Phenylobacterium composti sp. nov., isolated from cotton waste compost in Korea

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A light-yellow-coloured bacterium, designated strain 4T-6^T, was isolated from cotton waste compost in Korea. Cells of the strain were strictly aerobic, Gram-negative, motile (by means of single polar flagella) and rod-shaped. Optimal growth occurred at 30 °C and at pH 7.0–8.0. The major fatty acids were 11-methyl C_{18:1}ω7c (23.8 %), C_{18:1}ω7c (23.2 %), C_{16:0} (19.8 %) and C_{17:0} (14.8 %). The DNA G+C content was 67.5 mol%. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain 4T-6^T was a member of the genus *Phenylobacterium*, showing the highest sequence similarities with those of '*Phenylobacterium zucineum*' HLK1 (98.8 %) and *Phenylobacterium lituiforme* DSM 14363^T (98.4 %). However, the values for DNA–DNA relatedness between strain 4T-6^T and '*P. zucineum*' HLK1 and *P. lituiforme* DSM 14363^T were 45 and 43 %, respectively. Phylogenetic characteristics, physiological properties and DNA–DNA hybridization data indicate that strain 4T-6^T represents a novel species of the genus *Phenylobacterium*, for which the name *Phenylobacterium composti* sp. nov. is proposed. The type strain is 4T-6^T (=KACC 12597^T=DSM 19425^T).

The genus *Phenylobacterium* was first proposed by Lingens *et al.* (1985) for several soil isolates. At that time, the genus *Phenylobacterium* was described as consisting of aerobic, Gram-negative, non-motile, xenobiotic-compound-degrading bacteria having various cell shapes. Later, Kanso & Patel (2004) emended the genus as comprising strict aerobes or facultative anaerobes and motile or non-motile bacteria. Phylogenetically, members of this genus formed a monophyletic group within the order *Caulobacterales*, class *Alphaproteobacteria*. At the time of writing, this genus comprises four recognized species: *Phenylobacterium immobile* (Lingens *et al.*, 1985), *Phenylobacterium lituiforme* (Kanso & Patel, 2004), *Phenylobacterium falsum* (Tiago *et al.*, 2005) and *Phenylobacterium koreense* (Aslam *et al.*, 2005); and '*Phenylobacterium zucineum*' (Zhang *et al.*, 2007).

During a study on the bacterial diversity of cotton waste composts used for the cultivation of the oyster mushroom (*Pleurotus ostreatus*), we isolated a strain designated 4T-6^T. Strain 4T-6^T was isolated by means of the plating technique with trypticase soy agar (TSA; Difco) at 30 °C.

The methods described by Smibert & Krieg (1994) were used to test the following: Gram staining, catalase activity, oxidase activity and hydrolysis of casein, DNA, starch and Tween 80. Hydrolysis of carboxymethylcellulose (0.1 %, w/v), chitin (1 %, w/v), hypoxanthine (0.5 %, w/v), pectin (0.5 %, w/v), tyrosine (0.5 %, w/v) and xanthine (0.5 %, w/v) was also tested. Bacterial cells were observed under phase-contrast microscopy (AXIO; Zeiss). Transmission electron microscopy (model 912AB; LEO) observation was performed with cells grown for 2 days at 30 °C on R2A (Difco). Anaerobic growth was assessed on R2A incubated in a GasPak anaerobic system (BBL). Growth tests with L-phenylalanine (1.0 g l⁻¹), chloridazon (1.0 g l⁻¹) and antipyrin (1.0 g l⁻¹) as sole carbon sources were determined in minimal medium B (Lingens *et al.*, 1985) with vitamin B₁₂ (0.03 mg l⁻¹) at 30 °C. Other biochemical and physiological tests were performed using the commercial systems API 20NE, API ID 32GN and API ZYM according to the instructions of the manufacturer (bioMérieux). To determine the growth temperature, the strain was cultivated on R2A at 5, 10, 20, 30, 37, 40, 45 and 50 °C. NaCl tolerance was studied using R2A broth supplemented with different concentrations of NaCl. Growth at pH 4.0–10.0 was evaluated in R2A broth. To test antimicrobial susceptibility, discs containing the following

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 4T-6^T is EU022524.

antibiotics were used: amikacin (30 µg), ampicillin (10 µg), cephalothin (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), erythromycin (15 µg), fusidic acid (10 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), lincomycin (15 µg), meticillin (5 µg), nalidixic acid (30 µg), neomycin (30 µg), netilmicin (30 µg), penicillin (10 U), polymyxin B (300 U), rifampicin (5 µg), streptomycin (10 µg), tetracycline (30 µg) and vancomycin (30 µg).

