Litorimonas taeansensis gen. nov., sp. nov., isolated from a sandy beach

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A heterotrophic, Gram-negative, prosthecate bacterium, designated strain G5^T, was isolated from a sandy beach of Taeon in South Korea. Cells of strain G5^T were aerobic, catalase- and oxidase-positive, straight to slightly curved motile rods with a single flagellum and formed yellow–orange colonies on agar. Growth occurred at 15–40 °C (optimum 25–30 °C) and pH 6–9 (optimum pH 7–8). The major cellular fatty acids were C_{18:1ω7c}, C_{17:0}, C_{16:0}, 11-methyl C_{18:1ω7c}, C_{17:1ω8c} and C_{17:1ω6c}. The polar lipid pattern indicated the presence of phosphatidylglycerol, monoglycosyldiglyceride, glucuronopyranosyldiglyceride and two unidentified glycolipids. The G + C content of the genomic DNA was 63.6 mol% and the major quinone was Q-10. Comparative 16S rRNA gene sequence analysis showed that strain G5^T belonged to the branch containing the genera *Hellea*, *Robiginitomaculum* and *Hypomonas* within the family *Hyphomonadaceae*. Within this group, strain G5^T was most closely related to *Hellea balneolensis* 26III/A02/215^T with 95.8 % 16S rRNA gene sequence similarity. Based on its phylogenetic position and its phenotypic, chemotaxonomic and molecular properties, strain G5^T represents a novel species of a novel genus of the family *Hyphomonadaceae*, for which the name *Litorimonas taeansensis* gen. nov., sp. nov. is proposed. The type strain is G5^T (=KACC 13701^T =DSM 22008^T).

In 2005 the genera *Hyphomonas*, *Hirschia*, *Maricaulis* and *Oceanicaulis* were combined into a new family, *Hyphomonadaceae* Lee *et al.* (2005), in the order *Rhodobacterales* of the class *Alphaproteobacteria* based on phylogenetic analysis of 16S rRNA gene sequences. Since then, new members of the family *Hyphomonadaceae* have been isolated from marine environments, such as the genera *Robiginitomaculum* (Lee *et al.*, 2007), *Hellea* (Alain *et al.*, 2008) and *Maribaculum* (Lai *et al.*, 2009). Some members of the family *Hyphomonadaceae* possess one or more stalks called prosthecae (Abraham *et al.*, 1999; Weiner *et al.*, 2000; Strömpl *et al.*, 2003; Alain *et al.*, 2008), which are cytoplasmic extrusions that undoubtedly play a role in attachment. During the course of an investigation into the bacterial communities present in the seashore of the Yellow

Sea in Korea (Kim *et al.*, 2010), a novel Gram-reaction-negative bacterium bearing a stalk and belonging to the family *Hyphomonadaceae* was isolated. Here, we describe the taxonomic characterization of a novel species of a novel genus, *Litorimonas*, within the family *Hyphomonadaceae*.

Strain G5^T was isolated from sand-soil of a beach of the Yellow Sea in Taeon, South Korea using previously described procedures with some modifications (Kim *et al.*, 2008). Briefly, a sand-soil sample was serially diluted in marine broth (MB; Difco), spread on Marine agar 2216 (MA; Difco) and incubated at 25 °C for 5 days. Genomic DNA from selected colonies was prepared and PCR amplification of 16S rRNA genes was performed as described previously (Lu *et al.*, 2006). The amplicons were double-digested with *HaeIII* and *HhaI*, their fragment patterns were analysed on 2.5 % MetaPhore agarose (BioWhittaker) gels and PCR products containing distinct fragment patterns were sequenced. The resulting 16S rRNA gene sequences were analysed using the BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) in GenBank. From the analysis, a novel strain belonging to the family *Hyphomonadaceae*, designated strain G5^T, was selected to undergo further phenotypic and phylogenetic analysis. The novel strain was grown routinely on MA at

Abbreviations: GUDG, glucuronopyranosyldiglyceride; MGDG, monoglycosyldiglyceride; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PG, phosphatidylglycerol.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain G5^T is FJ230838.

