Stenotrophomonas daejeonensis sp. nov., isolated from sewage

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A Gram-stain-negative, motile, aerobic bacterial strain, designated MJ03^T, was isolated from sewage and was characterized taxonomically by using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that strain MJ03^T belongs to the family *Xanthomonadaceae*, class *Gammaproteobacteria*, and was related most closely to *Stenotrophomonas acidaminiphila* AMX 19^T (97.9% sequence similarity), *Stenotrophomonas humi* R-32729^T (97.1%), *Stenotrophomonas nitritireducens* L2^T (96.9%), *Stenotrophomonas maltophila* ATCC 13637^T (96.8%) and *Stenotrophomonas terrae* R-32768^T (96.7%). The G + C content of the genomic DNA of strain MJ03^T was 64.7 mol%. The detection of a quinone system with ubiquinone Q-8 as the predominant component and a fatty acid profile with iso-C_{15:0}, iso-C_{11:0}, iso-C_{14:0}, iso-C_{17:1ω9c}, iso-C_{11:0} 3-OH and iso-C_{13:0} 3-OH as major components supported the affiliation of strain MJ03^T to the genus *Stenotrophomonas*. However, levels of DNA–DNA relatedness between strain MJ03^T and the type strains of five closely related species of the genus *Stenotrophomonas* ranged from 11 to 34%, showing clearly that the isolate represents a novel genospecies. Strain MJ03^T could be differentiated clearly from its phylogenetic neighbours on the basis of several phenotypic, genotypic and chemotaxonomic features. Therefore, strain MJ03^T is considered to represent a novel species of the genus *Stenotrophomonas*, for which the name *Stenotrophomonas daejeonensis* sp. nov. is proposed. The type strain is MJ03^T (=KCTC 22451^T =JCM 16244^T).

The genus *Stenotrophomonas*, a member of the family *Xanthomonadaceae*, class *Gammaproteobacteria*, was created in 1993 with the reclassification of *Xanthomonas maltophila* as *Stenotrophomonas maltophila* (Palleroni & Bradbury, 1993). At the time of writing, the genus comprises eight recognized species: *S. maltophila* (Palleroni & Bradbury, 1993), *S. nitritireducens* (Finkmann *et al.*, 2000), *S. acidaminiphila* (Assih *et al.*, 2002), *S. rhizophila* (Wolf *et al.*, 2002), *S. koreensis* (Yang *et al.*, 2006), *S. humi* (Heylen *et al.*, 2007), *S. terrae* (Heylen *et al.*, 2007) and *S. ginsengisoli* (Kim *et al.*, 2010). Members of the genus *Stenotrophomonas* have the following characteristics: they are Gram-negative, non-spore-forming, motile or non-motile rods, are positive

for catalase and glucose fermentation, contain iso-C_{15:0} as the predominant cellular fatty acid and have DNA G + C contents in the range 64.0–69.1 mol%. Species of the genus *Stenotrophomonas* are common inhabitants of a wide variety of natural and artificial environments and geographical regions. In particular, the type strains of the above-mentioned species were isolated from soil, compost, plant rhizosphere, an anaerobic sludge blanket reactor, an ammonia-supplied bio-filter and the pleural fluid of a patient with oral carcinoma. During the course of screening micro-organisms from sewage, a Gram-stain-negative, rod-shaped bacterial strain, designated MJ03^T, was isolated. On the basis of 16S rRNA gene sequence analysis, this isolate was considered to be a *Stenotrophomonas*-like strain. To determine its exact taxonomic position, strain MJ03^T was subjected to a detailed investigation by using a polyphasic taxonomic approach, including genotypic, chemotaxonomic

Abbreviation: HE-cellulose, hydroxyethylcellulose.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain MJ03^T is GQ241320.

and classical phenotypic analyses. These results indicate that strain MJ03^T should be placed in the genus *Stenotrophomonas* as a representative of a novel species.

