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Sphingomonas rosea sp. nov. and *Sphingomonas swuensis* sp. nov., Rosy Colored β-Glucosidase-Producing Bacteria Isolated from Soil

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Two strains PB196^T and PB62^T of Gram-negative, non-motile, and non-spore-forming bacteria, were isolated from soil in South Korea and characterized to determine their taxonomic positions. 16S rRNA gene sequence analysis showed that the two strains belonged to the genus *Sphingomonas*. The highest degree of sequence similarity of strain PB196^T was found with PB62^T (98.9%), *Sphingomonas humi* PB323^T (98.9%), *Sphingomonas kaistensis* PB56^T (98.2%), and *Sphingomonas astaxanthinifaciens* TDMA-17^T (98.0%). The highest degree of sequence similarity of strain PB62^T was found with *Sphingomonas humi* PB323^T (98.8%), *Sphingomonas astaxanthinifaciens* TDMA-17^T (98.2%), and *Sphingomonas kaistensis* PB56^T (98.1%). Chemotaxonomic data revealed that they possessed ubiquinone-10 (Q-10) as common in the genus *Sphingomonas*, that the predominant fatty acids were summed feature 7 ($C_{18:1}\omega7c/\omega9t/\omega12t$), summed feature 4 ($C_{16:1}\omega7c/C_{15:0}$ iso 2OH), $C_{16:0}$, and $C_{17:1}\omega6c$, and that they contained sphingoglycolipid, phosphatidylglycerol (PG), and phosphatidylethanolamine (PE) in common but they showed difference for diphosphatidylglycerol (DPG). Based on these data, PB196^T (=KCTC 12339^T =JCM 16604^T) and PB62^T (=KCTC 12336^T =JCM 16605^T =KEMB 9004-005^T) should be classified as type strains of two novel species, for which the names *Sphingomonas rosea* sp. nov. and *Sphingomonas swuensis* sp. nov. are proposed, respectively.

***Keywords*:** taxonomy, 16S rRNA gene, *Sphingomonas rosea*, *Sphingomonas swuensis*

The genus *Sphingomonas* belongs to the family *Sphingomonadaceae*, the order *Sphingomonadales* and the class *Alphaproteobacteria*. The genus *Sphingomonas* was proposed by Yabuuchi *et al.* (1990) to identify Gram-negative, rod-shaped, yellow-pigmented bacteria. Subsequently, it has been divided into four genera, *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis* (Takeuchi *et al*., 1993, 2001; Yabuuchi *et al*., 2002; Busse *et al*., 2003). At the time of writing, the genus *Sphingomonas* comprises more than 40 species with validly published names, including the recently described *Sphingomonas changbaiensis* (Zhang *et al*., 2010), *Sphingomonas ginsenosidimutans* (Choi *et al*., 2010). *Sphingomonas glacialis* (Zhang *et al*., 2011), *Sphingomonas histidinilytica* (Nigam *et al*., 2010), *Sphingomonas humi* (Yi *et al*., 2010), *Sphingomonas rubra* (Huo *et al*., 2011), and *Sphingomonas xinjiangensis* (An *et al*., in press). The colors of *Sphingomonas* species are off-white, yellow, or orange. *Sphingomonas* species are non-fermentative and their cellular fatty acids contain 2-hydroxy fatty acid, but not 3-hydroxy fatty acid.

Microorganisms were isolated from soil to investigate both microbial community structure in soil (Duan *et al*., 2009; Kim *et al*., 2009) and the antimicrobial activities of plants against the microorganisms (Moon *et al*., 2009). In this study, two *Sphingomonas* strains PB196^T and PB62^T were isolated from soil near a pond located on the campus of the Korea Advanced Institute of Science and Technology (Daejeon, South Korea). The two strains were characterized by a polyphasic approach including phylogenetic analysis of 16S rRNA gene sequences, genomic relatedness, and chemotaxonomic and phenotypic properties. Results obtained in this study indicated that the strains should be assigned as new members of the genus *Sphingomonas*.

