Chitinophaga terrae sp. nov., isolated from soil

Myung Kyum Kim^{1,2} and Hee-Young Jung¹

Correspondence
Hee-Young Jung
heeyoung@knu.ac.kr

¹Division of Applied Biology and Chemistry, College of Agriculture and Life Sciences, Kyungpook National University, Daegu, South Korea

²Department of Oriental Medicinal Material and Processing, College of Life Sciences, Kyung Hee University, Kyunggi-do, South Korea

A novel strain, designated KP01^T, belonging to the class *Sphingobacteria* (phylum *Bacteroidetes*) was isolated from soil in South Korea and was characterized taxonomically using a polyphasic approach. The strain was found to comprise Gram-negative, aerobic, non-motile, non-spore-forming rods. A phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain belonged to the genus *Chitinophaga* but was clearly separated from established *Chitinophaga* species. The 16S rRNA gene sequence similarities between KP01^T and type strains of established *Chitinophaga* species ranged from 90.3 to 95.7 %. Phenotypic and chemotaxonomic data (major menaquinone, MK-7; major fatty acids, iso-C_{15:0} and C_{16:1}ω5c; major hydroxy fatty acid, C_{17:0} iso 3-OH) supported the affiliation of strain KP01^T with the genus *Chitinophaga*. Therefore strain KP01^T represents a novel species, for which the name *Chitinophaga terrae* sp. nov. is proposed. The type strain is KP01^T (=KCTC 12836^T =LMG 24015^T).

The genus *Chitinophaga*, the type species of which is *Chitinophaga pinensis*, was first described by Sangkhobol & Skerman (1981) as including strains of filamentous, chitinolytic, gliding bacteria that transform, upon ageing, into spherical bodies. Recently, Kämpfer *et al.* (2006) proposed five more *Chitinophaga* species by reclassifying [*Cytophaga*] *arvensicola*, [*Flexibacter*] *filiformis*, [*Flexibacter*] *japonensis* and [*Flexibacter*] *sancti* into the genus *Chitinophaga*, and by proposing *Chitinophaga skermanii*. At the time of writing, there are six *Chitinophaga* species with validly published names.

Strain KP01^T was originally isolated from soil from a field in Daejeon in South Korea. This soil sample was thoroughly suspended with 50 mM phosphate buffer (pH 7.0) and the suspension was spread on one-tenth-strength R2A agar (Difco) plates after serial dilution with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30 °C for 1 week. Single colonies were purified by transferring them onto new plates and were incubated once again, on full-strength R2A or Luria–Bertani (LB) agar (Difco) plates. Purified colonies were tentatively identified on the basis of partial sequences of the 16S rRNA gene (Im *et al.*, 2005). They were routinely cultured on LB agar at 30 °C and maintained as a glycerol suspension (20 %, w/v) at –70 °C.

A Gram reaction was performed using the non-staining method, as described by Buck (1982). Cell morphology was

observed under a Nikon light microscope at ×1000, with cells grown for 3 days at 30 °C on LB agar. Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂, and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Carbon-source utilization and enzyme activities were tested by using API 20NE, API ID 32 GN and API ZYM test kits (bioMérieux). Growth at different temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and various pH values (pH 5.0–11.0 at intervals of 0.5 pH units) was assessed after 5 days incubation. Growth on nutrient agar and trypticase soy agar (Difco) was also evaluated at 30 °C.

Extraction of genomic DNA was performed with a commercial genomic DNA-extraction kit (Solgent). PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with the CLUSTAL_X program (Thompson *et al.*, 1997), gaps were edited in the BioEdit program (Hall, 1999) and evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987) with the MEGA3 program (Kumar *et al.*, 2004); bootstrap percentages were based on 1000 replications (Felsenstein, 1985).

For the measurement of the G + C content of the chromosomal DNA, genomic DNA of the novel strain was

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain KP01^T is AB278570.

Table 1. Phenotypic characteristics of strain KP01^T and type strains of related *Chitinophaga* species