Strain MJ03^T was isolated from a sewage water sample collected from the inflow region of a municipal sewage treatment plant located in Daejeon, South Korea. The sample was a cloudy liquid with a pH of 7.2 and a temperature of 22 °C. It mainly comprised water (>99.9%) together with relatively small concentrations of suspended (150 mg l⁻¹) and dissolved (230 mg l⁻¹) organic and inorganic solids. The sample was suspended and spread on R2A agar plates (Difco) after being serially diluted with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30 °C for 2 weeks. Single colonies on the plates were purified by transferring them onto fresh plates and incubating them again under the same conditions. The isolate was routinely cultured on R2A agar at 30 °C and was maintained as a glycerol suspension (20%, w/v) at -70 °C. *S. acidaminiphila* KACC 11356^T, *S. humi* DSM 18929^T, *S. maltophilia* KCTC 1773^T, *S. nitritireducens* KACC 10891^T and *S. terrae* DSM 18941^T were used as reference strains for DNA–DNA hybridization and other experiments.

The Gram reaction was determined by using a Gram-stain kit (Difco) according to the manufacturer's instructions. Cell morphology and motility were observed under a Nikon light microscope (×1000 magnification) by using the hanging-drop technique with cells grown for 2 days at 30 °C on R2A agar. Endospores were stained negatively by using the method of Cappuccino & Sherman (2002) and were observed by light microscopy. Tests for anaerobic growth were performed in serum bottles containing R2A broth supplemented with thioglycolate (1 g l⁻¹) under a nitrogen atmosphere. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). Assimilation of single carbon sources, enzyme activities, acid production from substrates and other physiological characteristics were determined with the API ID 32 GN (48 h, 30 °C), API ZYM (4 h, 30 °C), API 20NE (48 h, 30 °C) and API 50CHB (48 h, 30 °C) galleries according to the manufacturer's instructions (bioMérieux). Acid production from *N*-acetylglucosamine, aesculin and glycogen was additionally determined by using O/F basal medium containing bromothymol blue (Atlas, 1993) supplemented with 1% carbohydrate (soft-agar stabs with a sterile mineral oil overlay). Reduction of nitrate and nitrite was determined by using serum bottles (25 ml) containing 12 ml R2A broth supplemented with KNO₃ (10 mM) and NaNO₂ (10 mM), respectively (Assih *et al.*, 2002). Reduction of nitrate and nitrite was monitored by ion chromatography on a model 790 personal chromatograph (Metrohm) equipped with a conductivity detector and an anion exchange column (Metrosep Anion Supp 4; Metrohm). Hydrolysis of casein was examined on nutrient agar supplemented with 1.5% dry skimmed milk (Tindall *et al.*, 2007). Chitinase activity was tested by using R2A agar medium supplemented with colloidal chitin (4 g l⁻¹;

Hsu & Lockwood, 1975). Hydrolysis of starch was tested on nutrient agar (Difco) plates supplemented with soluble starch (10 g l⁻¹). Starch hydrolysis was detected by flooding the plates with iodine solution (Tindall *et al.*, 2007). The test for degradation of DNA [DNase agar (Scharlau), with DNase activity detected by flooding plates with 1 M HCl] was performed according to Collins & Lyne (1984). Hydrolysis of xylan and hydroxyethylcellulose (HE-cellulose) was determined with a nutrient agar medium supplemented with a mixture of chromogenic substrates (xylan-red and HE-cellulose-blue), as described previously (Ten *et al.*, 2004). Biochemical tests for aesculin hydrolysis, indole production (method 1), gelatin hydrolysis (method 2), the Voges–Proskauer reaction, H₂S production from thiosulfate (method 1), hydrolysis of Tween 80, and acid phosphatase and alkaline phosphatase activities were performed according to standard methods as described by Tindall *et al.* (2007). Growth at 10, 15, 20, 25, 30, 37, 42 and 45 °C was assessed on R2A agar, nutrient agar, Luria–Bertani (LB) agar, trypticase soy agar (TSA; Difco) and MacConkey agar (Difco) after 5 days of incubation. Salt tolerance was tested on R2A agar medium supplemented with 1–5% (w/v) NaCl after incubation for up to 5 days. Growth at pH 5.0–11.0 (at intervals of 0.5 pH units) was assessed after incubation for up to 5 days at 30 °C. The effect of pH on growth was determined in LB broth media using four different buffers (final concentration, 50 mM): sodium acetate buffer (for pH 5.0–5.5), potassium phosphate buffer (for pH 6.0–8.0), Na₂CO₃/NaHCO₃ buffer (for pH 8.5–10.0) and Na₂HPO₄/NaOH buffer (for pH 10.5–11.0).

