

Luteimonas lutimaris sp. nov., isolated from a tidal flat

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A Gram-staining-negative, strictly aerobic bacterium, designated strain G3^T, was isolated from a tidal flat of the Taean coast in South Korea. Cells were moderately halotolerant and non-motile rods showing catalase- and oxidase-positive reactions. Growth of strain G3^T was observed between 15 and 40 °C (optimum 30 °C) and between pH 5.5 and 9.0 (optimum pH 6.5–7.5). Strain G3^T contained Q-8 as the predominant lipoquinone and iso-C_{15:0}, iso-C_{17:1}ω₉C, iso-C_{16:0} and iso-C_{11:0} as the major fatty acids. The G+C content of the genomic DNA was 69.6 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain G3^T formed a tight phylogenetic lineage with *Luteimonas mephitis* B1953/27.1^T within the genus *Luteimonas* and was most closely related to *L. mephitis* B1953/27.1^T with 98.0% 16S rRNA gene sequence similarity. The DNA–DNA relatedness between strain G3^T and *L. mephitis* B1953/27.1^T was 35.2 ± 3.3%. On the basis of chemotaxonomic data and molecular properties, strain G3^T represents a novel species of the genus *Luteimonas*, for which the name *Luteimonas lutimaris* sp. nov. is proposed. The type strain is G3^T (=KACC 14929^T=JCM 16916^T).

The genus *Luteimonas*, a member of the family *Xanthomonadaceae* of the *Gammaproteobacteria*, was first proposed by Finkmann *et al.* (2000) with a single species, *Luteimonas mephitis*, isolated from a biofilter. This genus is closely related phylogenetically to the genera *Lysobacter*, *Xanthomonas*, *Pseudoxanthomonas* and *Stenotrophomonas*, which share some characteristics such as the presence of branched-chain fatty acids and ubiquinone-8 (Q-8). Currently, the genus *Luteimonas* comprises only six recognized species, *L. mephitis* (Finkmann *et al.*, 2000), *L. aestuarii* (Roh *et al.*, 2008), *L. aquatica* (Chou *et al.*, 2008), *L. composti* (Young *et al.*, 2007), *L. marina* (Baik *et al.*, 2008) and *L. terricola* (Zhang *et al.*, 2010), but they have been isolated from diverse habitats such as a biofilter, tidal flat, fresh water, food waste and soil. The western and southern coastal seashores of the Korean peninsula consist of sea tidal flats, known as getbol in Korea, which are unique marine habitats alternately undergoing flooding with seawater and exposure to the atmosphere, and they contain valuable biological resources such as micro-organisms and marine animals. Therefore, efforts have

been made in our laboratory to isolate and characterize members of the bacterial community from tidal flats of the Yellow Sea in South Korea (Kim *et al.*, 2010a, c; Lee *et al.*, 2011). Here, we describe the taxonomic characterization of one more novel *Luteimonas* species isolated from a tidal flat, for which the name *Luteimonas lutimaris* sp. nov. is proposed.

Strain G3^T was isolated from a tidal flat of the Taean coast of the Yellow Sea, South Korea, using a previously described procedure with some modifications (Kim *et al.*, 2008). Briefly, a tidal flat sample was serially diluted with 0.85% (w/v) saline, spread on marine agar 2216 (MA; Difco) plates and incubated at 25 °C for 5 days. PCR amplifications of 16S rRNA genes from representative colonies were performed using the universal primers F1 (5'-AGAGT-TTGATCMTGGCTCAG-3') and R13 (5'-TACGGYTACC-TTGTTACGACTT-3') as described previously (Lu *et al.*, 2006). The amplicons were analysed on the basis of restriction fragment length polymorphism (RFLP) after their *Hae*III and *Hha*I double digestion with some modifications of a previously described procedure (Kim *et al.*, 2010b). All representative amplicons with unique RFLP patterns were partially sequenced with the F1 primer and the resulting 16S rRNA gene sequences were analysed using the BLAST program (<http://www.ncbi.nlm.nih.gov/>)

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain G3^T is GU199001.

A supplementary figure and a supplementary table are available with the online version of this paper.

