# Stenotrophomonas panacihumi sp. nov., Isolated from Soil of a Ginseng Field

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(Received January 6, 2010 / Accepted February 17, 2010)

The study isolated a Gram-negative, rod-shaped, non-motile bacterium from the soil of a ginseng field in Daejeon, South Korea and characterized it to determine its taxonomic position. Phylogenetic analysis, based on the 16S rRNA gene sequence, revealed that strain MK06<sup>T</sup> belongs to the family *Xanthomonadacea*, and showed the highest degree of sequence similarity to *Stenotrophomonas rhizophila* e-p10<sup>T</sup> (98.6%), *Xanthomonas campestris* LMG 568<sup>T</sup> (98.0%), *Stenotrophomonas maltophilia* ATCC 1d3637<sup>T</sup> (97.3%), and *Stenotrophomonas humi* R-32729<sup>T</sup> (96.9%). Chemotaxonomic data revealed that strain MK06<sup>T</sup> possesses ubiquinone Q-8 as the predominant respiratory lipoquinone, which is common in the genus *Stenotrophomonas*, and that the predominant fatty acids were 15:0 iso (41.1%), 15:0 anteiso (12.6%), and 17:1 iso  $\omega$ 9c (8.6%). The results of physiological and biochemical tests clearly demonstrated that strain MK06<sup>T</sup> represents a distinct species and supported its affiliation with the genus *Stenotrophomonas*. Based on these data, MK06<sup>T</sup> (KCTC, 22893<sup>T</sup>; JCM, 16536<sup>T</sup>; KEMB, 9004-002<sup>T</sup>) should be classified as the type strain for a novel species, for which we propose the name *Stenotrophomonas panacihumi* sp. nov.

Keywords: 16S rRNA gene, S. panacihumi, taxonomy, Xanthomonadaceae

The genus *Stenotrophomonas* came about when Palleroni and Bradbury (1993) proposed reclassifying *Xanthomonas maltophilia* as *Stenotrophomonas maltophilia*. *Stenotrophomonas* belongs to the class *Gammaproteobacteria* (Moore *et al.*, 1997). This genus comprises eight species with validly published names. Members of this genus have been isolated from various environmental sources. *Stenotrophomonas acidaminiphila* and *Stenotrophomonas nitritireducens* were isolated from waste (Finkmann *et al.*, 2000; Assih *et al.*, 2002); *Stenotrophomonas terrae* from soil (Heylen *et al.*, 2007; Kim *et al.*, 2009); *Stenotrophomonas rhizophila* from the rhizosphere (Wolf *et al.*, 2002); *Stenotrophomonas koreensis* from compost (Yang *et al.*, 2006); and *S. maltophilia* from clinical samples (Palleroni and Bradbury, 1993).

In a series of earlier studies, we had attempted to isolate microorganisms from soil in order to investigate their community structure based on a culture dependent method. In this study, we isolated a strain from soil in a ginseng field and characterized it using a polyphasic approach, which included analyses based on phylogenetic 16S rRNA gene sequences, genomic relatedness, and chemotaxonomic and phenotypic properties, in order to determine the precise taxonomic position of the strain MK06<sup>T</sup>. This study assigns MK06<sup>T</sup> as a new member of the genus *Stenotrophomonas*.

#### **Materials and Methods**

**Isolation of bacterial strains and culture conditions** We isolated the strain MK06<sup>T</sup> from soil in a ginseng field, via direct plating onto ten-times diluted R2A agar (Difco, USA), then purified single colonies from the plates by transferring them onto new plates and subjecting them to additional incubation for 5 days at 30°C. Using partial 16S rRNA gene sequences, we tentatively identified the purified colonies, cultured them, in the routine manner, on R2A agar (Difco) at 30°C, and preserved them in glycerol solution (20%, w/v) at -70°C. We then submitted this organism to the Korean Collection for Type Cultures and Japan Collection of Microorganisms (KCTC 22893<sup>T</sup> = JCM 16536<sup>T</sup>).