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Materials and Methods

Isolation of bacterial strains and culture conditions

Two strains PB196^T and PB62^T were isolated from soil via direct plating onto ten-fold diluted R2A agar (Difco, USA) and incubated at 30°C. The purified colonies were identified tentatively by partial 16S rRNA gene sequences and preserved in a glycerol solution (20%, w/v) at -70°C. These strains were deposited at the Korean Collection for Type Cultures (KCTC), Japan Collection of Microorganisms (JCM), and the Korea National Environmental Microorganism Bank (KEMB).

Phenotypic and biochemical characteristics

Gram reactions were conducted according to the non-staining method described by Buck (1982). The cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy (TEM, Call Zeiss LEO912AB) after the cells had grown for 5 days at 30°C on LB agar. Oxidase activity was evaluated via the oxidation of 1% (w/v) tetramethyl-p-phenylene diamine. Catalase activity was determined by measuring bubble production after applying 3% (v/v) hydrogen peroxide solution. Growth on different media was assessed on trypticase soy agar (TSA), Luria-Bertani agar (LB), nutrient agar (NA), and R2A agar. The API 20NE, API ID32GN, and API ZYM microtest systems were employed according to the recommendations of the manufacturer (bioMérieux, France) for studying carbon source

utilization and the enzyme activities of the strains. Growth at different temperatures (4, 25, 30, 37, and 42°C) was assessed on R2A agar for 5 days. Growth at various pH levels (5, 6, 7, 8, 9, 10, and 11) was assessed in R2A broth at 30°C.

16S rRNA gene sequencing and phylogenetic analysis

The 16S rRNA genes of strains PB196^T and PB62^T were amplified from chromosomal DNA using the 9F and 1512R universal bacterial primer set (Weisburg *et al*., 1991). Purified PCR products were sequenced by Genotech, Daejeon, Korea (Kim *et al*., 2005). The full sequences of the 16S rRNA genes were compiled with SeqMan software (DNASTAR Inc., USA). The 16S rRNA gene sequences of related taxa were obtained from GenBank and edited with the BioEdit program (Hall, 1999). Multiple alignments were performed with the CLUSTAL X program (Thompson *et al*., 1997). Pairwise distances for the neighbor-joining algorithm (Saitou and Nei, 1987) were calculated according to Kimura two-parameter model (Kimura, 1983) and a phylogenetic tree was constructed using the MEGA 3 program (Kumar *et al.*, 2004). A bootstrap analysis with 1,000 replicates was also conducted to obtain confidence levels for the branches (Felsenstein, 1985). Min-mini heuristic method with search factor of one was applied in maximum-parsimony analysis in the MEGA 3 Program and maximum-likelihood analysis was performed with phylip 3.69.

Determination of DNA G+C content

To determine G+C content, genomic DNA was extracted and purified with the QIAGEN Genomic-tip system 100/G (Tokyo, Japan) and enzymatically degraded into nucleosides. The nucleosides were then analyzed using reverse-phase high performance liquid chromatography (HPLC), as previously described (Tamaoka and Komagata, 1984; Mesbah *et al*., 1989).

DNA-DNA hybridization

DNA-DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al*. (1989), using photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was performed with five replications per sample. The highest and lowest values obtained for each sample were excluded and the remaining three values were utilized to calculate hybridization values. The DNA relatedness values were expressed as means of these three values. The experiments were done in triplicates.

