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

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A novel actinomycete, designated strain 5414T-18^T, was isolated from an air sample collected from the Taean region, Korea. The strain contained oxidase and grew in the presence of 7 % NaCl. A neighbour-joining tree constructed on the basis of the 16S rRNA gene sequence showed that strain 5414T-18^T is a member of the genus *Terrabacter*, sharing 97.8–98.3 % 16S rRNA gene sequence similarities to type strains of species of the genus *Terrabacter* (98.3 % sequence similarity with *Terrabacter lapilli* LR-26^T). It contained peptidoglycan containing LL-diaminopimelic acid of A3 γ type, with three glycine residues as the interpeptide bridge. Whole-cell sugars were glucose, mannose and ribose. Mycolic acids were absent. The predominant menaquinone was MK-8(H₄). The major fatty acids (>7 % of total fatty acids) were iso-C_{15:0}, iso-C_{16:0}, C_{17:1} ω 8c and iso-C_{14:0}. The major polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and an unidentified phosphoglycolipid. The DNA G + C content of the type strain was 73 mol%. Strain 5414T-18^T exhibited DNA–DNA relatedness levels of 44, 43, 39, 34 and 34 % to the type strains of *Terrabacter lapilli*, *Terrabacter aerolatus*, *Terrabacter terrae*, *Terrabacter tumescens* and *Terracoccus luteus*, respectively. These findings suggest that strain 5414T-18^T represents a novel species within the genus *Terrabacter*. The name *Terrabacter aeriphilus* sp. nov. is proposed for this novel species, with the type strain 5414T-18^T (=KACC 20693^T=DSM 18563^T).

The genus *Terrabacter* was proposed for the former *Pimelobacter tumescens* (Collins *et al.*, 1989). This genus was classified into the family *Intrasporangiaceae*, and is closely related to the genus *Terracoccus* (Prauser *et al.*, 1997). However, the former can be differentiated from the latter notably on the basis of fatty acid compositions. At the time of writing, the genus *Terrabacter* included four recognized species: *Terrabacter tumescens*, *Terrabacter aerolatus*, *Terrabacter terrae* and *Terrabacter lapilli* (Montero-Barrientos *et al.*, 2005; Weon *et al.*, 2007; Lee *et al.*, 2008).

Strain 5414T-18^T was isolated from an air sample collected in the Taean region, Republic of Korea. The air was

sampled using a MAS-100 air sampler (Merck; single-stage multiple-hole impactor) which contained Petri dishes with R2A agar (BBL) containing 200 μ g cycloheximide ml⁻¹ (Sigma). After sampling, plates were incubated at 30 °C for 5 days, and strain 5414T-18^T was recovered.

Sequencing of the 16S rRNA gene was performed as described previously (Kwon *et al.*, 2003). The sequence similarity levels were calculated by using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic analysis was performed by using MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of data by CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic dendrograms were constructed using the neighbour-joining (with Kimura's two-parameter correction) and maximum-parsimony (with heuristic search option) methods with bootstrap values based on 1000 replications. A continuous 1436 bp stretch of strain 5414T-18^T was used in the analysis.

Cell biomass for the analysis of cell-wall and isoprenoid quinones was obtained from cells grown in tryptic soy agar

Abbreviation: TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5414T-18^T is EU573947.

A supplementary figure of the maximum-parsimony tree based on almost-complete 16S rRNA gene sequences, showing the phylogenetic position of strain 5414T-18^T, is available with the online version of this paper.

(Difco) at 30 °C. Chemotaxonomic analyses for the following substrates were performed as described: peptidoglycan (Schleifer & Kandler, 1972), polar lipids and menaquinones (Minnikin *et al.*, 1984), whole-cell sugars (Staneck & Roberts, 1974), mycolic acids (Minnikin *et al.*, 1975) and fatty acids [according to the standard protocol of the Sherlock Microbial Identification System (Sasser, 1990)]. 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). DNA G+C content was determined using an HPLC method (Mesbah *et al.*, 1989).