For phylogenetic analysis of strain MJ03^T, genomic DNA was extracted by using a commercial genomic DNA-extraction kit (Solgent), and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled by using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed by using the program CLUSTAL X (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated by using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were generated by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms from MEGA4 (Tamura *et al.*, 2007) and the maximum-likelihood (Felsenstein, 1981) method from PHYLIP, version 3.69 (Felsenstein, 2009), with bootstrap values based on 1000 replications (Felsenstein, 1985).

For determination of chromosomal DNA G+C content, the genomic DNA of strain MJ03^T was extracted and purified as described by Moore & Dowhan (1995) and degraded enzymically into nucleosides; the DNA G+C content was determined as described by Mesbah *et al.* (1989) by using reversed-phase HPLC. Cell biomass for isoprenoid quinone analysis was obtained from culture

grown in LB broth for 2 days at 30 °C. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane/water (1:1, v/v). The crude quinone in n-hexane was purified by using Sep-Pak Vac silica cartridges (Waters) and then analysed by HPLC, as described by Hiraishi *et al.* (1996). Cellular fatty acids were analysed from cells grown on TSA for 48 h at 30 °C. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analysed by GC (model 6890; Hewlett Packard) by using the Microbial Identification software package (Sasser, 1990).

Cells of strain MJ03^T were Gram-stain-negative, aerobic, motile rods. Colonies grown on LB agar plates for 2 days at 30 °C were 2–4 mm in diameter, circular, smooth, convex, shiny, transparent and yellowish. On LB agar, strain MJ03^T was able to grow at 20–42 °C, but not at 15 or 45 °C. Normal growth was also observed on R2A agar, TSA and nutrient agar at 20–30 °C and weak growth occurred on these media at 37 and 42 °C. On MacConkey agar, growth occurred at 20–30 °C, but not at 37 °C. Strain MJ03^T and the type strains of five closely related species of the genus *Stenotrophomonas* (*S. acidaminiphila* KACC 11356^T, *S. humi* DSM 18929^T, *S. nitritireducens* KACC 10891^T, *S. terrae* DSM 18941^T and *S. maltophilia* KCTC 1773^T), used in this study as reference strains, were positive for catalase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, casein hydrolysis, assimilation of *N*-acetylglucosamine, L-alanine, L-proline and L-serine, and acid production from cellobiose, D-fructose, D-glucose, lactose, D-mannose and starch. All these strains were negative for cystine arylamidase, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, lipase (C14) and α -mannosidase, indole production, hydrolysis of xylan and HE-cellulose, H₂S production, assimilation of adipate, caprate, gluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, itaconate, 2-ketogluconate, 5-ketogluconate and phenylacetate, and acid production from a number of sugars (e.g. D-arabinose, L-arabinose, D-fucose and sucrose). These results are in good agreement with previously reported data and supported the affiliation of the new isolate to the genus *Stenotrophomonas* (Palleroni & Bradbury, 1993; Finkmann *et al.*, 2000; Assih *et al.*, 2002; Heylen *et al.*, 2007). Phenotypic and chemotaxonomic characteristics that differentiate strain MJ03^T from its closest neighbours in the genus *Stenotrophomonas* are listed in Table 1. In particular, strain MJ03^T could be differentiated readily from the above-mentioned species of the genus *Stenotrophomonas* based on its ability to utilize L-arabinose, L-fucose, D-mannitol, L-rhamnose, D-ribose and D-sorbitol and to produce acid from methyl α -D-glucopyranoside.

The 16S rRNA gene sequence of strain MJ03^T was a continuous stretch of 1420 bp. 16S rRNA gene sequence similarity calculations after a neighbour-joining analysis indicated that the closest relatives of strain MJ03^T were *S.*

