Terrabacter aerolatus sp. nov., isolated from an air sample

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A Gram-positive, strictly aerobic, motile, rod- or coccoid-shaped bacterium, strain 5516J-36^T, was isolated from an air sample from Jeju region, Korea, and its taxonomic position was investigated by a polyphasic approach. The organism grew optimally at 30 °C and pH 7.0–8.0. Comparative 16S rRNA gene sequencing studies demonstrated that this strain was highly related phylogenetically to *Terrabacter terrae* PPLB^T and *Terrabacter tumescens* DSM 20308^T, showing 98.9 % sequence similarity to both strains. However, the DNA–DNA reassociation values between 5516J-36^T and the type strains of *Terrabacter terrae* and *Terrabacter tumescens* were low (51 and 48 %, respectively). The peptidoglycan type was A3 γ , the predominant menaquinone was MK-8(H₄), the polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and an unidentified phosphoglycolipid and the whole-cell sugars were glucose, ribose, rhamnose, xylose and galactose. Mycolic acids were absent. The major fatty acids (>5 % of total fatty acids) were iso-C_{15:0}, iso-C_{16:0}, iso-C_{14:0}, iso-C_{17:0} and anteiso-C_{15:0}. The DNA G + C content was 71.7 mol%. On the basis of the above data, it is proposed that strain 5516J-36^T represents a novel species, *Terrabacter aerolatus* sp. nov. The type strain of *Terrabacter aerolatus* is 5516J-36^T (=KACC 20556^T =DSM 18562^T).

The genus *Terrabacter*, with the type species *Terrabacter tumescens*, was created by the transfer of *Pimelobacter tumescens* on the basis of phylogenetic and chemotaxonomic data (Collins *et al.*, 1989). Recently, another species *Terrabacter terrae* was proposed for an isolate from soil in Spain (Montero-Barrientos *et al.*, 2005). The genus was placed in the family *Intrasporangiaceae* and is characterized by MK-8(H₄) as the predominant isoprenoid quinone, A3 γ as the peptidoglycan type, iso-C_{15:0} as the major fatty acid and high DNA G + C contents (71–73 mol%).

Strain 5516J-36^T was isolated from an air sample from the Jeju region of Korea. The air sample was collected using an MAS-100 air sampler (Merck; single-stage multiple-hole impactor) which contained Petri dishes with R2A agar (BBL) amended with 200 μ g cycloheximide ml⁻¹ (Sigma). After sampling, plates were incubated at 30 °C for 5 days, and strain 5516J-36^T was recovered. *Terrabacter tumescens*

DSM 20308^T and *Terrabacter terrae* CECT 3379^T were used as reference strains. These strains were cultivated on R2A medium (pH 7.0; Difco) at 30 °C and maintained on R2A medium.

The 16S rRNA gene of the isolate was amplified by a PCR and sequenced directly using an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Phylogenetic analysis was carried out using MEGA3 (Kumar *et al.*, 2004) after multiple alignment of the sequences by CLUSTAL W (Thompson *et al.*, 1994). Distances using distance options according to the Kimura two-parameter model (Kimura, 1983) and clustering using neighbour-joining were determined by using bootstrap values based on 1000 replicates. DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG High Prime DNA labelling and detection starter kit II (Roche Molecular Biochemicals). Reassociation was conducted at 65 °C. The hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5516J-36^T is EF212039.

For strain 5516J-36^T, 1400 bp of the 16S rRNA gene sequence was determined. Comparative 16S rRNA gene sequence analysis showed that strain 5516J-36^T was most closely related to members of the genus *Terrabacter*. In a phylogenetic tree based on the neighbour-joining algorithm, strain 5516J-36^T joined the clade comprising *Terrabacter* species at a bootstrap confidence value of 100 % (Fig. 1). Strain 5516J-36^T exhibited 16S rRNA gene sequence similarity values of 98.9 % to both *Terrabacter terrae* PPLB^T and *Terrabacter tumescens* DSM 20308^T. It showed 97.4 and 96.9 % sequence similarity, respectively, to *Terracoccus luteus* DSM 44267^T and *Intrasporangium calvum* DSM 43043^T. DNA–DNA relatedness data demonstrated that strain 5516J-36^T represents a genomic species that is different from recognized *Terrabacter* species (Wayne *et al.*, 1987). DNA–DNA relatedness values between strain 5516J-36^T and *Terrabacter tumescens* DSM 20308^T, *Terrabacter terrae* CECT 3379^T and *Terracoccus luteus* DSM 44267^T were respectively 51, 48 and 32 %.