BLAST/) in GenBank. From the analysis, a novel strain belonging to the genus *Luteimonas*, designated strain G3^T, was selected, and its 16S rRNA gene was cloned into the pCR2.1 vector using a TOPO cloning kit (Invitrogen) according to the manufacturer's instructions for more complete sequencing.

Strain G3^T was routinely grown aerobically on MA at 30 °C for 3 days, except where indicated otherwise. The strain was stored at -80 °C in marine broth (MB; Difco) supplemented with 10 % (v/v) glycerol for preservation. *Luteimonas mephitis* KACC 11391^T, *Luteimonas aestuarii* KCTC 22048^T and *Luteimonas aquatica* LMG 24212^T were used as reference strains for DNA-DNA hybridization, phenotypic characterization and fatty acid analysis: *L. mephitis* KACC 11391^T was provided by the KACC (Korea) and *L. aestuarii* KCTC 22048^T and *L. aquatica* LMG 24212^T were purchased from the KCTC (Korea) and the BCCM/LMG (Belgium), respectively.

The 16S rRNA gene sequence (1467 nt) of strain G3^T was compared with available 16S rRNA gene sequences from GenBank using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) to determine an approximate phylogenetic affiliation. Sequence similarity values between the isolate and related bacteria were evaluated using the Nucleotide Similarity Search program (<http://147.47.212.35:8080>; Chun *et al.*, 2007) and sequences were aligned by using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees using the neighbour-joining and maximum-parsimony algorithms were constructed by the PHYLIP software (version 3.6; Felsenstein, 2002). The resulting tree topologies were evaluated using a bootstrap analysis based on 1000 resampled datasets with the PHYLIP package. Maximum-likelihood analysis was performed using RAxML-HPC in Abe (version 7.2.6) of the Cyberinfrastructure for Phylogenetic Research

project (CIPRES, www.phylo.org; Stamatakis *et al.*, 2005) at the San Diego Supercomputer Center.

Comparative analysis of the 16S rRNA gene sequences showed that strain G3^T was most closely related to *L. mephitis* B1953/27.1^T with 98.0 % similarity and its 16S rRNA gene sequence similarities with other related type species were less than 96.5 %. Phylogenetic analysis based on 16S rRNA gene sequence indicated that strain G3^T formed a tight phylogenetic cluster with the type species of the genus *Luteimonas*, *L. mephitis* B1953/27.1^T, within the genus *Luteimonas* with a 100 % bootstrap value (Fig. 1). Analysis with the maximum-likelihood and maximum-parsimony algorithms also showed that the strain formed a tight phylogenetic cluster with *L. mephitis* B1953/27.1^T within the genus *Luteimonas* (Fig. 1).

DNA-DNA hybridization was carried out to evaluate the level of DNA relatedness between strain G3^T and *L. mephitis* KACC 11391^T as described previously (Lee *et al.*, 2011). Briefly, extracted genomic DNAs were fragmented with a *Hae*III digestion and different amounts of the fragmented DNA were blotted onto Hybond-N+ nylon membranes (Amersham Pharmacia Biotech) in three replicates after denaturation using NaOH solution and heating (80 °C). Each DNA (4 µg) was used individually as a labelled DNA probe for cross-hybridization (Park *et al.*, 2007). Random primed DNA labelling with digoxigenin (DIG)-dUTP and detection of hybrids by enzyme immunoassay on nylon membrane were performed using a DIG High Prime DNA Labelling kit (Roche Applied Science) according to the manufacturer's instructions and standard procedures (Sambrook & Russell, 2001). The hybridization signals were captured and analysed with the Bio-Rad Quantity One software (version 4.62). The signal produced by hybridization