Chemotaxonomic analyses

Polar lipids were extracted according to the procedures described by Minnikin *et al*. (1984) and identified by two-dimensional thin-layer chromatography (TLC) followed by spraying with the appropriate detection reagents (Minnikin *et al*., 1984; Komagata and Suzuki, 1987). For TLC development, 1st mobile phase was chloroform, methanol and water (65:25:4, v/v/v) and 2nd mobile phase was chloroform, methanol, acetic acid and water (80:12:15:4, v/v/v/v). Total lipid profile was detected by spraying with phosphomolybdic acid solution (Sigma-Aldrich, USA) followed by heating at 150°C. Aminolipids by spraying with 0.2% (w/v) ninhydrin solution followed by heating at 105°C for 10 min; glycolipids 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; phospholipids by spraying with Zinzadze reagent; and phosphatidylcholine by spraying with Dragendorff reagent (Sigma-Aldrich).

Sphingolipid that is characteristic of the genus *Sphingomonas* was also analyzed according to Yabuuchi *et al.* (1990). A portion of the extracted lipids was subjected to alkaline hydrolysis with 1 M KOH in methanol (2:1, v/v) for 2 h at 40°C. The lipids were analyzed by TLC with a solvent system composed of chloroform:methanol:water (70:30:5, v/v/v). Lipid profile was detected by spraying with phosphomolybdic acid solution and 0.2% (w/v) ninhydrin solution followed by heating at 105°C for 10 min.

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified via TLC, evaporated under vacuum conditions, and re-extracted with n-hexane:water (1:1 v/v). The crude n-hexane quinone solution was purified and subsequently analyzed by HPLC, as previously described (Collins and Jones, 1981; Shin *et al*., 1996). The strains were allowed to grow on TSA for 3 days at 30°C to perform the fatty acid methyl ester analysis, and then two loops of well-grown cells were harvested. Fatty acid methyl esters were prepared, separated, and identified with the Sherlock Microbial Identification System (MIDI, Inc., USA) (Sasser, 1990).

Results and Discussion

Morphological and phenotypic characteristics

The colonies of the two strains PB196^T and PB62^T were orange-red colored when routinely cultured on LB and R2A agar (Difco) at 30°C. They were Gram-negative, non-motile bacteria. Cells of strain PB196^T are long rod-shaped but cells of strain PB62^T are short rod-shaped (Fig. 1). They grew at a temperatures ranging from 25 to 37°C. Strain PB196^T grew at 42°C but strain PB62^T did not. They grew well at pH 6-8. Results regarding the physiological characteristics of two strains are summarized in the species description, and a comparison of selective characteristics with closely related type strains of *Sphingomonas* species is shown in Table 1.

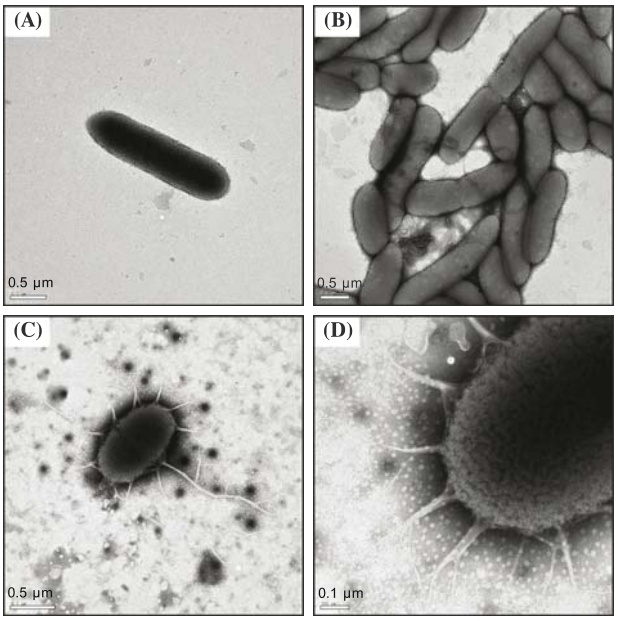

Fig. 1. Cell morphology of strains PB196^T (A, B) and PB62^T (C, D) as determined by a transmission electron microscope after growth on LB agar for 5 days at 30°C. Strain PB196^T is long rod-shaped (A, B) whereas strain PB62^T is short rod-shapes (C, D).

