

Rudaea cellulositytica gen. nov., sp. nov., isolated from soil

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A yellow-pigmented, Gram-negative, aerobic, rod-shaped bacterium, strain KIS3-4^T, was isolated from soil collected on Daechung Island in the West Sea of Korea. Phylogenetic analysis based on the 16S rRNA gene sequence placed strain KIS3-4^T in a distinct lineage in the family *Xanthomonadaceae*. Strain KIS3-4^T shared 87.3–93.7% sequence similarity with members of the family *Xanthomonadaceae*, and was related most closely to the genera *Dyella* and *Dokdonella*. In its biochemical characteristics, strain KIS3-4^T was clearly separable from other genera within the family *Xanthomonadaceae* on the basis of the hydrolysis of cellulose and urea, high G + C content (64 mol%) and fatty acid profile. Major fatty acids (>10% of the total fatty acids) were iso-C_{17:1ω9c} (32.8%), iso-C_{17:0} (18.0%) and iso-C_{16:0} (12.7%). Q-8 was the predominant respiratory quinone. Phosphatidylethanolamine and several unidentified aminophospholipids and phospholipids were present. Based on its unique phenotypic, genotypic and phylogenetic features, strain KIS3-4^T represents a novel genus and species, for which the name *Rudaea cellulositytica* gen. nov., sp. nov. is proposed. The type strain of *Rudaea cellulositytica* is KIS3-4^T (=KACC 12734^T =JCM 15422^T).

The family *Xanthomonadaceae* consists of Gram-negative, rod-shaped bacteria. Its members are characterized by positive catalase activity, negative nitrate reduction (with the exception of *Stenotrophomonas*) and variable oxidase activity. Chemotaxonomically, they have complex fatty acid profiles that include branched-chain and/or hydroxyl fatty acids, and contain ubiquinone Q-8 as the predominant isoprenoid quinone. According to Saddler & Bradbury (2005), the members of the family *Xanthomonadaceae* were *Xanthomonas*, *Frateuria*, *Fulvimonas*, *Luteimonas*, *Lysobacter*, *Nevskia*, *Pseudoxanthomonas*, *Rhodanobacter*, *Schineria*, *Stenotrophomonas*, *Thermomonas* and *Xylella*. Since then, the genera *Aquimonas* (Saha *et al.*, 2005), *Aspromonas* (Jin *et al.*, 2007), *Dokdonella* (Yoon *et al.*, 2006), *Dyella* (Xie & Yokota, 2005), *Luteibacter*

(Johansen *et al.*, 2005) and *Silanimonas* (Lee *et al.*, 2005) have been added to the family.

A soil sample was collected from Daechung Island in the West Sea, Republic of Korea. Soil (1 g) was suspended in 9 ml of 0.85% NaCl (w/v) and serially diluted solutions were plated on R2A agar (Reasoner & Geldreich, 1985). A strain, designated KIS3-4^T, was isolated after incubation at 30 °C for 4 days.

Phenotypic characteristics, including Gram-staining, catalase and oxidase activity and hydrolysis of CM-cellulose, casein, chitin, DNA, hypoxanthine, tyrosine, Tween 80, starch and xanthine, were determined using the methods of Smibert & Krieg (1994). The pH range (pH 4–10 at intervals of 1 pH unit) for growth was determined on R2A agar that was buffered with citrate/phosphate buffer or Tris/HCl (Breznak & Costilow, 1994). Growth with 1, 2, 3 and 5% NaCl (w/v), and at various temperatures (5–50 °C at intervals of 5 °C) was investigated in R2A broth. Tests with the API 20NE, API 20E, API ID 32GN and API ZYM systems (bioMérieux) were generally performed according to the manufacturer's instructions. The API ZYM test strip

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KIS3-4^T is EU741687.

Images of cells of strain KIS3-4^T and fatty acid composition by 2-D TLC and a table of fatty acid comparisons are available as supplementary material with the online version of this paper.

was read after 4 h incubation at 30 °C, whilst the other API strips were examined after 7 days at 30 °C. Cell morphology was observed by transmission electron microscopy (912AB; LEO) and phase-contrast microscopy (Axio Imager.A1; Zeiss) by using cells grown on R2A agar.

