Photobacterium gaetbulicola sp. nov., a lipolytic bacterium isolated from a tidal flat sediment

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A Gram-negative, motile, non-spore-forming and lipolytic bacterial strain, designated Gung47^T, was isolated from a tidal flat on the west coast of Korea. Strain Gung47^T grew optimally at 30 °C and with 2–5 % (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain Gung47^T belonged to the genus *Photobacterium*. Strain Gung47^T exhibited 98.1 % 16S rRNA gene sequence similarity with *Photobacterium rosenbergii* LMG 22223^T and 94.3–96.3 % similarity with other type strains of species of the genus *Photobacterium*. Strain Gung47^T exhibited 47 % DNA–DNA relatedness to *P. rosenbergii* LMG 22223^T. Strain Gung47^T contained Q-8 as the predominant ubiquinone and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, C_{16:0} and C_{18:1}ω7c as the major fatty acids. In this study, two closely related type strains, *P. rosenbergii* LMG 22223^T and *Photobacterium halotolerans* LMG 22194^T, were also found to have Q-8 as the predominant ubiquinone. The DNA G+C content of strain Gung47^T was 50.6 mol%. The differential phenotypic properties together with the phylogenetic and genetic distinctiveness of strain Gung47^T demonstrated that this strain is distinguishable from recognized *Photobacterium* species. Therefore, strain Gung47^T is considered to represent a novel species of the genus *Photobacterium*, for which the name *Photobacterium gaetbulicola* sp. nov. is proposed. The type strain is Gung47^T (=KCTC 22804^T =CCUG 58399^T).

The genus *Photobacterium* was first proposed by Beijerinck (1889) and belongs phylogenetically to the class *Gamma-proteobacteria* (Anzai *et al.*, 2000). At the time of writing, the genus *Photobacterium* comprised 16 species with validly published names: *Photobacterium phosphoreum* (Beijerinck, 1889) (the type species), *P. leiognathi* (Boisvert *et al.*, 1967), *P. fischeri* (Beijerinck, 1889; Reichelt & Baumann, 1973), *P. angustum* (Reichelt *et al.*, 1976), *P. damsela* (Smith *et al.*, 1991), *P. iliopiscarium* (Onarheim *et al.*, 1994; Urakawa *et al.*, 1999), *P. profundum* (Nogi *et al.*, 1998), *P. indicum* (Xie & Yokota, 2004), *P. rosenbergii* (Thompson *et al.*, 2005), *P. lipolyticum* (Yoon *et al.*, 2005), *P. frigidiphilum* (Seo *et al.*, 2005a), *P. aplysiae* (Seo *et al.*, 2005b), *P. ganghwense* (Park *et al.*, 2006), *P. halotolerans* (Rivas *et al.*, 2006), *P. lutimaris* (Jung *et al.*, 2007) and *P. kishitanii* (Ast *et al.*, 2007). Members of the genus *Photobacterium* have been isolated from marine organisms, marine sediments,

seawater and saline lakes (Ast *et al.*, 2007; Seo *et al.*, 2005a; Farmer & Hickman-Brenner, 2006; Park *et al.*, 2006; Rivas *et al.*, 2006; Thompson *et al.*, 2005). In this study, we describe a lipolytic bacterial strain, designated Gung47^T, which was isolated from a tidal flat sediment on the west coast of Korea.

Strain Gung47^T was isolated by means of the standard dilution plating technique at 25 °C on marine agar 2216 (MA; Difco). The type strains of three *Photobacterium* species were used as reference strains for phenotypic characterization, fatty acid analysis and DNA–DNA hybridization: *P. rosenbergii* LMG 22223^T and *P. halotolerans* LMG 22194^T were obtained from LMG, Ghent, Belgium, and *P. ganghwense* KCTC 12328^T was obtained from KCTC, Taejeon, Korea. The morphological, physiological and biochemical characteristics of strain Gung47^T were investigated using routine cultivation on marine agar 2216 (MA; Difco) at 30 °C. Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). Flagellation was determined by transmission electron microscopy with cells

Abbreviation: TCN, tricaprilyn.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gung47^T is GQ260188.

