Stenotrophomonas koreensis sp. nov., isolated from compost in South Korea

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A Gram-negative, rod-shaped, non-spore-forming bacterium (TR6-01^T) was isolated from compost near Daejeon city in South Korea. On the basis of 16S rRNA gene sequence similarity, strain TR6-01^T was shown to belong to the class *Gammaproteobacteria*, related to *Stenotrophomonas acidaminiphila* (97.1 %) and *Stenotrophomonas maltophilia* (96.9 %); the phylogenetic distance from any other established species within the genus *Stenotrophomonas* was less than 97.0 %. Phenotypic and chemotaxonomic data (major ubiquinone Q-8; fatty acid profile) supported the affiliation of strain TR6-01^T to the genus *Stenotrophomonas*. The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain TR6-01^T from the five *Stenotrophomonas* species with validly published names. TR6-01^T therefore represents a novel species, for which the name *Stenotrophomonas koreensis* sp. nov. is proposed, with the type strain TR6-01^T (=KCTC 12211^T=JCM 13256^T).

During a course of study on the culturable aerobic bacterial community in compost sampled near Daejeon city in Korea, a large number of novel bacterial strains were isolated (Im *et al.*, 2003). One of these isolates (strain TR6-01^T) was member of genus *Stenotrophomonas* in the class *Gammaproteobacteria* and was the subject of this taxonomic investigation.

The genus *Stenotrophomonas* was first described by Palleroni & Bradbury (1993) and, at present, the genus comprises the five species *Stenotrophomonas acidaminiphila* Assih *et al.* 2002, *S. africana* Drancourt *et al.* 1997, *S. maltophilia* (Hugh 1981) Palleroni and Bradbury 1993, *S. nitritireducens* Finkmann *et al.* 2000 and *S. rhizophila* Wolf *et al.* 2002.

In the present study, we conducted a phylogenetic analysis on the basis of 16S rRNA gene sequences, DNA–DNA hybridization tests and some important phenotypic characteristics to determine the precise taxonomic position of this strain. On the basis of the results obtained in this study, we propose that strain TR6-01^T should be placed in the genus *Stenotrophomonas* as the type strain of a novel species.

Strain TR6-01^T was originally isolated from a compost which was composed of cow dung and rice straw, near Daejeon city in South Korea. This compost sample was suspended with 50 mM phosphate buffer (pH 7.0) and the suspension was spread on R2A agar plates (Difco) after serial dilution 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 new plates and were incubated once again under the same conditions. The purified colonies were tentatively identified by partial sequences of the 16S rRNA gene (Im *et al.*, 2003). TR6-01^T was one of the dominant isolates on the R2A agar plates under aerobic conditions. This organism was deposited in the Korean Collection for Type Cultures as KCTC 12211^T (=JCM 13256^T). Other type strains of species of the genus *Stenotrophomonas* were obtained from the DSMZ or KCTC.

The Gram reaction was performed by the non-staining method as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at ×1000, with cells grown for 2 days at 30 °C on R2A agar. Catalase and oxidase tests were performed by the procedures outlined by Cappuccino & Sherman (2002). Substrate utilization as sole carbon source and some physiological characteristics were determined with API 32GN and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). Anaerobic growth was performed in serum bottles adding thioglycolate (1 g l⁻¹) to R2A broth and substituting the upper air layer with nitrogen gas. Nitrate and nitrite reduction were later confirmed by inoculating, in each case, into three serum bottles (25 ml) containing 13 ml R2A media,

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TR6-01^T is AB166885.

The fatty acid profile of *Stenotrophomonas koreensis* compared with other *Stenotrophomonas* species is available as a supplementary table in IJSEM online.

while nitrate and nitrite were added as KNO_3 and NaNO_2 at concentrations of 10 mM. The reduction of nitrate and nitrite was monitored by ion chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and anion exchange column (Metrosep Anion Supp 4; Metrohm). Degradation of DNA [using DNA agar (Difco) supplemented with 0.01 % toluidine blue (Merck)], degradation of casein, chitin, cellulose and starch (Atlas, 1993), degradation of lipids (Kouker & Jaeger, 1987) and degradation of xylan (Ten *et al.*, 2004) were also investigated; reactions were read after 5 days. Growth at different temperatures and pH was assessed after 5 days incubation. Salt tolerance was tested on R2A medium supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Duplicate antibiotic-sensitivity tests were done using filter-paper discs containing the following: streptomycin (5, 10 and 15 μg), tetracycline (5, 10 and 15 μg), kanamycin (1.0, 1.5 and 2.0 mg) and ampicillin (20, 30 and 50 μg) (Sigma). Discs were placed on R2A plates spread with TR6-01^T culture and were then incubated at 30 °C for 5 days.

