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TAXONOMIC DESCRIPTION

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Solimonas fluminis sp. nov., isolated from a freshwater river

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Abstract

A strictly aerobic, catalase-negative and oxidase-positive bacterium (HR-BB^T), isolated from a water sample of the Han River, was taxonomically studied using a polyphasic approach. Cells were Gram-stain-negative motile rods with a polar flagellum. The strain grew at 20–35 °C and pH 7–8 and in the absence of NaCl. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain HR-BB^T belonged to the family *Nevskiaceae* in the phylum *Proteobacteria* and formed a phylogenic lineage with members of the genus *Solimonas*. A comparison of the 16S rRNA gene sequences of strain HR-BB^T and the type strains of closely related species of the genus *Solimonas* showed that it shared highest sequence similarity with *Solimonas terrae* KIS83-12^T (94.9 %), *Solimonas soli* DCY12^T (94.8 %), *Solimonas variicoloris* MN28^T (94.4 %) and *Solimonas flava* CW-KD 4^T (94.2 %). The fatty acids of the strain consisted of summed features 8 (comprising $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$) and 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), $C_{16:0}$ and $C_{12:0}$ as major components. The polar lipids comprised phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, three unidentified phospholipids and an unidentified lipid. Ubiquinone-8 was detected as the sole respiratory quinone. The DNA G+C content of strain HR-BB^T was 68.5 mol%. Based on the genotypic, chemotaxonomic and phenotypic analyses, strain HR-BB^T represents a novel species of the genus *Solimonas*, for which the name *Solimonas fluminis* sp. nov. is proposed. The type strain is HR-BB^T (=KACC 19410^T=JCM 32268^T).

The genus *Solimonas*, belonging to the family *Nevskiaceae* of the phylum *Gammaproteobacteria*, was first proposed by Kim *et al.* in 2008 [1, 2] with *Solimonas soli* isolate from a soil sample in a ginseng field as the type species. Three species were added to the genus *Solimonas* by Sheu *et al.* [3]. *Solimonas aquatica* was isolated from freshwater spring and *Sinobacter flavus* [4] and *Singularimonas variicoloris* [5] were reclassified as *Solimonas flava* and *Solimonas variicoloris*, respectively [3]. Subsequently, one more species, *Solimonas terrae*, was isolated from soil [6]. Members of the genus *Solimonas* are Gram-stain-negative, non-motile rods, oxidase-negative, catalase-positive and contain $C_{16:0}$ and $C_{18:1}\omega7c$ as the major fatty acids. Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and an unidentified aminophospholipid are the major polar lipids and ubiquinone-8 (Q-8) is the major isoprenoid quinone [1, 3–6]. At the time of writing, the genus *Solimonas* comprises five species with the validly published names (www.bacterio.net/solimonas.html). In this study, we isolated one more putative novel species strain belonging to the genus *Solimonas*, designated strain HR-BB^T, from a freshwater sample of the Han River, Republic of Korea (37° 30′ 33.7″ N 126° 57′ 55.4″ E), and characterized it taxonomically byn using a polyphasic approach.

For the isolation of strain HR-BB^T, a water sample was collected and serially diluted in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.2). An aliquot (100 µl) of each dilution was plated on R2A agar (BD) and incubated aerobically at 25 °C for 5 days. For long-term preservation, strain HR-BB^T was routinely cultured on R2A agar at 25 °C for 5 days, except where indicated, and stored at −80 °C in R2A broth supplemented with 15 % (v/v) glycerol.