One supplementary table is available with the online version of this paper.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain G5^T and related taxa. Bootstrap values >50% (based on 1000 replicates) are shown at branch points. Filled circles indicate nodes that were also recovered in trees generated using the MP and ML algorithms. *Escherichia coli* ATCC 11775^T (X80725) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

30 °C for 3 days under aerobic conditions, except where indicated otherwise, and was stored at −80 °C in MB supplemented with 10% (v/v) glycerol for preservation. *Hellea balneolensis* DSM 19091^T and *Hyphomonas polymorpha* DSM 2665^T, purchased from DSMZ (Germany), and *Robiginitomaculum antarcticum* KCCM 42687^T, purchased from KCCM (South Korea), were used as reference strains for phenotypic characterization and fatty acid analysis.

The 16S rRNA gene sequence (1357 nt) of strain G5^T was compared with available 16S rRNA gene sequences from GenBank using the BLAST program. Sequence similarity values between the isolate and related taxa were evaluated using the EzTaxon nucleotide similarity search program (<http://147.47.212.35:8080/>; Chun *et al.*, 2007) and aligned by using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees were constructed by using the neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms available in PHYLIP software version 3.6 (Felsenstein, 2002). The resulting tree topology constructed by the NJ method was evaluated using bootstrap analysis based on 1000 resampled datasets in the PHYLIP software package. Comparative analysis of the 16S rRNA gene sequences showed that strain G5^T was most closely related to *H. balneolensis* 26III/A02/215^T and *Robiginitomaculum antarcticum* IMCC3195^T with sequence similarities of 95.8 and 94.3%, respectively. 16S rRNA gene sequence similarities with the other related type species were <92.3%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain G5^T formed a phyletic lineage with *H. balneolensis* 26III/A02/215^T within the family *Hyphomonadaceae* with 91% bootstrap support. (Fig. 1). Analysis with the ML and MP algorithms also showed that the strain formed a phyletic clade with *H. balneolensis* 26III/A02/215^T (Fig. 1).

Growth of strain G5^T at different temperatures and pH levels was determined by growing the isolate on MA at 5–45 °C with intervals of 5 °C and in marine broth (MB) adjusted to pH 5–10 with intervals of 0.5 pH units. pH was adjusted prior to sterilization by the addition of HCl or NaOH and measured again after sterilization. Gram staining was

performed using a bioMérieux Gram Stain kit according to the manufacturer's instructions. Cell morphology and the presence of flagella were determined using phase-contrast microscopy and transmission electron microscopy (JEM-1010, JEOL) using two-day-old cells grown in MB as described previously (Jeon *et al.*, 2004). Requirement and tolerance of NaCl were determined using nutrient broth (containing 3 g beef extract l^{−1} and 5 g peptone l^{−1}) supplemented with modified artificial seawater (ASW) comprising (l^{−1}) 0–10% (w/v) NaCl at 0.5% intervals, 5.94 g MgSO₄·7H₂O, 4.53 g MgCl₂·6H₂O, 0.64 g KCl and 1.3 g CaCl₂ (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) hydrogen peroxide solution (Smibert & Krieg, 1994). Nitrate reduction was assessed according to the method of Lanyi (1987). Antibiotic susceptibility tests were performed in duplicate using filter-paper discs (8 mm diameter) containing the following antibiotics (μg per disc unless stated otherwise): ampicillin (10), polymyxin B (100 U), streptomycin (50), penicillin G

Fig. 2. Transmission electron micrographs of strain G5^T negatively stained with uranyl acetate after growth for 3 days at 30 °C in MB. (a) Curved rod showing budding (arrow). (b) Cell possessing a thin prostheca (arrow). Bars: 0.2 μm (a), 1 μm (b).

