Pedobacter aquae sp. nov., a multi-drug resistant bacterium isolated from fresh water

Le Tran Tien Chau · Yong-Seok Kim · Chang-Jun Cha

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Abstract  A novel bacterial strain designated CJ43T was isolated from fresh water located in Gangwon-do, South Korea, displaying multi-drug resistance. The isolate was Gram-stain-negative, aerobic, orange-pigmented, and rod-shaped. Strain CJ43T grew optimally at 30 ºC and pH 7 on R2A agar in the absence of NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain CJ43T belonged to the genus Pedobacter in the family Sphingobacteriaceae and was most closely related to Pedobacter puniceum HX-22-1T and P. glucosidilyticus 1-2T (98.3 and 98.1% sequence similarity). The genome size of strain CJ43T was 3.9 Mb in a single contig with DNA G+C content of 34.9%. The genome included 3144 predicted protein-coding genes, as well as 55 tRNA, 9 rRNA and 3 ncRNA genes. The genome also contained 128 putative antibiotic resistance genes, reflecting its phenotypes. The average nucleotide identity values between strain CJ43T and two closely related strains P. puniceum HX-22-1T and P. glucosidilyticus 1-2T were 91.0 and 88.7%, respectively. In silico digital DNA-DNA hybridization results between strain CJ43T and the related strains were 42.8 and 38.6%, respectively. The major fatty acids of strain CJ43T were iso-C15:0, iso-C17:0 3-OH, and summed feature 3 (C16:1 ω6c and/or C16:1 ω7c). Strain CJ43T contained phosphatidylethanolamine as the major polar lipid and menaquinone-7 as the sole respiratory quinone. Based on the polyphasic taxonomy data, strain CJ43T represents a novel species of the genus Pedobacter, for which the name Pedobacter aquae sp. nov. is proposed with the type strain CJ43T (= KACC 21350T = JCM 33709T).

Keywords  Fresh water · Multidrug resistance · Pedobacter · Polyphasic taxonomy · Sphingobacteriaceae · Whole genome sequencing

Abbreviations
ANI  Average Nucleotide Identity
dDDH  Digital DNA-DNA hybridization
ML  Maximum-likelihood
MP  Maximum-parsimony
NJ  Neighbor-joining
SMRT  Single Molecule Real-Time
UBCG  Up-to-date bacterial core-gene

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Introduction

The genus Pedobacter, first proposed by Steyn et al. (1998), belongs to the family Sphingobacteriaceae. At the time of writing, 95 species in the genus Pedobacter are currently listed in the List of Prokaryotic names with Standing in Nomenclature (Parte et al. 2020) with validly published names. As suggested by the name, Pedobacter species have been predominantly isolated from soils (Steyn et al. 1998; Luo et al. 2010; Singh et al. 2015; Yang and Hong 2017; Cui et al. 2018; Hu et al. 2019). Several members of the genus Pedobacter were also recovered from various environmental habitats, including water (Gallego et al. 2006; Baik et al. 2007; An et al. 2009; Urios et al. 2013; Chun et al. 2014; Kang et al. 2014; Joung et al. 2018), plant rhizosphere (Kwon et al. 2007, 2011), sediment (Gordon et al. 2009), activated sludge (Zhang et al. 2018; Yang et al. 2020), glaciers (Shivaji et al. 2005; Qiu et al. 2014), and arctic tundra soil (Zhou et al. 2019). Pedobacter members are obligately Gram-stain-negative, oxidase- and catalase-positive, but negative for urease activity, nitrate reduction, and indole production (Gallego et al. 2006). Menaquinone-7 (MK-7) is the predominant respiratory quinone and phosphatidylethanolamine is the major polar lipid (Steyn et al. 1998; Hu et al. 2019). The major fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH, and summed feature 3 (C_{16:1} ω6c and/or C_{16:1} ω7c) (Hu et al. 2019). DNA G+C contents range from 33 to 45 mol% (Kang et al. 2014). Furthermore, Pedobacter spp. are considered multidrug-resistant environmental bacteria and excellent model organisms for studies on antibiotic resistance (Viana et al. 2018; Ullmann et al. 2020; Bjerketorp et al. 2021). In the present study, we established the taxonomic position of strain CJ43^T, isolated from fresh water at Gangwon-do, South Korea, using polyphasic taxonomy and genomics approaches.

