Paraperlucidibaca baekdonensis gen. nov., sp. nov., isolated from seawater

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A Gram-negative, non-spore-forming, rod-shaped bacterial strain, RL-2^T, was isolated from seawater of the East Sea in Korea and was subjected to a polyphasic taxonomic study. Strain RL-2^T grew optimally at pH 7.5–8.0, at 20 °C and in the absence of NaCl. Phylogenetic trees based on 16S rRNA gene sequence analysis showed that strain RL-2^T forms a cluster with *Perlucidibaca piscinae* IMCC1704^T and various uncultured and unidentified gammaproteobacteria. Strain RL-2^T exhibited 16S rRNA gene sequence similarity values of 96.1 % to *Perlucidibaca piscinae* IMCC1704^T and 93.7–99.7 % to the uncultured bacterial clones belonging to the cluster and an unidentified gammaproteobacterium. The fatty acid profile of strain RL-2^T was similar to that of *Perlucidibaca piscinae* IMCC1704^T, but the predominant ubiquinone type (Q-11) of strain RL-2^T was different from that (Q-8) of *Perlucidibaca piscinae* IMCC1704^T. The DNA G+C content of strain RL-2^T was 61.3 mol%. On the basis of phylogenetic, chemotaxonomic and phenotypic data, strain RL-2^T is considered to represent a novel species of a new genus in the family Moraxellaceae, for which the name *Paraperlucidibaca baekdonensis* gen. nov., sp. nov. is proposed. The type strain of *Paraperlucidibaca baekdonensis* is RL-2^T (=KCTC 23145^T =CCUG 59307^T).

The family Moraxellaceae belongs to the class Gammaproteobacteria and was first proposed by Rossau *et al.* (1991). The family initially consisted of the genera *Moraxella* (Lwoff, 1939), *Acinetobacter* (Brisou & Prévot, 1954) and *Psychrobacter* (Juni & Heym, 1986). Three other genera of the family were subsequently described, namely *Enhydrobacter* (Staley *et al.*, 1987), *Alkanindiges* (Bogan *et al.*, 2003) and *Perlucidibaca* (Song *et al.*, 2008). The genus *Perlucidibaca* was distinguished from other members of the family Moraxellaceae on the basis of high DNA G+C content and differences in fatty acid compositions and several phenotypic properties, and was proposed based on the description of a single recognized species, *Perlucidibaca piscinae* (Song *et al.*, 2008). In this study, we describe a novel bacterial strain, designated RL-2^T, which was isolated from seawater obtained from Baekdo Harbor, East Sea, Korea. Comparative 16S rRNA gene sequence analysis indicated that strain RL-2^T is phylogenetically most closely affiliated to the genus *Perlucidibaca* and several uncultured bacterial clones. The aim of the present work was to determine the exact taxonomic position of strain RL-2^T by using a polyphasic approach.

Strain RL-2^T was isolated by using standard dilution plating on R2A agar (Difco) at 20 °C. *Perlucidibaca piscinae*

IMCC1704^T, which was used as a reference strain for phenotypic characterization and fatty acid analysis, was obtained from the original authors and was cultivated routinely at 30 °C on R2A agar. Morphological, physiological and biochemical characteristics of strain RL-2^T were investigated by using routine cultivation at 20 °C on R2A agar. Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy. Flagellation was determined by using a Philips CM-20 transmission electron microscope with cells incubated for 5 days at 20 °C; 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 under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on R2A agar and on R2A agar supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under nitrogen atmosphere. Growth at 4, 10, 15, 20, 25, 30 and 37 °C was determined on R2A agar. Growth with 0–7.0 % (w/v) NaCl (increments of 1.0 %) was investigated on R2A agar. Growth in the absence of NaCl was also investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded. The optimal pH and pH range for growth were determined in R2A broth prepared according to the formula of the Difco

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain RL-2^T is GU731671.

medium except that agar was excluded. The medium was adjusted to pH 4.5–10.5 (intervals of 0.5 pH units) by using sodium acetate/acetic acid and Na₂CO₃ buffers. Catalase and oxidase activities were tested as described by Cowan & Steel (1965). Nitrate reduction and hydrolysis of aesculin, gelatin and urea were tested as described by Lányi (1987). Hydrolysis of casein, hypoxanthine, starch, tyrosine, xanthine, and Tweens 20, 40, 60 and 80 was tested on R2A agar by using the substrate concentrations described by Cowan & Steel (1965). Utilization of various substrates was tested as described by Baumann & Baumann (1981), by using supplementation with 2 % (v/v) Hutner's mineral salts solution (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). The carbon sources were added at a concentration of 0.2 % (w/v) after sterilization by filtration. Enzyme activities were determined by using the API ZYM system (bioMérieux).