(10 IU), gentamicin (30), chloramphenicol (100), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), carbenicillin (100) and novobiocin (50). Additional phenotypic properties of strain G5^T and the reference strains were determined using API ZYM and API 20 NE kits (bioMérieux) and the GN2 MicroPlate system (Biolog) according to the manufacturers' instructions. The test strips were incubated at 30 °C for 3 days except for *R. antarcticum* KCCM 42687^T (20 °C) and all suspension media were supplemented with ASW containing 2 % (w/v) NaCl. Growth was assessed under aerobic conditions (4–10 % CO₂) on MA by using the GasPak Plus system (BBL) at 30 °C for 20 days.

Growth of strain G5^T occurred at 15–40 °C (optimum 25–30 °C) and pH 6–9 (optimum of pH 7–8). Cells were Gram-reaction-negative and were straight to curved motile rods bearing a prostheca (0.4–0.6 × 1.6–2.0 µm) and a single polar flagellum when grown at 30 °C on MA (Fig. 2). Details of the phenotypic characteristics of strain G5^T and those of closely related taxa are given in the genus and species descriptions and in Table 1. Some of phenotypic features clearly differentiated strain G5^T from closely related type strains of members the

family *Hypomonadaceae*. Isoprenoid quinones were analysed using HPLC (model LC-20A, Shimadzu) equipped with a diode array detector (SPD-M20A, Shimadzu) and a reversed-phase column (250 × 4.6 mm, Kromasil, Akzo Nobel) as described previously (Komagata & Suzuki, 1987). For fatty acid methyl ester analysis, cells of strain G5^T and reference strains were harvested after incubation at 30 °C for 3 days on MA except for *R. antarcticum* KCCM 42687^T, which was cultivated on MA at 20 °C. Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI).

Polar lipids were determined by TLC as described by Minnikin *et al.* (1977). The DNA G + C content of strain G5^T was determined using HPLC fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE, GROM) according to the method of Tamaoka & Komagata (1984). The major respiratory quinone of strain G5^T was ubiquinone-10 (Q-10). The major cellular fatty acids (>5 % of the total fatty acids) were C_{18:1}ω7c (26.9 %), C_{17:0} (18.5 %), C_{16:0} (17.7 %), 11-methyl C_{18:1}ω7c (7.9 %), C_{17:1}ω8c (6.8 %) and C_{17:1}ω6c (6.5 %) (Table 2). Although the overall fatty acid

Table 1. Differential phenotypic characteristics of strain G5^T and closely related phylogenetic neighbours of the family *Hypomonadaceae*

Taxa: 1, strain G5^T (results from this study); 2, *Hellea balneolensis* DSM 19091^T (Alain *et al.*, 2008); 3, *Robiginitomaculum antarcticum* KCCM 42687^T (Lee *et al.*, 2007); 4, *Hyphomonas polymorpha* DSM 2665^T (Weiner *et al.*, 1985; Kang and Lee 2009; Abraham *et al.*, 1999). All strains were positive for the following characteristics: alkaline phosphatase, esterase, lipase (C8), leucine arylamidase, valine arylamidase and naphthol-AS-B1-phosphohydrolase activities (API ZYM). +, Positive; –, negative; (+), weakly positive; ND, no data available; tr, trace amount (<1 %); Tau, 1,2-diacyl-3- α -D-glucopyranosyl-*sn*-glycerol taurineamide; PL, unidentified phospholipid; GL, unidentified glycolipid.

Characteristics	1	2	3	4
Shape	Straight or curved rod	Rod or vibrioid	Rod or vibrioid	Spheroid
Pigmentation	Yellow–orange	Black–red	Rusty orange	Grey or colourless
Flagella	+	+	–	+
Budding	+	+	–	+
Oxidase	+	+	–	+
Growth at 6 % (w/v) NaCl	(+)	–	–	–
Optimum growth temperature (°C)	25–30	30	20	30–37
Enzyme activities (API ZYM)*				
Lipase (C14)	–	(+)	+	–
Cystine arylamidase	+	–	+	+
Acid phosphatase	+	+	–	+
Trypsin	+	(+)	–	+
α -Chymotrypsin	+	(+)	–	+
Utilization of (Biolog GN2)*:				
Tween 40	+	–	+	–
D-Fructose	–	–	+	–
D-Glucose	–	+	+	–
Raffinose	–	–	+	+
Polar lipids	PG, GL1, GL2, MGDG, GUDG	PG, GL, MGDG, GUDG, PL ₁ , PL ₂ , PL ₃	ND	MGDG, GUDG, PG, Tau
Major quinone	Q-10	Q-10	Q-10	Q-10/Q-11
Nitrate reduction	–	–	+	–
DNA G + C content (mol%)	63.6	47	60.3	60

