Paraperlucidibaca wandonensis sp. nov., isolated from seawater, and emended description of the genus *Paraperlucidibaca* Oh *et al.* 2011

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A Gram-stain-negative, aerobic, motile and rod-shaped bacterial strain, designated WT-RY4^T, was isolated from wood falls in the South Sea, South Korea, and its taxonomic position was investigated using a polyphasic approach. Strain WT-RY4^T grew optimally at 25 °C, at pH 7.0–7.5 and in the absence of NaCl. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that strain WT-RY4^T clustered with the type strain of *Paraperlucidibaca baekdonensis* with a bootstrap resampling value of 100 %. Strain WT-RY4^T exhibited 16S rRNA gene sequence similarity values of 98.8 % and 96.3 % to *Paraperlucidibaca baekdonensis* RL-2^T and *Perlucidibaca piscinae* IMCC 1704^T, respectively and less than 91.5 % to the type strains of other species used in the phylogenetic analysis. The DNA G+C content of strain WT-RY4^T was 52.4 mol% and the mean DNA–DNA relatedness value with *Paraperlucidibaca baekdonensis* RL-2^T was 25 %. Strain WT-RY4^T contained Q-11 as the predominant ubiquinone and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$ and $C_{12:0}$ 3-OH as the major fatty acids. Differential phenotypic properties, together with the phylogenetic and genetic distinctiveness, demonstrated that strain WT-RY4^T was distinguishable from *Paraperlucidibaca baekdonensis* RL-2^T. On the basis of the data presented, strain WT-RY4^T is considered to represent a novel species of the genus *Paraperlucidibaca*, for which the name *Paraperlucidibaca wandonensis* sp. nov. is proposed. The type strain is WT-RY4^T (=KCTC 32216^T=CCUG 63419^T). An emended description of the genus *Paraperlucidibaca* is also provided.

The genus *Paraperlucidibaca*, a member of the class *Gammaproteobacteria*, was recently proposed by Oh *et al.* (2011) with the description of *Paraperlucidibaca baekdonensis*, the sole recognized species of the genus, which was isolated from seawater. The genus *Paraperlucidibaca* was clearly distinguished from the genus *Perlucidibaca* by differences in predominant ubiquinone type and other phenotypes, including motility, optimal growth temperature, tolerance to NaCl and anaerobic growth (Oh *et al.*, 2011). In this study, we describe a bacterial strain, designated WT-RY4^T, which was isolated from a wood fall in the South Sea, South Korea. Comparative 16S rRNA gene sequence analysis indicated that strain WT-RY4^T is most phylogenetically closely related to the genus *Paraperlucidibaca*. The aim of the present work was to carry out a taxonomic study using a polyphasic approach to investigate the possibility that strain WT-RY4^T represents a second species of the genus *Paraperlucidibaca*.

Samples from wood falls, which is wood located in the coastal sea, were collected around Wando island in the South Sea, South Korea, and used as a source for the isolation of bacterial strains. Strain WT-RY4^T was isolated by the standard dilution plating technique at 25 °C on R2A agar (BD) and cultivated routinely under the same conditions. Strain WT-RY4^T was maintained on R2A agar at 4 °C for short-term preservation and as a glycerol suspension (20 %, w/v in distilled water) at −80 °C for long-term preservation. The DNA of *Paraperlucidibaca baekdonensis* RL-2^T, which was used for DNA–DNA hybridization, was obtained from the study of Oh *et al.* (2011). Cell morphology was examined by light microscopy (BX51; Olympus) and transmission electron microscopy (JEM1010; JEOL). Flagellation was examined by using transmission electron microscopy on cells from an

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WT-RY4^T is JX994294.

A supplementary figure is available with the online version of this paper.

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 investigated using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation for 10 days in an anaerobic jar (BBL) with AnaeroPack (MGC) on MA; the jar was kept overnight at 4 °C to produce anoxic conditions before incubation at 25 °C. Growth at various temperatures (4, 10, 15, 20, 25, 30, 37 and 40 °C) was measured on R2A agar. Growth in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0 % (w/v) NaCl was investigated by using R2A broth prepared according to the formula of the BD medium except that agar was excluded. The pH range for growth was determined in R2A broth adjusted to pH 4.5–9.5 (using increments of 0.5 pH units) by using sodium acetate/acetic acid and Na_2CO_3. The pH values were verified after autoclaving. Catalase and oxidase activities were determined as described by Barrow & Feltham (1993). Hydrolysis of casein, starch, hypoxanthine, L-tyrosine and xanthine was tested on R2A agar using the substrate concentrations described by Barrow & Feltham (1993). Hydrolysis of gelatin and urea was investigated by using nutrient gelatin and urea agar base media (BD), respectively. Nitrate reduction and hydrolysis of aesculin and Tween 80 were investigated as described previously (Lányí, 1987). Utilization of various substrates for growth was tested according to the methods of Baumann & Baumann (1981), using media supplemented with 1 % (v/v) vitamin solution (Staley, 1968) and 2 % (v/v) Hutner's mineral salts (Cohen-Bazire *et al.*, 1957). The carbon sources were added at a concentration of 0.2 % (w/v) after sterilization by filtration. Enzyme activities were determined, after incubation for 8 h at 25 °C, by using the API ZYM system (bioMérieux).