acidaminiphila AMX 19^T (97.9%), *S. humi* R-32729^T (97.1%), *S. nitritireducens* L2^T (96.9%), *S. maltophilia* ATCC 13637^T (96.8%) and *S. terrae* R-32768^T (96.7%). Lower levels of similarity (<96.5%) were found with the type strains of all other recognized species in the family *Xanthomonadaceae*, including *S. koreensis* TR6-01^T, *S. rhizophila* e-p10^T and *S. ginsengisoli* DCY01^T. The phylogenetic position of the new isolate, determined by using the various tree-making algorithms (neighbour-joining, maximum-parsimony and maximum-likelihood), revealed that strain MJ03^T appeared within the genus *Stenotrophomonas*, joining *S. acidaminiphila* L2^T with moderate bootstrap values of 61, 55 and 63%, respectively (Fig. 1). The generally accepted criteria for delineating bacterial species state that strains having a level of DNA–DNA relatedness below 70% (as measured from hybridization) or showing 16S rRNA gene sequence dissimilarity above 3% are considered to belong to separate species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002). In view of this definition, the above data indicate that strain MJ03^T can be considered to represent a novel species of the genus *Stenotrophomonas*. For further verification of the taxonomic position of strain MJ03^T, DNA–DNA hybridization was performed with its most closely related members of the genus *Stenotrophomonas*.

DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), by using photobiotin-labelled DNA probes (Sigma) and microdilution wells (Greiner), with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as the DNA–DNA relatedness. Strain MJ03^T exhibited relatively low levels of DNA–DNA relatedness with respect to *S. acidaminiphila* KACC 11356^T (34%), *S. humi* DSM 18929^T (19%), *S. nitritireducens* KACC 10891^T (15%), *S. maltophilia* KCTC 1773^T (12%) and *S. terrae* DSM 18941^T (11%), indicating that it is not related to them at the species level (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

Cellular fatty acid profiles of strain MJ03^T and the type strains of phylogenetically related species of the genus *Stenotrophomonas* are shown in Table 2. All strains contained iso-C_{15:0} as the dominant component, and branched fatty acids such as iso-C_{11:0} and anteiso-C_{15:0}, the monounsaturated fatty acid iso-C_{17:1} ω 9c and branched 3-hydroxyl fatty acids as the other major components. However, some minor qualitative and quantitative differences in fatty acid content could be observed between strain MJ03^T and its phylogenetically closest relatives. In particular, strain MJ03^T could be distinguished based on the presence of a small amount of iso-C_{13:0} and by the absence of C_{12:0} 3-OH. Strain MJ03^T contained Q-8 as the predominant ubiquinone. The DNA G + C content of strain MJ03^T was 64.7 mol%. These data are in good agreement with those of other members of the genus *Stenotrophomonas* (Finkmann *et al.*, 2000; Assih *et al.*, 2002; Wolf *et al.*, 2002; Yang *et al.*, 2006; Heylen *et al.*, 2007).

Table 1. Differential phenotypic characteristics between strain MJ03^T and its closest phylogenetic neighbours in the genus *Stenotrophomonas*

