Terrabacter terrigena sp. nov., isolated from soil

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A Gram-positive-staining, non-motile and rod-shaped bacterium, strain ON10T, was isolated from soil around a wastewater treatment plant in Korea and its taxonomic position was investigated by using a polyphasic approach. Strain ON10T grew optimally at pH 6.5–7.0 and 30 °C in the presence of 0.5 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain ON10T clustered with the clade comprising Terrabacter species, with which it exhibited 16S rRNA gene sequence similarity values of 98.4–98.8 %. The cell-wall peptidoglycan type was based on L-diaminopimelic acid and the cell-wall sugars were glucose, mannose, arabino and xylose. The predominant menaquinone was MK-8(H4). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, an unidentified phospholipid and an unidentified lipid. The major fatty acids were iso-C15 : 0 and iso-C14 : 0. The DNA G+C content was 71.6 mol%. Strain ON10T exhibited DNA–DNA relatedness levels of 17–28 % to the type strains of Terrabacter species and could also be differentiated from these species by differences in phenotypic characteristics. On the basis of the data obtained, strain ON10T was considered to represent a novel species of the genus Terrabacter, for which the name Terrabacter terrigena sp. nov. is proposed. The type strain is ON10T (=KCTC 19602T=CCUG 57508T).

The genus Terrabacter was created by Collins et al. (1989) through the reclassification of Pimelobacter tumescens as Terrabacter tumescens. Subsequently, three further species, Terrabacter terrae (Montero-Barrientos et al., 2005), Terrabacter aerolatus (Weon et al., 2007) and Terrabacter lapilli (Lee et al., 2008), were described. Phylogenetic analyses based on 16S rRNA gene sequences showed that the genus Terrabacter falls within the family Intrasporangiaceae in the order Actinomycetales (Stackebrandt et al., 1997; Lee et al., 2008). In this study, we describe a Terrabacter-like bacterial strain that was isolated from soil around a wastewater treatment plant in Korea.

Strain ON10T was isolated by using dilution plating onto nutrient agar (Difco) at 30 °C. The type strains of the four Terrabacter species were used as reference strains for DNA–DNA hybridization and phenotypic characterization. T. tumescens KCTC 9133T and T. lapilli KCTC 19199T were obtained from the Korean Collection for Type Cultures. T. terrae KACC 11642T and T. aerolatus KACC 20556T were obtained from the Korean Agricultural Culture Collection. The morphological, physiological and biochemical characteristics of strain ON10T were investigated using routine cultivation on trypticase soy agar (TSA; Difco) at 30 °C. Cell morphology was examined by light microscopy (Eclipse E600; Nikon) using cells from exponentially growing cultures. The Gram reaction was determined using the bioMérieux Gram Stain kit according to the manufacturer’s instructions. Growth at various temperatures (4, 10, 20, 25, 28, 30, 35, 37, 40 and 45 °C) was measured on TSA. The pH range for growth was determined in nutrient broth (Difco) with the pH adjusted prior to sterilization to various values (pH 4.5–10.5 at intervals of 0.5 units) by the addition of HCl or Na2CO3. Growth at various NaCl concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 %, w/v) was investigated using trypticase soy broth (TSB; Difco) prepared according to the manufacturer’s instructions except that NaCl was added to the desired final concentration. Growth under anaerobic conditions was determined by incubation in an anaerobic chamber on TSA and on TSA supplemented with potassium nitrate (0.1 %, w/v), which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tween 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were studied as described by Lányi (1987). Susceptibility to
antibiotics was tested on TSA plates using antibiotic discs containing the following (μg unless otherwise stated): polymyxin B (100 U), streptomycin (50), penicillin G (20 U), chloramphenicol (100), ampicillin (10), cephalothin (30), gentamicin (30), novobiocin (5), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), neomycin (30) and carbencillin (100). Utilization of a range of various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50 CH and API ZYM systems (bioMérieux). The cells were suspended in AUX medium, according to the manufacturer’s instructions, to inoculate the API 50 CH system.

Cell biomass of strain ON10T for DNA extraction and for the analyses of cell-wall composition, polar lipids and isoprenoid quinones was obtained after incubation in TSB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon et al. (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene sequence was amplified using PCR with two universal primers, 5'-GAGTTTGATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCCAGCC-3', as described by Yoon et al. (1998) and sequencing of the amplified sequence was performed as described by Yoon et al. (2003). Alignment of sequences was carried out with CLUSTAL W software (Thompson et al., 1994). Gaps at the 5' and 3' ends of the alignment were excluded from further analysis. Phylogenetic trees were inferred with three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods implemented within the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated with the algorithm of Jukes & Cantor (1969) using the program DNADIST. The stability of relationships was assessed with a bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

The isomer type of the diamino acid in the cell-wall peptidoglycan was analysed using TLC according to the method described by Komagata & Suzuki (1987). Whole-cell sugars were determined as described by Komagata & Suzuki (1987). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For cellular fatty acid analysis, cell mass was harvested from TSA plates after incubation for 7 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin et al. (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987).

The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) except that the DNA was first hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained with each sample were excluded, and the mean of the remaining three values was calculated for the DNA–DNA relatedness value.

