Pedobacter Aquae Sp. Nov., A Multi-Drug Resistant Bacterium Isolated from Fresh Water

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Research Article

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Abstract

A novel bacterial strain designated CJ43^T was isolated from fresh water located in Jeongseon-gun, Gangwon-do, South Korea, displaying multi-drug resistance. The isolate was observed to be Gram-stain-negative, aerobic, orange-pigmented, and rod-shaped. Strain CJ43^T grew optimally at 30 °C and pH 7 on R2A agar in the absence of NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain CJ43^T belongs to the genus *Pedobacter* in the family *Sphingobacteriaceae* and was the most closely related to *Pedobacter glucosidilyticus* 1-2^T (98.1% sequence similarity). The whole-genome sequencing of strain CJ43^T was performed using the PacBio RS II platform, revealing a genome size of 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 92 putative antibiotic resistance genes, reflecting its phenotypes. The average nucleotide identity and *in silico* digital DNA-DNA hybridization values between strain CJ43^T and *P. glucosidilyticus* 1-2^T were 88.7%, and 38.6%, respectively. The major fatty acids of strain CJ43^T were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and summed feature 3 ($C_{16:1}$ $\omega6c$ and/or $C_{16:1}$ $\omega7c$). Strain CJ43^T contained phosphatidylethanolamine as a major polar lipid and menaquinone-7 as a sole respiratory quinone. Based on the polyphasic taxonomy data, strain CJ43^T represents a novel species of the genus *Pedobacter*, for which the name *Pedobacter aquae* sp. nov. is proposed with the type strain CJ43^T (= KACC 21350^T = JCM 33709^T).

Introduction

The genus *Pedobacter*, first proposed by Steyn et al. (1998), belongs to the family *Sphingobacteriaceae* comprising four species. At the time of writing, 92 species in the genus *Pedobacter* are currently listed in the List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de/genus/pedobacter) 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). However, 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), glaciers (Shivaji et al. 2005; Qiu et al. 2014), and arctic tundra soil (Zhou et al. 2019). Species of the genus *Pedobacter* 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 a 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 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$) (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 establish 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 fresh water 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 at 30 ℃, and the obtained pure culture was preserved at -80 ℃ 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). Three closely related type strains, identified from 16S rRNA phylogenetic tree, were used as reference strains for phenotypic tests and chemotaxonomic analysis.

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 ℃. The optimum growth temperature was determined at 4, 15, 20, 25, 30, and 37 ℃ 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 ℃. 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-stain 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_2O_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 producer's prescriptions.

Chemotaxonomic analysis

The respiratory quinone and polar lipids were extracted and analysed from freeze-dried cells following methods described by Minnikin et al. (1984). Isoprenoid quinone was extracted using hexane:methanol (1:2, v/v) in darkness, evaporated using a vacuum rotary evaporator, separated by thin-layer chromatography (TLC) plate 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) *a*-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, strains 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 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. 71240 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-gene (UBCG) set and the UBCG pipeline version 3 (Na et al. 2018) were used for the reconstruction of phylogenomic tree. 92 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-Kolthoffet al. 2014) was used to assess digitalDNA-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 of type strains were downloaded from the EzBioCloud database (www.ezbiocloud.net) (Yoon et al. 2017). 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 played 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), Kyoto Encyclopedia of Genes and Genomes pathway database (Kanehisa and Goto 2000). The antibiotic resistance genes were determined using the comprehensive antibiotic resistance database (CARD) (Jia et al. 2017).

Antimicrobial susceptibility testing

The antibiotic susceptibility of strain CJ43^T was evaluated by the disk diffusion assay using antibiotic impregnated disks (Liofilchem, Italy) with amoxicillin (10 mg), cephalexin (30 mg), chloramphenicol (30 mg), ciprofloxacin (5 mg), clindamycin (2 mg), colistin sulfate (10 mg), fosfomycin (200 mg), gentamicin (10 mg), linezolid (10 mg), meropenem (10 mg), rifampicin (5 mg), streptomycin (10 mg), sulfamethoxazole (50 mg), tetracycline (30 mg), and trimethoprim (5 mg). Susceptibilities 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 25922 was used as a quality control strain.