Cell biomass of strain WT-RY4^T for DNA extraction and for isoprenoid quinone analysis was obtained from cultures grown for 6 days in R2A broth at 25 °C. Chromosomal DNA was extracted and purified according to the method described by Yoon *et al.* (1996), with the modification that RNase T1 was used in combination with RNase A to minimize contamination by RNA. The 16S rRNA gene was amplified by PCR as described previously (Yoon *et al.*, 1998) using two universal primers, forward primer (9F, 5′-GAGTTTGATCCTGGCTCAG-3′) and reverse primer (1512R, 5′-ACGGTTACCTTGTTACGACTT-3′). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The DNA G+C content was determined according to the method of Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The nucleotides were eluted by a mixture of 0.55 M $NH_4H_2PO_4$ (pH 4.0) and acetonitrile (40 : 1, v/v), using a flow rate of 1 ml min^{-1} at room temperature and detected by UV absorbance at 270 nm. Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987), using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. The isoprenoid quinones were eluted by a mixture of methanol/2-propanol (2 : 1, v/v) using a flow rate of 1 ml min^{-1} at room temperature and detected by UV absorbance at 275 nm. For cellular fatty acid analysis, cell mass of strain WT-RY4^T was harvested from MA plates after incubation for 6 days at 25 °C. Fatty acids were saponified, methylated and extracted using the standard protocol of the Sherlock Microbial Identification System, version 6.1 (MIDI). The fatty acids were analysed by GC (model 6890; Hewlett Packard) and identified by using the TSBA6 database of the Microbial Identification System (Sasser, 1990). DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes for cross-hybridization in microdilution wells. Hybridization was performed with five replications for each sample and the DNAs of strain WT-RY4^T and of *Paraperlucidibaca baekdonensis* RL-2^T were used as probes for reciprocal hybridization. The

Table 1. Differential phenotypic characteristics of strain WT-RY4^T and *Paraperlucidibaca baekdonensis* RL-2^T

Strains: 1, WT-RY4^T (data from this study); 2, *Paraperlucidibaca baekdonensis* RL-2^T (Oh *et al.*, 2011). Both strains are positive for oxidase activity; hydrolysis of Tween 80; utilization of acetate and pyruvate; and activity of esterase (C4), esterase lipase (C8) and leucine arylamidase (weak for WT-RY4^T). Both strains are negative for anaerobic growth; hydrolysis of aesculin, casein, gelatin, hypoxanthine, xanthine, L-tyrosine and urea; utilization of L-arabinose, cellobiose, D-galactose, D-xylose, citrate, formate, L-malate, succinate, L-glutamate and salicin; and activity of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. +, Positive reaction; −, negative reaction.

Characteristic	1	2
Motility	+	−
Catalase	+	−
Optimal growth temperature (°C)	25	20
NaCl range for growth (%)	0–2	0–5
Nitrate reduction	+	−
Utilization of:		
D-Fructose	−	+
D-Glucose	−	+
Maltose	−	+
D-Mannose	−	+
Sucrose	−	+
Trehalose	−	+
Benzoate	−	+
Enzyme activity (by API ZYM)		
Alkaline phosphatase	+	−
Acid phosphatase	+	−
Naphthol-AS-BI-phosphohydrolase	+	−
DNA G+C content (mol%)	52.4	61.3

highest and lowest values obtained for each sample were excluded and the mean of the remaining three values is quoted as the DNA–DNA relatedness value.