Materials and methods

Isolation and maintenance

In May 2018, an environmental bacterial strain, designated CJ43^T, was isolated from a fresh water sample of the Han River at Gangwon-do, South Korea (N37.483472, E128.657556), using the method described by Lee et al. (2020). Briefly, environmental antibiotic-resistant bacteria were isolated by the dilution-plating method on Mueller–Hinton agar (BD) containing 8 mg/L gentamicin (Sigma-Aldrich). Colonies were individually streaked on Mueller–Hinton agar and cultured at 30 °C, and the obtained pure culture was preserved at −80 °C with glycerol suspension (30%, w/v).

Phylogenetic analysis

The 16S rRNA gene of strain CJ43^T was amplified by PCR using the universal primers 27F, and 1492R for bacteria (Frank et al. 2008). The PCR product was purified and sequenced at Biofact (Daejeon, South Korea) using the sequencing primers 27F, 1492R, 785F, and 805R (Baker et al. 2003). The 16S rRNA gene sequence of strain CJ43^T was aligned using the multiple sequence alignment program clustal W (Thompson et al. 1994) with those of related type strains obtained from the EzBioCloud database (www.ezbiocloud.net) (Yoon et al. 2017). Phylogenetic analysis was conducted using MEGA X version 10.1.7 (Kumar et al. 2018). Evolutionary distances were calculated for the neighbor-joining (NJ) tree (Saitou and Nei 1987) using the Jukes-Cantor model (Jukes and Cantor 1969). The best-fit substitution model for the maximum-likelihood (ML) tree was determined to be Kimura two-parameter model (Kimura 1980) by model test option of MEGA X version 10.1.7 (Kumar et al. 2018). The maximum-parsimony (MP) tree was obtained using the subtree pruning and regrafting algorithm (Nei and Kumar 2000). The topology of NJ, ML, and MP trees were evaluated based on the bootstrap resampling method with 1000 replications (Felsenstein 1985). Four closely related type strains identified from the 16S rRNA phylogenetic tree were used as reference strains for phenotypic tests and chemotaxonomic analyses.

Physiological characterisation

Strain CJ43^T was examined to find out optimal media for growth on different media such as R2A agar (BD), tryptic soy agar (TSA, BD), Luria–Bertani (LB) agar (BD), nutrient agar (NA, BD), and marine agar (MA, BD) at 30 °C. The optimum growth temperature was determined at 4, 15, 20, 25, 30, and 37 °C on R2A agar. The pH range for growth was tested using
different pH buffers in R2A broth (MB cell), which were 0.1 M citrate buffer for pH 5, 0.2 M phosphate buffer for pH 6–8, and 0.1 M bicarbonate-carbonate buffer for pH 9. Salt tolerance was tested in R2A broth supplemented with 0–3% (w/v) NaCl (at 1% intervals). Cellular morphology of strain CJ43\(^T\) was observed by transmission electron microscopy (JEM 1010; JEOL) using three-day cultured cells on R2A agar at 30 °C. Gliding motility was tested by stab culture in semi-solid R2A medium [0.4% (w/v) agar]. Anaerobic growth was determined after two weeks of cultivation at 30 °C on R2A agar using the GasPak Anaerobe Pouch System (BD). Gram staining was carried out using a Gram-staining kit according to the manufacturer’s protocols (Sigma-Aldrich). Oxidase activity was assessed using oxidase reagent (bioMérieux) and catalase activity was evaluated by bubble production in a 3% (v/v) H\(_2\)O\(_2\) solution on several fresh colonies. Hydrolysis of starch, cellulose, casein, and deoxyribonucleic acid were tested using 1% (w/v) soluble starch, 0.2% (w/v) carboxymethyl cellulose, 3% (w/v) skimmed milk, and deoxyribonuclease (DNase) test agar (BD), respectively, after seven days of incubation, following methods described by Reichenbach (2006). The reactions in API 20NE, API ZYM, and API 50CH strips (bioMérieux) were incubated at 30 °C and assessed following the manufacturer’s instructions.