*Results from this study. Performed at 30 °C except for tests on *R. antarcticum* KCCM 42687^T which were performed at 20 °C.

profile of strain G5^T was similar to those of phylogenetically related species, significant differences in the respective proportions of several components, the presence of 11-methyl C_{18:1}ω7c and the absence of 10-methyl C_{19:0} clearly distinguished the strain from phylogenetically related type strains (Table 2). The major polar lipids were phosphatidylglycerol (PG), monoglycosyldiglyceride (MGDG), glucuronopyranosyldiglyceride (GUDG) and two unidentified glycolipids (GL1 and GL2) (Fig. 3). Among these polar lipids, the presence of MGDG and GUDG appears to be a characteristic of members of the family Hyphomonadaceae (Alain *et al.*, 2008; Kang & Lee 2009).

Although strain G5^T formed a phylogenetic group with *H. balneolensis* 26III/A02/215^T with 95.8% 16S rRNA gene sequence similarity, the G+C content of strain G5^T was 63.6 mol%, which is much higher than that of *H. balneolensis* 26III/A02/215^T (47 mol%) (Table 1, Rosselló-Mora & Amann, 2001). Based on these results and on its physiological, biochemical and phylogenetic properties, strain G5^T represents a novel species of a novel genus within the family Hyphomonadaceae of the class Alphaproteobacteria, for which the name *Litorimonas taeanensis* gen. nov., sp. nov. is proposed.

Description of *Litorimonas* gen. nov.

Litorimonas (Li.to.ri.mo'nas. L. n. *litus* -oris beach; L. fem. n. *monas* monad, unit; N.L. fem. n. *Litorimonas* beach bacterium).

Cells are Gram-reaction-negative, non-spore-forming, budding, straight to slightly curved rods. Usually non-stalked and motile by means of a polar flagellum; some cells possess one polar stalk (prostheca) and are non-motile. Oxidase- and catalase-positive. Neither nitrate nor nitrite is reduced. The only isoprenoid quinone detected is ubiquinone-10 (Q-10). The DNA G+C content of the type strain of the type species is 63.6 mol% (HPLC). Phylogenetically, the genus *Litorimonas* is a member of the family Hyphomonadaceae of the class Alphaproteobacteria. The type species is *Litorimonas taeanensis*.

Description of *Litorimonas taeanensis* sp. nov.

Litorimonas taeanensis (tae.an.en'sis. N.L. fem. adj. *taeanensis* of or belonging to Taean, from where the type strain was isolated).

In addition to the characteristics described for the genus, the species is characterized by the following properties. Cells are 0.4–0.6 × 1.6–2.0 μm, strictly aerobic, chemoheterotrophic and moderately halophilic. Colonies on MA are yellow-orange, convex and round with entire margins. Growth occurs at 15–40 °C (optimum 25–30 °C), at pH 6–9 (optimum pH 7–8) and in 1–6 % (w/v) NaCl (optimum 2–3 %). Positive for assimilation of D-glucose but negative for assimilation of L-arabinose, D-mannose, maltose, D-mannitol, malic acid, potassium gluconate, N-acetylglucosamine, capric acid, adipic acid, trisodium citrate and phenylacetic acid (API 20 NE). Positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase,

Table 2. Cellular fatty acid compositions (%) of strain G5^T and type strains of related members of the family Hyphomonadaceae

Taxa: 1, strain G5^T; 2, *Hellea balneolensis* DSM 19091^T; 3, *Robiginotomaculum antarcticum* KCCM 42687^T; 4, *Hyphomonas polymorpha* DSM 2665^T. All data from this study. Fatty acids <1% in all strains are not shown. tr, Trace amount (<1%); –, not detected. All strains were grown on MA at 30 °C except for *R. antarcticum* KCCM 42687^T (20 °C).