Table 1. Differential characteristics between novel strains and type strains of closely related *Sphingomonas* species
Strains: 1, PB196^T; 2, PB62^T; 3, *S. humi* KCTC 12341^T; 4, *S. kaistensis* KCTC 12334^T; 5, *S. astaxanthinifaciens* NBRC 102146^T.
All data were obtained from this study. All strains grew at 30°C and produced esterase (C4).
The strains did not grow at 4°C or reduce nitrate. They did not produce indole from tryptophan, nor did they produce N-acetyl-β-glucosaminidase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase, or protease (gelatin hydrolysis). They did not assimilate caprate, 3-hydroxybenzoate, 5-ketogluconate, L-arabinose, D-melibiose, D-sorbitol, or salicin.
+, positive; -, negative; w, weak positive; ++, strong positive

Characteristic	1	2	3	4	5
Motility	-	-	-	-	+
Acid production from glucose	-	-	w	w	-
Enzyme activity					
Acid phosphatase	+	w	+	w	w
Alkaline phosphatase	++	++	++	+	-
Arginine dihydrolase	-	+	-	-	-
Cystine arylamidase	+	-	w	-	w
Esterase lipase (C8)	+	+	+	+	-
Leucine arylamidase	++	+	+	++	-
Lipase (C14)	-	-	-	-	+
Naphtol-AS-BI-phosphohydrolase	+	w	+	w	+
Trypsin	+	+	++	w	w
Urease	-	+	-	-	-
Valine arylamidase	++	+	w	+	+
α-Chymotrypsin	-	-	+	-	+
α-Glucosidase	+	w	w	-	+
β-Clucosidase (esculin hydrolysis)	+	w	-	-	w
Assimilation test					
2-Ketogluconate	-	-	+	-	-
3-Hydroxybutyrate	-	-	-	++	w
4-Hydroxybenzoate	++	+	-	+	-
Acetate	-	+	-	-	-
Adipate	w	+	+	+	-
Citrate	-	-	+	-	-
D-Glucose	+	-	-	+	+
DL-Lactate	-	+	-	-	-
D-Mannose	+	+	-	+	-
D-Ribose	-	+	-	-	-
D-Sucrose	-	+	-	-	-
Gluconate	+	w	w	w	-
Glycogen	-	-	-	+	w
Itaconate	-	+	-	-	-
L-Alanine	-	+	-	-	-
L-Fucose	+	-	-	-	-
L-Histidine	-	-	+	-	-
L-Proline	++	+	-	++	w
L-Rhamnose	++	++	+	++	-
L-Serine	-	+	+	+	w
Malate	+	+	+	+	-
Malonate	-	+	-	-	-
Maltose	+	+	+	+	-
Mannitol	+	+	+	+	-
myo-Inositol	-	+	-	-	-
N-Acetyl-glucosamine	-	-	-	+	-
Phenyl acetate	w	-	-	-	-
Propionate	-	-	-	-	w
Suberate	-	+	-	-	-
Valerate	-	-	+	-	w
G+C content	69.9	71.1	69.0	69.9	67.7

