Luteimonas cucumeris sp. nov., isolated from a cucumber leaf

Zhan-Bin Sun,^{1†} Hui Zhang,^{2†} Xing-Fang Yuan,¹ Yin-Xian Wang,¹
Dong-Mei Feng,¹ Yi-Hua Wang¹ and Yong-Jun Feng¹

Correspondence
Yong-Jun Feng
fengyj@bit.edu.cn

¹School of Life Science, Beijing Institute of Technology, Beijing 100081, PR China

²School of Life Science, Liaocheng University, Liaocheng, Shandong 252059, PR China

A Gram-negative, aerobic and non-motile rod, designated Y4^T, was isolated from a cucumber leaf from Pinggu District, east Beijing, PR China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain Y4^T was most closely related to *Luteimonas aquatica* RIB1-20^T (96.7 % 16S rRNA gene sequence similarity). DNA–DNA relatedness between strain Y4^T and *L. aquatica* RIB1-20^T was 42.5 ± 3.9 %. The predominant fatty acids were iso-C_{15:0}, iso-C_{17:1ω9c}, iso-C_{16:0} and iso-C_{17:0}. The major ubiquinone was Q-8. The DNA G + C content of the type strain was 69.9 mol%. Based on the evidence above, strain Y4^T represents a novel species of the genus *Luteimonas*, for which the name *Luteimonas cucumeris* sp. nov. is proposed. The type strain is Y4^T (=CGMCC 1.10821^T=KCTC 23627^T).

The genus *Luteimonas*, in the family *Xanthomonadaceae* of the class *Gammaproteobacteria*, was first described by Finkmann *et al.* (2000) for aerobic, Gram-negative rods with Q-8 as the major ubiquinone and iso-C_{15:0} as the predominant fatty acid. At the time of writing, the genus *Luteimonas* comprises six species with validly published names: *Luteimonas mephitis* (Finkmann *et al.*, 2000), *L. composti* (Young *et al.*, 2007), *L. aestuarii* (Roh *et al.*, 2008), *L. aquatica* (Chou *et al.*, 2008), *L. marina* (Baik *et al.*, 2008) and *L. terricola* (Zhang *et al.*, 2010).

A yellow rod, designated Y4^T, was isolated from a cucumber leaf collected from Pinggu District, east Beijing, PR China, during the blossoming stage in May 2009. The leaf was surface sterilized (Rubini *et al.*, 2005) and a sample of crushed tissue was incubated on nutrient agar (NA; Difco) at 30 °C for 3 days and a pure culture of strain Y4^T was obtained after subcultivation. Strain Y4^T was routinely cultivated on NA at 30 °C and preserved in 25 % (w/v) glycerol at –80 °C.

Cells were grown for 72 h and observed by light microscopy (×1000; Olympus) and transmission electron microscopy (JEM-1400; JEOL) to ascertain their morphology and size. Motility was examined by the hanging drop method (Mackie & McCartney, 1989). The Gram-reaction was determined by using non-staining KOH method, as described by Powers (1995). Poly-β-hydroxybutyrate granule accumulation was tested by staining with Sudan black (Baomanbio) and observation by light microscopy. Tests for

catalase, oxidase and hydrolysis of casein, gelatin, starch, DNA and Tweens 20 and 60 were performed according to the methods of Smibert & Krieg (1994). Growth at 15, 20, 25, 30, 37, 42 and 45 °C was assessed in nutrient broth (NB; Difco). Growth at pH 4–10 (at intervals of one pH unit) was determined by measuring the OD₆₀₀ of cultures in NB adjusted with appropriate biological buffers (Chung *et al.*, 1995) prior to sterilization. Growth with 0, 0.5 and 1.0–10.0 % (w/v) NaCl (at intervals of 1.0 %) was tested in NB prepared according to the formula of the Difco medium. Anaerobic growth was determined after incubation in an anaerobic chamber (Thermo). Strain Y4^T was a Gram-negative, aerobic, and non-motile rod. Poly-β-hydroxybutyrate granules were not accumulated. The isolate was able to grow on NA, tryptic soy agar (TSA; BD, Difco) and R2A agar (BD Difco). After 72 h at 30 °C on NA, cells were 0.5–0.6 µm wide and 2.0–3.0 µm long and colonies were approximately 1.5–2.0 mm in diameter, yellow and round with umbonate elevations and smooth edges. Strain Y4^T was able to grow at 20–40 °C, at pH 6.0–8.0 and with 0–3 % NaCl. The optimum growth conditions were 30 °C, pH 7.0 and 0 % NaCl. The isolate was catalase- and oxidase-positive and able to hydrolyse gelatin, starch and Tween 60, but it could not hydrolyse casein, DNA or Tween 20.