Strain HR-BB^T was maintained and subcultivated on R2A at 30 °C for 5 days and subsequently used for 16S rRNA gene sequence analysis. The 16S rRNA gene sequence of strain HR-BB^T was amplified using the F1 (5′-AGA GTT TGA TCM TGG CTC AG-3′) and R13 (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) primer pair and subsequently sequenced using the universal primers, 340F (5′-CCT ACG GGA GGC AGC AG-3′), 518R (5′-ATT ACC GCG GCT GCT GG-3′) and 805F (5′-GAT TAG ATA CCC TGG TAG TC-3′) at Macrogen (Republic of Korea) to obtain an

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Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; Q-8, ubiquinone-8.
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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and the genome sequence of strain HR-BB^T are MF682434 and PSNW00000000, respectively.
One supplementary table and three supplementary figures are available with the online version of this article.

almost-complete 16S rRNA gene sequence [7]. The nearly full-length 16S rRNA gene sequence of strain HR-BB^T (1452 nucleotides) was compared with those of all reported type strains using the Nucleotide Similarity Search program in the EzTaxon-e server (www.ezbiocloud.net/) [8]. Reference strains for physiological and biochemical tests and fatty acid analysis (*S. terrae* KACC 16967^T, *S. soli* KACC 15377^T and *S. variicoloris* KACC 16991^T) were obtained from the Korean Agricultural Culture Collection (KACC) and were grown under the same conditions as the novel strain.

The identification of closely related taxa for the phylogenetic tree reconstructions and the calculations of pairwise 16S rRNA gene sequence similarities were accomplished by using the EzTaxon-e database [8]. The 16S rRNA gene sequences of strain HR-BB^T and closely related type strains were aligned using the fast secondary-structure aware infernal aligner in the Ribosomal Database Project (https://pyro.cme.msu.edu/aligner/form.spr) [9]. Phylogenetic relationships [neighbour-joining (NJ) and maximum-parsimony (MP) trees] of strain HR-BB^T with closely related type strains were inferred using the DNADIST and DNAPARS in the PHYLIP software (version 3.695) [10] and their tree topologies were evaluated through bootstrap analyses based on a 1000-resampled dataset. The maximum-likelihood (ML) analysis with bootstrap values was performed using RAxML-HPC BlackBox (version 8.2.9) available in the Cyber-Infrastructure for Phylogenetic Research project (www.phylo.org) [11]. The genomic DNA of strain HR-BB^T was extracted using the Wizard Genomic DNA Purification Kit (Promega) and sequenced by an Illumina HiSeq 2500 platform at Macrogen (Republic of Korea). The draft genome assembly was obtained from high-quality reads (final coverage 1414×) using SOAPdenovo2 (http://soap.genomics.org.cn/soapdenovo.html), and annotated with Prokka version 1.12 (www.vicbioinformatics.com/software.prokka.shtml). The draft assembly contained 34 contigs, with a total length of 4.67 Mb and N50 length of 321 084 bp.

The highest pairwise 16S rRNA gene sequence similarities of strain HR-BB^T were 94.9 % to *S. terrae* KIS83-12^T, 94.8 % to *S. soli* DCY12^T, 94.4 % to *S. variicoloris* MN28^T and 94.2 % to *S. flava* CW-KD 4^T. Sequence similarities to the type strains of all other bacteria with validly published names were below 93.9 %. The phylogenetic analysis using the NJ algorithm indicated that strain HR-BB^T clearly formed a phylogenic lineage within the genus *Solimonas* (Fig. 1), which was also supported by phylogenetic analyses using the ML and MP tree algorithms (Fig. S1, available in the online version of this article). It has been suggested that 98.65–98.70 % similarity of 16S rRNA gene sequences can be used as a new alternative threshold value for the requirement for DNA–DNA hybridization (DDH) in bacterial classification [12–14]. The similarities of 16S rRNA gene sequences between strain HR-BB^T and closely related type strains were clearly below the threshold value, which indicates that strain HR-BB^T at least represents a novel genotypic species of the genus *Solimonas* without the need for DDH experiments.