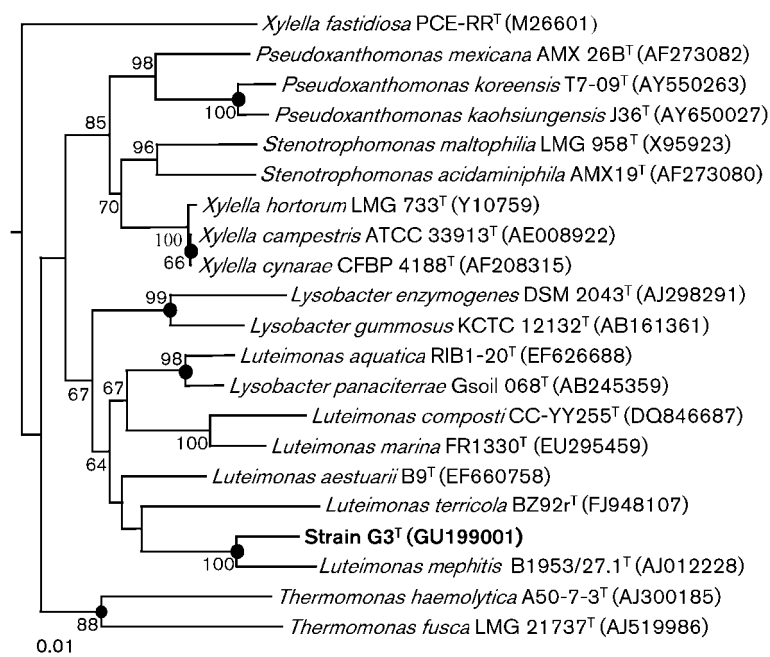


Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain G3^T and related taxa. Bootstrap values are shown in percentages of 1000 replicates, when greater than 50 %. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony and maximum-likelihood algorithms. *Escherichia coli* ATCC 11775^T (X80725) was used as an outgroup (not shown). Scale bar, 0.01 changes per nucleotide position.

of the probe to the homologous target DNA was taken to be 100% and signal intensities by the self-hybridization of the series of dilutions were used for the calculation of the levels of DNA relatedness between strain G3^T and *L. mephitis* KACC 11391^T. The hybridization experiment was confirmed by cross-hybridization. The DNA G+C content of strain G3^T was determined by HPLC using a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the method of Tamaoka & Komagata (1984). The DNA–DNA relatedness value between strain G3^T and *L. mephitis* KACC 11391^T was 35.2 ± 3.3%, which is clearly below the 70% threshold generally accepted for species delineation (Rosselló-Mora & Amann, 2001). The DNA G+C content of strain G3^T was 69.6 mol%, which was within the range of 67–72 mol% for the genus *Luteimonas*.

Growth was tested on R2A agar (Difco), laboratory-prepared Luria–Bertani (LB) agar, tryptic soy agar (TSA; Difco) and MA (Difco) at 30 °C. Growth temperatures and pH values for growth of strain G3^T were examined by growing the isolate on MA agar at 5–45 °C at 5 °C intervals and in MB adjusted to pH 5.0–10.0 at 0.5 pH unit intervals. Media with different pH values were prepared using appropriate biological buffers: Na₂HPO₄/NaH₂PO₄ buffer,

Na₂CO₃/NaHCO₃ buffer and Na₂HPO₄/NaOH buffer were used for pH values below 8.0, pH values of 8.0–10.0, and pH 10.0, respectively (Gomori, 1955). The pH was confirmed and adjusted again after sterilization. Gram staining was performed using a bioMérieux Gram Stain kit according to the instructions of the manufacturer. Cell morphology and the presence of flagella were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) with 2-day-old cells grown on MA agar as described previously (Jeon *et al.*, 2004). Requirement and tolerance of NaCl were determined in nutrient broth (NB: 3.0 g beef extract and 5.0 g peptone l⁻¹) supplemented with modified artificial seawater (ASW: 0–10%, w/v, NaCl at 1% intervals, 5.94 g MgSO₄·7H₂O, 4.53 g MgCl₂·6H₂O, 0.64 g KCl and 1.3 g CaCl₂ l⁻¹) (Kahng *et al.*, 2009). Oxidase activity was tested by oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck), and catalase activity was evaluated by the production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution (Smibert & Krieg, 1994). Additional enzymic activities, biochemical features and the utilization of carbon source of strain G3^T and other reference *Luteimonas* species were determined by using the API ZYM kit, API 20NE kit (bioMérieux) and GN2 MicroPlate system (Biolog) according to the manufacturer's