Polar lipids were analysed according to Minnikin *et al.* (1984). Fatty acid methyl esters were extracted and prepared by using the standard protocol of the Microbial Identification System (MIDI) after cells were grown on R2A agar for 2 days at 30 °C. Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). The DNA G + C content was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18-S; Supelco).

The 16S rRNA gene sequence was determined by PCR amplification (Kwon *et al.*, 2003) and direct sequencing (Hiraishi, 1992). A partial 16S rRNA gene sequence (1384

nt) was determined for strain KIS3-4^T. CLUSTAL W version 1.8 (Thompson *et al.*, 1994) was used to align the sequence of strain KIS3-4^T with corresponding sequences from related taxa retrieved from public databases. Phylogenetic analysis was performed using MEGA version 3.1 (Kumar *et al.*, 2004) with the neighbour-joining and maximum-parsimony methods. Bootstrap values were calculated from 1000 replications.

Strain KIS3-4^T grew on R2A agar but not on nutrient agar, trypticase soy agar or MacConkey agar (all from Difco). Cells of strain KIS3-4^T were aerobic, Gram-negative, motile with peritrichous flagella, rod-shaped (0.5 µm wide and 1.0–3.0 µm long) (see Supplementary Fig. S1, available in IJSEM Online). The phenotypic characteristics that differentiate strain KIS3-4^T from related genera are listed in Table 1.

Polar lipids of strain KIS3-4^T included phosphatidylethanolamine and several unidentified aminophospholipids

Table 1. Characteristics of strain KIS3-4^T and related members of the family *Xanthomonadaceae*

Taxa: 1, *Rudaea cellulositytica* gen. nov., sp. nov. KIS3-4^T; 2, *Aquimonas* (data from Saha *et al.*, 2005); 3, *Dokdonella* (Cunha *et al.*, 2006; Yoon *et al.*, 2006); 4, *Dyella* (An *et al.*, 2005; Kim *et al.*, 2006; Xie & Yokota, 2005); 5, *Frateriuria* (An *et al.*, 2005; Kim *et al.*, 2006; Saddler & Bradbury, 2005; Yoon *et al.*, 2006); 6, *Fulvimonas* (Mergaert *et al.*, 2002; Yoon *et al.*, 2006); 7, *Luteibacter* (Johansen *et al.*, 2005); 8, *Rhodanobacter* (De Clercq *et al.*, 2006; Im *et al.*, 2004; Lee *et al.*, 2007; Nalin *et al.*, 1999; Weon *et al.*, 2007). +, Positive; w, weak; v, variable among species or strains; –, negative; ND, no data available; i-, iso-branched. Data in parentheses are for the type strain of the type species.

Characteristic	1	2	3	4	5	6	7	8
Colony colour	Yellow	Yellowish brown	Yellow or greenish yellow	Yellow	Gold–yellow	Deep yellow	Yellow	Yellow
Motility	+	+	v	v	v (+)	+	+	v
Catalase	+	+	v	+ or w	+	+	+	+
Oxidase	+	+	+ or w	v	–	+	+	+
Acid production from glucose	–	–	–	+	+	–	–	–
Nitrate reduction	–	–	v	v	–	–	–	–*
H ₂ S production	–	–	–	–	+	ND	–	ND
Growth at pH 4.5	–	–	–	–	+	(+)	–	v
Growth at 4 % NaCl	–	–	–	v	(–)	ND	–	–
Arginine dihydrolase	+	ND	–	–	–	–	–	–
Hydrolysis of:								
Aesculin	+	–	–	v	(–)	+	+	v
Casein	–	+	+	v	(–)	(+)	+	v
Cellulose	+	ND	ND	v	–	ND	ND	–
Gelatin	+	+	+	v	–	–	+	v
Starch	–	+	–	–	(–)	+	+	–
Tween 80	+	+	+	ND	ND	ND	ND	+
Urea	+	–	–	–	(–)	–	ND	–
Major fatty acids (>10 % of total)	i-C _{17:1ω9c} , i-C _{17:0} , i-C _{16:0}	i-C _{15:0} , i-C _{17:1ω9c} , i-C _{16:0}	i-C _{17:1ω9c} , i-C _{17:0} , i-C _{15:0}	i-C _{17:1ω9c} , i-C _{16:0} , i-C _{15:0}	i-C _{15:0} , summed feature 3	i-C _{15:0} , i-C _{17:0} , i-C _{16:0} , i-C _{17:1ω9c}	i-C _{17:1ω9c} , i-C _{15:0} , i-C _{17:0}	i-C _{16:0} , i-C _{17:1ω9c}
DNA G + C content (mol%)	64	75	68.3–71	63–64	62–64	71.5–71.9	64.3	61.0–67.6