Morphological, cultural, physiological and biochemical characteristics of strain WT-RY4^T are given in the species description or in Table 1. The almost complete 16S rRNA gene sequence of strain WT-RY4^T comprised 1459 nt, equivalent to approximately 95 % of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree reconstructed using the neighbour-joining algorithm, strain WT-RY4^T clustered with *Paraperlucidibaca baekdonensis* RL-2^T with a bootstrap confidence value of 100 %, and this cluster joined *Perlucidibaca piscinae* IMCC 1704^T with a bootstrap confidence value of 100 % (Fig. 1). The relationships among strain WT-RY4^T, *Paraperlucidibaca baekdonensis* RL-2^T and *Perlucidibaca piscinae* IMCC 1704^T were also found in the trees reconstructed using the maximum-likelihood and the maximum-parsimony algorithms (Fig. 1). Strain WT-RY4^T exhibited 16S rRNA gene sequence similarity values of 98.8 and 96.3 % to *Paraperlucidibaca baekdonensis* RL-2^T and *Perlucidibaca piscinae* IMCC 1704^T, respectively and of less than 91.5 % to the sequences of other species used in the phylogenetic analysis. The DNA G+C content of strain WT-RY4^T was 52.4 mol%, a value lower than that (61.3 mol%) reported for *Paraperlucidibaca baekdonensis* RL-2^T.

The predominant isoprenoid quinone found in strain WT-RY4^T was ubiquinone-11 (Q-11), which is characteristic of the genus *Paraperlucidibaca* but is different from that (Q-8) of the genus *Perlucidibaca* (Song *et al.*, 2008; Oh *et al.*, 2011). In Table 2, the cellular fatty acid profile of strain WT-RY4^T was compared with that of *Paraperlucidibaca baekdonensis* RL-2^T analysed by Oh *et al.* (2011) using the cell mass obtained from a culture of similar age on the same medium at optimal growth temperature. The major fatty acids (>10 % of the total fatty acids) found in strain WT-RY4^T were summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$ and $C_{12:0}$ 3-OH (Table 2). The fatty acid profiles of strain WT-RY4^T and *Paraperlucidibaca baekdonensis* RL-2^T were similar, although there were differences in the proportions of some fatty acids (Table 2). These phylogenetic and chemotaxonomic data are likely to be sufficient to confirm that strain WT-RY4^T is a member of the genus *Paraperlucidibaca*.

Strain WT-RY4^T exhibited a mean DNA–DNA relatedness value of 25 % with *Paraperlucidibaca baekdonensis* RL-2^T. Strain WT-RY4^T was distinguishable from *Paraperlucidibaca baekdonensis* RL-2^T by differences in some

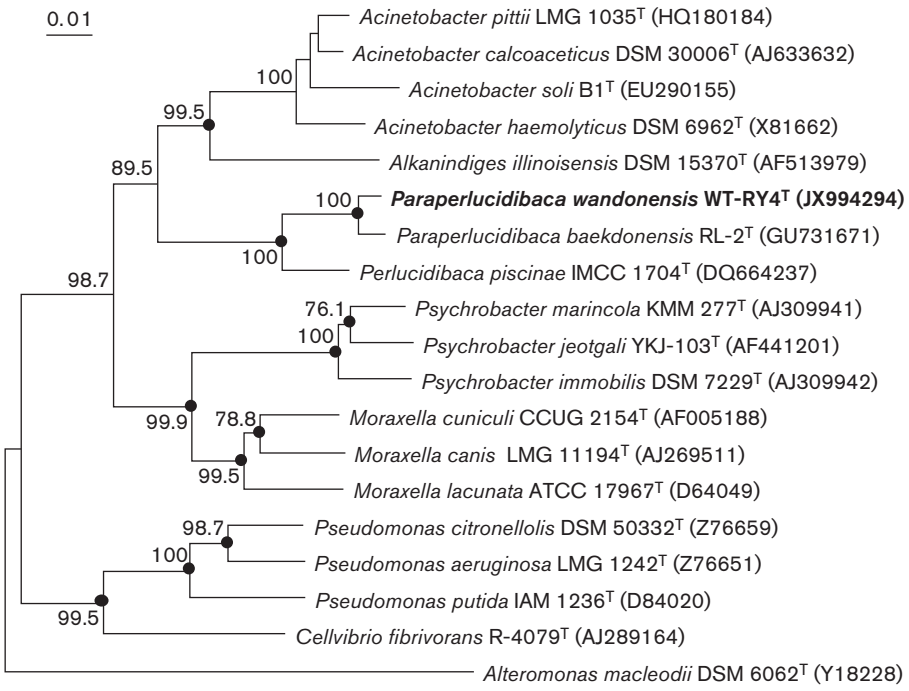

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain WT-RY4^T, *Paraperlucidibaca baekdonensis* RL-2^T and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >50 % are shown at branching 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 (GenBank accession number Y18228) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions (%) of strain WT-RY4^T and *Paraperlucidibaca baekdonensis* RL-2^T

Strains: 1, WT-RY4^T (data from this study); 2, *Paraperlucidibaca baekdonensis* RL-2^T (Oh *et al.*, 2011; 6 days on R2A agar at 20 °C). Fatty acids that represented <0.5 % in both strains were omitted. −, Not detected or not assigned; ECL, Equivalent chain-length.