Strains: 1, KP01^T (data from this study); 2, *C. arvensicola* IAM 12650^T; 3, *C. filiformis* NBRC 15056^T; 4, *C. japonensis* NBRC 16041^T; 5, *C. pinensis* ACM 2034^T; 6, *C. sancti* NBRC 15057^T; 7, *C. skermanii* CC-SG1B^T. Data for reference strains are from Kämpfer *et al.* (2006). All of the strains were Gram-negative and isolated from soil. All utilize maltose and D-mannose. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Gliding motility	–	–	+	+	+	+	–
Cell length (µm)	0.6–0.8	0.6–4	30–80	2–18	<40	2–15	1–2
Colony colour	Yellow	Yellow–orange	Golden yellow	Yellow–orange	Yellow	Golden yellow	Yellow
Oxidase	+	+	+	+	+	ND	+
Catalase	+	+	–	+	+	–	+
Growth at 37 °C	+	–	+	+	+	–	+
Chitinase	–	–	+	–	+	–	ND
Gelatin hydrolysis	–	–	+	+	+	+	+
Urease	–	–	ND	ND	+	ND	–
Assimilation of:							
Gluconate	–	–	–	–	–	+	–
L-Arabinose	+	+	–	+	+	+	–
D-Melibiose	+	+	+	+	–	+	+
L-Rhamnose	+	+	–	+	+	–	–
D-Ribose	–	+	–	–	–	–	–
Sucrose	+	+	+	+	+	–	–
Inositol	–	–	–	–	–	–	–
N-Acetylglucosamine	+	+	–	+	+	–	+
Salicin	+	+	+	+	+	–	+
DNA G + C content (mol%)	46.3	46.0	45.0	49.8	45.2	43.3	40.7

extracted and purified as described by Moore & Dowhan (1995) and enzymically degraded into nucleosides and the G + C content was determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane–quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and subsequently analysed by HPLC as described previously (Hiraishi *et al.*, 1996). Cellular fatty acid profiles were determined for strains grown on trypticase soy agar at 30 °C for 2 days. The cellular fatty acids were saponified,

methyated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed by gas chromatography (6890; Hewlett Packard) and identified using the Microbial Identification software package (Sasser, 1990).

Strain KP01^T was found to comprise Gram-negative, aerobic, non-motile, non-spore-forming rods. Colonies grown on LB agar plates for 3 days were smooth, circular and yellowish. The strain grew well on nutrient agar, trypticase soy agar and LB agar. The physiological characteristics of strain KP01^T are summarized in the

Fig. 1. Phylogenetic tree, constructed from a comparative analysis of 16S rRNA gene sequences, showing the relationships of strain KP01^T with related species. This tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with Kimura's two-parameter distance matrix (Kimura, 1983) and pairwise deletion. Bootstrap percentages are based on 1000 replications. Bar, 0.02 substitutions per nucleotide position.

species description, and differential characteristics are compared with those of *Chitinophaga* species in Table 1.

An almost-complete 16S rRNA gene sequence (1478 bp) for strain KP01^T was determined and subjected to a comparative analysis. A phylogenetic analysis based on 16S rRNA gene sequences indicated that strain KP01^T belongs to the genus *Chitinophaga* (Fig. 1), but the 16S rRNA gene sequence similarity with respect to the most closely related *Chitinophaga* species was 95.7 %, suggesting that it is different at the species level (Stackebrandt & Goebel, 1994). Strain KP01^T showed the highest level of 16S rRNA gene sequence similarity with respect to *C. arvensicola* IAM 12650^T (95.7 %), followed by *Chitinophaga ginsengisegetis* M1-09^T (95.6 %), *Chitinophaga japonensis* IFO 16041^T (93.3 %), *Chitinophaga sancti* IFO 15057^T (92.7 %), *Chitinophaga filiformis* IFO 15056^T (91.9 %), *Chitinophaga ginsengisoli* Gsoil 052^T (91.9 %), *C. skermanii* SG-1B^T (91.7 %), *C. pinensis* ACM 2034^T (90.3 %) and *Terrimonas lutea* 14990Y^T (90.4 %). These values (<97 %) were low enough, according to Stackebrandt & Goebel (1994), to justify the placement of strain KP01^T as a novel species within the genus *Chitinophaga*.

The DNA G+C content of strain KP01^T was 46.3 mol%. The major respiratory quinone was MK-7, as is the case for the known species of the genus *Chitinophaga* (Kämpfer *et al.*, 2006). The major fatty acids of the strain were C_{15:0} iso, C_{16:1}ω5c and hydroxy fatty acid C_{17:0} iso 3-OH, as shown in Table 2; this profile is consistent with those of known *Chitinophaga* species (Kämpfer *et al.*, 2006). Minor amounts of saturated fatty acids C_{14:0} (0.9 %), C_{15:0} iso 3-OH (3.4 %), C_{16:0} 3-OH (3.0 %), C_{17:0} iso (1.0 %) and summed features 3 (C_{16:1}ω7c/C_{15:0} iso 2-OH) (3.0 %) and 4 (C_{17:1} iso I /C_{17:1} anteiso B) (1.0 %) were present.

On the basis of the data and observations described above, strain KP01^T should be assigned to the genus *Chitinophaga* as the type strain of a novel species, for which the name *Chitinophaga terrae* sp. nov. is proposed.

Description of *Chitinophaga terrae* sp. nov.