Physiological and biochemical properties and enzyme activities were determined using the API 20 NE and API ZYM systems (bioMérieux), respectively, and utilization of carbohydrates and organic acids was investigated using the GN2 MicroPlate system (Biolog), according to the manufacturers' instructions. The results are listed in Table 1 together with those of members of the genus *Luteimonas* obtained using the same conditions and methodology.

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Y4^T is HQ874629.

Table 1. Differential phenotypic characteristics between strain Y4^T and its closest phylogenetic neighbours in the genus *Luteimonas*

Strains: 1, Y4^T; 2, *L. aquatica* LMG 24212^T (Chou *et al.*, 2008); 3, *L. composti* BCRC 17598^T (Young *et al.*, 2007); 4, *L. mephitis* DSM 12574^T (Finkmann *et al.*, 2000); 5, *L. marina* FR1330^T (Roh *et al.*, 2008); 6, *L. aestuarii* B9^T (Roh *et al.*, 2008). All strains grow under the same conditions and are positive for catalase, oxidase, gelatin hydrolysis, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, acid phosphatase, valine arylamidase, naphthol-AS-BI-phosphohydrolase and utilization of L-alanyl glycine, glycyl L-glutamic acid and L-proline. All strains are negative for lipase (C14), β -glucuronidase, α -fucosidase, glucose acidification, indole production, arginine dihydrolase, urease, assimilation of mannitol, gluconate, caprate, adipate and phenyl acetate and utilization of adonitol, myo-inositol, lactulose, raffinose, L-rhamnose, *cis*-aconitic acid, citric acid, D-glucuronic acid, 2-aminoethanol, 2,3-butanediol and DL- α -glycerol phosphate. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6
Isolation source	Cucumber leaf	Fresh water	Food waste	Ammonia biofilter	Seawater	Tidal flat
Nitrate reduction	+	–	+	–	–	–
Assimilation (API 20 NE)						
Glucose	–	+	+	–	+	+
Arabinose	–	–	+	–	+	–
Mannose	–	+	–	–	–	–
Maltose	–	+	+	–	+	+
Enzyme activities (API ZYM)						
Cystine arylamidase	+	+	–	–	–	+
α -Chymotrypsin	–	–	+	–	+	+
α -Glucosidase	–	+	+	–	+	–
N-Acetyl- β -glucosaminidase	–	+	+	–	+	+
Utilization (GN2 MicroPlate)						
Dextrin	+	+	+	–	+	+
D-Arabitol	–	–	+	+	–	–
Cellobiose	–	–	+	+	+	+
Maltose	–	+	+	–	+	+
D-Mannitol	–	–	+	+	–	–
D-Mannose	–	+	–	–	–	–
Trehalose	–	+	+	–	–	+
Acetic acid	–	+	–	–	w	+
Succinic acid	w	–	+	+	+	–
Bromosuccinic acid	–	+	+	+	w	–
L-Asparagine	w	–	+	+	–	+
L-Aspartic acid	–	+	+	+	–	–
L-Histidine	+	–	+	–	–	–
Inosine	–	+	–	–	–	+
Glucose 1-phosphate	–	+	+	+	–	–
DNA G + C content (mol%)	69.9	70.3	68.1	67.0	67.6	69.6