#### Phenotypic and biochemical characteristics

Using a Nikon light microscope  $(1,000 \times \text{magnification})$ , we observed the cells' morphology and motility, allowing the cells to grow for 3 days at 30°C on R2A agar. We tested the organism's Gram reactions according to the non-staining method described by Buck (1982), evaluated its oxidase activity via the oxidation of 1% (w/v) tetramethylp-phenylenediamine, and determined its catalase activity by measuring bubble production after the application of 3% (v/v) hydrogen peroxide solution. To determine the organism's anaerobic growth, we employed serum bottles containing R2A broth, supplemented with thioglycolate (1 g/L), in which we had replaced the upper air layer with nitrogen. We assessed its growth on LB agar at different temperatures (4, 10, 25, 30, 37, 40, 42, and 45°C) for 5 days. We also assessed its growth at various pHs (5, 6, 7, 8, 9, 10, and 11), in LB broth at 30°C, and on different media (TSA, LB, NA, and R2A agar). To adjust the pH of the LB broth, we added phosphate buffer and then used 1 N HCl or 1 N NaOH to achieve the desired pH. To study the carbon source utilization and assay the enzyme activity of strain MK06<sup>T</sup>, we employed the API 20NE, API ID32 GN, and API ZYM microtest systems according to the recommendations of the manufacturer (bioMérieux, France).

# Isoprenoid quinones and cellular fatty acids

We extracted isoprenoid quinones from the cells with chloroform/

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methanol (2:1, v/v), purified them via TLC (thin-layer chromatography), then used vacuum conditions to evaporate them and reextracted them in n-hexane:water (1:1, v/v). Then we purified the crude n-hexane quinine solution and analyzed it via HPLC, as previously described (Collins and Jones, 1981; Shin *et al.*, 1996). To perform fatty acid methyl ester analysis, we allowed the strain to grow on TSA for 48 h at 30°C, harvested two loops of the well-grown cells, and then prepared, separated, and identified the fatty acid methyl esters using the Sherlock Microbial Identification System (MIS), produced by MIDI Inc. (Newark, DE, USA) (Sasser, 1990).

### Xanthomonadin pigment analysis

Xanthomonadins are a unique class of carotenoid-like pigments, which are produced by members of the phytopathogenic genus *Xanthomonas*. We extracted these pigments from strain  $MK06^{T}$  and analyzed them according to Goel *et al.* (2001).

# Determination of DNA G+C content

To determine the strain's G+C content, we extracted genomic DNA, purified it via the QIAGEN Genomic-tip system 100/G (QIAGEN, Japan), and enzymatically degraded it into nucleosides. We analyzed the nucleosides using reverse-phase HPLC, as previously described (Tamaoka and Komagata, 1984; Mesbah *et al.*, 1989).

# PCR amplification, 16S rRNA sequencing and phylogenetic analysis

Using the universal bacterial primer set, 9F and 1512R (Weisburg et al., 1991) we amplified the 16S rRNA gene from the chromosomal DNA, and Genotech, in Daejeon, Korea, sequenced the purified PCR product (Kim et al., 2005). We compiled the full sequence of the 16S rRNA gene using SeqMan software (DNASTAR Inc., USA), obtaining the 16S rRNA gene sequences of the related taxa from GenBank and editing them using the BioEdit program (Hall, 1999). We performed multiple alignments with the CLUSTAL X program (Thompson et al., 1997), calculated evolutionary distances using the Kimura twoparameter model (Kimura, 1983), and constructed the phylogenetic tree via the neighbor-joining method (Saitou and Nei, 1987) in the MEGA 2 Program (Kumar et al., 2001). Furthermore, we conducted b ootstrap analyses, with 1,000 replicates, to obtain confidence leve ls for the branches (Felsenstein, 1985) and constructed a maximumlikelihood tree using the PHYLIP Program (Choi et al., 2000; Brinkman et al., 2001). We included in the phylogenetic tree the closest relatives of the strain MK06<sup>T</sup>, all the *Stenotrophomonas* species strains, and the closely-related type species in the family Xanthomonadaceae.

#### **DNA-DNA hybridization**

We performed the DNA-DNA hybridization fluorometrically, according to the method developed by Ezaki *et al.* (1989), using photobiotinlabelled DNA probes and micro-dilution wells, with five replications per sample. We excluded each sample's highest and lowest values, using only the remaining three and taking their mean as our DNArelatedness value for each.