Table 1. Differential phenotypic characteristics of strain Gung47^T and its closest phylogenetic neighbours

Strains: 1, *Photobacterium gaetbulicola* sp. nov. Gung47^T; 2, *P. rosenbergii* LMG 22223^T (unless indicated, data from Thompson *et al.*, 2005); 3, *P. lutimaris* DF-42^T (Jung *et al.*, 2007); 4, *P. halotolerans* LMG 22194^T (Rivas *et al.*, 2006); 5, *P. ganghwense* KCTC 12328^T (Park *et al.*, 2006). All strains were positive for motility, nitrate reduction, catalase (data for *P. rosenbergii* LMG 22223^T from this study; weak for *P. ganghwense* KCTC 12328^T), oxidase, hydrolysis* of tyrosine and Tweens 20, 40, 60 and 80, utilization* of D-galactose, D-glucose, maltose, trehalose, citrate, L-malate, pyruvate and succinate, acid production* from D-fructose and D-ribose, activity* of alkaline phosphatase, esterase (C4) and esterase lipase (C8) and susceptibility* to chloramphenicol, gentamicin, neomycin and streptomycin. All strains were negative for Gram-staining, H₂S production (data for *P. rosenbergii* LMG 22223^T from this study), hydrolysis of urea*, utilization* of benzoate, formate and L-glutamate, acid production* from melezitose and D-sorbitol, activity* of valine arylamidase, trypsin, α -chymotrypsin, α - and β -galactosidase, β -glucuronidase, α - and β -glucosidase, α -mannosidase and α -fucosidase and susceptibility* to oleandomycin. +, Positive; w, weakly positive; -, negative.

Characteristic	1	2	3	4	5
Growth at:					
4 °C	—	—	+	+	—
40 °C	+	—	+	—	+
Hydrolysis of:*					
Aesculin	+	+	+	—	—
Casein	—	—	—	+	+
Gelatin	—	—	—	+	+
Starch	+	—	+	—	+
Xanthine	—	—	—	+	—
Hypoxanthine	—	—	—	+	+
Utilization of:*					
L-Arabinose	—	+	—	+	—
Cellobiose	+	+	+	—	+
D-Fructose	—	+	+	+	+
D-Mannose	+	+	+	—	—
Sucrose	+	+	+	+	—
D-Xylose	—	—	+	+	—
Acetate	+	+	—	+	+
Salicin	—	+	+	—	+
Acid production from:*					
L-Arabinose	—	—	—	+	—
Cellobiose	+	+	+	—	+
D-Galactose	+	+	—	+	+
D-Glucose	+	+	—	+	+
myo-Inositol	+	+	+	—	+
Lactose	+	+	—	+	—
Maltose	+	+	—	+	+
D-Mannitol	+	+	—	+	+
D-Mannose	+	+	—	—	—
Melibiose	+	+	—	—	—
Raffinose	+	—	—	—	—
L-Rhamnose	—	+	—	—	+
Sucrose	+	+	+	+	—
Trehalose	+	+	—	+	+

Table 1. cont.

Characteristic	1	2	3	4	5
D-Xylose	—	—	+	+	—
Enzyme activity (API ZYM)*					
Lipase (C14)	+	—	—	+	—
Leucine arylamidase	—	—	+	+	—
Cystine arylamidase	—	—	+	—	—
Acid phosphatase	—	—	+	+	—
Naphthol-AS-BI-phosphohydrolase	—	—	+	+	—
N-Acetyl- β -glucosaminidase	—	+	—	—	—
Susceptibility to:*					
Ampicillin	—	—	+	—	—
Carbenicillin	+	+	+	—	+
Cephalothin	—	—	+	—	—
Kanamycin	w	+	+	—	+
Lincomycin	+	—	+	—	—
Novobiocin	+	—	+	—	—
Penicillin G	—	—	+	—	—
Polymyxin B	+	+	+	—	+
Tetracycline	w	w	—	w	w
DNA G + C content (mol%)	50.6	47.6–47.9	48.3	49.8	44

*Data were taken from this study.

from an exponentially growing culture. For this purpose, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at 4, 10, 20, 25, 28, 30, 35, 37, 40 and 45 °C was measured on MA. Growth with 0, 0.5, 1.0, 2.0 and 3.0 % (w/v) NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was omitted and 0.45 % (w/v) MgCl₂·6H₂O or 0.06 % (w/v) KCl was added. Growth with 2.0–10.0 % (w/v) NaCl (in increments of 1.0 %) was investigated in marine broth 2216 (MB; Difco). Growth at pH 4.5–9.5 (in increments of 0.5 pH units) was investigated in MB with the pH adjusted after autoclaving with HCl and Na₂CO₃. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Lipolytic activity was determined by the formation of a halo on MA supplemented with tricaprillin (TCN), which was prepared as follows: a TCN emulsion (50 ml) was made by emulsifying 5 ml TCN in 45 ml gum arabic solution (200 mM NaCl, 10 mM CaCl₂ and 5 %, w/v, gum arabic) for 2 min using a Waring blender and mixed with 450 ml MA. Nitrate reduction and hydrolysis of aesculin, gelatin, urea and Tweens 20, 40, 60 and 80 were investigated as described by Lányi (1987) with the