Fatty acid	1	2	3	4
Saturated				
C _{15:0}	2.7	tr	tr	8.9
C _{16:0}	17.7	2.6	tr	3.3
C _{17:0}	18.5	7.4	8.2	27.0
C _{18:0}	3.2	2.0	6.4	tr
C _{19:0}	–	1.7	2.4	tr
Unsaturated				
C _{16:1} ω5c	2.6	–	–	tr
C _{17:1} ω8c	6.8	2.1	8.6	17.9
C _{17:1} ω6c	6.5	6.2	3.8	16.5
11-methyl C _{18:1} ω7c	7.9	1.3	–	8.4
C _{18:1} ω9c	tr	–	2.7	–
C _{18:1} ω7c	26.9	26.5	46.9	10.5
10-methyl C _{19:0}	–	21.2	tr	–
C _{20:1} ω9c	–	1.4	–	–
Branched				
iso-C _{19:0}	–	1.6	–	–
Hydroxyl				
C _{11:1} 3-OH	–	1.0	tr	tr
C _{18:1} 2-OH	–	9.0	–	–
10-methyl C _{18:0} (TBSA)	–	5.4	–	–
Unknown ECL 18.814	–	–	14.5	–
Summed features*				
3	tr	tr	2.8	–
7	tr	6.6	tr	2.4

*Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 7 comprises C_{19:0} cyclo ω10c and/or C_{19:1}ω6c and/or ECL (equivalent chain-length) 18.846.

trypsin, α-chymotrypsin, acid phosphatase and α-glucosidase activities but negative for lipase (C14), α- and β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. Weak enzymic activities are observed for esterase (C4), β-glucosidase and naphthol-AS-B1-phosphohydrolase (API ZYM). Positive for (Biolog GN2) utilization of Tweens 40 and 80, N-acetyl-D-galactosamine, D-arabitol, cellobiose, D-mannitol, D-mannose, pyruvic acid methyl ester, succinic acid mono-methyl ester, formic acid, D-gluconic acid, β- and γ-hydroxybutyric acid, DL-lactic acid, sebacic acid, succinic acid, L-asparagine, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-ornithine, L-serine, DL-carnitine, γ-amino butyric acid, inosine, uridine, 2-aminoethanol, glycerol, α-DL-glycerol phosphate, α-D-glucose-1-phosphate and D-glucose-6-phosphate. Other organic substrates included in Biolog GN2 microplates are not utilized. Resistant

Fig. 3. Thin-layer chromatogram sprayed with molybdatophosphoric acid reagent showing the polar lipid profile of strain G5^T following separation by two-dimension TLC. Ascending solvent system: (I) chloroform–methanol–water (65:25:4, v/v/v.); (II) chloroform–acetic acid–methanol–water (80:15:12:4, v/v/v/v.). For detection of the polar lipids, phosphomolybdic acid (for PG, MGDG, GUDG, GL1 and GL2), ninhydrin (no detection), α -naphthol (for MGDG, GUDG, GL1 and GL2) and Dittmer–Lester reagent (for PG) were applied. GL, unidentified glycolipid.

to polymyxin B, streptomycin, gentamicin, kanamycin, novobiocin, oleandomycin, lincomycin, ampicillin, tetracycline, penicillin G and carbenicillin but sensitive to chloramphenicol. PG, MGDG, GUDG and two unidentified glycolipids are the polar lipids detected. The major cellular fatty acids are C_{18:1} ω 7c, C_{17:0}, C_{16:0}, 11-methyl C_{18:1} ω 7c, C_{17:1} ω 8c and C_{17:1} ω 6c.

The type strain, G5^T (=KACC 13701^T =DSM 22008^T), was isolated from beach sand in Taejeon, South Korea. The DNA G + C content of the type strain is 63.6 mol%.

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