The NCBI GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MK06<sup>T</sup> (=KCTC 22893<sup>T</sup> =JCM 16536<sup>T</sup> =KEMB 9004-002<sup>T</sup>) is GQ856217.

# **Result and Discussion**

# Morphological and phenotypic characteristics

When cultured on R2A agar (Difco) at 30°C, strain MK06<sup>T</sup> yields yellow, circular colonies. The cells were Gram-negative,

non-motile, and rod-shaped. Strain  $MK06^T$  grew in a temperature range of 4-40°C, but not at 45°C. Its optimum-growth temperature was 25-30°C. We summarize the physiological characteristics of the strain  $MK06^T$  in the species description, and Table 1 shows a comparison of selected characteristics of the strain with related strains of *Stenotrophomonas* species.

#### Cellular fatty acids and isoprenoid quinones

Table 2 shows the cellular fatty acid profile of strain MK06<sup>T</sup>. The cellular fatty acids included 15:0 iso (41.1%), 15:0 anteiso (12.6%), 17:1 iso  $\omega$ 9c (8.6%), summed feature 4 (16:1)ω7c/15:0 iso 2OH) (7.5%), 16:0 (6.1%), 11:0 (4.5%), and 13:0 iso 3OH (4.3%). The fatty acid profile of strain MK06<sup>T</sup> actually differs from those of other Stenotrophomonas species. Strain MK06<sup>T</sup> contains no 10:0 iso, 11:0 iso, 12:0 iso 3OH, 13:0 iso, 15:0, 15:1 w8c, 6:0 10 methyl, 17:0, 17:0 iso, 17:0 anteiso, 17:1 w8c, or 17:0 cyclo. Another major difference is the presence of fatty acid 11:0, which is not common in other Stenotrophomonas species. Strain MK06<sup>T</sup> can be differentiated from the genus Xanthomonas by the presence of higher amounts of 15:0 iso, as seen in other Stenotrophomonas species. In addition, strain MK06<sup>T</sup> contained less hydroxy fatty acid 13:0 iso 3OH and lacked a branched fatty acid, 17:0 iso, that is common in the genus Xanthomonas (Lee et al., 2008). Strain MK06<sup>T</sup> contained ubiquinone Q-8 as the predominant respiratory lipoquinone, which is commonly found in the family Xanthomonadaceae (Yang et al., 2006; Yoon et al., 2006).

### Xanthomonadin pigment analysis

Xanthomonadins are a unique class of carotenoid-like pigments, produced by members of the phytopathogenic genus *Xanthomonas* (Palleroni and Bradbury, 1993). Xanthomonadins can be used as characteristic markers, differentiating *Xanthomonas* species from *Stenotrophomonas* species. The pigment analysis (Fig. 2) showed *Xanthomonas* campestris KCTC 22159<sup>T</sup> (type species of the genus *Xanthomonas*) was a strong xanthomonadin producer, whereas *Stenotrophomonas* species, including *S. maltophilia* ATCC 1d3637<sup>T</sup> (type species) produced no pigment. Strain MK06<sup>T</sup> did not produce as much xanthomonadin as *X. campestris* KCTC 22159<sup>T</sup>, showing little pigment production at all. This supports the assertion that strain MK06<sup>T</sup> belongs to the genus *Stenotrophomonas* and not to the genus *Xanthomonas*.

### **Phylogenetic analysis**

We found the 16S rRNA gene sequence of strain MK06<sup>T</sup> was a continuous stretch of 1476 nucleotides. The 16S rRNA gene sequences of the related taxa were obtained from GenBank. The strain MK06<sup>T</sup> belonged to the class *Gammaproteobacteria*, the order Xanthomonadales, and the family Xanthomonadaceae. The 16S rRNA gene sequence of strain MK06<sup>T</sup> showed the highest degree of sequence similarity to Stenotrophomonas *rhizophila* e-p10<sup>T</sup> (98.6%), *X. campestris* LMG 568<sup>T</sup> (98.0%), S. maltophilia ATCC 1d3637<sup>T</sup> (97.3%), and S. humi R-32729<sup>T</sup> (96.9%). In all three phylogenetic trees, constructed via Neighbor-Joining, Maximum-Parsimony, and Maximum-Likelihood algorithms, respectively, strain MK06<sup>T</sup> was clearly affiliated to the branch of the Stenotrophomonas species (Fig. 1). Based on 16S rRNA gene sequencing, the phylogenetic position of strain MK06<sup>T</sup> among members of the family Xanthomonadaceae was unique and distinct.