modification that artificial seawater (1^{-1} distilled water: 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2 \cdot 6H_2O$, 5.94 g $MgSO_4 \cdot 7H_2O$ and 1.3 g $CaCl_2 \cdot 2H_2O$; Bruns *et al.*, 2001) was used for preparation of media. Hydrolysis of casein, starch, hypoxanthine, tyrosine and xanthine was tested on MA, using the substrate concentrations described by Cowan & Steel (1965). H_2S production was tested as described by Bruns *et al.* (2001). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981) using supplementation with 2 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). Susceptibility to antibiotics was tested on MA plates using antibiotic discs containing (μ g, unless otherwise indicated) ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), novobiocin (5), oleandomycin (15), penicillin G (20 U), polymyxin B (100 U), streptomycin (50) and tetracycline (30). Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux).

Cell mass of strain Gung47^T and *P. rosenbergii* LMG 22223^T for DNA extraction and isoprenoid quinone analysis and cell mass of *P. halotolerans* LMG 22194^T for isoprenoid quinone analysis were obtained from cultures grown for 3 days in MB at 30 °C. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The identification of phylogenetic neighbours was achieved using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid methyl ester analysis, cell mass of strain Gung47^T, *P. rosenbergii* LMG 22223^T, *P. halotolerans*

LMG 22194^T and *P. ganghwense* KCTC 12328^T was harvested from MA plates after incubation for 3 days at 30 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain Gung47^T are given in the species description and Table 1. The almost-complete 16S rRNA gene sequence of strain Gung47^T, comprising 1474 nt (approx. 96 % of the *Escherichia coli* 16S rRNA gene sequence), was determined in this study. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain Gung47^T fell within the clade comprising *Photobacterium* species and formed a cluster with *P. rosenbergii* LMG 22223^T, *P. lutimaris* DF-42^T, *P. halotolerans* MACL01^T and *P. ganghwense* FR1311^T (Fig. 1). This cluster was also observed in phylogenetic trees constructed using the maximum-likelihood and maximum-parsimony algorithms (not shown). Strain Gung47^T exhibited 16S rRNA gene sequence similarity of 98.1, 96.3, 96.2 and 95.8 % with *P. rosenbergii* LMG 22223^T, *P. lutimaris* DF-42^T, *P. halotolerans* MACL01^T and *P. ganghwense* FR1311^T, respectively, and 94.3–95.5 % with other type strains of species of the genus *Photobacterium*.

The predominant isoprenoid quinone detected in strain Gung47^T was ubiquinone-8 (Q-8), which has been found in only some members of the genus *Photobacterium* (Park *et al.*, 2006; Nogi *et al.*, 1998; Jung *et al.*, 2007). In this study,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain Gung47^T and type strains of species of the genus *Photobacterium* and some other related taxa. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

P. rosenbergii LMG 22223^T and *P. halotolerans* LMG 22194^T were also found to have Q-8 as the predominant isoprenoid quinone (Table 1). The cellular fatty acid profile of strain Gung47^T is shown in Table 2, together with those of *P. rosenbergii* LMG 22223^T, *P. halotolerans* LMG 22194^T and *P. ganghwense* KCTC 12328^T analysed in this study and that of *P. lutimaris* DF-42^T taken from Jung *et al.* (2007). The fatty acid profiles of the five strains were essentially similar in that C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, C_{16:0} and C_{18:1}ω7c were the major fatty acids, although there were differences in the proportions of some fatty acids. The DNA G + C content of strain Gung47^T was 50.6 mol%. The results from the chemotaxonomic analyses were in agreement with the result of the phylogenetic analysis, that strain Gung47^T is a member of the genus *Photobacterium* (Park *et al.*, 2006; Nogi *et al.*, 1998; Jung *et al.*, 2007; Tables 1 and 2).