For the phenotypic characterization, R2A medium was used as the basal medium. Gram staining, catalase, oxidase and hydrolysis of casein, carboxymethylcellulose, chitin, DNA, hypoxanthine, pectin, starch, Tween 80, tyrosine and xanthine were determined by using the methods described by Smibert & Krieg (1994). Temperature, salinity and pH ranges for growth were tested at 5, 10, 20, 25, 30, 35, 40 and 45 °C, at 0, 1, 3, 5, 7 and 10 % (w/v) NaCl, and at pH 4, 5, 6, 7, 8, 9 and 10, respectively. Cell morphology and dimensions were examined by using phase-contrast microscopy with a Zeiss Axio Imager. Motility was checked by transmission electron microscopy (TEM) with culture grown for 24 h on R2A agar. For TEM observations, cells were negatively stained with 0.5 % (w/v) uranyl acetate and examined with an LEO model 912AB electron microscope. Anaerobic growth was checked on R2A agar medium using the BBL anaerobic jar (Becton Dickinson; >15 % CO₂). Other physiological properties, carbohydrate assimilation

and enzymic activities were tested using API 20NE, API ID 32GN and API ZYM (bioMérieux). Test strips were used according to the manufacturer's instructions. API 20NE, API ID 32GN test strips were observed after 7 days incubation, and API ZYM test strips were checked after 4 h incubation.

Sequence similarities between the new isolate and currently described species of the genus *Terrabacter* ranged from 97.8 to 98.3 %, with the highest sequence similarity to *Terrabacter lapilli* LR-26^T. Furthermore, 5414T-18^T showed a high sequence similarity (98.1 %) with *Terrabacter aerolatus* KACC 20556^T. According to the neighbour-joining tree, all the species of the genus *Terrabacter*, including 5414T-18^T were clustered into the same group with a relatively low bootstrap value (48 %) (Fig. 1). However, the maximum-parsimony tree showed different phylogenetic relationships in which species of the genus *Terrabacter*, including 5414T-18^T, did not form one independent cluster (see Supplementary Fig. S1, available in IJSEM Online). Both trees indicate that the genera *Humihabitans*, *Intrasporangium*, *Humibacillus*, *Terracoccus* and *Terrabacter* are closely related, but phylogenetic positions among some of these genera or member species could not be resolved clearly solely on the basis of 16S rRNA gene sequence analysis.

From the quantitative analysis of the peptidoglycan amino acids by gas chromatography and analysis of the 2D TLC pattern of peptides in the partial peptidoglycan hydrolysate, strain 5516 J-36^T showed peptidoglycan type A3_γ as described by Schleifer & Kandler (1972) (type A41.1 according to http://www.dsmz.de/microorganisms/main.php?content_id=35). Phospholipids comprised phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol as the major polar lipids, and a small amount of phosphatidylglycerol. The predominant menaquinone was

Fig. 1. Neighbour-joining tree based on almost-complete 16S rRNA gene sequences showing the phylogenetic position of strain 5414T-18^T. Numbers at nodes indicate the percentages of 1000 bootstrap resamplings; only values >40 % are given. Bar, 0.01 substitutions per nucleotide position.

MK-8(H₄). Whole-cell sugars were glucose, mannose and ribose. Mycolic acids were absent. The fatty acids (>5 % of total fatty acids) contained iso-C_{15:0} (40.1 %), iso-C_{16:0} (11.9 %), C_{17:1ω8c} (9.0 %), iso-C_{14:0} (7.6 %), anteiso-C_{15:0} (6.9 %) and iso-C_{16:1} H (5.4 %) (Table 1). DNA–DNA hybridization values between 5414T-18^T and *Terrabacter lapilli* LR-26^T, *Terrabacter aerolatus* KACC 20556^T, *Terrabacter terrae* CECT 3379^T, *Terrabacter tumescens* DSM 20308^T and *Terracoccus luteus* DSM 44267^T were 44, 43, 39, 34 and 34 %, respectively. The DNA G + C content of strain 5414T-18^T was 73 mol%.

Strain 5414T-18^T formed visible colonies on R2A agar after 48 h at 30 °C. The strain was able to grow on nutrient agar (Difco) and tryptic soy agar (Difco), but not on MacConkey agar (Difco). The physiological and biochemical characteristics are summarized in Table 2.

Despite the high sequence similarities with members of the genus *Terrabacter*, the phylogenetic position of strain 5414T-18^T was not clear, in that the novel isolate also showed a relatively high sequence similarity (97.9 %) with *Terracoccus*