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**Table 1.** Differential phenotypic characteristics between strain MK06<sup>T</sup> and related strains

Strains: 1, S. panacihumi MK06<sup>T</sup>; 2, S. acidaminiphila DSM 13117<sup>T</sup>; 3, S. ginsengisoli DCY01<sup>T</sup>; 4, S. humi DSM 18929<sup>T</sup>: 5, S. koreensis KCTC 12211<sup>T</sup>; 6, S. maltophilia DSM 50170<sup>T</sup>; 7, S. nitritireducens DSM 12575<sup>T</sup>; 8, S. rhizophila e-p10<sup>T</sup>; 9, S. terrae DSM 18941<sup>T</sup>; 10, X. campestris KCTC 22159<sup>T</sup>; 11, Xanthomonas cynarae CFBP 4188<sup>T</sup> (Trébaol et al., 2000); 12, Xanthomonas populi CFBP 1818<sup>T</sup> (Ridé and Ridé, 1992), and 13, Pseudomonas aeruginosa KCTC 1750<sup>T</sup>.

All strains are Gram-negative and are non-producers of indole.

+, positive; -, negative; w, weak positive; R, rod; S/C-R, straight or curved rods; CR, curved rod; W, white; Y, yellow; PY, pale yellow; CY, cream yellow; BY, bright yellow; B/BG, brown/ blue green

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Motility	-	+	-	+	-	+	-	-	+	+	+	+	+
Characteristic morphology	R	S/C-R	R	R	CR	S/C-R	S/C-R	S/C-R	R	R	Straight rod	SR	R
Colony color	pale Y	PY	Y	Y	Y	PY	Y	Y	Y	BY	Yellow	Y	BG
Production of Xanthomonadin	-	ND	ND	-	-	-	-	-	-	+	+	+	-
Oxidase	+	+	+	+	+	-	-	-	+	-	-	-	+
Nitrate reduction to NO <sub>2</sub>	-	+	-	+	-	+	-	+	+	-	-	ND	+
Enzyme activity													
Esculin hydrolysis	-	-	-	-	-	+	-	+	-	+	+	+	-
Gelatin hydrolysis	w	-	w	w	+	+	-	+	+	+	+	-	+
Urease	-	-	-	-	-	+	+	-	-	-	-	-	-
Fermentation of sugar													
Glucose	-	w	-	+	-	-	-	+	+	+	+	+	+
D-Arabinose	-	-	w	+	+	+	-	-	w	w	-	-	-
Assimilation test													
2-Ketogluconate ( $\alpha$ )	+	-	-	-	-	-	-	-	-	+	+	-	+
D,L-3-Hydroxybutyrate	+	+	+	-	+	-	+	-	-	-	+	-	+
4-Hydroxybenzoate	w	-	-	-	-	-	-	-	-	+	+	-	-
Acetate	+	+	-	w	+	+	+	+	w	-	+	-	-
Adipate	w	-	-	-	-	-	-	-	-	-	-	ND	+
Citrate	+	-	-	+	-	+	+	+	+	+	+	-	+
Malonate	+	-	-	w	-	+	-	+	w	+	-	ND	+
Propionate	w	w	-	-	-	-	-	-	+	w	-	-	+
Suberate	-	-	-	-	-	-	-	+	+	+	-	-	W
n-Valerate	+	w	-	-	-	-	-	w	+	+	-	-	W
D-Glucose	+	+	+	-	-	+	+	+	+	+	+	+	+
D-Maltose	-	+	+	w	-	+	-	+	+	+	+	ND	-
D-Mannose	+	+	-	+	-	w	-	+	+	w	+	+	-
L-Rhamnose	+	-	+	-	-	-	-	-	-	-	-	-	-
D-Sucrose	w	-	-	-	-	+	-	+	w	w	+	+	W
D-Mannitol	-	-	+	-	-	-	-	-	-	-	-	-	-
L-Alanine	w	+	-	w	+	+	+	-	w	+	+	-	W
L-Histidine	w	+	-	-	-	+	+	+	w	+	+	-	W
L-Serine	w	+	-	w	-	+	w	-	+	-	+	-	W
N-Acetyl-D-glucosamine	w	+	+	+	-	+	+	+	+	+	+	+	+
Salicin	-	-	-	-	-	-	-	+	-	-	-	-	+
G+C content	67.77	66.4- 67.4	65.5	64	66	66.7	69.1	67.9	65	65-66	63	62-65	66.6