Genomic DNA for amplification was prepared as described by Rainey *et al.* (1996). The 16S rRNA gene was amplified using the universal bacterial primers 27F and 1492R (Lane, 1991). The PCR products were purified and sequenced using procedures described elsewhere (Heery *et al.*, 1990; Chun & Goodfellow, 1995). The 16S rRNA gene sequence was compiled using Chromas version 1.43 (Conor McCarthy; Griffith University, Brisbane, Queensland, Australia). Sequences from related taxa were obtained from GenBank using BLAST (<http://www.ncbi.nlm.nih.gov/>) and aligned using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analysis was performed using MEGA version 4.1 (Tamura *et al.*, 2007). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1980) using neighbour joining (Saitou & Nei, 1987) and maximum parsimony (Fitch, 1971). A phylogenetic tree was constructed using neighbour joining and bootstrap values

were based on 1000 replications (Felsenstein, 1985). Similarity values were calculated using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The 16S rRNA gene sequence obtained from strain Y4^T was a continuous stretch of 1454 bp. In the neighbour-joining tree (Fig. 1), strain Y4^T fell within the genus *Luteimonas*, which formed a distinct cluster that included the type strain of the type species of the genus. This cluster was supported by the maximum-parsimony analysis (data not shown). Strain Y4^T was most closely related to *L. aquatica* RIB1-20^T (96.7 % 16S rRNA gene sequence similarity).

Genomic DNA for the measurement of G + C content and DNA–DNA relatedness was extracted according to the method described by Marmur (1961). The G + C content was determined by the thermal denaturation method (Sly *et al.*, 1986), using a model Lambda 35 UV/Vis spectrometer

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain Y4^T and members of related species. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Bar, 0.02 substitutions per nucleotide position.

equipped with a thermometer controller (Perkin Elmer) and DNA from *Escherichia coli* K-12 as a control. The DNA G + C content of strain Y4^T was 69.9 mol%, which is in the range for the genus *Luteimonas* (64.7–72 mol%). DNA–DNA relatedness was determined using a reassociation-rate method, as suggested by Dong *et al.* (2000), and calculated according to De Ley *et al.* (1970). DNA–DNA relatedness between strain Y4^T and *L. aquatica* RIB1-20^T was $42.5 \pm 3.9\%$ (mean $\pm$ standard deviation of six replicates, $P=0.01$). According to the threshold of 70 % DNA–DNA relatedness for the definition of bacterial species (Wayne

et al., 1987), this value indicated that strain Y4^T did not belong to any known species.

For cellular fatty acid analysis, strain Y4^T was cultivated on TSA at 28 °C for 48 h. Fatty acids were extracted, prepared, identified and quantified according to the Microbial Identification System (Sasser, 1990). The major fatty acids of strain Y4^T were iso-C_{15:0} (33.41 %), iso-C_{17:1ω9c} (20.75 %), iso-C_{16:0} (9.27 %) and iso-C_{17:0} (8.89 %), which were in large agreement with those determined for members of the genus *Luteimonas* under the same conditions and

methodology (Table 2). Some differences with the reference strains were observed, especially in the proportions of iso-C_{17:0} in the isolate and *L. aquatica* RIB1-20^T.

Isoprenoid quinones were extracted and purified as described by Collins *et al.* (1987) and subsequently analysed by HPLC (Wu *et al.* 1989). The predominant ubiquinone in strain Y4^T was Q-8, which is as expected for members of the genus *Luteimonas*.

Sensitivity to antibiotics of strain Y4^T was tested using the method described by Chou *et al.* (2008) for the following (µg per disc, unless otherwise indicated): ampicillin (10), chloramphenicol (30), gentamicin (10), kanamycin (30), nalidixic acid (30), novobiocin (30), penicillin G (10), rifampicin (5), streptomycin (10), sulfamethoxazole (30) and tetracycline (30). After 3 days of cultivation, strain Y4^T was assessed to be resistant to penicillin G and sulfamethoxazole, but susceptible to kanamycin, chloramphenicol, gentamicin, rifampicin, novobiocin, nalidixic acid, streptomycin, tetracycline and ampicillin.

On the basis of phylogenetic, phenotypic and chemotaxonomic data, strain Y4^T represents a novel species of the

genus *Luteimonas*, for which the name *Luteimonas cucumeris* sp. nov. is proposed.

Description of *Luteimonas cucumeris* sp. nov.