Chemotaxonomic analyses

The respiratory quinone and polar lipids were extracted and analysed from freeze-dried cells following methods described by Minnikin et al. (1984). Iso-prenoid quinone was extracted using hexane:methanol (1:2, v/v) in darkness, evaporated using a vacuum rotary evaporator, separated by thin-layer chromatography (TLC) using petroleum ether:acetone (95:5, v/v), and detected by UV absorbance at 254 nm. The purified quinones were detected by high performance liquid chromatography (Collins and Jones 1981). The polar lipids of strain CJ43\(^T\) were analysed by two-dimensional TLC using chloroform:methanol:water (65:25:4, v/v/v) for the first-dimension solvent and chloroform:methanol:acetic acid:water (80:12:15:4, v/v/v/v) for the second-dimension solvent. Appropriate detection reagents were used to identify the spots; 5% (w/v) phosphomolybdic acid, 0.25% (w/v) ninhydrin, molybdenum blue spray reagent (Sigma-Aldrich), and 15% (w/v) α-naphthol reagent were used to detect total polar lipids, amino lipids, phospholipids, and glycolipids, respectively (Costa et al. 2011). To carry out whole cell fatty acid methyl esters analysis, strain CJ43\(^T\) and the related type strains were grown on R2A at 30 °C and harvested at the mid-exponential phase. Cells were saponified, methylated, and extracted following the instruction of the standard Microbial Identification system version 6.1. The fatty acids were analysed by gas chromatography (HP 6890 Series GC System; Hewlett Packard) and identified using the RTSBA6 6.10 database of the Microbial Identification system (Sasser 1990).

Whole genome sequencing and genomic analyses

The genomic DNA of strain CJ43\(^T\) was extracted using DNeasy Powersoil Kit (Qiagen) according to the manufacturer’s instructions. Thereafter, the whole genome sequencing was performed using the PacBio RS II (Pacific Biosciences) Single Molecule Real-Time (SMRT) platform with 20 kb SMRTbell\(^TM\) template library. 71,240 sequencing reads were obtained and assembled de novo using the Hierarchical Genome Assembly Process implemented in the PacBio SMRT analysis software version 2.3.0. The up-to-date bacterial core-genes 2 (UBCG2) and the UBCG2 phylogenomic pipeline (Kim et al. 2021) were used for the reconstruction of phylogenomic tree. 81 UBCGs were concatenated for alignments and the phylogenomic tree was inferred by the approximate ML method using FastTree (version 2.1.3) (Price et al. 2010). The Genome-to-Genome Distance Calculator 3.0 (http://ggdc.dsmz.de/distcalc2.php) (Meier-Kolthoff et al. 2014) was used to assess digital DNA-DNA hybridization (dDDH). Average nucleotide identity (ANI) values between genomic sequences of strain CJ43\(^T\) and its closest phylogenomic neighbors were calculated using the OrthoANI tools (Lee et al. 2016). Reference genome sequences were downloaded from the EzBioCloud database (www.ezbiocloud.net) (Yoon et al. 2017) and the NCBI genome database (https://www.ncbi.nlm.nih.gov/genome). Annotation was performed using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline software revision 4.11 (Tatusova et al. 2016), and NCBI reference sequences (Haft et al. 2018). PRODIGAL version 2.6.3 (Hyatt et al. 2010) was
used for finding protein-coding sequences (CDSs). Functional annotations were performed against various databases, including Clusters of Orthologous Groups (COGs) categories based on the COG database (Tatusov et al. 2000), SEED subsystem (Overbeek et al. 2014), and Kyoto Encyclopedia of Genes and Genomes pathway database (Kanehisa and Goto 2000). Pan-genome and core-genome analyses of seven genome sequences of Pedobacter species including CJ43T were performed using PIRATE v1.0.4 (Bayliss et al. 2019). Venn diagram was visualized in R using package venn (Dusa 2021). Antibiotic resistance genes were determined using the comprehensive antibiotic resistance database (CARD) (Jia et al. 2017).