Strain 4T-6^T grew on R2A, TSA and nutrient agar (Difco). However, no growth was observed on MacConkey agar (Difco). The cells were aerobic, Gram-negative, motile (by means of single polar flagella) rods 0.5 µm in width and 0.8–1.8 µm in length. Colonies were light yellow in colour and irregular in shape on R2A. Phenotypically, strain 4T-6^T and *P. lituiforme* DSM 14363^T were similar and could be differentiated from other members of the genus *Phenylobacterium* on the basis of the yellow colour of colonies, the presence of single polar flagella and the lack of oxidase activity and nitrate reduction. However, strain 4T-6^T could be distinguished from *P. lituiforme* DSM 14363^T in that the former was strictly aerobic, did not hydrolyse gelatin and utilized different substrates (including anti-

pyrin and chloridazon). Strain 4T-6^T could be also differentiated from the close phylogenetic relative '*P. zucineum*' HLK1 on the basis of the former's yellow pigmentation, absence of oxidase activity and nitrate reduction, hydrolysis of aesculin and gelatin and assimilation of sodium acetate, glycogen and lactic acid (Table 1).

The DNA G + C content of the isolate was determined with an HPLC method, as described by Mesbah *et al.* (1989), by using a reversed-phase column (Supelcosil LC-18 S; Supelco). The cellular fatty acid profile was determined by using the standard protocol of the Microbial Identification System (MIDI; Microbial ID) with cells that had been grown on R2A for 3 days at 30 °C. The predominant fatty acids of strain 4T-6^T were 11-methyl C_{18:1ω7c} (23.8%), C_{18:1ω7c} (23.2%), C_{16:0} (19.8%) and C_{17:0} (14.8%) (Table 2). The fatty acid composition of strain 4T-6^T was unique in having such a large proportion (23.8%) of 11-methyl C_{18:1ω7c}. The DNA G + C content was 67.5 mol%.

The 16S rRNA gene sequence of strain 4T-6^T was amplified by means of a PCR with conserved primers, as described by Kwon *et al.* (2003), and direct sequencing (Hiraishi, 1992). Multiple alignments performed with sequences of isolate 4T-6^T and those of members of the genera *Phenylo-*

Table 1. Differential phenotypic characteristics of strain 4T-6^T and recognized *Phenylobacterium* species

Strains: 1, 4T-6^T (*P. composti* sp. nov.); 2, *P. falsum* DSM 18556^T (data from Tiago *et al.*, 2005 and this study); 3, *P. immobile* DSM 1986^T (Lingens *et al.*, 1985 and this study); 4, *P. lituiforme* DSM 14363^T (Kanso & Patel, 2004 and this study); 5, *P. koreense* KACC 11507^T (Aslam *et al.*, 2005 and this study); 6, '*P. zucineum*' HLK1 (Zhang *et al.*, 2007 and this study). For all strains, nitrate reduction, urease, hydrolysis of aesculin and gelatin and utilization tests (with the exception of antipyrin, chloridazon and L-phenylalanine) were conducted using API 20NE and API ID 32GN in this study. All strains are negative for indole production, glucose fermentation, arginine dihydrolase and urease. +, Positive; (+), weakly positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6
Source	Compost	Groundwater	Soil	Water	Sludge	Human cell line
Pigmentation	Light yellow	Colourless	Colourless	Yellow to pale yellow	Colourless	Grey
Anaerobic growth	–	–	–	+	–	–
Motility	+	–	–	+	–	+
Oxidase	–	+	(+)	–	+	+
Nitrate reduction	–	+	–	–	+	+
Hydrolysis of:						
Aesculin	–	–	–	–	–	+
Gelatin	–	–	–	+	–	(+)
Utilization of:						
Sodium acetate	+	–	–	–	+	–
N-Acetylglucosamine	–	–	–	+	–	–
Adipic acid	+	+	–	+	+	+
Antipyrin	(+)	–	+	–*	–	ND
Chloridazon	(+)	ND	+	–*	–	ND
Potassium gluconate	+	+	–	+	–	+
Glycogen	+	–	–	–	–	–
Lactic acid	+	–	–	–	+	–
Maltose	–	–	–	+	–	–
L-Phenylalanine	(+)	–	+	+	+	+
DNA G + C content (mol%)	67.5	66.9	65.5	66.5 ± 0.5	68.1	71.2 ± 0.2

*Data from Aslam *et al.* (2005).

Table 2. Cellular fatty acid compositions (%) of strain 4T-6^T and related strains

Strains: 1, 4T-6^T (*P. composti* sp. nov.); 2, *P. falsum* DSM 18556^T; 3, *P. immobile* DSM 1986^T; 4, *P. lituiforme* DSM 14363^T; 5, *P. koreense* KACC 11507^T; 6, '*P. zucineum*' HLK1. Cells were harvested after growth on R2A for 3 days. –, Less than 1 % or not detected.