Fatty acid	1	2
Straight-chain fatty acids		
$C_{10:0}$	1.4	4.6
$C_{12:0}$	8.9	3.1
$C_{14:0}$	4.0	4.6
$C_{16:0}$	21.1	12.0
$C_{18:0}$	1.9	5.7
Unsaturated fatty acids		
$C_{18:1}\omega7c$	5.0	17.3
$C_{18:1}\omega9c$	−	2.6
Hydroxy fatty acids		
$C_{10:0}$ 2-OH	2.7	1.8
$C_{10:0}$ 3-OH	−	1.7
$C_{12:0}$ 3-OH	11.2	10.0
11 methyl $C_{18:1}$ $\omega7c$	−	1.4
Summed feature 3*	43.7	31.1
Unknown fatty acids		
ECL 11.799	−	1.4
ECL 12.484	−	0.7

*Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. In the study of Oh *et al.* (2011), summed feature 3 is described as containing $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; in this study, summed feature 3 is described as containing $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$.

phenotypic properties, including motility, catalase activity, tolerance to NaCl, nitrate reduction, utilization of some substrates, activity of some enzymes and DNA G+C contents (Table 1). These differences, in combination with phylogenetic and genetic distinctiveness between strain WT-RY4^T and *Paraperlucidibaca baekdonensis* RL-2^T, are sufficient to show that the novel strain is separate from *Paraperlucidibaca baekdonensis* (Wayne *et al.*, 1987). Therefore, on the basis of the data presented, strain WT-RY4^T represents a novel species of the genus *Paraperlucidibaca*, for which the name *Paraperlucidibaca wandonensis* sp. nov. is proposed. An emended description of the genus *Paraperlucidibaca* is also provided.

Emended description of the genus *Paraperlucidibaca* Oh *et al.* 2011

The description of the genus *Paraperlucidibaca* is as given by Oh *et al.* (2011) with the following amendments. Motility is variable. Catalase activity is variable. Reduction of nitrate to nitrite is variable. The DNA G+C content is 52.4–61.3 mol%.

Description of *Paraperlucidibaca wandonensis* sp. nov.

Paraperlucidibaca wandonensis (wan.do.nen′sis. N.L. fem. adj. *wandonensis* pertaining to Wando, an island of South Korea, from where the type strain was isolated).

Cells are Gram-stain-negative, aerobic, rod-shaped, approximately 0.2–0.4 μm in diameter and 0.5–4.0 μm in length, and motile by means of a single polar flagellum (Fig. S1, available in IJSEM Online). Colonies on R2A agar are circular, slightly convex, smooth, glistening, milky white and 0.6–1.2 mm in diameter after incubation for 7 days at 25 °C. Optimal growth temperature is 25 °C; growth occurs at 4 and 30 °C, but not at 37 °C. Optimal pH for growth is between 7.0 and 7.5; growth occurs at pH 6.0 and 9.0, but not at pH 5.5 and 9.5. Growth occurs in the presence of 0–2.0 % (w/v) NaCl with optimal growth in the absence of NaCl. Catalase- and oxidase-positive. Urease-negative. Nitrate is reduced to nitrite. Tween 80 is hydrolysed, but aesculin, casein, gelatin, hypoxanthine, starch, L-tyrosine and xanthine are not. Acetate and pyruvate are utilized, but L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, sucrose, trehalose, D-xylose, benzoate, citrate, formate, L-malate, succinate, salicin and L-glutamate are not. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present and leucine arylamidase activity is weakly present, but lipase (C14), valine arylamidase, cystine arylamidae, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. The predominant ubiquinone is Q-11. The major fatty acids (>10 % of the total fatty acids) are summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{16:0}$ and $C_{12:0}$ 3-OH.

The type strain, WT-RY4^T (=KCTC 32216^T=CCUG 63419^T), was isolated from wood falls collected from coast at Wando, an island of South Korea. The DNA G+C content of the type strain is 52.4 mol% (standard deviation, 0.06).

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