Antimicrobial susceptibility testing
The antibiotic susceptibility of strain CJ43T was evaluated by the disk diffusion assay using antibiotic impregnated disks (Liofilchem, Italy) with amoxicillin (10 μg), cephalaxin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), colistin sulfate (10 μg), fosfomycin (200 μg), gentamicin (10 μg), linezolid (10 μg), meropenem (10 μg), rifampicin (5 μg), streptomycin (10 μg), sulfamethoxazole (50 μg), tetracycline (30 μg), and trimethoprim (5 μg). Susceptibility results were recorded as susceptible (S) or resistant (R) based on breakpoints for Escherichia coli according to the EUCAST QC breakpoint table (version 11.0) (www.eucast.org/clinical_breakpoints). E. coli ATCC 25,922 was used as a quality control strain.

Results and discussion
Isolation and 16S rRNA phylogeny

Strain CJ43T displaying antibiotic resistance against gentamicin was isolated from a freshwater sample of the Han River. The comparison of the 16S rRNA gene sequence of strain CJ43T with those of the related type strains of bacterial species available from the EzBioCloud database revealed that strain CJ43T was affiliated with the genus Pedobacter. As inferred from the phylogenetic tree (Fig. 1), strain CJ43T formed a robust cluster with Pedobacter puniceum HX-22-1T and Pedobacter glucosidilyticus 1-2T, which had the high 16S rRNA gene sequence similarities (98.3% and 98.1%, respectively), followed by P. daechungensis Dae 13T (94.9%). Based on the observed pairwise 16S rRNA gene sequence identities below the established threshold (Stackebrandt and Ebers 2006; Kim et al. 2014; Rosselló-Móra and Amann 2015), strain CJ43T represents a novel species within the genus Pedobacter.

Genomic features
The final assembly of strain CJ43T resulted in a single chromosomal contig, corresponding to a genome size of 3,854,044 bp. No plasmid sequence was found. The predicted genes were 3144 CDSs, 55 tRNA genes, 9 rRNA genes (including three genes from each of 5S, 16S, and 23S rRNA), and 3 ncRNA genes. The genomic DNA G+C content of strain CJ43T was 34.9%, which was within the G+C content range (33–45 mol%) of the genus Pedobacter (Kang et al. 2014). The ML phylogenomic tree of strain CJ43T and 52 Pedobacter type strains was reconstructed using the concatenated sequences of 81 UBCGs (Fig. 2). Phylogenomic analysis revealed that strain CJ43T was most closely related to P. puniceum HX-22-1T. To further establish the phylogenomic relationship of strain CJ43T with other Pedobacter strains, we compared the whole genome of strain CJ43T to other available genomes of related type strains of Pedobacter by calculating dDDH and ANI values. The in silico dDDH values between strain CJ43T and P. puniceum HX-22-1T, P. glucosidilyticus 1-2T, P. cryophilus AR-3-7T, P. arcticus A12T, and P. psychrophilus P4487AT were 42.8%, 38.6%, 18.9%, 19.5%, and 19.1%, respectively, which were clearly below the 70% dDDH threshold for species delineation (Chun et al. 2018). ANI values of strain CJ43T with the related type strains mentioned above were 91.0, 88.7, 73.5, 71.7, and 72.5%, respectively, which were clearly below the 70% dDDH threshold for species delineation (Chun et al. 2018). ANI values of strain CJ43T with the related type strains mentioned above were 91.0, 88.7, 73.5, 71.7, and 72.5%, respectively, which also exhibited values lower than the threshold of 95–96% ANI for species demarcation at the genomic level (Richter and Rosselló-Móra 2009). These data indicated that strain CJ43T should be considered as a separate species of the genus Pedobacter.