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of purified PCR product were carried out according to Im *et al.* (2004). The 16S rRNA gene sequences of related taxa were obtained from GenBank. The multiple alignments were performed by CLUSTAL_X program (Thompson *et al.*, 1997). Gaps were edited using the BIOEDIT program (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) using the MEGA2 program (Kumar *et al.*, 2001) with bootstrap values based on 1000 replications (Felsenstein, 1985) (Fig. 1).

The G+C content of the chromosomal DNA was determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Quinones were extracted from cells grown on nutrient broth (Difco) and analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. Cellular fatty acids were analysed in organisms grown on a trypticase soy agar (TSA; Difco) for 2 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed by a gas chromatograph (Hewlett Packard 6890) were identified by the Microbial ID software package (Sasser, 1990).

Cells of strain TR6-01^T were aerobic, Gram-negative, non-motile rods. Colonies grown on R2A agar plates (Difco) for 2 days were smooth, circular, non-glossy, yellowish and 2–4 mm in diameter. On R2A agar, TR6-01^T was able to grow at 20–37 °C, but not at 4 or 45 °C. Physiological characteristics of strain TR6-01^T are summarized in the species description and comparisons of selective characteristics with related type strains are shown in Table 1.

The 16S rRNA gene sequence of the strain TR6-01^T was a continuous stretch of 1456 bp. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain TR6-01^T were *S. acidaminiphila* (97.1 %) and *S. maltophilia* (96.9 %). Lower sequence similarities (<97.0 %) were found with all recognized species of the genus *Stenotrophomonas*.

The DNA G+C content of strain TR6-01^T was 66.0 mol%. Characterization of the respiratory quinone system supports the affiliation of TR6-01^T with the *Gammaproteobacteria*, where the majority of species in the genus *Xanthomonas* and *Stenotrophomonas* have Q-8 as the major quinone (Finkmann *et al.*, 2000). The fatty acid profile of strain TR6-01^T (available in Supplementary Table S1 in IJSEM Online) was mainly composed of $\text{C}_{15:0}$ iso (34.0 %), $\text{C}_{15:1}$ iso F (18.1 %), $\text{C}_{13:0}$ iso (8.9 %), $\text{C}_{11:0}$ iso (6.8 %), $\text{C}_{11:0}$ iso 3-OH (4.8 %), $\text{C}_{14:0}$ iso (4.2 %), iso $\text{C}_{17:1}$ $\omega 9c$ (3.3 %) and $\text{C}_{13:0}$ iso 3-OH (3.1 %). A significant difference was found compared with other *Stenotrophomonas* species: the production of a large amount of the fatty acid $\text{C}_{15:1}$ iso F (18.1 %). Moreover, *S. acidaminiphila* produced significantly larger amounts of $\text{C}_{14:0}$ iso (17.8 %) and $\text{C}_{16:0}$ iso (14.3 %) and *S. rhizophila* produced a significantly larger amount of $\text{C}_{15:0}$ anteiso (23.8 %). Thus, fatty acid profiles can be used to differentiate the members of the genus *Stenotrophomonas* at the species level.

DNA–DNA hybridization experiments were performed between TR6-01^T and type strains of the genus *Stenotrophomonas* with the method described by Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. DNA–DNA relatedness values of strain TR6-01^T to the other species of genus *Stenotrophomonas* were 25–42 % (40 % to *S. acidaminiphila* DSM 13117^T, 25 % to *S. maltophilia* KCTC 1773^T, 42 % to *S. nitritireducens* DSM 12575^T and 25 % *S. rhizophila* DSM 14405^T), which is low enough to assign strain TR6-01^T as a novel species of the genus *Stenotrophomonas*. On the basis of the data and

Fig. 1. Phylogenetic tree constructed by the neighbour-joining method from a comparative analysis of 16S rRNA gene sequences showing the relationships of *S. koreensis* sp. nov. TR6-01^T with other related species. Bar, 0.01 substitutions per nucleotide position. Bootstrap values (expressed as percentages of 1000 replications) greater than 65 % are shown at the branch points.