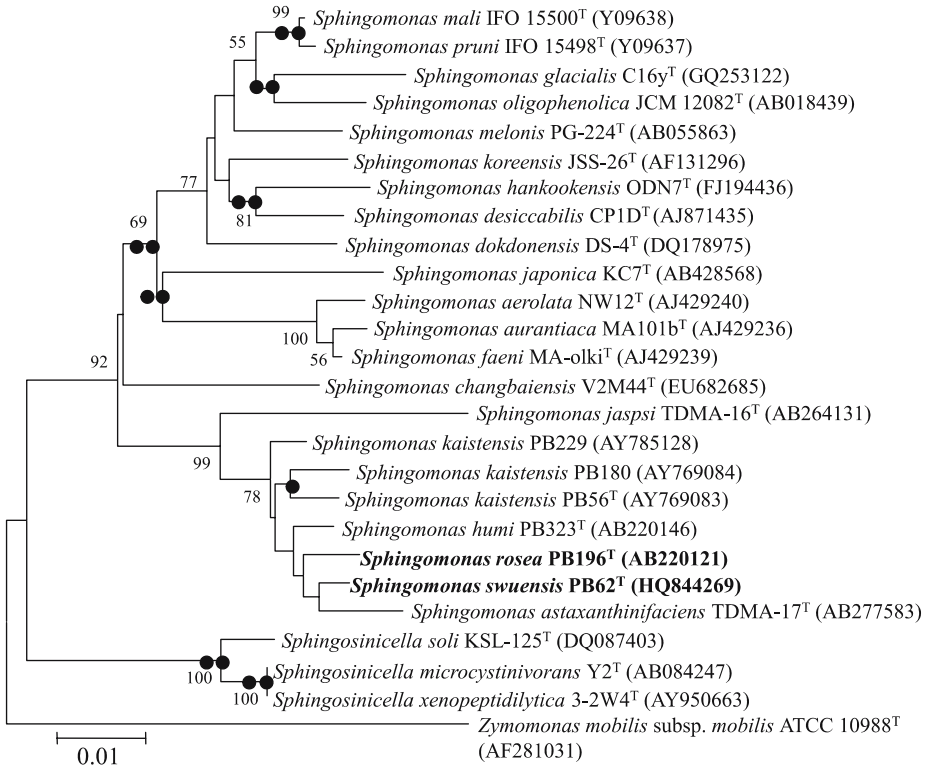

Fig. 2. A phylogenetic tree based on the 16S rRNA gene sequences of strains PB196^T and PB62^T and representatives of related taxa. The neighbor-joining method was used. The bar represents 0.01 substitutions per nucleotide position. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50% are shown at the branch points. A black circle indicates the common nodes recovered from either the maximum-parsimony algorithm or the maximum-likelihood tree. Black double-circles indicate that the corresponding nodes were recovered in both the maximum-parsimony tree and the maximum likelihood tree.

Phylogenetic analysis

The 16S rRNA gene sequences of the two strains were continuous stretches of 1,380 nucleotides. Both strains belonged to the class *Alphaproteobacteria*, the order *Sphingomonadales* and the family *Sphingomonadaceae*. The highest degree of sequence similarity for strain PB196^T was found with PB62^T (98.9%), *Sphingomonas humi* PB323^T (98.9%) (Yi *et al*., 2010), *Sphingomonas kaistensis* PB56^T (98.2%) (Kim *et al*., 2007), and *Sphingomonas astaxanthinifaciens* TDMA-17^T (98.0%) (Asker *et al*., 2007). The highest degree of sequence similarity for strain PB62^T was found with *S. humi* PB323^T (98.8%), *S. astaxanthinifaciens* TDMA-17^T (98.2%), and *S. kaistensis* PB56^T (98.1%). Lower sequence similarities were found with other described *Sphingomonas* species (<96.2%). In the phylogenetic tree (Fig. 2), both strains clearly belonged to the genus *Sphingomonas* lineage based on 16S rRNA gene sequences and the phylogenetic positions of the strains among members of the genus *Sphingomonas* was unique and distinct.