For comparative genomic analysis, the whole genome sequence of strain CJ43T was compared with those of six Pedobacter species whose genome sequences are available: P. puniceum HX-22-1T, P. glucosidilyticus 1-2T, P. arcticus A12T, P.
Fig. 1 Maximum-likelihood phylogenetic tree of strain CJ43\textsuperscript{T} and related type strains based on 16S rRNA gene sequences. Closed circles indicate nodes recovered in all three trees generated by neighbor-joining, maximum-parsimony, and maximum-likelihood methods. Bootstrap values greater than 70% are shown at branch points based on maximum-likelihood analysis of 1000 replicated datasets. Sphingobacterium spiritivorum NCTC 11386\textsuperscript{T} was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.
Fig. 2 Maximum-likelihood phylogenomic tree inferred using UBCGs showing the position of strain CJ43T and related type strains within the genus Pedobacter. The number of single gene trees supporting a branch in a UBCG tree is calculated and designated the gene support index (GSI). The GSIs are given at branching points. Sphingobacterium spiritivorum NCTC 11386T was used as an outgroup. Bar, 0.1 substitutions per nucleotide position.
psychrophilus P4487AT, P. cryophilus AR-3-17T, and P. heparinus DSM 2366T. General genomic features of strain CJ43T and these strains were summarized in Table 1. The Venn diagram of the seven compared species showed that the core-genome of the Pedobacter species included 1227 genes and 358 unique genes from strain CJ43T were not shared with other compared species (Fig. S1). A majority of these unique genes encode proteins of unknown function. Strain CJ43T contained crtB, crtI, and crtY genes encoding phytoene synthase, phytoene desaturases, and lycopene cyclase, respectively. These enzymes are involved in the carotenoid biosynthesis, which might be responsible for the orange color of this organism. In addition, 128 antibiotic resistance genes, including a variety of resistance genes against β-lactam, aminoglycoside, tetracycline, macrolide, lincosamide, rifamycin, glycopeptide, sulfonamide, amphenicol, and fluoroquinolone antibiotics, were identified in the genome of strain CJ43T after search against CARD (Jia et al. 2017). These genotypic results coincided with the previous findings that most of environmental Pedobacter species were multidrug-resistant (Viana et al. 2018; Ullmann et al. 2020).

Phenotypic and biochemical features

Strain CJ43T was Gram-stain-negative, orange-pigmented, aerobic, nonmotile, rod-shaped, 0.4 µm in width, and 1.5 µm in length (Fig. S2). Bacterial colonies were approximately 0.5–1 mm in diameter, light orange, smooth, circular and convex with regular edges after three days of cultivation on R2A agar at 30 °C. Strain CJ43T grew well on R2A agar, TSA, LB agar, and NA but not on MA. Optimal growth occurred at 30 °C and pH 7 in the absence of NaCl. Detailed biochemical and physiological characteristics of strain CJ43T that differentiated from closely related type strains (P. puniceum HX-22-1T, P. glucosidilyticus 1-2T, P. rivuli HME8457T, and P. pituitosus MIC2002T) are summarized in Table 2.

The only respiratory quinone identified in strain CJ43T was menaquinone-7 (MK-7), which is in line with those of other Pedobacter species. The polar lipids of strain CJ43T contained phosphatidylethanolamine, two unidentified amino lipids and four unidentified lipids (Fig. S3). Strain CJ43T shared the same major polar lipid with most members of the genus Pedobacter. The presence and absence of some unidentified aminolipids and lipids varied among Pedobacter species. Glycolipids were not detected in strain CJ43T. As the results shown in Table 3, the major cellular fatty acids of strain CJ43T were iso-C15:0 (29.5%), summed feature 3 (C16:1ω6c and/or C16:1ω7c) (17.7%), and iso-C17:0 3-OH (11.2%), which were similar to those of related type strains of Pedobacter species with only minor differences.