Phenotypic characteristics of strain HR-BB^T were investigated as follows. Growth of strain HR-BB^T was assessed at 25 °C for 5 days on several bacteriological agar media: R2A agar, Luria–Bertani agar (MP Biomedicals), nutrient agar (BD), and tryptic soy agar (BD). Growth of strain HR-BB^T at different temperatures (5–40 °C at 5 °C intervals) and different pH values (4.0–9.0 at 1.0 pH unit intervals) was tested in R2A broth. The R2A broths with pH below 5.0 and at 6.0–7.0, 8.0–9.0 were prepared using sodium citrate, Na_2HPO_4-NaH_2PO_4 and Tris-HCl buffers, respectively, according to the method of Lányí [15]. NaCl tolerance was determined in R2A broths with different NaCl concentrations (0–5 % at 1 % intervals). Gram staining was conducted by using the Gram-stain kit (bioMérieux) according to the manufacturer's instructions. Anaerobic growth was assessed on R2A agar at 25 °C for 3 weeks under the anaerobic condition (with 4–10 % CO_2) using the GasPak Plus system (BBL). Cell morphology and motility were analysed by nusing transmission electron microscopy (JEM-1010, JEOL) and phase contrast microscopy using cells grown in R2A broth at 25 °C for 3 days. The following properties of strain HR-BB^T and two reference strains were investigated in parallel under the same conditions in this study. Hydrolysis of Tween 20, Tween 80, casein, starch, tyrosine and aesculin was checked on R2A agar following the methods described by Lányí [15] and Smibert and Krieg [16]. Additional enzymatic activities, biochemical features and oxidation of carbon compounds were tested using the API ZYM and API 20NE kits (bioMérieux) and the GN2 MicroPlate system (Biolog), respectively, according to the manufacturers' instructions.

Colonies of strain HR-BB^T were white, circular, smooth and convex on R2A agar after 5 days of incubation and its cells were approximately 0.3–0.4 µm wide and 1.0–1.3 µm long (Fig. S2). Strain HR-BB^T oxidized dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, *m*-inositol, lactose, maltose, D-mannitol, β-methyl-D-glucoside, raffinose, D-sorbitol, sucrose, trehalose, methyl pyruvate, acetic acid, *cis*-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, β-hydroxy butyric acid, α-keto glutaric acid, D,L-lactic acid, propionic acid, quinic acid, D-saccharic acid, bromo succinic acid, glucuronamide, L-alaninamide, D-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, hydroxyl-L-proline, L-proline, D-serine, L-serine, inosine, thymidine, glycerol, D,L-α-glycerol phosphate, glucose-1-phosphate, glucose-6-phosphate, phenylethylamine, 2,3-butanediol, *N*-acetyl-D-galactosamine, L-arabinose, D-arabitol, D-mannose, melibiose, L-rhamnose, turanose, D-glucuronic acid, *p*-hydroxy phenylacetic acid, itaconic acid, succinic acid, L-alanine, L-histidine, L-ornitine, L-pyroglutamic acid, D,L-carnitine, γ-amino butyric acid, urocanic acid, uridine, putrescine,