DNA-DNA hybridization

The G+C content of genomic DNA from strains PB196^T and PB62^T were 69.9 mol% and 71.1%, respectively. Strain PB196^T exhibited DNA-DNA relatedness with the closely related strains PB62^T (24.1±1.1%), *S. humi* PB323^T (38.4±4.9%), *S. kaistensis* PB56^T (41.6±3.9%), *S. kaistensis* PB180 (48.1±4.7%), and *S. astaxanthinifaciens* TDMA-17^T (21.0±3.1%). In addition, strain PB62^T exhibited DNA-DNA relatedness with PB196^T (24.5±2.5%), *S. humi* PB323^T (18.6±5.9%), *S. kaistensis* PB56^T (35.4±4.4%), *S. kaistensis* PB180 (25.6±5.2%), and *S. astaxanthinifaciens* TDMA-17^T (19.1±3.8%). So, DNA-DNA hybridization levels between the two strains PB196^T and PB62^T were less than 70% and DNA-DNA hybridization levels between the two strains and other type strains were also less than 70%, which is the threshold for delineating a genomic species (Wayne *et al*., 1987; Stackebrandt and Goebel, 1994). Thus, our results support the placement of the two strains PB196^T and PB62^T as representative of two separate and previously unrecognized genomic species, respectively.

Chemotaxonomic analysis

The predominant cellular fatty acids of strain PB196^T were summed feature 7 ($C_{18:1}\omega7c/\omega9t/\omega12t$) (39.9%), summed feature 4 ($C_{16:1}\omega7c/C_{15:0}$ iso 2OH) (35.3%), $C_{16:0}$ (12.8%), and $C_{17:1}\omega6c$ (5.3%). Strain PB196^T contained minor fatty acids such as $C_{16:1}\omega5c$ (2.5%), $C_{18:1}$ 2OH (1.7%), $C_{18:1}\omega5c$ (1.0%), $C_{15:0}$ (0.8%), and $C_{17:1}\omega8c$ (0.9%). Strain PB62^T showed a similar fatty acid profile to strain PB196^T: its predominant cellular fatty acids were summed feature 7 ($C_{18:1}\omega7c/\omega9t/\omega12t$) (39.5%), summed feature 4 ($C_{16:1}\omega7c/C_{15:0}$ iso 2OH) (36.6%), $C_{17:1}\omega6c$ (10.9%), and $C_{16:0}$ (6.4%) and minor cellular fatty

acids were $C_{16:1}\omega5c$ (2.7%), $C_{17:1}\omega8c$ (1.7%), $C_{14:0}$ (1.3%), and $C_{15:0}$ (1.1%). The fatty acid profiles of the two strains were similar to those of closely related *Sphingomonas* species (Table 2). Strains PB196^T and PB62^T contained ubiquinone-10 (Q-10) as the predominant respiratory quinone, which is common with that of the class *Alphaproteobacteria*. Alkaline stable sphingoglycolipid reported for the genus *Sphingomonas* (Busse *et al.*, 1999) was also detected from strains PB196^T and PB62^T; which clearly showing that the strains belong to the genus *Sphingomonas* (data is not shown). Major polar lipids found in strains PB196^T and PB62^T were sphingoglycolipid (SGL) and phosphatidylglycerol (PG). Minor amounts of unidentified lipids were also found (Fig. 3). Strain PB62^T showed diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) as major polar lipids whereas strain PB196^T showed no diphosphatidylglycerol (DPG).

Taxonomic conclusion

The results obtained in this study clearly showed that the two new isolates PB196^T and PB62^T belong to two new species in the genus *Sphingomonas*. They showed lower DNA-DNA relatedness value (<25%). In morphology, strain PB196^T showed round and convex colonies with long rod-shaped cells whereas strain PB62^T showed dry and raised colonies with short rod-shaped cells (Fig. 1). Moreover, strains PB196^T and PH62^T have a major difference in their polar lipid profiles. Strain PB196^T showed major amounts of PG and SGL with no DPG whereas strain PB62^T showed DPG, PG, PE, and SGL as major polar lipids.

Based on the results, we propose that the strains PB196^T and PB62^T represent two separate novel species of the genus *Sphingomonas*, for which we propose the names *Sphingomonas rosea* sp. nov. and *Sphingomonas swuensis* sp. nov., respectively.

