Photobacterium ganghwense sp. nov., a halophilic bacterium isolated from sea water

Yoon-Dong Park,¹ Keun Sik Baik,² Chi Nam Seong,² Kyung Sook Bae,³ Sanggoo Kim¹ and Jongsik Chun¹

Correspondence

Jongsik Chun

jchun@snu.ac.kr

¹School of Biological Sciences and Institute of Microbiology, Seoul National University, 56-1 Shillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea

²Department of Biology, College of Natural Sciences, Sunchon National University, Sunchon 540-742, Republic of Korea

³Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, Taejeon 305-600, Republic of Korea

A marine bacterial strain, designated FR1311^T, was isolated from a sea-water sample from Ganghwa Island, South Korea. Cells were Gram-negative, facultatively anaerobic, catalase- and oxidase-positive, motile, oval or rod-shaped and halophilic (optimum sea-salt concentration for growth of 5–6 %). Phylogenetic analysis of its 16S rRNA gene sequence revealed that it represented a distinct line of descent within the genus *Photobacterium*. The major fatty acids were straight-chain saturated (C_{16:0}) and monounsaturated fatty acids (C_{16:1ω7c} and C_{18:1ω7c}). The predominant respiratory lipoquinone was Q-8. The DNA G + C content was 44 mol%. The phenotypic features of strain FR1199^T were similar to those of *Photobacterium damsela* subsp. *damsela* and *Photobacterium damsela* subsp. *piscicida*, but several physiological and chemotaxonomic properties readily distinguish the new isolate from them. On the basis of the polyphasic results revealed in this study, FR1311^T is considered to be the type strain of a novel species, for which the name *Photobacterium ganghwense* sp. nov. is proposed. The type strain is FR1311^T (=IMSNU 60287^T=KCTC 12328^T=JCM 12487^T).

The genus *Photobacterium* was first described by Beijerinck (1889) and at the time of writing the genus comprises 11 recognized species: *Photobacterium phosphoreum* (Reichelt & Baumann, 1973) (the type species), *P. fischeri* (Beijerinck, 1889; Reichelt & Baumann, 1973), *P. leiognathi* (Boisvert *et al.*, 1967; Ast & Dunlap, 2004), *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. lipolyticum* (Yoon *et al.*, 2005), *P. rosenbergii* (Thompson *et al.*, 2005) and *P. frigidiphilum* (Seo *et al.*, 2005). Phylogenetic analyses based on 16S rRNA gene sequences have shown that the genus *Photobacterium* is closely related to the genus *Vibrio* (Nogi *et al.*, 1998; Anzai *et al.*, 2000), and has Q-8 as the predominant respiratory lipoquinone and C_{16:1} and C_{16:0} as the major fatty acids (Nogi *et al.*, 1998). During the course of a study on marine microbial diversity, a halophilic

bacterium, designated strain FR1311^T, was isolated from a sea-water sample and was the subject of a taxonomic investigation. On the basis of the polyphasic evidence presented, this strain is considered to represent a novel species of the genus *Photobacterium*.

A sample was collected in coastal surface sea water from Ganghwa Island, South Korea (37° 35' 39.1" N 126° 27' 24.5" E). The sample was diluted with sterilized artificial sea water (ASW; Lyman & Fleming, 1940), spread onto a plate containing marine agar 2216 (MA) (Becton Dickinson) and incubated at 25 °C for 3 weeks. The isolate was routinely cultured on MA and maintained as a glycerol suspension (20 %, w/v) at –80 °C.

The 16S rRNA gene sequence of this strain, designated FR1311^T, was determined using universal primers (Lane, 1991) as described by Chun & Goodfellow (1995), and an almost complete sequence was obtained (1462 bp). Phylogenetic analyses were performed using the Fitch–Margoliash (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The topology of the resultant neighbour-joining tree

Published online ahead of print on 18 November 2005 as DOI 10.1099/ijs.0.63811-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FR1311^T is AY960847.