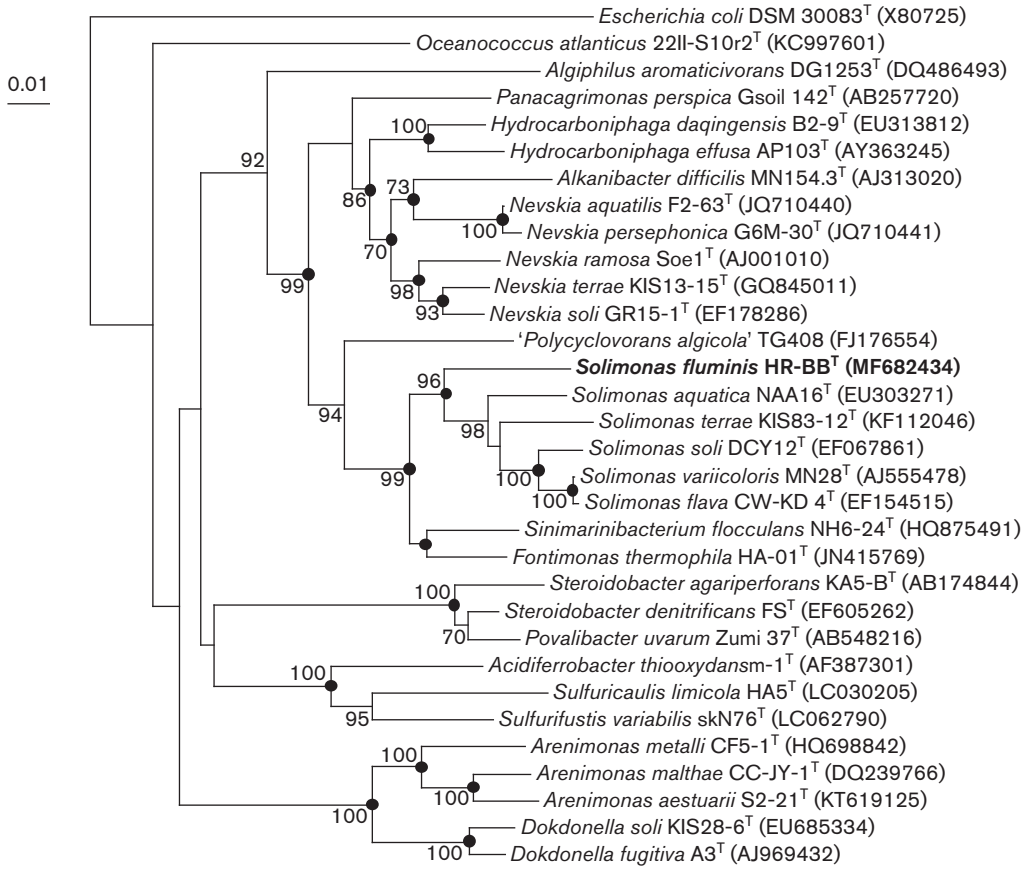

Fig. 1. An NJ tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain HR-BB^T and related taxa. Bootstrap values with more than 70 % are shown on the nodes as percentages of 1000 replicates. Filled circles (●) indicate that the corresponding nodes were also recovered in the trees reconstructed by the ML and MP algorithms. *Escherichia coli* DSM 30083^T (X80725) was used as an outgroup. Bar, 0.01 changes per nucleotide position.

2-aminoethanol and mono-methyl-succinate, but did not oxidize other carbon compounds in the Biolog GN2 MicroPlate (Table S1). Many characteristics of HR-BB^T such as Gram reaction, catalase and oxidase activities, and nitrogen production in nitrate reduction were in good agreement with those of the reference strains, whereas other properties such as colony colour, motility, indole production and glucose fermentation and many other phenotypic properties allowed differentiation of strain HR-BB^T from other closely related *Solimonas* species (Table 1). Phenotypic characteristics of strain HR-BB^T are presented in the species description and compared with those of the closely related type strains in Tables 1 and S1.

Isoprenoid quinones of strain HR-BB^T were analysed with an high-performance liquid chromatography (model LC-20A, Shimadzu) system equipped with a reversed-phase column (250 Í 4.6 mm, Kromasil, Akzo Nobel) and a diode array detector (SPD-M20A, Shimadzu) using methanol–isopropanol (2 : 1, v/v) as an eluent (1 ml min^{-1}), as described by Komagata and Suzuki [17]. For the analysis of cellular fatty acids, strain HR-BB^T and reference strains were cultivated in R2A broth at 25 °C. The fatty acids were saponified, methylated, extracted and washed using the standard MIDI protocol and the fatty acid methyl esters were analysed by using a gas chromatography system (model 6890; Hewlett Packard) based on the TSBA6 database in the Microbial Identification System (Sherlock version 6.2B) [18]. The polar lipids were analysed by thin-layer chromatography using cells harvested at the exponential growth phase according to the method described previously [19]. The following reagents were used to detect different polar lipids: 10 % ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer–Lester reagent (for phospholipids) and α-naphthol (for glycolipids). The DNA G+C content of strain HR-BB^T was calculated using the EditSeq module of the Lasergene package based on its genome sequence.