Chitinophaga terrae (ter'rae. L. gen. n. *terrae* of the earth).

Cells are Gram-negative, aerobic, non-motile rods that are 0.3–0.5 µm in width and 0.6–0.8 µm in length after 3 days culture on R2A agar. Colonies grown on LB agar for 3 days are smooth, circular, convex and yellowish. Grows at 15–42 °C and at pH 6.0–9.0, but grows slowly at 42 °C and pH 9.0. Can reduce nitrate to nitrite but not to nitrogen gas. Enzyme activities, substrate assimilation and other physiological characteristics are indicated in Table 1. Produces *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase (C8), α-fucosidase, α-galactosidase, α-glucosidase, β-galactosidase, β-glucosidase, leucine arylamidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase. Does not produce arginine dihydrolase, β-glucuronidase, lipase

Table 2. Cellular fatty acid profiles of strain KP01^T and type strains of related *Chitinophaga* species

Strains: 1, KP01^T (data from this study); 2, *C. arvensicola* DSM 3695^T; 3, *C. filiformis* CCUG 12809^T; 4, *C. japonensis* DSM 13484^T; 5, *C. pinensis* DSM 2588^T; 6, *C. sancti* DSM 784^T; 7, *C. skermanii* CC-SG1B^T. Data for reference strains are from Kämpfer *et al.* (2006). Major components are in bold.

Fatty acid	1	2	3	4	5	6	7
C _{14:0}	0.9	1.4	0.9	0.5	0.7	0.7	1.8
C _{15:0}		0.4			0.4		0.4
C _{15:0} 2-OH		0.3					
C _{15:0} anteiso		0.6		0.5			
C _{15:0} iso	42.2	35.3	37.3	40.0	30.4	44.0	47.3
C _{15:0} iso 3-OH	3.4	3.0	3.3	2.4	3.1	3.4	2.6
C _{16:0}	3.2	5.1	4.3	3.0	4.2	4.2	3.5
C _{16:0} 10-methyl		0.4		0.9			
C _{16:0} 2-OH			1.1	3.0	0.7	0.7	
C _{16:0} 3-OH	3.0	1.7	1.2	0.8	1.2	0.5	1.6
C _{16:0} iso		0.3					
C _{16:0} iso 3-OH		0.5		0.4	0.4		0.4
C _{16:1} ω11c		0.5		1.0	1.9	0.9	0.4
C _{16:1} ω5c	25.0	33.6	25.5	22.2	33.2	13.5	24.4
C _{17:0} 2-OH		0.4		0.4			
C _{17:0} anteiso				0.4			
C _{17:0} iso	1.0	0.4		1.8	0.4	0.6	0.6
C _{17:0} iso 3-OH	12.3	5.9	11.8	13.0	11.5	9.1	5.0
C _{17:1} iso ω9c		0.3				1.1	
C _{18:0}							
C _{18:3} ω6c							
Summed features*							
2		0.4		0.5			0.4
3	3.0	3.7	11.2	3.3	7.7	16.0	3.5
4	1.0			1.3		0.8	0.4
Unknown fatty acids†							
ECL 11.543		0.5					0.8
ECL 13.565	3.2	3.6	2.5	3.1	2.6	3.2	4.4
ECL 14.959		0.3			0.3		0.4
ECL 16.582	1.1	0.8	0.9	1.1	1.1	1.3	0.7

*Summed feature 2 contains one or more of C_{12:0} aldehyde, C_{14:0} 3-OH and C_{16:1} iso; summed feature 3 contains C_{16:1}ω7c and/or C_{15:0} iso 2-OH; summed feature 4 contains C_{17:1} iso I and/or C_{17:1} anteiso B.

†ECL, Equivalent chain length.

(C14), protease (gelatin hydrolysis) or urease. Assimilates L-arabinose, L-fucose, D-glucose, maltose, D-mannose, D-melibiose, L-rhamnose, sucrose, *N*-acetyl-D-glucosamine and salicin. Does not assimilate acetate, adipate, caprate, citrate, gluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, itaconate, 2-ketogluconate, 5-keto-gluconate, lactate, L-malate, malonate, phenylacetate, propionate, suberate, *n*-valerate, D-ribose, *myo*-inositol, D-mannitol, D-sorbitol, L-alanine, L-histidine, L-proline, L-serine or glycogen. MK-7 is the predominant menaquinone and C_{15:0} iso, C_{16:1}ω5c and C_{17:0} iso 3-OH are the

predominant cellular fatty acids. The G + C content of the genomic DNA of the type strain is 46.3 mol%.

The type strain, KP01^T (=KCTC 12836^T=LMG 24015^T), was isolated from soil from a field near Daejeon, South Korea.

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