Cell biomass of strain RL-2^T for DNA extraction and for isoprenoid quinone analysis was harvested from cultures grown for 9 days on R2A agar at 20 °C. Chromosomal DNA was extracted and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination of RNA. The 16S rRNA gene was amplified by PCR using two universal primers (5'-GAGTTTGATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCCAGCC-3') as described previously (Yoon *et al.*, 1998) and was purified by using a QIAquick purification

kit (Qiagen). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed according to the methods of Yoon *et al.* (2003). 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. Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and were analysed by using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For cellular fatty acid analysis, cell mass of strain RL-2^T and *Perlucidibaca piscinae* IMCC1704^T was harvested from R2A agar plates after incubation for 6 days at 20 °C. Fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990).

Morphological, cultural, physiological and biochemical characteristics of strain RL-2^T are given in the genus and species descriptions below and in Table 1. The almost-complete 16S rRNA gene sequence of strain RL-2^T determined in this study comprised 1460 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic trees reconstructed with the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms, strain RL-2^T fell within a cluster comprising *Perlucidibaca piscinae* IMCC1704^T and various uncultured and unidentified gammaproteobacteria (Fig. 1). Strain RL-2^T exhibited highest 16S rRNA gene sequence

Table 1. Differential phenotypic characteristics between strain RL-2^T and *Perlucidibaca piscinae* IMCC1704^T

Data for *Perlucidibaca piscinae* IMCC1704^T are from Song *et al.* (2008) and this study. w, Weakly positive reaction. The two strains are positive for activity of oxidase, esterase (C4) and esterase lipase (C8), but negative for catalase, hydrolysis of casein†, gelatin, hypoxanthine†, xanthine†, L-tyrosine† and urea, and activity of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase.

Characteristic	RL-2 ^T	<i>Perlucidibaca piscinae</i> IMCC1704 ^T
Cell size (μm)	0.3–0.4 × 1.0–3.5	0.5–0.7 × 0.7–1.2
Motility	–	+
Optimal growth temperature (°C)	20	30
Growth at 37 °C	–	+
NaCl range for growth (%)	0–5	0–1
Anaerobic growth	–	+
Nitrate reduction	–	w
Hydrolysis of:		
Aesculin	–	–*
Tween 80	+	+†
Enzyme activity (API ZYM)		
Alkaline phosphatase	–	+
Leucine arylamidase	+	–
Predominant ubiquinone	Q-11	Q-8
DNA G+C content (mol%)	61.3	63.1

*Weakly positive in Song *et al.* (2008).

†Data for *P. piscinae* IMCC1704^T are from this study.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain RL-2^T, uncultured bacterial clones and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Alteromonas macleodii* DSM 6062^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

similarity (99.7%) to uncultured bacterial clone IHE3_080 and 93.7–96.4% to three other uncultured bacterial clones belonging to the same cluster. Strain RL-2^T exhibited 16S rRNA gene sequence similarity values of 96.1 and 94.5% to *Perlucidibaca piscinae* IMCC1704^T and gammaproteobacterium JAM-GA1201, respectively, and less than 91.5% to the type strains of other species used in the phylogenetic analysis. The predominant isoprenoid quinone detected in strain RL-2^T was ubiquinone-11 (Q-11). The cellular fatty acid profile of strain RL-2^T is shown in Table 2, together with that of *Perlucidibaca piscinae* IMCC1704^T. The major fatty acids (>10% of the total) found in strain RL-2^T were C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (31.1%), C_{18:1}ω7c (17.3%), C_{16:0} (12.0%) and C_{12:0} 3-OH (10.0%). The DNA G+C content of strain RL-2^T was 61.3 mol%.

In this study, strain RL-2^T, *Perlucidibaca piscinae* IMCC1704^T, an unidentified gammaproteobacterium and several uncultured bacterial clones were found to form a phylogenetic cluster that is independent of all other members of the class Gammaproteobacteria (Fig. 1). The fatty acid profile of strain RL-2^T was essentially similar to that of *Perlucidibaca piscinae* IMCC1704^T, even though there were differences in the proportions of some components (Table 2). However, the predominant ubiquinone (Q-11) of strain RL-2^T was different from those of other members of the family Moraxellaceae as well as from *Perlucidibaca piscinae* IMCC1704^T, which contain Q-8 or Q-9 as the predominant ubiquinone (Song *et al.*, 2008). Strain RL-2^T could also be differentiated from *Perlucidibaca piscinae* IMCC1704^T based on differences in several phenotypic properties, including motility, optimal growth temperature, NaCl tolerance, anaerobic growth and nitrate reduction, as shown in Table 1. The phylogenetic data and differential chemotaxonomic and phenotypic properties suggest that strain RL-2^T represents a novel species of a new genus within the family Moraxellaceae, class Gammaproteobacteria, for

which the name *Paraperlucidibaca baekdonensis* gen. nov., sp. nov. is proposed.