Cell morphology was examined by phase-contrast microscopy (AXIO; Zeiss). The isolate was tested for a number of key characteristics, such as Gram staining, catalase, oxidase and hydrolysis of casein, DNA, hypoxanthine, gelatin, starch, Tween 80 and xanthine, by using standard procedures (Smibert & Krieg, 1994). Growth was assessed at 5, 10, 20, 25, 30, 35 and 40 °C, at pH 4–10 (increments of 1 pH unit) and at 0, 1, 3, 5, 7 and 10 % NaCl. Hydrolysis of CM-cellulose (Sigma) (0.1 %), chitin from crab shells (1 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) was checked. Motility was examined on 1/10-strength R2A medium. Anaerobic growth was checked using a BBL anaerobic jar (Becton Dickinson). Other physiological properties and enzyme activities were tested using the API 20NE, API ID 32GN and API ZYM systems (bioMérieux). The results of API 20NE and API ID 32GN were recorded after 5 days incubation.

Strain 5516J-36^T formed visible colonies on R2A agar after 48 h at 28 °C. The strain grew on R2A, nutrient agar (Difco) and tryptic soy agar (TSA; Difco), but did not grow on MacConkey agar (Difco). The colonies were white, convex and round. Cells were strictly aerobic, Gram-positive, motile, catalase-positive and oxidase-negative. Cells are cocci (diameter 0.9–1.0 µm) that occur in pairs,

or rods (0.9 × 1.5–3.5 µm). The physiological and biochemical characteristics are summarized in Table 1.

Biomass for chemotaxonomic studies was grown in shake flasks of R2A broth for 4 days at 28 °C, checked for purity, harvested by centrifugation and freeze-dried. The peptidoglycan was analysed as described by Schleifer & Kandler (1972). The phospholipids and menaquinones were analysed by the method of Minnikin *et al.* (1984). Sugar analysis of whole cells and mycolic acid determinations were carried out as described by Stanek & Roberts (1974) and Minnikin *et al.* (1975), respectively. For fatty acid methyl ester analysis, cell mass of strain 5516J-36^T was harvested from TSA plates after incubation for 3 days at 28 °C. Analyses of the whole-cell fatty acid pattern followed described methods using the MIDI system (Microbial ID, Inc.) (Kroppenstedt, 1985; Sasser, 1990). The G + C content of the DNA was determined according to Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18S; Supelco).

From quantitative analysis of the peptidoglycan amino acids by gas chromatography, strain 5516J-36^T contained glycine, alanine, glutamic acid and LL-diaminopimelic acid in an approximate molar ratio of 4.0:1.8:1.0:1.0. From the two-dimensional TLC pattern (not shown) of peptides in the partial peptidoglycan hydrolysate, strain 5516J-36^T showed peptidoglycan type A3γ as described by Schleifer & Kandler (1972) (type A41.1 according to <http://www.dsmz.de/species/murein.htm>). The predominant menaquinone was MK-8(H₄). The polar lipids phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and a phosphoglycolipid of unknown structure were detected. The peptidoglycan structure, isoprenoid quinone and polar lipids of strain 5516J-36^T were congruent with those of the two recognized species of the genus *Terrabacter*. The predominant fatty acids were iso-C_{15:0} (49.0 %), iso-C_{16:0} (14.5 %), iso-C_{14:0} (7.9 %), iso-C_{17:0} (5.8 %) and anteiso-C_{15:0} (5.2 %) (Table 2). The fatty acid profile of strain 5516J-36^T was similar to that of *Terrabacter terrae* CECT 3379^T, which was considerably different from that of *Terrabacter tumescens* DSM 20308^T (Montero-Barrientos *et al.*, 2005). The whole-cell sugar profile contained glucose, ribose, rhamnose, xylose and galactose (in decreasing order of abundance). Mycolic

Fig. 1. 16S rRNA gene sequence-based dendrogram obtained by distance matrix (neighbour-joining) analysis showing the position of strain 5516J-36^T. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets. Bootstrap values below 50 % are not indicated. Bar, 0.01 substitutions per position.

Table 1. Differential characteristics between strain 5516J-36^T and type strains of *Terrabacter* species

Strains: 1, 5516J-36^T; 2, *Terrabacter tumescens* DSM 20308^T; 3, *Terrabacter terrae* CECT 3379^T. Data were obtained in this study unless indicated. According to API 20NE and API ZYM strips, all strains are positive for aesculin hydrolysis, gelatin hydrolysis and β -galactosidase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase and α -glucosidase activities. All strains are negative for indole production, glucose fermentation and arginine dihydrolase, urease, alkaline phosphatase, cystine arylamidase, trypsin, α -chymotrypsin, β -glucuronidase, *N*-acetyl- β -glucosaminidase and α -fucosidase activities. According to API 20NE and API ID32GN strips, all strains assimilate D-glucose, D-mannose, *N*-acetylglucosamine, maltose, potassium gluconate, malic acid, inositol, sucrose, sodium malonate, sodium acetate, L-alanine, glycogen, propionic acid, valeric acid, L-histidine and 3-hydroxybutyric acid. None of the strains assimilate capric acid, trisodium citrate, phenylacetic acid, L-rhamnose, itaconic acid, suberic acid, potassium 5-ketogluconate, salicin, L-fucose, potassium 2-ketogluconate or 4-hydroxybenzoic acid. +, Positive; (+), weakly positive; –, negative.