Table 1. Phenotypic characteristics of strain G3^T and related *Luteimonas* species

Strains: 1, G3^T (this study); 2, *L. mephitis* KACC 11391^T (Finkmann *et al.*, 2000; Lipski & Stackebrandt, 2005; Young *et al.*, 2007); 3, *L. aestuarii* KCTC 22048^T (Roh *et al.*, 2008); 4, *L. aquatica* LMG 24212^T (Chou *et al.*, 2008). All strains were positive for the following characteristics (results from this study): hydrolysis of aesculin and gelatin, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. All of these strains were negative for Gram staining, nitrate reduction, indole production, acidification of glucose, arginine dihydrolase, hydrolysis of urea, β-glucuronidase, α-mannosidase, α-fucosidase and assimilation of L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid (all results from this study except Gram staining). +, Positive; –, negative; w, weakly positive.

Characteristic	1*	2	3	4
Isolation source	Tidal flat	Ammonia biofilter	Tidal flat	Fresh water
Colony colour	Yellow to brown	Yellow	Yellow	Yellow
Growth at 37 °C	+	–	+	+
NaCl range (%) for growth	0–9	0–6*	0–3	0–3
Assimilation of (API 20NE)*:				
D-Glucose	–	–	+	w
D-Mannose	–	–	–	w
Maltose	–	–	+	w
Malic acid	–	–	+	+
Enzyme activity (API ZYM)*				
Lipase (C14)	+	+	–	–
Trypsin	+	w	+	w
α-Galactosidase	–	w	–	–
β-Galactosidase	–	w	–	–
N-Acetyl-β-glucosaminidase	–	–	+	+
Major cellular fatty acids (>10%)*	iso-C _{15:0} , iso-C _{17:1} ω9c, iso-C _{16:0} and iso-C _{11:0}	iso-C _{15:0} , iso-C _{17:1} ω9c	iso-C _{15:0} , iso-C _{17:1} ω9c, iso-C _{11:0} and iso-C _{17:0}	iso-C _{15:0} , iso-C _{17:1} ω9c, iso-C _{11:0} 3-OH
DNA G+C content (mol%)	69.6	67.0	64.7	70.3

*These results were from this study (30 °C).

instructions, except that the inocula were prepared by suspending cells in 0.85% (w/v) saline. Anaerobic growth was assessed on MA under anaerobic (with 4–10% CO₂) conditions using the GasPak Plus system (BBL) at 30 °C for 20 days.

Strain G3^T grew well at 30 °C on R2A agar, LB agar, TSA and MA. When tested on MA, growth of strain G3^T was observed at temperatures between 15 and 40 °C, with an optimum growth temperature of 30 °C and at the range of pH 5.5–9.0, with an optimum of pH 6.5–7.5. Strain G3^T grew in NB supplemented with modified ASW containing 0–9% (w/v) NaCl; optimal growth occurred in the range of 0–3% NaCl. Bacterial cells were Gram-negative, strictly aerobic, non-motile rods, 0.3–0.5 µm wide and 0.7–1.2 µm long (see Supplementary Fig. S1 in IJSEM Online). More physiological and biochemical characteristics of strain G3^T are described in Table 1 and in the species description. Some of them are in accordance with characteristics of members of the genus *Luteimonas*, whereas others allow the differentiation of strain G3^T from other *Luteimonas* species (Table 1).

Isoprenoid quinone was analysed by HPLC using a chromatograph (model LC-20A; Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250 by 4.6 mm; Kromasil, Akzo Nobel) as described previously (Komagata & Suzuki, 1987). For analysis of fatty acid methyl esters, cells of strain G3^T and other reference *Luteimonas* species were harvested from TSA plates after incubation at 30 °C for 3 days. Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). The major respiratory lipoquinone detected in strain G3^T was ubiquinone-8 (Q-8). The major cellular fatty acids (>10.0% of the total fatty acids) of strain G3^T were iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{16:0} and iso-C_{11:0}. The overall fatty acid profile of strain G3^T was similar to those of other *Luteimonas* species, but the amounts of respective fatty acids, especially major cellular fatty acids, distinguished the strain from other *Luteimonas* species (Table 2). Therefore, the physiological, biochemical and phylogenetic properties of strain G3^T support its description as a novel species within the genus *Luteimonas* for which the name *Luteimonas lutimaris* sp. nov. is proposed.