A transmission electron micrograph of a negatively stained cell of strain FR1311^T is available as supplementary material in IJSEM Online.

was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program (available at <http://chunlab.snu.ac.kr/jphydit/>) and PAUP 4.0 (Swofford, 1998) as described by Chun *et al.* (2000).

Preliminary sequence comparison with 16S rRNA gene sequences held in GenBank indicated that the new isolate belongs within the genus *Photobacterium*. The newly determined sequence was then aligned manually against representatives of the family *Vibrionaceae* using bacterial 16S rRNA gene secondary structure information. Domains used to construct the phylogenetic trees were the regions available for all sequences (positions 85–1417; *Escherichia coli* numbering system). On the basis of 16S rRNA gene sequence similarity, the closest cultured bacterial relatives were the type strains of recognized *Photobacterium* species (95.5–92.7%) and *Vibrio* species (92.7–91.1%). Strain FR1131^T showed highest 16S rRNA gene sequence similarity to *P. damsela* subsp. *damsela* ATCC 33539^T (95.5%), followed by *P. damsela* subsp. *piscicida* NCIMB 2058^T (95.4%), *P. rosenbergii* LMG 22223^T (94.9%) and *P. leiognathi* ATCC 25521^T (94.4%). These values are well below the cut-off value of 97% suggested for bacterial species definition (Stackebrandt & Goebel, 1994). As shown in the neighbour-joining phylogenetic tree (Fig. 1), strain FR1131^T formed a distinct phyletic subline within the genus *Photobacterium*; this relationship was confirmed using the other tree-making algorithms. On the basis of pairwise 16S rRNA gene sequence similarity and phylogenetic analysis, it is clear that the new isolate represents a novel species in the genus *Photobacterium*.

For phenotypic tests, strain FR1131^T was grown on MA at 30 °C. Cellular morphologies were observed by use of a

differential interference microscope (Axioskop 40; Zeiss) and a transmission electron microscope (JEM1010; JEOL) using cells grown at 25 °C for 3 days. Motility was examined using wet mounts. The pH range (pH 3–12) for growth was determined using MA. The requirement of NaCl (0–10%) for growth was tested using synthetic ZoBell medium (ZoBell, 1941; 15 g Bacto agar, 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate in 1000 ml distilled water). Growth at various temperatures was examined on MA at 4–50 °C. Accumulation of poly-β-hydroxybutyrate was determined by the Sudan black staining method (Smibert & Krieg, 1994). Biochemical tests were performed using the API 20NE, API 20E and API ZYM kits (bioMérieux). Strips were inoculated with a heavy bacterial suspension in ASW or AUX medium (bioMérieux) supplemented with 2% sea salts. Catalase and oxidase activities were determined using 3% (v/v) hydrogen peroxide and Kovacs reagent (Kovacs, 1956), respectively. Cell biomass of strain FR1131^T for respiratory lipoquinone analysis and for DNA extraction was obtained from cultivation in marine broth 2216 (Becton Dickinson) at 25 °C. Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987) using reversed-phase TLC. Cellular fatty acids were analysed as methyl esters by GLC according to the instructions of the Microbial Identification System (MIDI). Fatty acid methyl esters were prepared from biomass grown on MA at 30 °C for 2 days. DNA G + C content was determined according to the thermal denaturation method described by Marmur & Doty (1962).