Results And Discussion

16S rRNA phylogeny

The comparison of the 16S rRNA gene sequence of strain CJ43^T with those of the related type strains of bacterial species available from the the EzBioCloud database revealed that strain CJ43^T was affiliated with the genus *Pedobacter*. As inferred from the phylogenetic tree (Fig. 1), strain CJ43^T formed a robust cluster with *Pedobacter glucosidilyticus* 1-2^T, which has the highest 16S rRNA gene sequence similarity (98.1%), followed by *P. rivuli* HME8457^T (94.4%) and *P. pituitosus* MIC2002^T (93.6%). 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 CJ43^T represents a novel species within the genus *Pedobacter.*

Genomic features

The final assembly of strain CJ43^T resulted in a single contig, corresponding to a genome size of 3,854,044 bp. The predicted genes were 3144 CDSs, 55 tRNA genes, 9 rRNA genes (including three genes from each of 5S, 16S, 23S rRNA), and 3 ncRNA genes. The genomic DNA G+C content of strain CJ43^T was determined to be 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 CJ43^T and 48 *Pedobacter* type strains was reconstructed using the concatenated sequences of core genes (Fig. 2). Phylogenomic analysis revealed that strain CJ43^T was most closely related to *P. glucosidilyticus* DSM 23534^T, and also formed a cluster with *P. cryophilus* AR-3-7^T, *P. arcticus* A12^T, and *P. psychrophilus* CCM 8644^T. To further establish the phylogenomic relationship of strain CJ43^T with other *Pedobacter* strains, we compared the whole genome of strain CJ43^T to other available genomes of related type strains of *Pedobacter* by calculating dDDH and ANI. The *in silico* dDDH values between strain CJ43^T and *P. glucosidilyticus* DSM 23534^T, *P. cryophilus* AR-3-7^T, *P. arcticus* A12^T, *P. psychrophilus* CCM 8644^T were 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 CJ43^T with the related type strains mentioned above were 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 CJ43^T should be considered as a separate species of the genus *Pedobacter*.

For comparative genomic analysis, the whole-genome sequence of strain CJ43^T was compared with those of five *Pedobacter* species whose genome sequences are available: *P. glucosidilyticus* DSM 23534^T, *P. arcticus* A12^T, *P. psychrophilus* CCM8644^T, *P. cryophilus* AR-3-17^T, and *P. heparinus* DSM 2366^T. General genomic features of strain CJ43^T and these strains were summarized in Table 1. The Venn diagram of the six compared species showed that the core-genome of the *Pedobacter* species included 1599 genes and 538 unique genes from strain CJ43^T were not shared with other compared species (Fig. S1), a majority of these unique genes encode proteins of unknown function. Strain CJ43^T contained *crtB*, *crt1*, and *crtY* genes encoding phytoene synthase, phytoene desaturases, and lycopene cyclase, respectively. These enzymes were involved in the carotenoid biosynthesis, which might be responsible for the orange color of this organism. In addition, 92 antibiotic resistance genes, including a variety of resistance genes against *b*-lactam, aminoglycoside, tetracycline, macrolide, lincosamide, rifamycin, glycopeptide, sulfonamide, amphenicol, and fluoroquinolone antibiotics, were identified in the genome of strain CJ43^T 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 CJ43^T was observed to be Gram-stain-negative, orange-pigmented, aerobic, nonmotile, rod-shaped, 0.4 μm in width, and 1.5 μm 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 cultivation on R2A agar at

30 °C. Strain CJ43^T 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 strainCJ43^T that differentiated from closely related type strains (*P. glucosidilyticus* 1-2^T, *P. rivuli* HME8457^T, and *P. pituitosus* MIC2002^T) are summarized in Table 2.