Description of *Luteimonas lutimaris* sp. nov.

Luteimonas lutimaris (lu.ti.mar'is. L. n. *lutum* mud; L. gen. n. *maris* of the sea; N.L. gen. n. *lutimaris* of sea mud).

Cells are Gram-staining-negative, strictly aerobic, non-motile rods, 0.3–0.5 µm wide and 0.7–1.2 µm long. The colonies are yellow to brown, convex, round with entire margins on MA. Growth occurs at 15–40 °C (optimum 30 °C), at pH 5.0–9.0 (optimum pH 6.5–7.5) and in the presence of 0–9.0% (w/v) NaCl (optimum 0–3%). Catalase- and oxidase-positive. In the API 20NE kit, negative for nitrate reduction, indole production, glucose acidification and arginine dihydrolase activities. Aesculin

Table 2. Cellular fatty acid composition (%) of strain G3^T and related *Luteimonas* species grown on TSA at 30 °C for 3 days

Strains: 1, G3^T; 2, *L. mephitis* KACC 11391^T; 3, *L. aestuarii* KCTC 22048^T; 4, *L. aquatica* LMG 24212^T. All data are from this study. Data are expressed as percentages of total fatty acids. Fatty acids amounting to less than 0.5% in all strains are not shown. Major components (>10.0%) are highlighted in bold. tr, Trace amount (<0.5%); –, not detected.

Fatty acid	1	2	3	4
Saturated straight-chain				
C _{14:0}	0.96	0.76	0.61	tr
C _{16:0}	3.81	1.17	2.01	5.69
Saturated branched				
iso-C _{10:0}	0.52	tr	tr	–
iso-C _{11:0}	11.19	8.64	12.93	7.86
iso-C _{13:0}	tr	0.67	tr	tr
iso-C _{14:0}	1.20	2.14	0.52	0.54
iso-C _{15:0}	29.67	48.31	27.55	36.81
iso-C _{16:0}	11.59	5.09	5.65	2.93
iso-C _{17:0}	7.12	2.94	12.24	6.43
anteiso-C _{15:0}	1.92	3.56	5.86	2.80
anteiso-C _{17:0}	tr	tr	0.73	tr
Unsaturated				
iso-C _{17:1} ω9c	17.01	13.07	20.32	16.18
iso-C _{15:1} F	1.41	3.03	–	0.57
Hydroxy				
C _{16:0} 3-OH	–	0.68	tr	–
iso-C _{11:0} 3-OH	8.07	6.36	7.09	10.27
iso-C _{12:0} 3-OH	1.54	tr	tr	tr
iso-C _{17:0} 3-OH	–	tr	tr	1.38
Summed features*				
3	0.88	1.06	1.16	1.54
Unknown†				
ECL 11.799	–	–	–	3.18
ECL 14.263	tr	–	tr	0.93

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1}ω7c and/or C_{16:1}ω6c.

†ECL, Equivalent chain-length.

and gelatin are hydrolysed. Urea is not hydrolysed. In the API ZYM kit, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol AS-BI-phosphohydrolase activities are present and weak enzymic activities are observed for trypsin, α-chymotrypsin and β-glucuronidase, but α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-fucosidase and α-mannosidase enzymic activities are absent. The carbon utilizations in the Biolog GN2 MicroPlate system are available in Supplementary Table S1 in IJSEM Online). The major isoprenoid quinone is Q-8. The major cellular fatty acids are iso-C_{15:0}, iso-C_{17:1}ω9c, iso-C_{16:0} and iso-C_{11:0}. The DNA G + C content is 69.6 mol% (HPLC).

The type strain, G3^T (=KACC 14929^T=JCM 16916^T), was isolated from a tidal flat of the Taean coast in South Korea.

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