Strain FR1131^T was halophilic; it required 1–7% (w/v) NaCl for growth (optimum 2%) and was unable to grow on ZoBell medium without NaCl. Results of the biochemical and physiological tests are given in the species description

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain FR1131^T and members of the genus *Photobacterium*. Numbers at nodes indicate levels of bootstrap support (%) for branch points based on 1000 resamplings. *Alteromonas macleodii* DSM 6062^T was used as an outgroup. Bar, 0.01 nucleotide substitutions per position.

and in Table 1. Strain FR1311^T had an unsaturated ubiquinone with eight isoprene units (Q-8), in agreement with previous findings that photobacteria contain Q-8 as the predominant respiratory lipoquinone (Nogi *et al.*, 1998). Strain FR1311^T had large amounts of straight-chain and unsaturated fatty acids: the major components were C_{16:0} (21%), C_{14:0} (3.6%), C_{12:0} (3.4%), C_{18:1 ω 7c} (29.6%), C_{12:0} 3-OH (2.1%), C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH (27.8%) and C_{14:0} 3-OH and/or iso-C_{16:1} (2.7%). This fatty acid profile is similar to those of recognized *Photobacterium* species (Nogi *et al.*, 1998).

The detailed results of biochemical, substrate utilization and DNA G + C content determinations are given in Table 1 and the species description. It is evident from Table 1 that there are several phenotypic characters that readily separate strain FR1311^T from phylogenetically related species. Together with 16S rRNA gene and chemotaxonomic analyses, it is evident that strain FR1311^T should be classified as a novel

species in the genus *Photobacterium*, for which the name *Photobacterium ganghwense* sp. nov. is proposed.

Description of *Photobacterium ganghwense* sp. nov.

Photobacterium ganghwense (gang.hwen'se. N.L. neut. adj. *ganghwense* pertaining to Ganghwa Island, Korea, the geographical origin of the type strain of the species).

Gram-negative, oxidase- and catalase-positive and facultatively anaerobic. Colonies on MA are circular, smooth, convex with entire margins, slightly cream-coloured and approximately 3 mm in diameter after 3 days at 30 °C. Cells are motile by means of a polar flagellum (see Supplementary Fig. S1 in IJSEM Online), are oval or rod-shaped and 0.8–1.2 × 1.3–2.0 µm in size. Spores are not formed. Growth occurs in 1–7% (w/v) NaCl (optimum 2%). Growth occurs at pH 5–11 (optimum pH 8–9) and at 10–45 °C (optimum

Table 1. Differential physiological and biochemical characteristics between strain FR1311^T and other *Photobacterium* species

Strains: 1, strain FR1311^T; 2, *P. damsela* subsp. *damsela* ATCC 33539^T (data from Smith *et al.*, 1991; Nogi *et al.*, 1998); 3, *P. damsela* subsp. *piscicida* NCIMB 2058^T (Gauthier *et al.*, 1995); 4, *P. rosenbergii* LMG 22223^T (Thompson *et al.*, 2005); 5, *P. leiognathi* ATCC 25521^T (Baumann & Baumann, 1984; Nogi *et al.*, 1998); 6, *P. angustum* ATCC 25915^T (Baumann & Baumann, 1984; Nogi *et al.*, 1998); 7, *P. iliopiscarium* ATCC 51760^T (Onarheim *et al.*, 1994); 8, *P. phosphoreum* ATCC 11040^T (Reichelt & Baumann, 1973; Baumann & Baumann, 1984; Nogi *et al.*, 1998); 9, *P. frigidophilum* SL13^T (Seo *et al.*, 2005); 10, *P. indicum* NBRC 14233^T (Xie & Yokota, 2004); 11, *P. profundum* JCM 10084^T (Nogi *et al.*, 1998); 12, *P. lipolyticum* DSM 16190^T (Yoon *et al.*, 2005). +, Positive reaction or production; –, negative reaction or no production; W, weakly positive; V, variable; ND, data not available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Motility	+	+	–	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	ND	–	–	+	+	+	–	+	+
Oxidase	+	+	+	+	+	+	ND	+	+	–	+	+
Optimum growth temperature (°C)	35	22–26	25–35	20–30	26	25	20	18	14	25	10	25–28
Growth at:												
4 °C	–	–	–	–	–	V	+	+	–	+	+	+
35 °C	+	+	+	+	+	+	–	–	–	–	–	–
Lysine decarboxylase	–	–	–	–	–	–	+	–	ND	–	–	–
Citrate utilization	+	–	–	+	–	–	–	–	–	–	–	–
Production of H ₂ S	–	–	–	ND	–	–	–	–	ND	+	–	–
Indole production	+	–	–	–	–	–	–	–	+	+	+	+
Acetoin production	–	+	+	–	+	+	+	+	ND	ND	+	–
Reduction of nitrate to nitrite	+	+	–	+	+	–	+	+	+	+	+	+
Arginine dihydrolase	+	+	+	+	+	+	+	+	+	ND	–	–
Hydrolysis of gelatine	+	–	–	–	–	V	–	–	ND	–	ND	–
β-Galactosidase	–	–	–	+	+	ND	W	+	+	ND	+	–
Fermentation of:												
D-Mannitol	+	–	–	+	–	–	–	–	+	ND	+	–
D-Sorbitol	–	–	+	–	–	–	–	–	ND	ND	–	–
L-Rhamnose	W	–	–	+	–	–	–	–	ND	ND	–	–
Sucrose	–	–	W	+	–	+	–	–	+	+	–	+
Melibiose	–	+	–	+	–	–	–	–	ND	ND	–	–
L-Arabinose	–	–	+	–	–	–	–	–	ND	–	–	–
Assimilation of maltose	+	–	–	+	+	+	+	+	+	+	+	+
DNA G + C content (mol%)	44	41	41	48	40	40	38–40	39–42	44	40	42	47