luteus DSM 44267^T and its phylogenetic affiliation was not strongly supported by bootstrap values of either phylogenetic trees (Fig. 1 and Supplementary Fig. S1). Furthermore, chemotaxonomic properties such as polar lipids, whole cell sugar contents, peptidoglycan structure and isoprenoid quinones could not differentiate the genera *Terrabacter* and *Terracoccus*. However, the fatty acid contents of species of the genus *Terrabacter* including 5414T-18^T contained lower amounts of anteiso-C_{15:0} and C_{16:0}, and higher amounts of iso-C_{16:0} and C_{17:1ω8c} compared with *Terracoccus luteus* (Table 1). Considering the phylogenetic and chemotaxonomic data presented, strain 5414T-18^T should be classified into the genus *Terrabacter*. Strain 5414T-18^T can also be differentiated from the most closely related species, *Terrabacter lapilli*, by the presence of oxidase and growth on 7 % NaCl, and a lack of nitrate reduction in addition to the differences in the assimilation and enzymic activity profiles (Table 2). Also, strain 5414T-18^T can be distinguished from *Terracoccus luteus* on the basis of the presence of oxidase, and the disparities in the assimilation and enzymic activity patterns (Table 2).

On the basis of the data presented, strain 5414T-18^T represents a novel species of the genus *Terrabacter*, for which the name *Terrabacter aeriphilus* sp. nov. is proposed.

Table 1. Cellular fatty acid contents of strain 5414T-18^T and related species

Strains: 1, 5414T-18^T; 2, *Terrabacter aerolatus* KACC 20556^T; 3, *Terrabacter lapilli* LR-26^T; 4, *Terrabacter terrae* CECT 3379^T; 5, *Terrabacter tumescens* DSM 20308^T; 6, *Terracoccus luteus* DSM 44267^T. All were grown at 28 °C for 2 days on TSA medium. Values are percentages of total fatty acids; fatty acids that represented <1.0 % in all strains are omitted. —, Not detected.

Fatty acid	1	2	3	4	5	6
C _{14:0}	—	—	—	1.8	5.8	3.0
iso-C _{14:0}	7.6	7.9	3.1	3.1	5.5	2.5
anteiso-C _{15:0}	6.9	5.2	4.9	19.6	6.5	24.1
iso-C _{15:0}	40.1	49.0	44.7	32.0	17.2	30.9
C _{15:1ω6c}	1.7	—	1.4	—	—	—
C _{16:0}	1.6	3.3	1.2	5.6	11.5	20.0
C _{16:0} 10-methyl	—	—	—	—	1.6	—
iso-C _{16:0}	11.9	14.5	8.0	5.2	6.1	2.0
iso-C _{16:1} H	5.4	—	3.3	—	—	—
C _{17:0}	2.1	1.4	4.2	1.6	5.5	2.5
C _{17:0} 10-methyl	—	—	1.4	—	—	—
anteiso-C _{17:0}	1.7	3.1	1.6	7.2	2.0	4.2
iso-C _{17:0}	1.0	5.8	1.8	2.0	—	1.6
C _{17:1ω8c}	9.0	—	10.9	2.4	7.3	—
anteiso-C _{17:1ω9c}	1.6	—	1.5	2.3	—	—
iso-C _{17:1ω9c}	2.0	—	3.7	1.6	—	—
C _{18:0}	—	3.2	—	2.3	5.4	5.7
C _{18:1ω9c}	1.3	—	3.1	4.3	13.1	—
Summed feature 1*	—	—	1.2	—	—	—
Summed feature 3*	2.6	1.0	1.9	7.1	7.1	2.0

*Summed feature 1 comprises C_{13:0} 3-OH and/or iso-C_{15:1} H; summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1ω7c}.

Description of *Terrabacter aeriphilus* sp. nov.

Terrabacter aeriphilus (aer.i'phil.us. L. masc. n. *aer*, *aeris* air; Gr. adj. *philos* loving; N.L. masc. adj. *aeriphilus* air-loving).

Cells are strictly aerobic, Gram-, catalase- and oxidase-positive, non-motile rods or cocci (0.9 × 1.0–2.5 µm). Cells are white, convex and circular with entire margins. Grows at 5–35 °C (optimum, 30 °C), pH 4–9 (optimum pH 6–7) and in the range of 0–7 % (w/v) NaCl. Hydrolyses casein, starch, tyrosine and Tween 80 but does not hydrolyse chitin, CM-cellulose, DNA, hypoxanthine, pectin or xanthine. Positive for aesculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. Assimilates sucrose, sodium acetate, lactic acid, L-alanine, glycogen, melibiose, propionic acid, valeric acid, L-histidine, 3-hydroxybutyric acid and L-proline, but does not assimilate L-rhamnose, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, L-fucose, D-sorbitol, potassium 2-ketogluconate and 4-hydroxybenzoic acid (API ID 32GN test strip). Results of other assimilation and enzymic activity assays are shown in Table 2. Peptidoglycan containing LL-diaminopimelic acid, A3γ type has three glycine residues as the interpeptide bridge. Whole-cell sugars are glucose, mannose and ribose. Mycolic acids are absent. The predominant menaquinone is MK-8(H₄). The major fatty acids (>7 % of total fatty acids) are iso-C_{15:0}, iso-C_{16:0}, C_{17:1ω8c} and iso-C_{14:0}. The major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and an unidentified phosphoglycolipid.