Fatty acid	1	2	3	4	5	6
C _{12:0}	2.2	3.1	3.0	1.6	2.5	8.3
C _{12:0} 3-OH	1.8	1.1	1.0	–	1.0	6.1
C _{12:1} 3-OH	–	2.9	2.5	1.0	2.3	4.8
C _{16:0}	19.8	8.6	16.5	13.4	18.3	9.9
C _{16:1} ω11 <i>c</i>	–	2.7	–	1.6	1.9	–
C _{17:0}	14.8	8.7	12.5	4.6	10.2	1.4
iso-C _{17:0}	–	2.8	–	–	–	–
C _{17:1} ω6 <i>c</i>	3.0	5.4	3.7	2.1	3.7	1.6
C _{17:1} ω8 <i>c</i>	2.1	3.9	2.9	1.4	2.3	–
C _{18:0}	1.8	–	1.9	1.0	–	–
C _{18:1} 2-OH	2.5	–	–	5.4	3.2	–
C _{18:1} ω7 <i>c</i>	23.2	55.7	43.6	58.3	30.3	59.6
11-Methyl C _{18:1} ω7 <i>c</i>	23.8	5.1	1.7	5.7	16.1	–
C _{19:0} ω8 <i>c</i> cyclo	1.3	–	4.6	–	–	1.2
C _{20:0}	–	–	–	–	–	5.2
Summed feature 3*	–	–	1.7	–	4.2	2.1

*Summed feature 3 comprises C_{16:1}ω7*c* and/or iso-C_{15:0} 2-OH.

bacterium, *Caulobacter* and other related species were carried out with the CLUSTAL W program (Thompson *et al.*, 1994). The evolutionary tree for the datasets was inferred with the neighbour-joining and maximum-parsimony methods by using MEGA, version 3.1 (Kumar *et al.*, 2004). The stability of relationships was assessed by performing bootstrap analyses based on 1000 resamplings. DNA–DNA hybridization was carried out using the filter hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted using the non-radioactive DIG-High Prime system (Roche) and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).

Strain 4T-6^T showed sequence similarities of 94.9–98.4 % with respect to the type strains of the four recognized species of the genus *Phenylobacterium*, *P. lituiforme* DSM 14363^T (98.4 %), *P. koreense* KACC 11507^T (95.5 %), *P. falsum* DSM 18556^T (95.3 %) and *P. immobile* DSM 1986^T (94.9 %). The sequence similarity with '*P. zucineum*' HLK1 was 98.8 %. Strain 4T-6^T showed less than 95 % 16S rRNA gene sequence similarity with respect to all of the other species of the genera included in this analysis. In the neighbour-joining phylogenetic tree (Fig. 1), strain 4T-6^T formed a robust cluster with the members of the genus *Phenylobacterium*, with 99 % bootstrap support. The maximum-parsimony tree also supported the clear affiliation of strain 4T-6^T with the genus *Phenylobacterium*. The values for DNA–DNA relatedness between strain 4T-6^T and '*P. zucineum*' HLK1 and *P. lituiforme* DSM 14363^T were 45 and 43 %, respectively.

Therefore, the physiological, biochemical and phylogenetic properties of strain 4T-6^T support its description as representing a novel species within the genus *Phenylobacterium*, for which the name *Phenylobacterium composti* sp. nov. is proposed.

Description of *Phenylobacterium composti* sp. nov.

Phenylobacterium composti (com.pos'ti. N.L. gen. n. *composti* of compost).

Cells are motile (with single polar flagella), Gram-negative, rod-shaped bacteria (0.5 × 0.8–1.8 μm). Colonies on R2A are light yellow and irregular. Growth occurs at 15–45 °C (optimally at 30 °C) and at pH 6.0–9.0 (optimally at pH 7.0–8.0). Optimum NaCl concentration is 0–1 % (w/v); no growth occurs at ≥3 % NaCl. Catalase-positive and oxidase-negative. Nitrate is not reduced and glucose is not fermented. Hydrolyses Tween 80 and tyrosine, but not casein, chitin, carboxymethylcellulose, DNA, hypoxanthine, pectin, starch or xanthine. Negative for indole production, arginine dihydrolase, urease, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase (API 20NE). Weakly utilizes antipyrin, chloridazon and L-phenylalanine. Utilizes potassium gluconate, adipic acid, suberic

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain 4T-6^T and closely related species. Asterisks indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bootstrap values below 50 % are not shown. Bar, 0.01 changes per nucleotide position.

acid, sodium acetate, lactic acid, glycogen, valeric acid, 3-hydroxybutyric acid and L-proline, but not D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, capric acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, sucrose, itaconic acid, sodium malonate, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, melibiose, L-fucose, D-sorbitol, propionic acid, L-histidine, potassium 2-ketogluconate or 4-hydroxybenzoic acid (API 20NE and API ID 32GN). Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase, but negative for lipase (C14), α -chymotrypsin, α -galactosidase, β -glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase (API ZYM). Resistant to fusidic acid, lincomycin, meticillin and nalidixic acid. Sensitive to amikacin, ampicillin, cephalothin, cefotaxime, ciprofloxacin, chloramphenicol, erythromycin, gentamicin, imipenem, kanamycin, neomycin, netilmicin, penicillin, polymyxin B, rifampicin, streptomycin, tetracycline and vancomycin. Major cellular fatty acids are 11-methyl $C_{18:1\omega 7C}$, $C_{18:1\omega 7C}$, $C_{16:0}$ and $C_{17:0}$. The DNA G+C content of the type strain is 67.5 mol% (HPLC).

The type strain, 4T-6^T (=KACC 12597^T=DSM 19425^T), was isolated from cotton waste compost, in Korea.

Acknowledgements

This study was supported by the National Institute of Agricultural Biotechnology (grant no. 06-4-11-19-1), Rural Development Administration, Republic of Korea.

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