Characteristic	1	2	3
Cell shape	Rod/coccus	Rod/coccus*	Long rods*
Colony colour	White	White/grey*	Yellow*
Motility	+	(+)*	–*
Growth on 7% NaCl	–	–*	+*
Nitrate reduction	+	+	–
Assimilation of:			
L-Arabinose	–	+	+
D-Mannitol	+	+	–
Adipic acid	(+)	(+)	–
D-Ribose	–	–	+
Lactic acid	–	–	+
3-Hydroxybenzoic acid	–	+	–
L-Serine	–	–	+
D-Melibiose	+	+	–
D-Sorbitol	+	+	–
L-Proline	–	+	–
Enzyme activities			
Lipase (C14)	+	–	–
Valine arylamidase	+	–	–
β -Glucosidase	(+)	–	–
α -Mannosidase	(+)	–	–
DNA G + C content (mol%)	71.7	73	71

*Data from Montero-Barrientos *et al.* (2005).

acids were absent. The DNA G + C content of strain 5516J-36^T was 71.7 mol%.

On the basis of the data presented, strain 5516J-36^T should therefore be placed in the genus *Terrabacter* within a novel species, for which the name *Terrabacter aerolatus* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions of strain 5516J-36^T and type strains of *Terrabacter* species

Strains: 1, 5516J-36^T; 2, *Terrabacter tumescens* DSM 20308^T; 3, *Terrabacter terrae* CECT 3379^T. All strains were grown for 2 days on TSA. Values are percentages of total fatty acids; fatty acids that represented <0.5% in all strains are omitted. –, Not detected.

Fatty acid	1	2	3
iso-C _{13:0}	0.7	0.2	0.3
C _{14:0}	0.6	5.8	1.8
iso-C _{14:0}	7.9	5.5	3.1
iso-C _{15:0}	49.0	17.2	32.0
anteiso-C _{15:0}	5.2	6.5	19.6
C _{16:0}	3.3	11.5	5.6
C _{16:0} 10-methyl	–	1.6	–
iso-C _{16:0}	14.5	6.1	5.2
iso-C _{16:1} H	0.5	0.6	0.8
C _{17:0}	1.4	5.5	1.6
C _{17:0} 10-methyl	0.1	0.6	–
iso-C _{17:0}	5.8	0.9	2.0
anteiso-C _{17:0}	3.1	2.0	7.2
C _{17:1} ω 8c	0.8	7.3	2.4
iso-C _{17:1} ω 9c	0.9	–	1.6
anteiso-C _{17:1} ω 9c	0.3	–	2.3
C _{18:0}	3.2	5.4	2.3
iso-C _{18:0}	0.8	0.3	–
C _{18:1} ω 9c	0.9	13.1	4.3
C _{18:1} ω 7c	–	0.5	–
Summed feature 3*	1.0	7.1	7.1
Summed feature 6*	–	0.9	–

*Summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c; summed feature 6 comprises C_{19:1} ω 11c and/or C_{19:1} ω 9c.

Description of *Terrabacter aerolatus* sp. nov.

Terrabacter aerolatus (ae.ro.la'tus. Gr. n. *aer* air; L. part. adj. *latus* carried; N.L. masc. part. adj. *aerolatus* airborne).

Cells are strictly aerobic, Gram-positive, motile rods or cocci (0.9–1.0 $\times$ 1.0–3.5 μ m), catalase-positive and oxidase-negative. Colonies are white, convex and round. Optimal temperature for growth is 30 °C. Growth occurs in the range 5–35 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs in the range pH 4–9. Growth occurs in the presence of 0–5% (w/v) NaCl. Casein, starch, tyrosine and Tween 80 are hydrolysed, but chitin, CM-cellulose, DNA, pectin, hypoxanthine and xanthine are not. LL-Diaminopimelic acid-containing peptidoglycan A3 γ has three glycine residues as the interpeptide bridge. Whole-cell sugars are glucose, ribose, rhamnose, xylose and galactose (in decreasing order of abundance). Mycolic acids are absent. The predominant menaquinone is MK-8(H₄). The major fatty acids (>5% of total fatty acids) are iso-C_{15:0} (49.0%), iso-C_{16:0} (14.5%), iso-C_{14:0} (7.9%), iso-C_{17:0} (5.8%) and anteiso-C_{15:0} (5.2%). The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol,

phosphatidylinositol and an unidentified phosphoglycolipid. The DNA G+C content of the type strain is 71.7 mol%.

The type strain, 5516J-36^T (=KACC 20556^T =DSM 18562^T), was isolated from an air sample in Jeju, Korea.

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