Strain CJ43T showed resistance phenotypes to gentamicin, streptomycin, fosfomycin, and colistin (Table S1). These results were consistent with the presence of several antibiotic resistance genes related to these antibiotics in the genome of strain CJ43T. Some phenotypic results, such as resistance to amoxicillin, cephalaxin, chloramphenicol, ciprofloxacine, clindamycin, meropenem, rifampicin, sulfamethoxazole, tetracycline and trimethoprim, did not coincide with the related genotypes, which may be because the

| Genomic features of strain CJ43T and related type strains | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---------------------------------------------------------|---|---|---|---|---|---|---|
| Genome size (Mb)                                         | 3.9 | 3.9 | 4.0 | 4.2 | 4.0 | 4.0 | 5.2 |
| Number of contigs                                        | 10 | 62 | 16 | 33 | 16 | 1 |
| G+C content (%)                                          | 34.9 | 34.3 | 34.3 | 36.9 | 32.5 | 33.9 | 42.0 |
| Protein coding genes                                     | 3144 | 3470 | 3577 | 3518 | 3432 | 4266 |
| tRNA genes                                              | 55 | 43 | 41 | 39 | 34 | 40 | 45 |
| rRNA genes                                              | 9 | 5 | 7 | 3 | 3 | 5 | 9 |
| ANI (%)a                                                 | 91.0 | 88.7 | 71.7 | 72.5 | 73.5 | 67.9 |
| dDDH (%)b                                                | 42.8 | 38.6 | 19.5 | 19.1 | 18.9 | 20.1 |

a,b Data were calculated between strain CJ43T and each of Pedobacter species.
### Table 2  Differential characteristics of strain CJ43\textsuperscript{T} and related type strains of the genus *Pedobacter*

| Characteristics                     | 1  | 2  | 3  | 4  | 5  |
|-------------------------------------|----|----|----|----|----|
| **Isolation source**                | Fresh water from river | Sludge | Soil from riverbed | Fresh water from stream | Waterfall |
| **Cell shape**                      | Rod | Rod | Rod | Rod | Rod |
| **Growth**                          |    |    |    |    |    |
| Temperature (°C)                    | 20–37 | 15–37 | 15–30 | 15–30 | 20–37 |
| pH                                  | 6–8 | 6–8 | 6–7 | 5–7 | 6–7 |
| NaCl (%, w/v)                       | 0–2 | 0–1 | 0–2 | 0–1 | 0–2 |
| **Hydrolysis of**                  |    |    |    |    |    |
| DNA                                 | +  | –  | + (−) | – | – |
| Starch                              | +  | –  | + | – | – |
| Carboxymethyl cellulose             | +  | –  | + | – | – |
| Gelatin (bovine origin)             | –  | –  | – | + | – |
| **Assimilation**                    |    |    |    |    |    |
| d-glucose                           | +  | –  | + | – | – |
| l-arabinose                         | +  | –  | + | – | – |
| d-mannose                           | +  | –  | + | – | – |
| N-acetyl glucosamine                | +  | –  | + | – | – |
| d-maltose                           | +  | –  | – | – | – |
| **Enzyme activities**               |    |    |    |    |    |
| Alkaline phosphatase                | +  | –  | + | – | – |
| Lipase (C14)                        | +  | –  | + | – | – |
| Valine arylamidase                  | +  | –  | + | – | – |
| Crystine arylamidase                | +  | –  | + | – | – |
| Trypsine                            | –  | +  | – | – | – |
| β-galactosidase                     | +  | –  | + | – | – |
| α-glucosidase                       | +  | –  | + | – | – |
| β-glucosidase                       | +  | –  | + | – | – |
| N-acetyl-β-glucosaminidase          | +  | –  | + | – | – |
| α-fucosidase                        | –  | +  | – | – | – |
| **Acid production from**            |    |    |    |    |    |
| Glycerol                            | +  | –  | + | – | – |
| Erythritol                          | –  | –  | – | + | – |
| d-Arabinose                         | +  | –  | + | – | – |
| l-Arabinose                         | +  | –  | + | – | – |
| d-Ribose                            | –  | +  | – | + | + |
| d-Xylose                            | +  | –  | + | – | – |
| d-Adonitol                          | –  | –  | – | + | – |
| Methyl β-d-xylopyranoside           | –  | –  | – | + | – |
| d-Galactose                         | +  | –  | + | – | – |
| d-Glucose                           | +  | –  | + | – | – |
| d-Fructose                          | +  | –  | + | – | – |
| d-Manose                            | +  | –  | + | – | – |
| Rhamnose                            | +  | –  | + | – | – |
| Methyl α-d-mannoside                | –  | –  | + | – | – |
| Methyl α-d-glucoside                | +  | –  | + | – | – |
| N-acetyl-glucosamine                | +  | –  | + | – | – |
| Amygdalin                           | –  | –  | + | – | – |
susceptibility guideline specific for *Pedobacter* species was not available and the definition of resistance for such environmental bacteria has not been established (Viana et al. 2018).