The only respiratory quinone identified in strain CJ43^T was menaquinone-7 (MK-7), which is in line with those of other *Pedobacter* species. The polar lipids of strain CJ43^T contained phosphatidylethanolamine (PE), two unidentified amino lipids, and four unidentified lipids (Fig. S3). Strain CJ43^T shared the same major polar lipid (PE) with most members of the genus *Pedobacter*. As the results shown in Table 3, the major cellular fatty acids of strain CJ43^T were iso-$C_{15:0}$(29.5%), summed feature 3 ($C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*) (17.7%), and iso-$C_{17:0}$ 3-OH (11.2%), which were similar to those of related type strains of *Pedobacter* species with only minor differences.

Strain CJ43^T 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 CJ43^T. Some phenotypic results did not coincide with the related genotypes, which may be because the 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 results of 16S rRNA sequence similarities, phylogenetic trees (based on 16S rRNA or concatenated the up-to-date bacterial core-gene), and associated with morphological, phenotypic characterisation data that isolate CJ43^T belongs to the *Pedobacter* genus. In addition, the whole genome sequence data show that strain CJ43^T can be distinguished from the type strains of *P. glucosidilyticus*, *P. cryophilus*, *P. arcticus*, and *P. psychrophilus* by low dDDH and ANI values. It is, therefore, proposed that isolate CJ43^T 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 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, crystine arylamidase, acid phosphatase, naphthol-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 strips, acid is produced from glycerol, d-arabinose, l-arabinose, d-xylose, d-galactose, d-glucose, d-fructose, d-manose, 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 fatty acid profile includes major amounts of iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and summed feature 3 ($C_{16:1}$ *ω6c* and/or $C_{16:1}$ *ω7c*). The genomic DNA G + C content of the strain is 34.9%, and its approximate genomic size 3.9 Mb.

The type strain, CJ43^T (= KACC 21350^T = JCM 33709^T), was isolated from fresh water in Gangwon-do, South Korea (N37.483472, E128.657556). The GenBank accession numbers of 16S rRNA gene sequence and the whole-genome sequence of strain CJ43^T are MK129424 and CP043329, respectively.

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.

Declarations

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Conflicts of interest

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

Availability of data and material

The 16S rRNA gene and whole genome sequences of strain CJ43^T that support the findings of this study have been deposited in the GenBank/EMBL/DDBJ database with the accession numbers MK129424 and CP043329, respectively.

Code availability

Not applicable.

Authors' contribution

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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Tables

Table 1 Genomic features of strain CJ43^T and related type strains

Strains: 1, *Pedobacter aquae* CJ43^T; 2, *P. glucosidilyticus* DSM 23534^T; 3, *P. arcticus* A12^T; 4, *P. psychrophilus* CCM8644^T; 5, *P. cryophilus* AR-3-17^T; 6, *P. heparinus* DSM 2366^T.

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

[a, b] Data were calculated between strain CJ43^T and each of *Pedobacter* species.

Table 2 Differential charateristics of strain CJ43^T and related type strains of the genus *Pedobacter*

Strains: 1, CJ43^T; 2, *P. glucosidilyticus* 1-2^T; 3, *P. rivuli* HME8457^T; 4, *P. pituitosus* MIC2002^T. +, positive; -, negative. All data are from this study unless indicated otherwise. Data taken from Luo et al. (2010), Kang et al. (2014) and Chun et al. (2014) that differed from our own data are shown in parentheses.

Characteristics	1	2	3	4
Hydrolysis of				
DNase	+	+ (-)	-	-
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	-	-	+	-
b-galactosidase	+	-	+	-
a-glucosidase	+	+	+	-
b-glucosidase	+	+	-	-
N-acetyl-*b*-glucosaminidase	+	+	+	-
a-fucosidase	-	+	-	-
Acid production from				
Glycerol	+	+	-	-
Erythritol	-	-	-	+
d-Arabinose	+	+	+	-
l-Arabinose	+	+	-	+
d-Ribose	-	-	+	+