*Positive for *Rhodanobacter thiooxydans* (Lee *et al.*, 2007).

(APLs) and phospholipids (PLs) (Supplementary Fig. S2a). Despite the similarities of the major components, strain KIS3-4^T could be distinguished from *Dokdonella koreensis* DSM 17203^T (Supplementary Fig. S2b) by the presence of APL1, PL1 and PL2, and the absence of APL2, PL4 and an unidentified polar lipid. The predominant fatty acids (>10% of total fatty acids) of strain KIS3-4^T were iso-C_{17:1}ω₉c (32.8%), iso-C_{17:0} (18.0%) and iso-C_{16:0} (12.7%) (Supplementary Table S1). The major quinone in strain KIS3-4^T was ubiquinone 8 (Q-8), which is also found in all the members of the family *Xanthomonadaceae*. The G+C content of the DNA was 64 mol%.

It is evident from the phylogenetic tree (Fig. 1) that strain KIS3-4^T belongs to a distinct lineage in the family *Xanthomonadaceae*. The strain formed a monophyletic clade with the genera *Aquimonas*, *Dokdonella*, *Dyella*, *Frateriia*, *Fulvimonas*, *Luteibacter* and *Rhodanobacter* with 95% bootstrap support, which was confirmed by the maximum-parsimony tree. Strain KIS3-4^T revealed highest sequence similarities, with the exception of uncultured environmental clones (Costello & Schmidt, 2006; Lozada *et al.*, 2006), with *Dyella* species (93.1–93.7%).

Strain KIS3-4^T can be differentiated from closely related genera by the presence of arginine dihydrolase, the hydrolysis of cellulose and urea, the polar lipid pattern and the major fatty acid composition (Table 1, Supplementary Table S1 and Supplementary Fig. S2). Moreover, the similarity level (<94%) of the 16S rRNA

gene sequence of strain KIS3-4^T with closely related genera within the family *Xanthomonadaceae* is much lower than 95%, a value that has been proposed as a 'practicable border zone for genus definition' (Ludwig *et al.*, 1998).

On the basis of these results, it is proposed that strain KIS3-4^T represents a novel species in a new genus, *Rudaea cellulosilytica* gen. nov., sp. nov.

Description of *Rudaea* gen. nov.

Rudaea (Ru.da.e'a. N.L. fem. n. *Rudaea* an arbitrary name after RDA, Rural Development Administration, where taxonomic studies of this taxon were conducted).

Cells are Gram-negative, strictly aerobic, motile rods. Catalase- and oxidase-positive. Nitrate is not reduced. Glucose is not fermented. H₂S is not produced. Arginine dihydrolase is produced. The major isoprenoid quinone is ubiquinone-8. The major fatty acids (>10%) are iso-C_{17:1}ω₉c, iso-C_{17:0} and iso-C_{16:0}. The DNA G+C content of the type strain of the type species is 64 mol% (HPLC). Phylogenetically, the genus belongs to the family *Xanthomonadaceae*. The type species is *Rudaea cellulosilytica*.

Description of *Rudaea cellulosilytica* sp. nov.

