 0.5–0.6 × 0.9–1.0 µm. Colonies grown on R2A agar at 30 °C for 3 days are 0.5–1.0 mm in diameter, dark-yellow, smooth, convex and circular. Grows on R2A agar, NA, LB agar and 1/10-strength TSA (Difco) at 30 °C but not on MacConkey agar or Czapek-dox agar. Oxidase-positive and catalase-negative. Grows at 20–40 °C (optimum 25–30 °C) and pH 6.0–10.5 (optimum pH 8–8.5). Optimal growth occurs in the absence of NaCl. Sensitive to (µg per disk) ampicillin (10), chloramphenicol (30), erythromycin (15), oleandomycin (15) and streptomycin (10) but resistant to penicillin (10), gentamicin (10), kanamycin (30), vancomycin (30), tetracycline (10) and rifampicin (10). Hydrolyses aesculin but not casein, Tweens 20 or 80, L-tyrosine, starch, gelatin or urea. Nitrate is not reduced to nitrite or nitrogen. Positive in tests for activity of α-glucosidase but not for alkaline phosphatase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase or α-glucosidase activities. Utilizes D-glucose, aesculin ferric citrate, maltose, starch and glycogen as sole carbon sources but not cellobiose, gentiobiose, lactose, L-arabinose, melibiose, D-fructose, D-galactose, D-mannose, D-mannitol or D-glucose. The major isoprenoid quinone is ubiquinone Q-10. The major polyamine is *sym*-homospermidine. The polar lipid profile comprises phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid, phosphatidyltrimethylethanolamine, three unknown phosphoglycolipids (PGL1, PGL2 and PGL5) and three unknown lipids (L1, L2 and L3). The major fatty acids are C_{18:1ω7c}, summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH) and C_{16:0}.

The type strain, YC6722^T (=KCTC 22476^T =DSM 21455^T), was isolated from the rhizosphere of a rice field managed under no-tillage in Jinju, Korea. The DNA G+C content of the type strain is 63.3 mol%.

Description of *Sphingomonas jinjuensis* sp. nov.

Sphingomonas jinjuensis (jin.ju.en'sis. N.L. adj. *jinjuensis* referring to the Jinju Region of Korea, from where the type strain was found).

Cells are Gram-reaction-negative, aerobic, non-motile rods, $0.6\text{--}0.8 \times 1.4\text{--}1.6\ \mu\text{m}$. Colonies grown on R2A agar at $30\ ^\circ\text{C}$ for 3 days are 1.0–1.5 mm in diameter, dark-orange, smooth, convex and circular. Grows on R2A agar, NA, LB agar and 1/10-strength TSA at $30\ ^\circ\text{C}$ but not on MacConkey agar or Czapek-dox agar. Oxidase-negative and catalase-positive. Grows at $20\text{--}35\ ^\circ\text{C}$ (optimum $25\text{--}30\ ^\circ\text{C}$) and pH 5.0–10.5 (optimum pH 7–8). Optimal growth occurs in the absence of NaCl. Sensitive to (μg per disc) ampicillin (10), chloramphenicol (30), erythromycin (15), oleandomycin (15), gentamicin (10), kanamycin (30), vancomycin (30) and streptomycin (10) but resistant to penicillin (10), tetracycline (10) and rifampicin (10). Hydrolyses Tween 20, L-tyrosine, aesculin and urea but not casein, Tween 80, starch or gelatin. Nitrate is not reduced to nitrite or nitrogen. Positive in tests for activities of α -chymotrypsin, α -galactosidase and α -glucosidase, but not for esterase lipase (C8), cystine arylamidase, naphthol-AS-BI-phosphohydrolase or β -glucuronidase. Utilizes glycerol, L-rhamnose, melibiose, trehalose, melezitose, raffinose, starch and turanose as sole carbon sources but not D-xylose, D-mannitol, arbutin or salicin. Assimilates 4-nitrophenyl- β -D-galactopyranoside, but not glucose, arabinose, mannose, maltose, caprate or N-acetylglucosamine. The major isoprenoid quinone is ubiquinone Q-10. The major polyamine is *sym*-homospermidine. The polar lipid profile comprises phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid, phosphatidylmethylethanolamine, four unknown phosphoglycolipids (PGL3, PGL4, PGL5 and PGL6) and two unknown lipids (L1 and L2). The major fatty acids are $\text{C}_{18:1\omega7c}$, $\text{C}_{14:0}$ 2-OH and summed feature 3 ($\text{C}_{16:1\omega7c}$ and/or iso- $\text{C}_{15:0}$ 2-OH).

The type strain, YC6723^T (=KCTC 22477^T =DSM 21457^T), was isolated from the rhizosphere of a rice field managed under conventional practice in Jinju, Korea. The DNA G+C content of the type strain is 61.0 mol%.

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