d-Xylose	+	+	-	-
d-Adonitol	-	-	-	+
Methyl *b*-d-xylopyranoside	-	-	-	+
d-Galactose	+	+	+	-
d-Glucose	+	+	+	-
d-Fructose	+	+	-	-
d-Manose	+	+	+	-
Rhamnose	+	+	+	-
Methyl *a*-d-mannoside	-	+	-	-
Methyl *a*-d-glucoside	+	+	-	-
N-acetyl-glucosamine	+	+	-	-
Amygdalin	-	+	-	-
Arbutin	+	+	-	-
Esculin	+	+	+	-
Salicin	-	+	-	-
Cellobiose	+	+	-	-
Maltose	+	+	+	-
Lactose	+	+	+	-
Melibiose	+	+	-	-
Trehalose	+	+	-	-
Inulin	+	-	-	-
Melezitose	+	+	-	-
Raffinose	+	+	-	-
Starch	+	+	+	-
Glycogen	+	-	+	-
Gentiobiose	+	+	+	-
d-Turanose	-	+	-	-
d-Tagatose	-	-	+	+
l-Fucose	+	+	+	-

DNA G+C content*	34.9%	37.2 mol%[a]	33.3 mol%[b]	34.7 mol%[c]

*Data were taken from a [Luo et al. 2010], b [Kang et al. 2014], and c [Chun et al. 2014].

Table 3 Fatty acid composition (%) of strain CJ43^T and related type strains of the genus *Pedobacter*

Strains: 1, strain CJ43^T; 2, *P. glucosidilyticus* 1-2^T; 3, *P. rivuli* HME8457^T; 4, *P. pituitosus* MIC2002^T. Fatty acids found in amounts < 1% are trace (tr); -, not detected. All data were obtained in this study.

Fatty acid	1	2	3	4
Straight-chain				
$C_{14:0}$	1.7	1.2	1.3	tr
$C_{16:0}$	4.1	4.4	2.6	7.6
$C_{17:0}$	tr	tr	tr	1.0
Branched				
iso-$C_{10:0}$	1.7	2.5	3.5	3.1
iso-$C_{11:0}$	1.1	1.4	1.6	tr
iso-$C_{14:0}$	1.2	tr	1.3	1.5
iso-$C_{15:0}$	29.5	28.5	23.4	9.3
iso-$C_{16:0}$	1.4	tr	1.2	2.4
iso-$C_{17:0}$	-	tr	-	1.0
anteiso-$C_{15:0}$	5.6	4.1	7.5	6.1
anteiso-$C_{17:0}$	tr	tr	tr	1.7
Hydroxy				
$C_{15:0}$ 2-OH	1.4	1.5	1.6	1.9
$C_{17:0}$ 2-OH	1.0	tr	1.6	2.8
$C_{16:0}$ 3-OH	2.1	1.3	1.2	1.6
iso-$C_{15:0}$ 3-OH	2.1	2.8	3.1	3.6
iso-$C_{16:0}$ 3-OH	2.5	1.1	3.6	2.2
iso-$C_{17:0}$ 3-OH	11.2	12.5	12.3	21.1
Unsaturated				
$C_{15:1}$ *ω6c*	1.6	3.0	2.6	tr
$C_{16:1}$ *ω5c*	3.2	2.1	2.1	-
$C_{17:1}$ *ω6c*	tr	tr	tr	1.1
$C_{17:1}$ *ω8c*	tr	1.1	1.7	1.1

Summed feature*				
3	17.7	13.5	13.1	15.5
5	tr	1.4	tr	-
9	1.8	3.6	2.5	3.0

*Summed features represent groups of two or three fatty acids that could not be separated by MIDI system. Summed feature 3 comprised $C_{16:1}$ *ω*6*c* and/or $C_{16:1}$ *ω*7*c*, summed feature 5 comprised anteiso-$C_{18:0}$ and/or $C_{18:2}$ *ω*6,9*c* and summed feature 9 comprised 10-methyl $C_{16:0}$ and/or iso-$C_{17:1}$ *ω*9*c*.