Table 1. Physiological characteristics of *S. koreensis* TR6-01^T and related type strains of species of genus *Stenotrophomonas*

Strains: 1, *S. koreensis* TR6-01^T; 2, *S. acidaminiphila* DSM 13117^T; 3, *S. maltophilia* KCTC 1773^T; 4, *S. nitritireducens* DSM 12575^T; 5, *S. rhizophila* DSM 14405^T. All analyses were done in this study. +, Positive; –, negative. All strains were positive for utilization of propionate, valerate, L-proline and acetate as carbon sources. All strains were negative for utilization of L-fucose, D-sorbitol, L-arabinose, 2-ketogluconate, 4-hydroxybenzoate, D-ribose, inositol, itaconate, 5-ketogluconate, 3-hydroxybenzoate, mannitol, caprate, adipate, phenylacetate and glycogen as carbon sources. All strains were positive for catalase activity and negative for glucose acidification and indole production.

Characteristic	1	2	3	4	5
Nitrate reduction to nitrite	–	+	+	–	+
Nitrite reduction	–	+	+	+	–
Enzyme activity:					
Oxidase	+	+	–	–	–
Arginine dihydrolase	–	–	+	+	–
Urease	–	–	+	+	–
β-Glucosidase (aesculin hydrolysis)	–	+	+	–	–
Protease (gelatin hydrolysis)	+	–	+	–	+
β-Galactosidase	–	–	+	–	+
Utilization as a sole carbon source:					
D-Glucose	–	+	+	+	+
Mannose	–	+	+	–	+
N-Acetylglucosamine	–	+	+	+	+
Maltose	–	+	+	–	+
Malate	–	–	+	–	+
Citrate	–	–	+	+	+
Histidine	–	+	+	+	+
Salicin	–	–	–	–	+
D-Melibiose	–	–	–	–	+
3-Hydroxybutyrate	+	+	–	+	–
Sucrose	–	–	+	–	+
Maltose	–	+	+	–	+
Suberate	–	–	–	–	+
Malonate	–	–	+	–	+
DL-Lactate	–	+	+	+	+
L-Alanine	+	+	+	+	–
L-Serine	+	+	+	+	–

observations described above, strain TR6-01^T should be assigned to the genus *Stenotrophomonas* as the type strain of a novel species, for which the name *Stenotrophomonas koreensis* sp. nov. is proposed.

Description of *Stenotrophomonas koreensis* sp. nov.

Stenotrophomonas koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* pertaining to Korea, the location of the compost sample from which the type strain was isolated).

Cells are Gram-negative, strictly aerobic, non-motile, slightly curved rods, 0.2–0.4 µm in diameter and 1.5–2.0 µm in length. Colonies grown on R2A agar (Difco) for 2 days are smooth, circular, non-glossy, yellowish and convex. Grows well at pH 6.0–8.5 and at 20–37 °C, but does not grow at 4 or 45 °C. Growth occurs in the presence of 0–2.0 % (w/v) NaCl, but not 4 % (w/v) NaCl. It cannot reduce nitrate and nitrite or grow anaerobically. It cannot degrade xylan, chitin, cellulose or starch but can degrade DNA. Substrate utilization, enzyme production, acid production and other physiological characteristics are shown in Table 1. Resistant to discs containing 15 µg tetracycline and 15 µg streptomycin, but sensitive to 20 µg ampicillin and 1.0 mg kanamycin. Q-8 is the predominant ubiquinone and C_{15:0} iso, C_{15:1} iso F, C_{13:0} iso and C_{11:0} iso are the major cellular fatty acids. The G+C content of genomic DNA is 66.0 mol% (as determined by HPLC).

The type strain, TR6-01^T (= KCTC 12211^T = JCM 13256^T), was isolated from a compost consisting of cow dung and rice straw, near Daejeon City, Korea.

Acknowledgements

This work was supported by the 2005 Research Program of the Rural Development Administration, Republic of Korea.

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