Q-8 was detected as the sole respiratory quinone, which was in line with all other members of the genus *Solimonas* [1–6]. Major fatty acids (>5 %) of strain HR-BB^T were summed

Table 1. Phenotypic comparisons of strain HR-BB^T and the type strains of closely related *Solimonas* species

Taxa: 1, strain HR-BB^T; 2, *S. terrae* KACC 16967^T [6]; 3, *S. soli* KACC 15377^T [1]; 4, *S. variicoloris* KACC 16991^T [3]. All strains are positive for the following characteristics: anaerobic growth, activity* of alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phospatase, naphtol-AS-BI-phosphohydrolase and β-galactopyranosidase, assimilation* of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, adipic acid and trisodium citrate, and hydrolysis* of aesculin. All strains are negative for the following characteristics: Gram-staining, nitrogen production in nitrate reduction*, activity* of urease, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase, and hydrolysis* of casein, tyrosine, starch and Tween 20. +, Positive; –, negative.

Characteristic	1	2	3	4
Isolation source	River	Soil	Soil	Industrial biofilter
Colony colour	White	Light yellow	Light yellow	Yellow
Cell morphology	Rod	Long rod	Rod	Long rod
Catalase activity	–	–	+	+
Oxidase activity	+	+	–	+
Indole production	+	+	–	–
Glucose fermentation	+	+	–	–
Motility	+	+	–	–
Temperature range for growth (°C)	25–35	15–33	25–37	20–42
Growth on 1 % NaCl (w/v)	–	–	+	+
Hydrolysis* of:				
Gelatin	+	+	–	+
Tween 80	+	–	+	–
Assimilation (API 20NE)* of:				
Capric acid	+	+	–	–
Malic acid and phenylacetic acid	+	+	–	+
Enzyme activity (API ZYM)* of:				
Lipase (C14)	+	–	–	–
Cystine arylamidase	–	+	–	–
Arginine dihydrolase	+	+	–	–
Leucine arylamidase and valine arylamidase	–	–	+	+
β-Glucosidase	+	+	–	+
DNA G+C content (mol%)	68.5†	67.9	68.5†	64.9

*These analyses were conducted under the same conditions in this study.

†The DNA G+C contents were calculated based on their genomes in this study.

features 8 (comprising $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$, 40.4 %) and 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$, 17.3 %), $C_{16:0}$ (10.2 %) and $C_{12:0}$ (6.1 %). The detection of $C_{16:0}$ and $C_{18:1}\omega7c$ (summed feature 8) as the major fatty acids was in good agreement with the previous reports, but summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and $C_{12:0}$ were also detected as major fatty acids, which allowed the differentiation of strain HR-BB^T from other closely related *Solimonas*

Table 2. Cellular fatty acid compositions (%) of strain HR-BB^T and the type strains of closely related *Solimonas* species

Taxa: 1, strain HR-BB^T; 2, *S. terrae* KACC 16967^T; 3, *S. soli* KACC 15377^T; 4, *S. variicoloris* KACC 16991^T. All data were from this study. The data are expressed as percentages of the total fatty acids and fatty acids amounting to less than 0.5 % in all strains are not shown. Major fatty acid components (>5.0 %) are highlighted in bold. –, not detected; TR, trace amount (<0.5 %).

