Terrabacter terrigena sp. nov., isolated from soil

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A Gram-positive-staining, non-motile and rod-shaped bacterium, strain ON10^T, was isolated from soil around a wastewater treatment plant in Korea and its taxonomic position was investigated by using a polyphasic approach. Strain ON10^T 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 ON10^T 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 LL-diaminopimelic acid and the cell-wall sugars were glucose, mannose, arabinose 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-C_{15:0} and iso-C_{14:0}. The DNA G + C content was 71.6 mol%. Strain ON10^T 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 ON10^T 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 ON10^T (=KCTC 19602^T=CCUG 57508^T).

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 ON10^T 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 9133^T and *T. lapilli* KCTC 19199^T were obtained from the Korean Collection for Type Cultures. *T. terrae* KACC 11642^T and *T. aerolatus* KACC 20556^T were obtained from the Korean Agricultural Culture Collection. The morphological, physiological and biochemical characteristics of strain ON10^T 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, 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, Tweens 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

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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 carbenicillin (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 ON10^T 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 $\times$ 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^T comprised 1477 nucleotides. Comparative sequence analysis showed that strain ON10^T is phylogenetically most closely related to the family *Intrasporangiaceae*. In the neighbour-joining phylogenetic tree (Fig. 1), strain ON10^T clustered with the clade comprising *Terrabacter* species, with a bootstrap value of 96.0%. Strain ON10^T 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 ON10^T exhibited DNA–DNA relatedness of 17–28% to the type strains of *Terrabacter* species: *T. tumescens* KCTC 9133^T (28%), *T. terrae* KACC 11642^T (19%), *T. aerolatus* KACC 20556^T (17%) and *T. lapilli* KCTC 19199^T (20%). These values indicated that strain ON10^T represents a genomic species different from the currently recognized *Terrabacter* species (Wayne *et al.*, 1987).

Strain ON10^T 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 ON10^T 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. ON10^T 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. *Arthrobacter globiformis* DSM 20124^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

found in strain ON10^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, an unidentified phospholipid and an unidentified lipid. The fatty acid profile of strain ON10^T 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_{17:1}ω9c (1.1 %), anteiso-

C_{17:1}ω9c (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_{15:1}ω6c (2.8 %) and C_{17:1}ω8c (2.6 %); summed feature 3 (C_{16:1}ω7c 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 ON10^T and the type strains of *Terrabacter* species

Taxa: 1, strain ON10^T (*Terrabacter terrigena* sp. nov.); 2, *T. tumescens* (data from Collins *et al.*, 1989; Montero-Barrientos *et al.*, 2005; Weon *et al.*, 2007); 3, *T. terrae* (Montero-Barrientos *et al.*, 2005; Weon *et al.*, 2007); 4, *T. aerolatus* (Weon *et al.*, 2007); 5, *T. lapilli* (Lee *et al.*, 2008; this study). All taxa are Gram-positive-staining and positive for catalase, hydrolysis of aesculin and gelatin (not determined for *T. lapilli*), utilization of D-glucose, maltose, sucrose, inositol, N-acetylglucosamine, gluconate, glycogen and malate, and activities of esterase lipase (C8), leucine arylamidase, acid phosphatase, α- and β-galactosidase and α-glucosidase. All taxa are negative for oxidase, utilization of salicin, L-fucose, caprate, citrate and phenylacetate, and activities of alkaline phosphatase, trypsin, α-chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase and α-fucosidase. +, Positive; –, negative; w, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5
Cell morphology	Short rods/rods	Rods/cocci	Long rods	Rods/cocci	Short rods
Motility	–	w	–	+	–
Colony colour*	Greyish yellow	White–grey	Yellow	White	Bright yellow
Growth at 7 % NaCl	–	–	+	–	–
Nitrate reduction	–	+	–	+	+
Hydrolysis of:					
Casein	+	+	–	+	+
Starch	–	+	+	+	+
Urea	–	–	+	–	ND
Utilization of:†					
L-Arabinose	–	+	+	–	–
D-Mannose	+	+	+	+	–
Melibiose	+	+	–	+	+
L-Rhamnose	+	–	–	–	–
D-Ribose	w	–	+	–	–
D-Mannitol	+	+	–	+	–
D-Sorbitol	+	+	–	+	–
Adipate	w	w	–	w	–
API ZYM†					
Esterase (C4)	–	+	+	+	+
Lipase (C14)	–	–	–	+	–
Valine arylamidase	–	–	–	+	–
Cystine arylamidase	+	–	–	–	+
Naphthol-AS-BI-phosphohydrolase	–	+	+	+	w
β-Glucosidase	–	–	–	w	–
α-Mannosidase	–	–	–	w	–
Whole-cell sugars‡	Glc, Man, Ara, Xyl	ND	Fuc, Gal	Glc, Rib, Rha, Xyl, Gal	Glc, Rha, Rib, Xyl, Ara
Polar lipids§	DPG, PG, PE, PI, PL, L	DPG, PE, PI, APGL	DPG, PE, PI, PGL	DPG, PE, PI, PL	DPG, PE, PG, PI, PL
DNA G + C content (mol%)	71.6	73	71	71.7	72.6

*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 × 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. H₂S and indole are not produced. Hypoxanthine and Tweens 20, 40 and 60 are hydrolysed, but xanthine, tyrosine and Tween 80 are not. D-Xylose, galactose, fructose, arbutin, aesculin, cellobiose, lactose, trehalose, melezitose, raffinose, starch, turanose, D-lyxose and D-arabitol are utilized as sole carbon and energy sources, but glycerol, erythritol, D-arabinose, L-xylose, adonitol, methyl β-D-xyloside, sorbose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, inulin, xylitol, gentiobiose, D-tagatose, D-fucose, L-arabitol and 2- and 5-ketogluconate are not. Susceptible to cephalothin, chloramphenicol, lincomycin, neomycin, oleandomycin, penicillin G, streptomycin and tetracycline, but not to ampicillin, carbenicillin, gentamicin, kanamycin, novobiocin or polymyxin B. The cell-wall peptidoglycan contains LL-2,6-diaminopimelic acid as the diagnostic diamino acid. Cell-wall sugars are glucose, mannose, arabinose and xylose. Predominant menaquinone is MK-8(H₄). Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, an unidentified phospholipid and an unidentified lipid. Major fatty acids (>10 % of total fatty acids) are iso-C_{15:0} and iso-C_{14:0}. Other phenotypic characteristics are given in Table 1. The DNA G+C content of the type strain is 71.6 mol% (HPLC).

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

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