Table 2. Differential characteristics between strain 5414T-18^T and related species

Strains: 1, strain 5414T-18^T; 2, *Terrabacter aerolatus* KACC 20556^T (data from Weon *et al.*, 2007); 3, *Terrabacter lapilli* LR-26^T (Lee *et al.*, 2008); 4, *Terrabacter terrae* CECT 3379^T (Weon *et al.*, 2007); 5, *Terrabacter tumescens* DSM 20308^T (Weon *et al.*, 2007); 6, *Terracoccus luteus* DSM 44267^T (data from this study, except where indicated). All are positive for aesculin hydrolysis, gelatin hydrolysis, β -galactosidase and esterase lipase (C8), and all assimilate D-glucose and maltose. All are negative for indole production, glucose fermentation, arginine dihydrolase, alkaline phosphatase, trypsin, α -chymotrypsin, β -glucuronidase, N-acetyl- β -glucosaminidase and α -fucosidase. All except for *Terrabacter lapilli* LR-26^T (no data available) are negative for urease. All except for *Terrabacter lapilli* LR-26^T (no data available) do not assimilate capric acid, trisodium citrate and phenylacetic acid. Ara, arabinose; Fuc, fucose; Gal, galactose; Glc, glucose; Rha, rhamnose; Rib, ribose; Man, mannose; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid; PGL, unknown phosphoglycolipid; APGL, unknown amino-containing phosphoglycolipid; +, positive; –, negative; (+), weakly positive; ND, not determined or no data available.

Characteristic	1	2	3	4	5	6
Catalase/oxidase	+ / +	+ / –	+ / –	+ / –*	+ / –*	+ / –*
Growth on 7% NaCl	+	–	–	+	–	ND
Nitrate reduction	–	+	+	–	+	–
Assimilation:						
L-Arabinose	–	–	–	+	+	+
D-Mannose	+	+	–	+	+	(+)
D-Mannitol	+	+	–	–	+	+
N-Acetylglucosamine	+	+	+	+	+	–
Potassium gluconate	+	+	+	+	+	–
Adipic acid	+	(+)	ND	–	(+)	–
Malic acid	+	+	–	+	+	–
Enzymic activity:						
Esterase (C4)	+	+	–	+	+	+
Lipase (C14)	–	+	–	–	–	–
Leucine arylamidase	+	+	ND	+	+	–
Valine arylamidase	+	+	+	–	–	–
Cystine arylamidase	+	–	+	–	–	–
Acid phosphatase	+	+	+	+	+	–
Naphthol-AS-BI-phosphohydrolase	+	+	–	+	+	+
α -Galactosidase	+	+	+	+	+	–
α -Glucosidase	+	+	ND	+	+	–
β -Glucosidase	–	(+)	–	–	–	–
α -Mannosidase	–	(+)	–	–	–	–
Whole-cell sugars†	Glc, Man, Rib	Glc, Rib, Rha, Xyl, Gal	Glc, Rha, Rib, Xyl, Ara	Fuc, Gal	ND	Rha, Rib, Gal, Glc, Man*
Polar lipids†	DPG, PE, PI, PG, PGL	DPG, PE, PI, PL	DPG, PE, PG, PI, PL	DPG, PE, PI, PGL	DPG, PE, PI, APGL	DPG, PG, PI, PE*
DNA G + C content (mol%)	73	71.7	72.6	71	73	73*

*Data for *Terrabacter terrae* CECT 3379^T are from Montero-Barrientos *et al.* (2005), for *Terrabacter tumescens* DSM 20308^T are from Collins *et al.* (1989) and for *Terracoccus luteus* DSM 44267^T are from Prauser *et al.* (1997).

†Data for *Terrabacter terrae* CECT 3379^T and *Terrabacter lapilli* LR-26^T are from Lee *et al.* (2008) and for *Terrabacter tumescens* DSM 20308^T are from Collins *et al.* (1989).

The type strain, 5414T-18^T (=KACC 20693^T=DSM 18563^T), was isolated from an air sample in Jeju, Korea. The DNA G + C content of the type strain is 73 mol%.

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