Table 2. Cellular fatty acid profiles of novel strains and type strains of closely related *Sphingomonas* species

Strains: 1, PB196^T; 2, PB62^T; 3, *S. humi* KCTC 12341^T; 4, *S. kaistensis* KCTC 12334^T; 5, *S. astaxanthinifaciens* NBRC 102146^T.

All strains were grown in this study on TSA at 30°C for 3 days for fatty acid analysis. The position of the double bond was located by counting from the methyl (ω) end of the carbon chain for unsaturated fatty acids. The *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. †Summed feature contained fatty acids, which could not be separated by GLC with the Microbial Identification System. ND, not detected; -, trace (<1.0%)

Fatty acid	1	2	3	4	5
Saturated fatty acids					
14:0	ND	1.3	-	ND	-
15:0	-	1.1	1.4	ND	ND
16:0	12.8	6.4	7.6	8.6	12.6
17:0	ND	ND	-	1.1	-
Unsaturated fatty acids					
16:1 $\omega5c$	2.5	2.7	2.0	4.0	2.6
17:1 $\omega6c$	5.3	10.9	8.2	7.6	3.6
17:1 $\omega8c$	-	1.7	1.6	2.0	-
18:1 $\omega5c$	1.0	ND	-	1.2	-
18:1 $\omega7c$ 11-methyl	ND	ND	ND	ND	3.1
Hydroxy fatty acids					
14:0 2OH	ND	ND	1.2	-	-
18:1 2OH	1.7	ND	-	1.3	1.2
†Summed feature 4 (16:1$\omega7c$ / 15:0 iso 2OH)	35.3	36.6	33.5	29.9	30.2
†Summed feature 7 (18:1$\omega7c$/$\omega9t$/$\omega12t$)	39.9	39.5	42.5	43.2	44.2

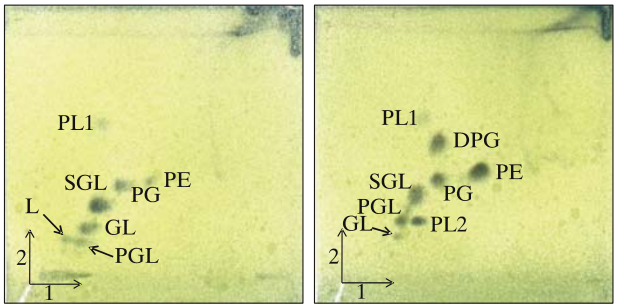

Fig. 3. Two dimensional thin-layer chromatograms of polar lipids from strains PB196^T (A) and PB62^T (B). The characteristic polar lipid of *Sphingomonas* species, sphingoglycolipid (SGL), is clearly shown in both chromatograms.
Ascending solvent system: (I) chloroform/methanol/water (65:25:4, v/v/v); (II) chloroform/ methanol/acetic acid/water (80:12:15:4, v/v/v/v). For detection of the polar lipids, molybdatophosphoric acid (PE, DPG, PG, SGL, PGL, GL, PL, and L), ninhydrin (PE), Zinzadze reagent (PE, DPG, PG, PGL, PL, and L) and α-naphthol reagent (SGL, PGL and GL) were applied.
Abbreviations: PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol PG, phosphatidylglycerol; SGL, sphingoglycolipid; PGL, Phosphoglycolipid; GL, unknown glycolipid; PL, unknown phospholipid; L, unknown lipid.

Description of *Sphingomonas rosea* sp. nov.

Sphingomonas rosea (ro'se.a. L. fem. adj. *rosea* rose-colored, rosy).

Strain PB196^T is 0.3-0.5 μm wide and 1.0-2.5 μm long, Gram-negative, non-motile, and long rod-shaped when grown on LB agar (Difco) at 30°C for 5 days. Colonies are orange-red colored, circular, raised, and convex with entire margin on LB or R2A agar, which also shows uniform suspension during growth in LB or R2A broth. It can grow at a temperature range of 25-42°C, but not at 4°C or 60°C. The optimum growth temperature was 37°C. Acid is not produced from D-glucose and indole is not produced. No reduction of nitrate to nitrite or nitrogen is observed.