### DNA G+C content

The G+C content of the genomic DNA of the strain  $MK06^{T}$  was 67.8 mole percentage, which was similar to that of other *Stenotrophomonas* species (65-69 mol %).

# **DNA-DNA hybridization**

Strain MK06<sup>T</sup> exhibited relatively low levels of DNA-DNA relatedness values to the type strains, *S. acidaminiphila* DSM  $13117^{T}$  (7%), *S. ginsengisoli* DCY01<sup>T</sup> (8%), *S. humi* DSM

18929<sup>T</sup> (16%), *S. koreensis* KCTC 12211<sup>T</sup> (14%), *S. maltophilia* DSM 50170<sup>T</sup> (25%), *S. nitritireducens* DSM 12575<sup>T</sup> (15%), *S. rhizophila* e-p10<sup>T</sup> (46%), *S. terrae* DSM 18941<sup>T</sup> (19%), *X. campestris* KCTC 22159<sup>T</sup> (7%), and *P. aeruginosa* KCTC 1750<sup>T</sup> (2%).

We determined the DNA-DNA hybridization levels to other type strains were less than 70% (Wayne *et al.*, 1987; Stackebrandt and Goebel, 1994), which is the threshold that delineates a genomic species. Thus, our results support the placement of Table 2. Cellular fatty acid profiles of strain MK06<sup>T</sup> and related species

Strains: 1, *S. panacihumi* MK06<sup>T</sup>; 2, *S. acidaminiphila* DSM 13117<sup>T</sup>; 3, *S. ginsengisoli* DCY01<sup>T</sup>; 4, *S. humi* DSM 18929<sup>T</sup>: 5, *S. koreensis* KCTC 12211<sup>T</sup>; 6, *S. maltophilia* DSM 50170<sup>T</sup>; 7, *S. nitritireducens* DSM 12575<sup>T</sup>; 8, *S. rhizophila* e-p10<sup>T</sup>; 9, *S. terrae* DSM 18941<sup>T</sup>; 10, *X. campestris* KCTC 22159<sup>T</sup>, and 11, *P. aeruginosa* KCTC 1750<sup>T</sup>.

All species grew on TSA agar at 30°C for 3 days before we obtained their data.

Fatty acids that are less than 1.0% of the total fatty acids are noted as -. Therefore, the percentages do not add up to 100%. For unsaturated fatty acids, locate the position of the double bond by counting from the methyl ( $\omega$ ) end of the carbon chain. The *cis* 7 and *trans* isomers are indicated by the suffixes *c* and *t*, respectively.