**Conclusion**

It can be concluded from the results of 16S rRNA sequence similarities, phylogenetic trees (based on 16S rRNA gene and concatenated UBCG), and phenotypic characterisation data that strain CJ43<sup>T</sup> belonged to the genus *Pedobacter*. In addition, the whole genome sequence data showed that strain CJ43<sup>T</sup> can be distinguished from the five type strains of *P. puniceum*, *P. glucosidilyticus*, *P. cryophilus*, *P. arcticus*, and *P. psychrophilus* by low dDDH and ANI values. Therefore, it is proposed that strain CJ43<sup>T</sup> represents a novel species within the genus *Pedobacter*, for which the name *Pedobacter aquae* sp. nov. is proposed.

**Description of *Pedobacter aquae* sp. nov.**

*Pedobacter aquae* (a’quae. L. gen. n. aquae, of water). Cells are Gram-stain-negative, orange-pigmented, aerobic, nonmotile and rod-shaped (0.4 µm wide, 1.5 µm long). Colonies are approximately 0.5–1 mm in diameter, light orange, smooth, circular and convex with regular edges after three days of cultivation on R2A agar at 30 °C. Optimum temperature and pH for growth are at 30 °C and at pH 7 in the absence of NaCl. Catalase and oxidase activities are positive. DNA, starch, and carboxymethyl cellulose are hydrolysed, but...
Casein and gelatin are not. Nitrate is not reduced. According to the API 20NE test, assimilation is positive for D-glucose, D-mannose, D-maltose, L-arabinose, and N-acetyl glucosamine, and negative for L-arginine, D-mannitol, malate, gluconate, caprate, adipate, citrate, and phenylacetate. Enzyme activities for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, napthol-AS-BI-phosphohydrolase, β-glucosidase, β-galactosidase, α-glucosidase, and N-acetyl-β-glucosaminidase are positive, but trypsin, α-chymotrypsin, urease, α-galactosidase, β-glucuronidase, α-mannosidase, and α-fucosidase activities are negative. Based on API 50CH test, acid is produced from glycerol, D-arabinose, L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, rhamnose, methyl α-D-glucoside, N-acetyl-glucosamine, arbutin, esculin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, gentiobiose, L-fucose, and 5-ketogluconate. The major polar lipid is phosphatidylethanolamine. The sole respiratory quinone is menaquinone-7 (MK-7). The major fatty acids are iso-C₁₅:0, iso-C₁₇:0 3-OH, and summed feature 3 (C₁₆:1 ω₆c and/or C₁₆:1 ω₇c). The genomic DNA G+C content of the type strain is 34.9% and its approximate genomic size is 3.9 Mb. The type strain, CJ43ᵀ (= KACC 21350ᵀ = JCM 33709ᵀ), was isolated from the Han River at Gangwon-do, South Korea (N37.483472, E128.657556). The GenBank accession numbers of the 16S rRNA gene sequence and the whole genome sequence of strain CJ43ᵀ are MK129424 and CP043329, respectively.

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Author contributions Chang-Jun Cha designed the study. Material preparation, data collection and analysis were performed by Le Tran Tien Chau and Yong-Seok Kim. The first draft of the manuscript was written by Le Tran Tien Chau and all authors revised the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflicts of interest The authors declare that they do not have any conflicts of interest.

Data availability The 16S rRNA gene and the whole genome sequences of strain CJ43ᵀ that support the findings of this study are available at DDBJ/EMBL/GenBank under accession numbers MK129424 and CP043329, respectively.
have been deposited in the GenBank/EMBL/DDJB database with the accession numbers MK129424 and CP043329, respectively.

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