Rudaea cellulosilytica (cel.lu.lo.si.ly'ti.ca. N.L. n. *cellulosum* cellulose; N.L. adj. *lyticus* dissolving; N.L. fem. adj. *cellulosilytica* cellulose-dissolving).

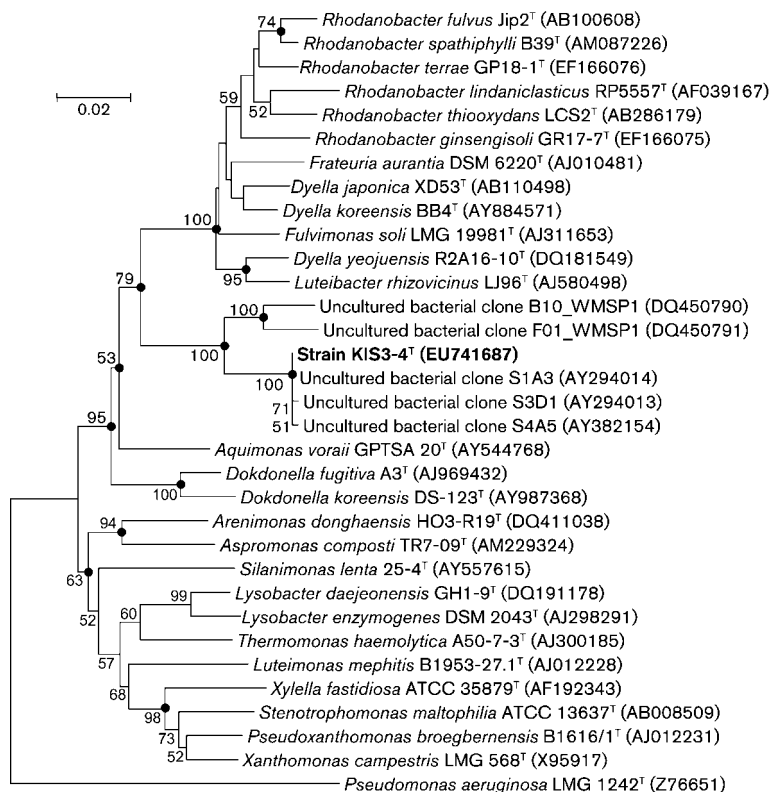


Fig. 1. Neighbour-joining tree based on partial 16S rRNA gene sequences showing the phylogenetic position of strain KIS3-4^T. Bootstrap values (>50%) based on 1000 resamplings are shown at branch nodes. Solid circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. The sequence of *Pseudomonas aeruginosa* LMG 1242^T was used as the outgroup. Bar, 0.02 substitutions per nucleotide position.

Displays the following properties in addition to those given in the genus description. Cells are 0.5 µm wide and 1.0–3.0 µm long. Colonies are yellow, round or irregular on R2A medium. Growth occurs at 5–35 °C (optimum 28–30 °C), at pH 5–8 (optimum pH 6–7) and at 0–1 % NaCl, but not at 2 % NaCl. Hydrolyses CM-cellulose, Tween 80 and tyrosine, but not casein, chitin, DNA, hypoxanthine, starch or xanthine. Positive for urease, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for indole production and glucose fermentation (API 20NE). Assimilates D-glucose, D-mannose, N-acetylglucosamine, maltose, inositol, sucrose, sodium acetate, lactic acid, salicin, melibiose, L-fucose, 3-hydroxybutyric acid and L-proline, but not L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, itaconic acid, suberic acid, sodium malonate, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate or 4-hydroxybenzoic acid (API 20NE and API ID 32GN strips). Acid is not produced from any substrates in the API 20E system. Produces alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α- and β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase; weakly produces esterase (C4), cystine arylamidase, trypsin and α-fucosidase; does not produce lipase (C14), α-chymotrypsin or β-glucuronidase.

The type strain is KIS3-4^T (=KACC 12734^T =JCM 15422^T), isolated from soil from Daechung Island of the West Sea of Korea.

Acknowledgements

This work was supported by a grant (no. 20080401034028) from the BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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