Fatty acids	1	2	3	4	5	6	7	8	9	10	11
Saturated											
$C_{10:0}$	1.6	-	-	ND	ND	ND	-	-	ND	-	-
$C_{11:0}$	4.5	ND	-	ND	ND	ND	-	-	ND	ND	nd
C <sub>12:0</sub>	ND	3.7									
$C_{14:0}$	3.3	-	1.3	2.0	2.1	ND	3.1	2.4	1.5	-	1.5
$C_{15:0}$	ND	ND	7.5	1.7	2.1	3.3	1.0	1.2	1.8	-	-
$C_{16:0}$	6.1	1.8	2.0	3.5	1.8	6.5	1.4	4.6	2.8	4.2	21.3
$C_{17:0}$	ND	-	-	ND	ND	ND	-	1.2	ND	ND	-
Unsaturated											
$C_{15:1} \omega 8c$	ND	-	1.6	ND	ND	ND	1.3	-	ND	-	ND
$C_{16:1} \omega 9c$	2.2	1.0	ND	1.4	ND	3.7	ND	ND	2.4	ND	ND
C <sub>17:1</sub> ω8c	ND	-	1.8	8.9	ND	ND	ND	ND	-	1.4	ND
C <sub>17:1</sub> iso ω9c	8.6	13.0	ND	4.6	3.3	4.5	6.3	7.8	9.5	9.3	ND
$C_{18:1} \omega 9c$	ND	37.2									
Branched chain											
C <sub>10:0</sub> iso	ND	2.4	-	ND	-	ND	1.2	-	-	ND	ND
C <sub>11:0</sub> iso	ND	5.4	4.8	4.2	6.8	3.4	1.6	10.6	5.8	4.6	ND
C <sub>13:0</sub> iso	ND	-	1.1	2.5	8.9	-	3.1	1.8	1.6	ND	ND
C <sub>14:0</sub> iso	-	8.7	3.9	2.3	4.2	4.5	4.6	3.5	7.8	-	ND
C <sub>15:0</sub> anteiso	12.6	4.9	1.3	7.8	1.6	7.0	5.0	3.8	5.3	16.6	ND
C <sub>15:0</sub> iso	41.1	24.0	28.2	25.4	34.0	36.7	34.6	30.9	37.4	24.6	ND
C <sub>15:1</sub> iso F	-	2.6	10.8	12.1	18.2	7.3	8.6	8.8	4.9	-	ND
C <sub>16:0</sub> iso	-	13.3	4.5	2.6	1.4	3.6	4.9	3.6	5.5	3.8	ND
C <sub>17:0</sub> iso	ND	2.7	2.5	1.8	-	3.1	1.6	2.6	1.3	10.6	ND
C <sub>16:0 10</sub> methyl	ND	ND	13.2	ND	ND	ND	ND	-	ND	ND	ND
Hydroxy											
C <sub>10:0</sub> 3OH	ND	3.4									
C <sub>11:0</sub> 3OH	-	-	1.2	1.2	1.2	ND	0.6	1.6	-	ND	ND
C <sub>12:0</sub> 3OH	1.9	2.1	-	2.1	0.9	3.0	1.2	-	-	ND	ND
C <sub>11:0</sub> iso 3OH	3.3	2.7	3.1	2.6	4.8	1.7	3.8	1.2	2.7	3.6	ND
C <sub>12:0</sub> iso 2OH	ND	3.5									
C <sub>12:0</sub> iso 3OH	ND	3.8	-	1.5	ND	ND	-	ND	1.4	ND	4.8
C <sub>13:0</sub> iso 3OH	4.3	1.7	1.6	2.2	3.1	3.5	4.2	2.6	2.3	8.7	ND
C <sub>17:0</sub> cyclo	ND	ND	3.2	ND	-	ND	1.6	1.0	1.8	ND	1.7
Summed feature <sup>a</sup>											
2; C <sub>15:1</sub> iso H / C <sub>13:0</sub> 3OH	ND	ND	ND	-	ND	4.4	ND	0.6	ND	ND	ND
4; $C_{16:1}\omega7c$ / $C_{15:0}$ iso 2OH	7.5	6.3	2.4	7.6	2.8	ND	5.6	4.6	1.3	8.4	19.7
7; $C_{18:1} \omega$ 7c / $\omega$ 9t / $\omega$ 12t	ND	ND	ND	1.1	ND	2.2	3.2	2.6	ND	ND	ND

<sup>a</sup> The summed feature contained fatty acids, which could not separated by GLC with the Microbial Identification System (MIDI). ND=not determined.

strain MK06<sup>T</sup> as a separate and previously unrecognized species.

# **Taxonomic conclusion**

The genus *Stenotrophomonas* originated from the genus *Xanthomonas*, thus their genetic and phenotypic properties are highly homologous (Hugh, 1981; Hauben *et al.*, 1999). The new isolate, strain MK06<sup>T</sup>, can be differentiated from genus

*Xanthomonas* because it produces no xanthomonadin pigment, contains higher amounts of 15:0 iso fatty acids, and produces oxidase (Palleroni and Bradbury, 1993). Based on the phenotypic, chemotaxonomic, and phylogenetic data, we concluded that the strain MK06<sup>T</sup> is a novel species of the genus *Stenotrophomonas*, for which we propose the name *S. panacihumi* sp. nov.