The mean DNA–DNA relatedness between strain Gung47^T and *P. rosenbergii* LMG 22223^T was 47 %, which indicated that the two strains are members of different genomic species (Wayne *et al.*, 1987). Strain Gung47^T was distinguishable from its closest phylogenetic neighbours by differences in several phenotypic characteristics, most of which were determined using the same methods in this study and Jung *et al.* (2007). The phylogenetic distinctiveness of strain Gung47^T, together with the genetic distinctiveness and differential phenotypic properties, are sufficient to show that this strain is separate from recognized *Photobacterium* species (Stackebrandt & Goebel, 1994). Therefore, strain Gung47^T is considered to represent a novel species of the genus *Photobacterium*, for which the name *Photobacterium gaetbulicola* sp. nov. is proposed.

Description of *Photobacterium gaetbulicola* sp. nov.

Photobacterium gaetbulicola [gaet.bu.li.co'la. N.L. n. *gaetbulum* -i gaetbul, the Korean name for a tidal flat; L. suff. -cola (from L. n. *incola*) a dweller, inhabitant; N.L. fem. n. *gaetbulicola* a dweller of a tidal flat].

Cells are Gram-negative, motile, non-spore-forming and rod- or oval-shaped (0.4–1.0 × 1.0–5.0 μm). After incubation on MA at 30 °C for 3 days, colonies are circular to irregular, flat, smooth, glistening, greyish-yellow and 4.0–6.0 mm in diameter. Growth occurs at 10 and 40 °C (optimum 30 °C), but not at 4 or 45 °C. Growth occurs at pH 5.0 (optimum pH 7.0–8.0), but not at pH 4.5. Growth occurs with 0–8.0 % (w/v) NaCl (optimum 2.0–5.0 %); growth occurs without NaCl if Mg²⁺ ions are added. Growth occurs under anaerobic conditions on MA and on MA supplemented with nitrate. Catalase- and oxidase-positive. Nitrate reduction is positive. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. The predominant ubiquinone is Q-8. The major fatty acids (>10 % of total fatty acids) are C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, C_{16:0} and C_{18:1}ω7c. Other phenotypic properties are shown in Table 1. The DNA G + C content of the type strain is 50.6 mol% (HPLC).

Table 2. Cellular fatty acid compositions of strain Gung47^T and its closest phylogenetic neighbours

Strains: 1, *P. gaetbulicola* sp. nov. Gung47^T; 2, *P. rosenbergii* LMG 22223^T; 3, *P. lutimaris* DF-42^T; 4, *P. halotolerans* LMG 22194^T; 5, *P. ganghwense* KCTC 12328^T (data from Jung *et al.*, 2007). Data were taken from this study unless indicated. Values are percentages of total fatty acids; fatty acids that represented <1.0 % in all strains were omitted.

Fatty acid	1	2	3	4	5
Straight-chain					
C _{12:0}	1.7	2.1	2.4	6.3	3.1
C _{14:0}	4.2	3.5	2.4	1.1	4.0
C _{15:0}	2.9	3.3	2.1	0.8	0.8
C _{16:0}	14.0	15.5	13.1	21.5	22.3
C _{16:0} N alcohol	0.5	0.3	—	—	1.3
C _{17:0}	1.0	1.9	—	1.5	1.0
Branched					
iso-C _{15:0}	6.9	3.9	5.0	0.1	0.5
iso-C _{16:0}	0.4	1.9	—	2.1	0.4
iso-C _{17:0}	3.5	5.1	1.8	0.4	1.0
iso-C _{17:1} ω9c	1.4	1.8	1.8	—	—
Unsaturated					
C _{16:1} ω9c	—	—	—	1.5	—
C _{17:1} ω8c	0.6	1.1	—	1.6	0.5
C _{18:1} ω6c	3.1	—	—	3.4	—
C _{18:1} ω7c	10.3	11.9	13.0	16.3	21.3
C _{16:1} ω7c alcohol	0.7	0.6	1.2	—	2.1
Hydroxy					
C _{11:0} 2-OH	2.8	—	—	—	—
C _{12:0} 3-OH	2.8	2.3	2.6	6.2	2.6
Summed features*					
2	3.7	3.4	3.0	6.0	3.9
3	33.6	35.6	43.3	24.5	31.0

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 2 consisted of iso-C_{16:1} and/or C_{14:0} 3-OH. Summed feature 3 consisted of C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

The type strain, Gung47^T (=KCTC 22804^T =CCUG 58399^T), was isolated from a tidal flat of Gung harbour on the west coast of Korea.

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