Strains: 1, MJ03^T; 2, *S. acidaminiphila* KACC 11356^T; 3, *S. humi* DSM 18929^T; 4, *S. nitritireducens* KACC 10891^T; 5, *S. maltophilia* KCTC 1773^T; 6, *S. terrae* DSM 18941^T. All data are from the present study except where indicated. All strains are positive for casein hydrolysis but negative for indole and H₂S production, and hydrolysis of DNA, HE-cellulose and xylan. In API ID 32 GN, API 20NE (assimilation of carbon sources), API ZYM and API 50CHB tests, all strains showed identical biochemical characteristics except those indicated here (see species description). +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6
Reduction of nitrite	–	–*	–	+	–*	–
Voges–Proskauer reaction	+	+	+	+	+	–
Growth with/at:						
5 % NaCl	+	–	–	–	+	+
42 °C	+	+	–	–	–	–
Hydrolysis of:						
Aesculin	+	+	–	–	+	–
Gelatin	+	–	–	–	+	+
Tween 80	+	+	–	+	+	+
Enzyme activities (API ZYM)						
<i>N</i> -Acetyl-β-glucosaminidase	+	+	+	+	–	–
Alkaline phosphatase†	+	+	+	+	–	+
Acid phosphatase†	+	+	+	+	–	+
α-Chymotrypsin	–	+	–	+	–	–
α-Glucosidase	+	+	+	–	+	+
β-Glucosidase	–	–	–	–	w	w
Leucine arylamidase	+	–	–	+‡	–	+
Trypsin	+	+	+	w	–	+
Valine arylamidase	+	–	w	–	–	–
Assimilation of (API ID 32GN and API 20 NE):						
Acetate	+	+	+	w	+	+
L-Arabinose	+	–	–	–	–	–
Citrate	+	–	+	+	+	+
L-Fucose	+	–	–	–	–	–
D-Glucose	+	+	–	–*	+	+
L-Histidine	+	+	–	+	+	–
DL-3-Hydroxybutyrate	+	+	–	–	–	–
Inositol	+	–	–	–	–	–
Lactate	–	+	+	+	–*	+
Malate	–	–	+	–	+	+‡
Malonate	–	–	w	–	w	–
Maltose	+	+	+	–	+	+
D-Mannitol	+	–	–	–	–	–
D-Mannose	+	+	+	–	w	+
Propionate	+	+§	–	+	–	w
L-Rhamnose	+	–	–	–	–	–
D-Ribose	+	–	–	–	–	–
Salicin	–	–	–	–	+*	–
D-Sorbitol	+	–	–	–	–	–
Suberate	–	–	–	–	–	w
Sucrose	–	–	–	–	+	–
Valerate	+	+	–	+	–	–
Acid production from (API 50 CHB):						
Amygdalin	–	+	–	–	+	+
D-Galactose	–	+	–	–	–	+
Gentiobiose	+	+	–	–	–	+
Glycogen	+	–	–	–	+	+
D-Lyxose	+	–	+	–	+	+
Maltose	+	–	+	–	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6
Melibiose	—	+	—	+	+	+
Methyl α -D-glucopyranoside	+	—	—	—	—	—
Methyl β -D-xylopyranoside	+	—	—	—	+	—
Raffinose	—	—	+	—	+	+
D-Ribose	—	+	+	+	—	+
Salicin	—	+	—	—	+	+
L-Sorbose	—	—	+	—	—	+
Trehalose	—	—	—	—	—	+
D-Xylose	—	—	+	+	+	+
DNA G + C content (mol%)	64.7	66.9 ^a	64.0 ^b	69.1 ^c	66.7 ^c	65.0 ^b

*Opposite results were obtained by Yang *et al.* (2006).

†Same results were obtained using the methods described by Tindall *et al.* (2007).

‡Opposite results were obtained by Heylen *et al.* (2007).

§Opposite result was obtained by Assih *et al.* (2002).

||Data from: a, Assih *et al.* (2002); b, Heylen *et al.* (2007); c, Yoon *et al.* (2006).

The phenotypic and phylogenetic data presented indicate that strain MJ03^T belongs to the genus *Stenotrophomonas*. The phylogenetic distinctiveness of strain MJ03^T, together with DNA–DNA hybridization data, confirmed that it represents a species that is distinct from recognized species of this genus. Strain MJ03^T can be differentiated from phylogenetically related species of the genus *Stenotrophomonas* based on several phenotypic characteristics (Table 1). Therefore, on the basis of the data presented, strain MJ03^T should be classified within the genus *Stenotrophomonas* as the type strain of a novel species, for which the name *Stenotrophomonas daejeonensis* sp. nov. is proposed.

Description of *Stenotrophomonas daejeonensis* sp. nov.

Stenotrophomonas daejeonensis (dae.je.on.en'sis. N.L. fem. adj. *daejeonensis* pertaining to Daejeon, a city in South Korea, from where the type strain was isolated).

Cells are Gram-stain-negative, aerobic, non-spore-forming rods, 0.3–0.4 μ m in diameter and 1.3–1.6 μ m in length. After 2 days of incubation at 30 °C on LB agar, colonies are 2–4 mm in diameter, circular, smooth, convex, shiny, transparent and yellowish. Grows at 20–42 °C (optimum, 30 °C) and at pH 6.0–10.0 (optimum, pH 7.0–7.5). Nitrate and nitrite are not reduced. Able to hydrolyse aesculin, casein, gelatin and Tween 80 but not HE-cellulose, chitin, starch, xylan or DNA. Positive for the Voges–Proskauer reaction, acid phosphatase, alkaline phosphatase, catalase and oxidase but negative for urease, indole and H₂S production. In API ZYM tests, positive for *N*-acetyl- β -glucosaminidase, esterase (C4), esterase lipase (C8), α -glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but negative for α -chymotrypsin, cystine arylamidase, α -fucosidase, α -galactosidase, β -galactosidase, β -glucosidase, β -glucuronidase, lipase (C14) and α -mannosidase. In API ID 32 GN and API 20NE tests, utilizes acetate, *N*-acetylglucosamine, L-alanine, L-arabinose, citrate, L-fucose, D-glucose,

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain MJ03^T among recognized species of the genus *Stenotrophomonas* and other related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony and maximum-likelihood algorithms. Bar, 0.005 substitutions per nucleotide position.