Fatty acid	1	2	3	4
Saturated:				
$C_{9:0}$	TR	0.5	TR	–
$C_{10:0}$	–	1.0	–	TR
$C_{12:0}$	**6.1**	4.2	4.8	**9.3**
$C_{14:0}$	2.3	4.1	**6.0**	**11.6**
$C_{16:0}$	**10.2**	**10.9**	**16.0**	**9.4**
$C_{17:0}$	TR	TR	0.9	–
$C_{18:0}$	0.9	0.7	0.5	TR
$C_{20:0}$	0.5	0.5	TR	–
Branched:				
iso-$C_{10:0}$	2.1	4.6	1.6	–
iso-$C_{12:0}$	–	1.3	–	2.3
iso-$C_{14:0}$	TR	1.5	TR	TR
iso-$C_{16:0}$	0.5	**8.6**	0.5	1.7
Hydroxy:				
$C_{8:0}$ 3-OH	0.7	1.4	0.6	–
$C_{12:0}$ 2-OH	2.1	–	3.5	1.2
$C_{12:0}$ 3-OH	4.6	1.1	0.7	0.9
$C_{14:0}$ 2-OH	–	1.0	–	–
$C_{16:0}$ 2-OH	–	0.5	0.6	–
iso-$C_{11:0}$ 3-OH	TR	0.8	TR	–
iso-$C_{14:0}$ 3-OH	–	1.1	–	0.6
iso-$C_{17:0}$ 3-OH	0.5	0.9	TR	0.7
Unsaturated:				
$C_{12:1}$	0.5	1.0	TR	–
$C_{16:1}\omega5c$	0.5	3.6	3.7	4.8
$C_{16:1}\omega11c$	–	2.3	TR	2.4
$C_{17:1}\omega6c$	TR	–	0.8	–
$C_{18:1}\omega5c$	TR	0.7	0.8	TR
$C_{18:1}\omega9c$	1.4	0.8	–	–
$C_{20:2}\omega6,9c$	–	TR	1.4	–
iso-$C_{17:1}\omega5c$	TR	0.7	TR	–
iso-$C_{18:1}$ H	TR	1.8	TR	TR
11-methyl $C_{18:1}\omega7c$	–	0.8	2.9	–
cyclo-$C_{19:0}\omega8c$	–	TR	4.0	–
Summed features*:				
2	4.0	4.2	**10.4**	**10.2**
3	**17.3**	2.5	4.5	**5.8**
5	–	0.5	TR	–
7	–	TR	0.7	–
8	**40.4**	**30.2**	**28.0**	**36.1**

*Summed features represent groups of two or three fatty acids that cannot be separated by gas–liquid chromatography with the MIDI system. Summed feature 2, $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; summed feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

species (Table 2). The DNA G+C content of strain HR-BB^T was 68.5 mol%, which is in the range of DNA G+C contents in *Solimonas* species [1–6]. Polar lipids of strain HR-BB^T contained phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, three unidentified phospholipids and an unidentified lipid (Fig. S3). The presence of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and three unidentified phospholipids was in good agreement with those of closely related *Solimonas* species [3, 6], but the absence of amino phospholipids in strain HR-BB^T allowed the differentiation of the strain from other closely related *Solimonas* species.

In conclusion, the phenotypic and chemotaxonomic features of strain HR-BB^T and its phylogenetic inference support its assignment to a novel species of the genus *Solimonas,* for which the name *Solimonas fluminis* sp. nov. is proposed.

DESCRIPTION OF *SOLIMONAS FLUMINIS* SP. NOV.

Solimonas fluminis (flu′mi.nis. L. gen. n. *fluminis* of a river).

Cells are Gram-stain-negative, strictly aerobic, catalase-negative, oxidase-positive and motile rods with a polar flagellum. Growth occurs at 20–35 °C (optimum, 25–30 °C), in the absence of NaCl and at pH 7.0–8.0. Aesculin, gelatin and Tween 80 are hydrolysed, but casein, tyrosine, starch and Tween 20 are not. Reduces nitrate to nitrite, but does not produce nitrogen. Indole is produced. Glucose is fermented. Alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactopyranosidase, β-glucosidase and arginine dihydrolase activities are positive, but urease, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, cystine arylamidase, leucine arylamidase and valine arylamidase activities are negative. Assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, adipic acid, trisodium citrate, capric acid, malic acid and phenylacetic acid is positive. Major fatty acids are summed features 8 (comprising $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$) and 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), $C_{16:0}$ and $C_{12:0}$. The polar lipids comprise phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, three unidentified phospholipids and an unidentified lipid. Only Q-8 is the respiratory quinone detected.

The type strain is HR-BB^T (=KACC 19410^T=JCM 32268^T), isolated from a freshwater river, the Han River, in the Republic of Korea. The DNA G+C content is 68.5 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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