The almost-complete 16S rRNA gene sequence of strain ON10 comprised 1477 nucleotides. Comparative sequence analysis showed that strain ON10T is phylogenetically most closely related to the family Intrasporangiaceae. In the neighbour-joining phylogenetic tree (Fig. 1), strain ON10T clustered with the clade comprising Terrabacter species, with a bootstrap value of 96.0%. Strain ON10T exhibited 16S rRNA gene sequence similarity values of 98.4–98.8% with the type strains of Terrabacter species and less than 97.7% with the other species used in the phylogenetic analysis. Strain ON10T exhibited DNA–DNA relatedness of 17–28% to the type strains of Terrabacter species: T. tumescens KCTC 9133T (28%), T. terrae KACC 11642T (19%), T. aerolatus KACC 20556T (17%) and T. lapilli KCTC 19199T (20%). These values indicated that strain ON10T represents a genomic species different from the currently recognized Terrabacter species (Wayne et al., 1987).

Strain ON10T had LL-2,6-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The cell-wall sugars were glucose, mannose, arabinose and xylose. The predominant isoprenoid quinone detected in strain ON10T was menaquinone-8(H4). Major polar lipids

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of Terrabacter terrigena sp. nov. ON10T and some other related taxa. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Terrabacter globiformis* DSM 20124T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

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found in strain ON10T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, an unidentified phospholipid and an unidentified lipid. The fatty acid profile of strain ON10T was composed of the following (each constituting >0.5% of total fatty acids): branched fatty acids iso-C 15 : 0 (48.2%), iso-C 14 : 0 (13.5%), anteiso-C 15 : 0 (8.2%), iso-C 16 : 1 (6.2%), iso-C 16 : 0 (5.9%), iso-C 17 : 1ω9c (1.1%), anteiso-C 17 : 1ω9c (0.9%), iso-C 13 : 0 (0.8%) and anteiso-C 17 : 0 (0.6%); summed feature 1 (iso-C 15 : 1 and/or C 13 : 0 3-OH; 2.9%); unsaturated fatty acids C 15 : 1ω6c (2.8%) and C 17 : 1ω8c (2.6%); summed feature 3 (C 16 : 1ω7c and/or iso-C 15 : 0 2-OH; 1.9%); and straight-chain fatty acids C 15 : 0 (1.8%) and C 16 : 0 (0.6%). This fatty acid profile was similar to those of other Terrabacter species, although there may be differences in the proportions of some fatty acids.

Table 1. Differential phenotypic characteristics of strain ON10T and the type strains of Terrabacter species

<table>
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<td>Cystine arylamidase</td>
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<tr>
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<tr>
<td>β-Glucosidase</td>
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<tr>
<td>α-Mannosidase</td>
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<td>w</td>
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<td>Whole-cell sugars‡</td>
<td>Glc, Man, Ara, Xyl</td>
<td>ND</td>
<td>Fuc, Gal</td>
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<td>Polar lipids§</td>
<td>DPG, PG, PE, PI, APGL</td>
<td>DPG, PE, PI, PGL</td>
<td>DPG, PE, PI, PGL</td>
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<td>DNA G+C content (mol%)</td>
<td>71.6</td>
<td>73</td>
<td>71</td>
<td>71.7</td>
<td>72.6</td>
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</table>

* Differences may be observed with different cultivation conditions.
† Data for T. tumescens and T. terrae were taken from Weon et al. (2007); data for T. lapilli were taken from this study.
‡ Ara, Arabinose; Fuc, fucose; Gal, galactose; Glc, glucose; Man, mannose; Xyl, xylose; Rha, rhamnose; Rib, ribose.
§APGL, Unknown amino-containing phosphoglycolipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PGL, unknown phosphoglycolipid; PL, unidentified phospholipid; L, unknown lipid.
due to differences in cultivation and extraction conditions (Weon et al., 2007; Lee et al., 2008). The DNA G+C content of strain ON10\(^T\) was 71.6 mol%.

Strain ON10\(^T\) showed the closest phylogenetic affiliation to members of the genus Terrabacter. Moreover, there were no distinct chemotaxonomic properties that differentiated strain ON10\(^T\) from members of the genus Terrabacter (Montero-Barrientos et al., 2005; Weon et al., 2007; Lee et al., 2008). Accordingly, it is reasonable to place strain ON10\(^T\) within the genus Terrabacter. Strain ON10\(^T\) was distinguishable from recognized Terrabacter species by differences in several phenotypic characteristics, as shown in Table 1. Therefore, the phylogenetic and genetic distinctiveness and the differential phenotypic properties suggest that strain ON10\(^T\) represents a novel species of the genus Terrabacter, for which the name Terrabacter terrigena sp. nov. is proposed.

**Description of Terrabacter terrigena sp. nov.**

*Terrabacter terrigena* (ter.’ri.ge.na. L. masc. or fem. n. *terrigena* child of the earth, earth-born, referring to the isolation of the type strain from soil).

Cells are aerobic, Gram-positive-staining, non-spore-forming, non-motile rods or short rods, 0.3–0.6 x 1.0–5.0 µm. Colonies on TSA are circular, convex, sticky, glistening and greyish yellow, 1.5–2.0 mm in diameter after incubation for 7 days at 30 °C. Growth occurs at 10 and 37 °C, but not at 4 or 40 °C, at pH 5.0 and 8.5 but not at pH 4.5 or 9.0 (optimum, pH 6.5–7.0) and in the presence of 0–3 % (w/v) NaCl (optimum, 0.5 % NaCl). Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. H\(_2\)S and indole are not produced.

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**References**


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**Terrabacter terrigena sp. nov.**

The type strain, ON10\(^T\) (=KCTC 19602\(^T\)=CCUG 57508\(^T\)), was isolated from soil around a wastewater treatment plant in Korea.


