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

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A Gram-negative, rod-shaped, non-spore-forming bacterium (TR6-01T) was isolated from compost near Daejeon city in South Korea. On the basis of 16S rRNA gene sequence similarity, strain TR6-01T 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 TR6-01T to the genus Stenotrophomonas. The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain TR6-01T from the five Stenotrophomonas species with validly published names. TR6-01T therefore represents a novel species, for which the name Stenotrophomonas koreensis sp. nov. is proposed, with the type strain TR6-01T (= KCTC 12211T = JCM 13256T).

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 TR6-01T) was member of genus Stenotrophomonas in the class Gammaproteobacteria and was the subject of this taxonomic investigation.


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 TR6-01T should be placed in the genus Stenotrophomonas as the type strain of a novel species.

Strain TR6-01T 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). TR6-01T 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 12211T (= JCM 13256T). 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,
while nitrate and nitrite were added as KNO₃ and NaNO₂ at concentrations of 10 mM. The reduction of nitrate and nitrite was monitored by ion chromatograph (model 790 personal IC; Metrotm) equipped with a conductivity detector and anion exchange column (Metrosep Anion Supp 4; Metrotm). 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 TRANS 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., 1994). The 16S rRNA gene sequence of the strain TR6-01 TRANS was deposited at GenBank under the accession number DQ151433. The DNA–DNA relatedness values of strain TR6-01 TRANS (available in Supplementary Table S1 in IJSEM Online) with the closest relatives of the genus Stenotrophomonas have Q-8 as the major quinone (Finkmann et al., 2000). The fatty acid profile of strain TR6-01 TRANS (available in Supplementary Table S1 in IJSEM Online) was mainly composed of C₁₅:0 iso (34.0 %), C₁₅:1 iso F (18.1 %), C₁₃:0 iso (8.9 %), C₁₁:0 iso (6.8 %), C₁₄:0 iso 3-OH (4.8 %), C₁₄:0 iso (4.2 %), C₁₇:1 iso 9c (3.3 %) and C₁₃:0 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 C₁₅:1 iso F (18.1 %). Moreover, S. acidaminiphila produced significantly larger amounts of C₁₄:0 iso (17.8 %) and C₁₆:0 iso (14.3 %) and S. rhizophila produced a significantly larger amount of C₁₅:0 anteiso (23.8 %). Thus, fatty acid profiles can be used to differentiate the members of the genus Stenotrophomonas at the species level.

Cells of strain TR6-01 TRANS 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 TRANS was able to grow at 20–37 °C, but not at 4 or 45 °C. Physiological characteristics of strain TR6-01 TRANS 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 TRANS was a continuous stretch of 1456 bp. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain TR6-01 TRANS 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.

DNA–DNA hybridization experiments were performed between TR6-01 TRANS 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 TRANS to the other species of genus Stenotrophomonas were 25–42 % (40 % to S. acidaminiphila DSM 13117 TRANS, 25 % to S. maltophilia KCTC 1773 TRANS, 42 % to S. nitritireducens DSM 12575 TRANS and 25 % S. rhizophila DSM 14405 TRANS), which is low enough to assign strain TR6-01 TRANS as a novel species of the genus Stenotrophomonas. On the basis of the data and
observations described above, strain TR6-01\textsuperscript{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% (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\textsubscript{15}:0 iso, C\textsubscript{15}:1 iso F, C\textsubscript{13}:0 iso and C\textsubscript{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\textsuperscript{T} (=KCTC 12211\textsuperscript{T} =JCM 13256\textsuperscript{T}), was isolated from a compost consisting of cow dung and rice straw, near Daejeon City, Korea.

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**References**