Figures

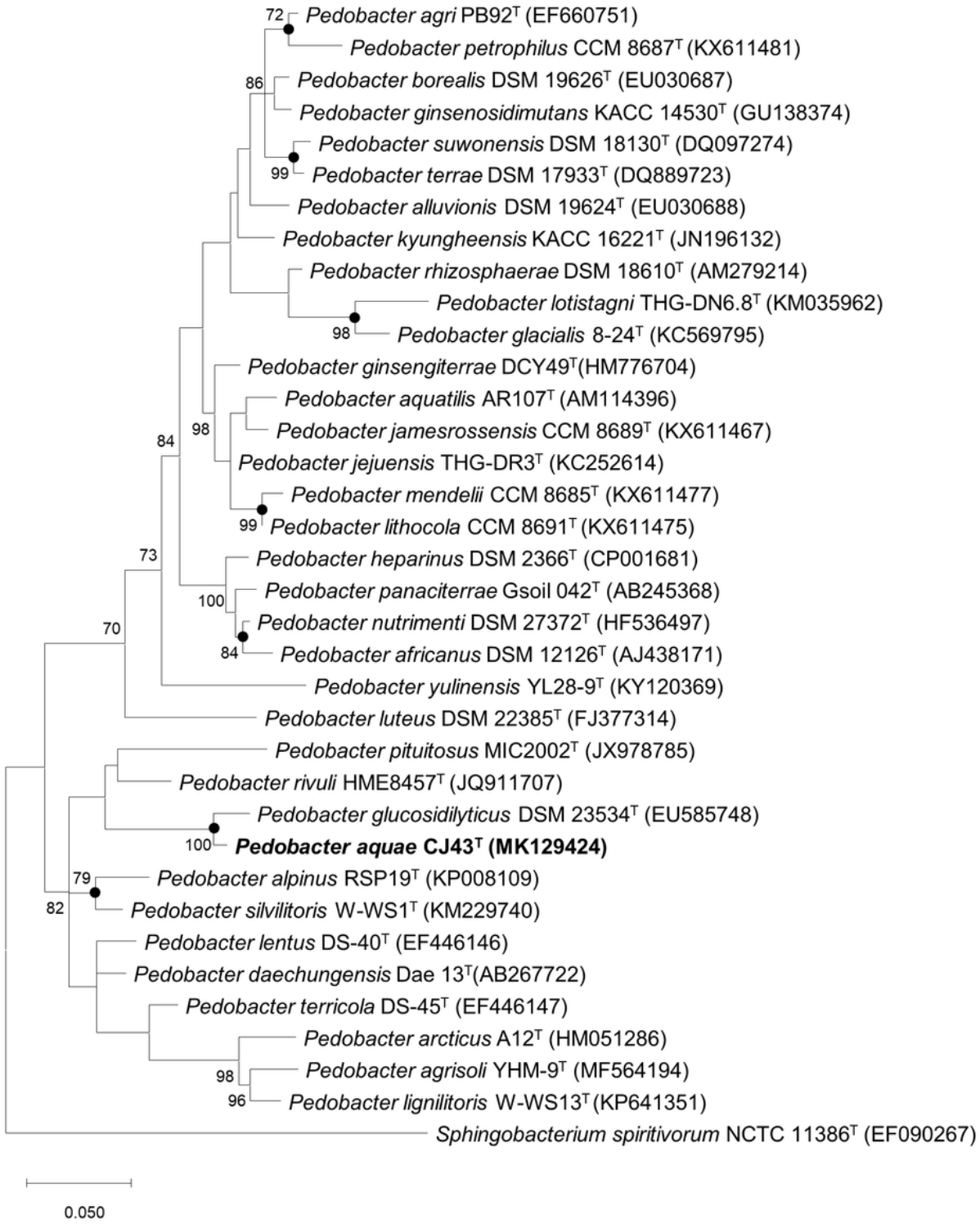

Figure 1

Maximum-likelihood phylogenetic tree of strain CJ43T 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 spiritivoum ATCC 33861T was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