Growth of strain PB196^T is positive with fucose, D-glucose, maltose, D-mannose, L-rhamnose, adipate, gluconate, 4-hydroxybenzoate, malate, phenyl acetate, L-proline, and L-mannitol.

Growth of strain PB196^T is negative with L-arabinose, glycogen, D-melibiose, D-ribose, D-sucrose, acetate, caprate, citrate, 3-hydroxybenzoate, 3-hydroxybutyrate, itaconate, 2-ketogluconate, 5-ketogluconate, DL-lactate, malonate, propionate, salicin, suberate, valerate, N-acetyl-glucosamine, L-alanine, L-histidine, L-serine, myo-inositol, and D-sorbitol.

In tests with the API Zym system, strain PB196^T is positive for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase (C8), α-glucosidase, β-glucosidase (esculin hydrolysis), leucine arylamidase, naphtol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase.

In tests with the API Zym system, strain PB196^T is negative for N-acetyl-β-glucosaminidase, arginine hydrolase, α-chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, lipase (C14), α-mannosidase, protease (gelatin hydrolysis), and urease.

The predominant cellular fatty acids of strain PB196^T is summed feature 7 ($C_{18:1}\omega7c/\omega9t/\omega12t$), summed feature 4 ($C_{16:1}\omega7c/C_{15:0}$ iso 2OH), $C_{16:0}$, and $C_{17:1}\omega6c$. The major polar lipids are sphingoglycolipid (SGL) and phosphatidylglycerol (PG).

Description of *Sphingomonas swuensis* sp. nov.

Sphingomonas swuensis (swu.en'sis. N.L. f. adj. *swuensis* of or belonging to SWU, Seoul Women's University, where taxonomic study was performed for the new organisms).

Strain PB62^T is 0.4-0.6 μm wide and 0.7-0.9 μm long, Gram-negative, non-motile, and short rod-shaped when grown on LB agar (Difco) at 30°C for 5 days. Colonies are orange-red colored, dry, and raised on LB or R2A agar, which also shows heterogeneous small granular or sandy suspension during growth in LB or R2A broth. Acid is not produced from D-glucose and indole is not produced. No reduction of nitrate to nitrite or nitrogen is observed.

Growth of strain PB62^T is positive with maltose, D-mannose, L-rhamnose, ribose, sucrose, acetate, adipate, gluconate, 4-hydroxybenzoate, itaconate, DL-lactate, malate, malonate, suberate, L-alanine, L-proline, L-serine, myo-inositol, and mannitol.

Growth of strain PB62^T is negative with L-arabinose, L-fucose, D-glucose, glycogen, D-melibiose, caprate, citrate, 3-hydroxybenzoate, 3-hydroxybutyrate, 2-ketogluconate, 5-ketogluconate, phenyl acetate, propionate, salicin, valerate, N-acetyl-glucosamine, L-histidine, and D-sorbitol.

In tests with the API Zym system, strain PB62^T is positive for acid phosphatase, alkaline phosphatase, arginine dihydrolase, esterase (C4), esterase (C8), α-glucosidase, β-glucosidase (esculin hydrolysis), leucine arylamidase, naphtol-AS-BI-phosphohydrolase, trypsin, urease, and valine arylamidase.

In tests with the API Zym system, strain PB62^T is negative for N-acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, lipase (C14), α-mannosidase, and protease (gelatin hydrolysis).

The predominant cellular fatty acids of strain PB62^T are summed feature 7 ($C_{18:1}\omega7c/\omega9t/\omega12t$), summed feature 4 ($C_{16:1}\omega7c/C_{15:0}$ iso 2OH), $C_{16:0}$, and $C_{17:1}\omega6c$. The major polar lipids are sphingoglycolipid (SGL), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE).

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