Luteimonas cucumeris (cu.cu'me.ris. L. gen. n. *cucumeris* of a cucumber).

Cells are Gram-negative, aerobic and non-motile rods. After 72 h at 30 °C on NA, cells are 0.5–0.6 µm wide and 2.0–3.0 µm long. Colonies are approximately 1.5–2.0 mm in diameter, yellow and round with umbonate elevations and smooth edges. Grows at 20–40 °C (optimum 30 °C), with 0–3 % NaCl (optimum 0 % NaCl) and at pH 6–8 (optimum pH 7). Poly-β-hydroxybutyrate granules are not accumulated. Nitrate is reduced to nitrite. Catalase- and oxidase-positive. Hydrolyses gelatin, starch and Tween 60, but not casein, DNA or Tween 20. With GN2 MicroPlate, utilizes dextrin, Tween 40, D-fructose, D-glucose, methylpyruvate, succinic acid monomethyl ester, D-galactonic acid lactone, β-hydroxybutyric acid, α-ketoglutaric acid, alaninamide, D-alanine, L-alanine, L-alanyl glycine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, L-threonine, urocanic acid, putrescine, glycerol and L-proline, weakly utilizes glycogen, succinic acid and L-asparagine, but does not utilize the other substrates in the GN2 system. With API 20 NE, positive result in tests for hydrolysis of aesculin and β-galactosidase, but negative result for indole production, glucose acidification, arginine dihydrolase, urease and assimilation of glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, phenylacetate and citrate. With API ZYM, positive result for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, α-galactosidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and β-glucosidase, but negative result in tests for lipase (C14), trypsin, α-chymotrypsin, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Resistant to penicillin G and sulfamethoxazole, but sensitive to gentamicin, rifampicin, ampicillin, novobiocin, kanamycin, chloramphenicol, nalidixic acid, tetracycline and streptomycin. The predominant fatty acids are iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{16:0} and iso-C_{17:0}. The major ubiquinone is Q-8.

The type strain, Y4^T (=CGMCC 1.10821^T=KCTC 23627^T), was isolated from a cucumber leaf from east Beijing, People's Republic of China. The DNA G+C content of the type strain is 69.9 mol%.

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Table 2. Cellular fatty acid compositions of strain Y4^T and related *Luteimonas* species.

Strains: 1, Y4^T; 2, *L. aquatica* RIB1-20^T (Chou *et al.*, 2008); 3, *L. composti* CC-YY255^T (Young *et al.*, 2007); 4, *L. mephitis* DSM 12574^T (Young *et al.*, 2007); 5, *L. marina* FR1330^T (Baik *et al.*, 2008); 6, *L. aestuarii* B9^T (Roh *et al.*, 2008). All data were taken after cultivation on TSA at 28 °C for 48 h. ECL, Equivalent chain-length; –, not detected <1 %.

Fatty acid (%)	1	2	3	4	5	6
Saturated straight-chain						
C _{14:0}	–	–	–	–	–	1.7
C _{16:0}	2.1	2.4	4.7	1.6	5.9	3.6
C _{18:0}	–	–	–	–	2.8	–
Saturated branched						
iso-C _{11:0}	6.0	4.0	6.5	5.2	6.4	16.0
iso-C _{14:0}	1.7	–	1.9	1.5	–	–
iso-C _{15:0}	33.4	25.3	25.5	47.4	26.2	16.8
anteiso-C _{15:0}	4.8	6.2	2.7	3.0	4.5	4.0
iso-C _{16:0}	9.3	4.4	13.8	5.6	1.7	8.7
iso-C _{17:0}	8.9	21.5	5.5	5.5	14.5	10.7
anteiso-C _{17:0}	–	1.9	–	–	–	–
Unsaturated						
iso-C _{15:1}	–	–	1.9	3.0	1.2	–
iso-C _{17:1} ω9c	20.8	22.3	25.5	18.7	26.0	19.2
Hydroxy						
iso-C _{11:0} 3-OH	6.6	6.7	5.5	5.3	4.7	10.1
Summed feature 3*	3.0	1.4	5.5	–	2.2	–
Unknown ECL 11.799	–	1.5	–	–	–	–

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

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