Description of *Paraperlucidibaca* gen. nov.

Paraperlucidibaca (Pa.ra.per.lu.ci.di.ba'ca. Gr. prep. *para* like, beside; N.L. fem. n. *Perlucidibaca* a bacterial generic name; N.L. fem. n. *Paraperlucidibaca* resembling *Perlucidibaca*).

Cells are Gram-stain-negative, non-motile and rod-shaped. Strictly aerobic. Catalase-negative and oxidase-positive. Nitrate is not reduced to nitrite. The predominant ubiquinone is Q-11. The major fatty acids (>10% of the total) are C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, C_{18:1}ω7c, C_{16:0} and C_{12:0} 3-OH. The type species is *Paraperlucidibaca baekdonensis*.

Description of *Paraperlucidibaca baekdonensis* sp. nov.

Paraperlucidibaca baekdonensis (baek.do.nen'sis. N.L. fam. adj. *baekdonensis* of Baekdo, from where the type strain was isolated).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.3–0.4 μm wide and 1.0–3.5 μm long. Colonies are circular, raised, smooth, semi-transparent, yellowish white and 0.5–1.0 mm in diameter after incubation on R2A agar at 20 °C for 7 days. Optimal growth is at 20 °C; growth occurs at 10–30 °C but not at 4 or 37 °C. Optimal growth is at pH 7.5–8.0; growth occurs at pH 6.0 but not at pH 5.5. Growth occurs in the presence of 0–5% (w/v) NaCl with optimal growth in the absence of NaCl. Hydrolyses Tweens 20, 40, 60 and 80, but not aesculin, casein, gelatin, hypoxanthine, starch, L-tyrosine, xanthine or urea. Utilizes D-glucose, D-fructose, maltose, D-mannose, sucrose, trehalose, acetate, benzoate and pyruvate as carbon and energy sources, but not L-arabinose, cellobiose, D-galactose, D-xylose, citrate, L-malate, formate, salicin,

Table 2. Cellular fatty acid compositions (%) of strain RL-2^T and *Perlucidibaca piscinae* IMCC1704^T

Strains: 1, RL-2^T (grown for 6 days on R2A agar at 20 °C); 2, *Perlucidibaca piscinae* IMCC1704^T (6 days on R2A agar at 20 °C; data from this study); 3, *Perlucidibaca piscinae* IMCC1704^T (3 days on R2A agar at 30 °C; data from Song *et al.*, 2008). Fatty acids that represented <0.5 % in all columns were omitted. tr, Trace (<0.5 %). –, Not detected.

Fatty acid	1	2	3
Straight-chain			
C _{10:0}	4.6	4.2	3.4
C _{11:0}	–	0.7	–
C _{12:0}	3.1	10.2	10.1
C _{14:0}	4.6	3.7	3.1
C _{15:0}	tr	0.6	tr
C _{16:0}	12.0	12.9	5.8
C _{17:0}	–	1.1	0.7
C _{18:0}	5.7	2.7	2.7
Branched			
iso-C _{16:0}	–	1.0	–
iso-C _{17:0}	–	0.6	0.9
Unsaturated			
C _{17:1ω8c}	tr	3.7	5.5
C _{18:1ω7c}	17.3	14.3	12.8
C _{18:1ω9c}	2.6	4.6	9.2
Hydroxy			
C _{10:0} 2-OH	1.8	tr	1.5
C _{10:0} 3-OH	1.7	tr	0.6
C _{12:0} 3-OH	10.0	8.6	12.3
11 methyl C _{18:1ω7c}	1.4	1.3	–
Summed feature 3*	31.1	24.6	21.2
Unknown†			
ECL 11.799	1.4	–	–
ECL 12.484	0.7	0.7	1.4

*Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 comprised C_{16:1ω7c} and/or iso-C_{15:0} 2-OH.

†ECL, equivalent chain-length.

succinate or L-glutamate. In assays with the API ZYM system, positive for activities of esterase (C4), esterase lipase (C8) and leucine arylamidase, but negative for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. The DNA G+C content of the type strain is 61.3 mol%.

The type strain, RL-2^T (=KCTC 23145^T =CCUG 59307^T), was isolated from seawater of Baekdo Harbor in the East Sea, Korea.

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