Table 2. Cellular fatty acid profiles of strain MJ03^T and its closest phylogenetic neighbours in the genus *Stenotrophomonas*

Strains: 1, MJ03^T; 2, *S. acidaminiphila* KACC 11356^T; 3, *S. humi* DSM 18929^T; 4, *S. nitritireducens* KACC 10891^T; 5, *S. maltophilia* KCTC 1773^T; 6, *S. terrae* DSM 18941^T. All data are from the present study. All strains were grown on TSA at 30 °C for 48 h prior to fatty acid analysis. Strain MJ03^T and the type strains of *S. acidaminiphila*, *S. humi* and *S. maltophilia* contained small amounts of iso-C_{13:0} (2.1%), anteiso-C_{11:0} (2.0%), iso-C_{12:0} (0.9%) and C_{16:1ω9c} (2.8%), respectively. Values are percentages of total fatty acids; –, not detected.

Fatty acid	1	2	3	4	5	6
Saturated						
C _{10:0}	—	—	1.1	—	—	1.7
C _{14:0}	1.6	—	1.1	—	2.7	2.5
C _{15:0}	1.6	—	1.1	2.3	1.6	1.8
C _{16:0}	2.5	3.1	4.0	5.1	6.1	5.2
iso-C _{10:0}	1.9	1.9	1.8	—	—	—
iso-C _{11:0}	10.1	11.5	7.0	12.0	7.2	14.4
iso-C _{14:0}	9.6	5.0	16.3	6.6	0.6	6.0
iso-C _{15:0}	33.7	26.8	14.6	27.7	39.9	18.1
iso-C _{16:0}	2.7	3.4	16.4	4.0	2.7	2.4
anteiso-C _{15:0}	4.2	8.6	5.4	8.4	10.1	4.7
Unsaturated						
iso-C _{15:1} F	4.6	4.1	1.8	5.1	2.1	5.8
iso-C _{17:1ω9c}	7.3	7.9	6.3	9.6	4.9	6.7
Hydroxy						
C _{12:0} 3-OH	—	2.5	2.5	2.9	2.7	3.3
C _{13:0} 2-OH	—	1.9	—	—	—	2.1
iso-C _{11:0} 3-OH	6.4	7.6	2.2	5.9	4.3	6.5
iso-C _{12:0} 3-OH	4.1	5.1	8.5	5.1	3.1	5.7
iso-C _{13:0} 3-OH	6.1	5.6	1.7	5.3	6.2	5.9
Summed feature 4*	1.5	3.0	7.3	—	3.0	7.2

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 4 comprised C_{16:1ω7c} and/or iso-C_{15:1} 2-OH.

L-histidine, DL-3-hydroxybutyrate, inositol, maltose, D-mannitol, D-mannose, L-proline, propionate, L-rhamnose, D-ribose, L-serine, D-sorbitol and valerate for growth, but not adipate, caprate, gluconate, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, itaconate, 2-ketogluconate, 5-ketogluconate, lactate, malate, malonate, melibiose, phenyl acetate, salicin, suberate or sucrose. In API 50 CHB tests, acid is produced without gas from *N*-acetylglucosamine, aesculin, cellobiose, D-fructose, gentiobiose, D-glucose, glycogen, lactose, D-lyxose, maltose, D-mannose, methyl α-D-glucopyranoside, methyl β-D-xylopyranoside and starch, but not from adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, dulcitol, erythritol, D-fucose, D-galactose, gluconate, inositol, inulin, 2-ketogluconate, 5-ketogluconate, D-mannitol, melezitose, melibiose, methyl α-D-mannopyranoside, raffinose, L-rhamnose, D-ribose, salicin, sorbitol, L-sorbose, sucrose, D-tagatose, trehalose, turanose, xylitol,

D-xylose or L-xylose. Q-8 is the predominant ubiquinone. The major cellular fatty acids are iso-C_{15:0}, iso-C_{11:0}, iso-C_{14:0}, iso-C_{17:1ω9c}, iso-C_{11:0} 3-OH and iso-C_{13:0} 3-OH. The DNA G+C content of the type strain is 64.7 mol%.