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**Fig. 1.** Phylogenetic relationships between the strain  $MK06^{T}$  and related species (all *Stenotrophomonas* species and related genera in the *Xanthomonas* branch), using the neighbor-joining method, with one bar representing 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 filled circle indicates the common nodes recovered from either the Maximum-Parsimony algorithm or the maximum-likelihood tree. Filled double-circles indicate that the corresponding nodes were recovered in both the maximum-parsimony tree and the maximum likelihood tree.

#### Description of S. panacihumi sp. nov.

*S. panacihumi* (pana.ci.humi. N.L. n. *Panax*, scientific name of ginseng; L. n. *humus*, soil; N.L. gen. n. *panacihumi*, of soil of a ginseng field).

MK06<sup>T</sup> is Gram-negative, aerobic, non-motile rod, 0.2-0.5  $\mu$ m wide and 0.8-3.0  $\mu$ m long when grown on LB agar (Difco) at 30°C for 5 days. The colonies are circular and pale yellow in color. Optimal growth temperature is 25-30°C, and the optimum pH is 6.0-8.0. It grows better on TSA agar, NA agar, and LB agar than on R2A agar. It is oxidase and catalase



**Fig. 2.** Xanthomonadin pigment analyses of strain MK06<sup>T</sup> and related taxa. Lanes: 1, *X. campestris* KCTC 22159<sup>T</sup> (type species); 2, *S. panacihumi* MK06<sup>T</sup>; 3, *S. maltophilia* ATCC 1d3637<sup>T</sup> (type species); 4, *S. rhizophila* e-p10<sup>T</sup>; 5, *S. humi* R-32729<sup>T</sup>.

positive and does not produce acid from D-glucose. It also does not produce indole. The organism did not reduce nitrate to nitrite or nitrogen.

Furthermore, MK06<sup>T</sup> assimilates acetate, citrate, D-glucose, gluconate, D,L-3-hydroxybutyrate, 2-ketogluconate (a), D,Llactate, malonate, D-mannose, L-proline, L-rhamnose, and nvalerate and weakly assimilates N-acetyl-D-glucosamine, adipate, L-alanine, L-histidine, 4-hydroxybenzoate, itaconate, D-melibiose, phenyl acetate, propionate, D-sucrose, and Lserine. It does not assimilate L-arabinose, caprate, L-fucose, 3-hydroxybenzoate, 5-ketogluconate, D-maltose, L-malate, Dmannitol, myo-inositol, D-ribose, salicin, D-sorbitol, or Suberate. Strain MK06<sup>T</sup> produces acid phosphatase, alkaline phosphatase, esterase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and valine arylamidase, and is a weak producer of N-acetyl- $\beta$ -glucosaminidase, cystine arylamidase, and protease (gelatin hydrolysis). It does not produce arginine dihydrolase, a-chymotrypsin, esterase lipase (C4), a-fucosidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -glucosidase (esculin hydrolysis),  $\beta$ -glucuronidase,  $\alpha$ -glucosidase, lipase (C14),  $\alpha$ -mannosidase, trypsin, or urease.

The DNA GC content of MK06<sup>T</sup> is 67.8 mol%, and it uses ubiquinone Q-8 as the predominant respiratory lipoquinone. Its cellular fatty acids include 15:0 iso (41.1%), 15:0 anteiso (12.6%), 17:1 iso  $\omega$ 9c (8.6%), summed feature 4 (16:1  $\omega$ 7c/15:0 iso 2OH) (7.5), 16:0 (6.1%), 11:0 (4.5%), and 13:0 iso 3OH (4.3%). The type strain, MK06<sup>T</sup>, was isolated from the soil of a ginseng field in Daejeon, South Korea and deposited with the KCTC (KCTC 22893<sup>T</sup>), JCM (JCM 16536<sup>T</sup>) and Korea National Environmental Microorganism Bank (KEMB 9004-002<sup>T</sup>).

### Acknowledgements

This work was supported by a special research grant from Seoul Women's University (2009).

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