35 °C). Bioluminescence is observed. No gas is produced from D-glucose under aerobic conditions. Growth occurs on thiosulfate/citrate/bile salts/sucrose medium (TCBS agar; Oxoid), producing green colonies. Acid is formed from glucose. Does not produce ornithine decarboxylase, urease or tryptophan deaminase. Does not hydrolyse aesculin. Positive for fermentation of glucose, D-inositol and amygdalin. Positive for assimilation of glucose, mannitol, *N*-acetyl-D-glucosamine, gluconate, malate, citrate and phenylacetate. Mannose, caprate and adipate are weakly utilized. Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase, but not lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase or β -fucosidase. Other physiological and biochemical characteristics are given in Table 1. Major fatty acids are C_{18:1} ω 7c (29.6%), C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH (27.8%) and C_{16:0} (21.1%). The DNA G + C content is 44 mol%.

The type strain, FR1311^T (=IMSNU 60287^T=KCTC 12328^T=JCM 12487^T), was isolated from sea water from Ganghwa Island, South Korea.

Acknowledgements

This work was supported, in part, by the Korea Ministry of Science and Technology under National Research Laboratory Program (M10500000110-05J0000-11010) and 21C Frontier Microbial Genomics and Applications Center Program (MG05-0101-2-0).

References

- Anzai, Y., Kim, H., Park, J.-Y., Wakabayashi, H. & Oyaizu, H. (2000). Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* **50**, 1563–1589.
- Ast, J. C. & Dunlap, P. V. (2004). Phylogenetic analysis of the *lux* operon distinguishes two evolutionarily distinct clades of *Photobacterium leiognathi*. *Arch Microbiol* **181**, 352–361.
- Baumann, P. & Baumann, L. (1984). Genus II. *Photobacterium* Beijerinck 1889, 401^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 539–545. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Beijerinck, M. W. (1889). Le *Photobacterium luminosum*. Bactérie luminosum de la Mer Nord. *Arch Néerl Sci* **23**, 401–427 (in French).
- Boisvert, H., Chatelain, R. & Bassot, J.-M. (1967). Etude d'un *Photobacterium* isolé de l'organe lumineux de poissons Leiognathidae. *Ann Inst Pasteur* **112**, 520–524 (in French).
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Chun, J., Bae, K. S., Moon, E. Y., Jung, S. O., Lee, H. K. & Kim, S. J. (2000). *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. *Int J Syst Evol Microbiol* **50**, 1909–1913.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP – phylogenetic inference package, version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome *c* sequences is of general applicability. *Science* **155**, 279–284.
- Gauthier, G., Lafay, B., Ruimy, R., Breitmayer, V., Nicolas, J. L., Gauthier, M. & Christen, R. (1995). Small-subunit rRNA sequences and whole DNA relatedness concur for the reassignment of *Pasteurella piscicida* (Snieszko *et al.*) Janssen and Surgalla to the genus *Photobacterium* as *Photobacterium damsela* subsp. *piscicida* comb. nov. *Int J Syst Bacteriol* **45**, 139–144.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.
- Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* **178**, 703.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by M. Goodfellow. Chichester: Wiley.
- Lyman, J. & Fleming, R. H. (1940). Composition of sea water. *J Mar Res* **3**, 134–146.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Nogi, Y., Masui, N. & Kato, C. (1998). *Photobacterium profundum* sp. nov., a new, moderately barophilic bacterial species isolated from a deep-sea sediment. *Extremophiles* **2**, 1–7.
- Onarheim, A. M., Wiik, R., Burghardt, J. & Stackebrandt, E. (1994). Characterization and identification of two *Vibrio* species indigenous to the intestine of fish in cold sea water; description of *Vibrio iliopiscarius* sp. nov. *Syst Appl Microbiol* **17**, 370–379.
- Reichelt, J. L. & Baumann, P. (1973). Taxonomy of the marine, luminous bacteria. *Arch Microbiol* **94**, 283–330.
- Reichelt, J. L., Baumann, P. & Baumann, L. (1976). Study of genetic relationships among marine species of the genera *Beneckea* and *Photobacterium*. *Arch Microbiol* **110**, 101–120.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Seo, H. J., Bae, S. S., Lee, J. H. & Kim, S. J. (2005). *Photobacterium frigidophilum* sp. nov., a psychrophilic, lipolytic bacterium isolated from deep-sea sediments of Edison Seamount. *Int J Syst Evol Microbiol* **55**, 1661–1666.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Microbiology*, pp. 611–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Smith, S. K., Sutton, D. C., Fuerst, J. A. & Reichelt, J. L. (1991). Evaluation of the genus *Listonella* and reassignment of *Listonella damsela* (Love *et al.*) MacDonell and Colwell to the genus *Photobacterium* as *Photobacterium damsela* comb. nov. with an emended description. *Int J Syst Bacteriol* **41**, 529–534.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Swofford, D. L. (1998). PAUP – phylogenetic analysis using parsimony, version 4. Sunderland, MA: Sinauer Associates.

- Thompson, F. L., Thompson, C. C., Naser, S., Hoste, B., Vandemeulebroecke, K., Munn, C., Bourne, D. & Swings, J. (2005). *Photobacterium rosenbergii* sp. nov. and *Enterovibrio coralii* sp. nov., vibrios associated with coral bleaching. *Int J Syst Evol Microbiol* **55**, 913–917.
- Urakawa, H., Kita-Tsukamoto, K. & Ohwada, K. (1999). Reassessment of the taxonomic position of *Vibrio iliopiscarius* (Onarheim *et al.* 1994) and proposal for *Photobacterium iliopiscarium* comb. nov. *Int J Syst Bacteriol* **49**, 257–260.
- Xie, C.-H. & Yokota, A. (2004). Transfer of *Hyphomicrobium indicum* to the genus *Photobacterium* as *Photobacterium indicum* comb. nov. *Int J Syst Evol Microbiol* **54**, 2113–2116.
- Yoon, J.-H., Lee, J.-K., Kim, Y.-O. & Oh, T.-K. (2005). *Photobacterium lipolyticum* sp. nov., a bacterium with lipolytic activity isolated from the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **55**, 335–339.
- ZoBell, C. E. (1941). Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J Mar Res* **4**, 42–75.