The type strain, MJ03^T (=KCTC 22451^T =JCM 16244^T), was isolated from a sewage sample collected from a municipal sewage treatment plant in Daejeon, South Korea.

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References

- Assih, E. A., Ouattara, A. S., Thierry, S., Cayol, J.-L., Labat, M. & Macarie, H. (2002). *Stenotrophomonas acidaminiphila* sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. *Int J Syst Evol Microbiol* **52**, 559–568.
- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: a Laboratory Manual*, 6th edn. Menlo Park, CA: Benjamin/Cummings.
- Collins, C. H. & Lyne, P. M. (1984). *Microbiological Methods*, 5th edn. London: Butterworth.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric DNA–DNA hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Felsenstein, J. (1985). Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (2009). PHYLIP (phylogeny inference package), version 3.69. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Finkmann, W., Altendorf, K., Stackebrandt, E. & Lipski, A. (2000). Characterization of N₂O-producing *Xanthomonas*-like isolates from biofilters as *Stenotrophomonas nitritireducens* sp. nov., *Luteimonas mephitis* gen. nov., sp. nov. and *Pseudoxanthomonas broegbernensis* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **50**, 273–282.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Heylen, K., Vanparys, B., Peirsegeale, F., Lebbe, L. & De Vos, P. (2007). *Stenotrophomonas terrae* sp. nov. and *Stenotrophomonas humi* sp. nov., two nitrate-reducing bacteria isolated from soil. *Int J Syst Evol Microbiol* **57**, 2056–2061.
- Hiraishi, A., Ueda, Y., Ishihara, J. & Mori, T. (1996). Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* **42**, 457–469.

- Hsu, S. C. & Lockwood, J. L. (1975). Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Appl Microbiol* **29**, 422–426.
- Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005). *Sphingopyxis granuli* sp. nov., a beta-glucosidase-producing bacterium in the family *Sphingomonadaceae* in alpha-4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.
- Kim, H.-B., Srinivasan, S., Sathiyaraj, G., Quan, L.-H., Kim, S.-H., Bui, T. P. N., Liang, Z.-Q., Kim, Y.-J. & Yang, D.-C. (2010). *Stenotrophomonas ginsengisoli* sp. nov., isolated from a ginseng field. *Int J Syst Evol Microbiol* **60**, 1522–1526.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Moore, D. D. & Dowhan, D. (1995). Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, pp. 2–11. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- Palleroni, N. J. & Bradbury, J. F. (1993). *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings *et al.* 1983. *Int J Syst Bacteriol* **43**, 606–609.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). Identification of bacteria through fatty acid analysis. In *Methods in Phytobacteriology*, pp. 199–204. Edited by Z. Klement, K. Rudolph & D. C. Sands. Budapest: Akademiai Kiado.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M.-S. & Lee, S.-T. (2004). Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, J. B., Sikorski, J., Simbert, A. R. & Krieg, R. N. (2007). Phenotypic characterization and the principles of comparative systematics. In *Methods for General and Molecular Microbiology*, 3rd edn, pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. A. Marzluf, T. M. Schmidt & L. R. Snyder. Washington, DC: American Society for Microbiology.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Wolf, A., Fritze, A., Hagemann, M. & Berg, G. (2002). *Stenotrophomonas rhizophila* sp. nov., a novel plant-associated bacterium with antifungal properties. *Int J Syst Evol Microbiol* **52**, 1937–1944.
- Yang, H.-C., Im, W.-T., Kang, M. S., Shin, D.-Y. & Lee, S.-T. (2006). *Stenotrophomonas koreensis* sp. nov., isolated from compost in South Korea. *Int J Syst Evol Microbiol* **56**, 81–84.
- Yoon, J.-H., Kang, S.-J., Oh, H. W. & Oh, T.-K. (2006). *Stenotrophomonas dokdonensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 1363–1367.