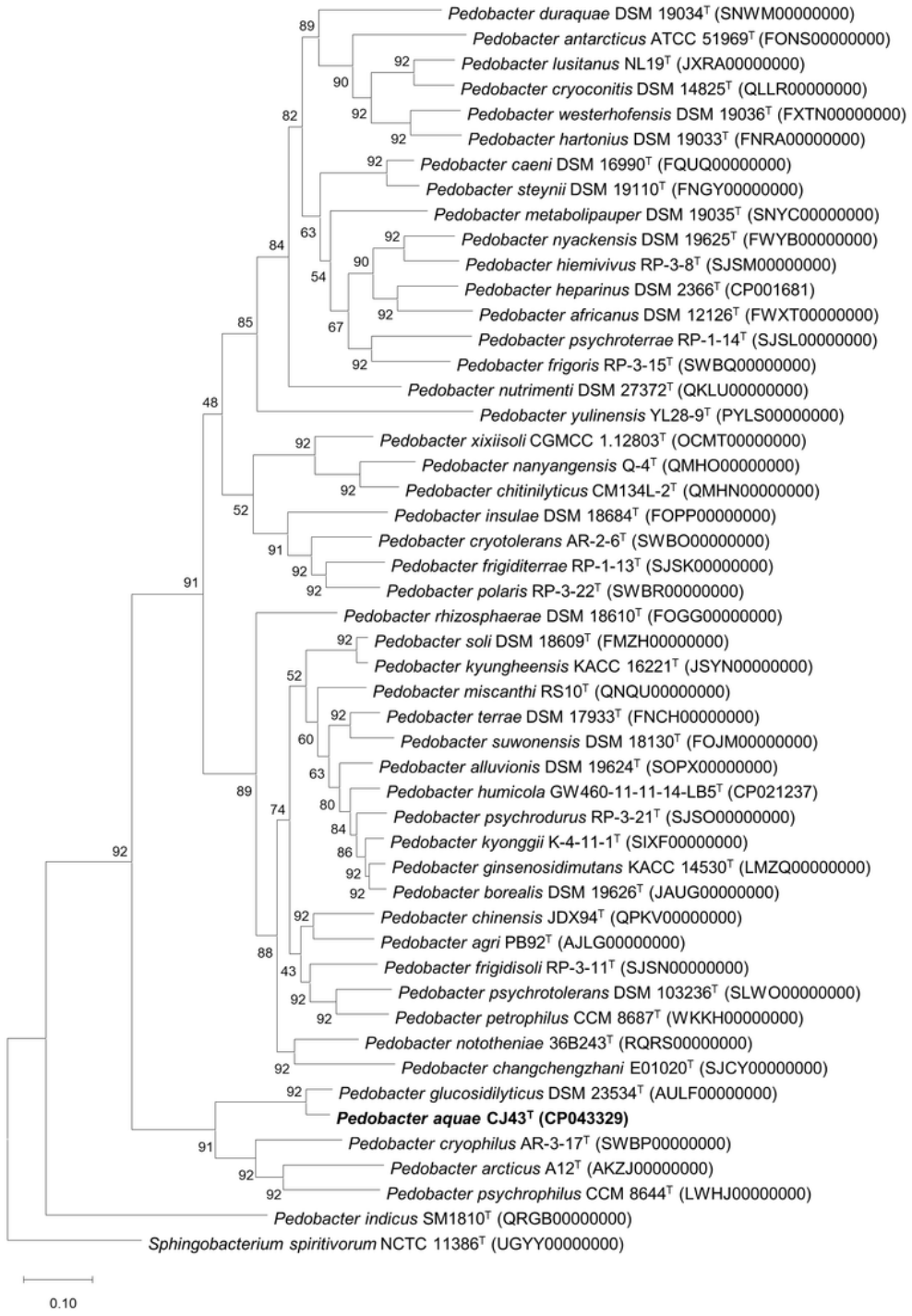

Figure 2

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 spiritivoum NCTC 11386T was used as an outgroup. Bar, 0.1 substitutions per nucleotide position.

Supplementary Files

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- CJ43SupplementaryAVLSubmitted.docx