Archives of Microbiology (2021) 203:3591–3604
https://doi.org/10.1007/s00203-021-02349-9

ORIGINAL PAPER

Peteryoungia gen. nov. with four new species combinations and description of *Peteryoungia desertarenae* sp. nov., and taxonomic revision of the genus *Ciceribacter* based on phylogenomics of *Rhizobiaceae*

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Received: 12 November 2020 / Revised: 22 April 2021 / Accepted: 24 April 2021 / Published online: 9 May 2021

Abstract

A novel bacterial strain designated as ADMK78^T was isolated from the saline desert soil. The cells were rod-shaped, Gram-stain-negative, and non-motile. The strain ADMK78^T grows best at 28 °C. Phylogeny of 16S rRNA gene placed the strain ADMK78^T with the members of genera *Ciceribacter* and *Rhizobium*, while the highest sequence similarity was with *Rhizobium wuzhouense* W44^T (98.7%) and *Rhizobium ipomoeae* shin9-1 ^{T} (97.9%). Phylogenetic analysis based on 92 core-genes extracted from the genome sequences and average amino acid identity (AAI) revealed that the strain ADMK78^T forms a distinct cluster including five species of *Rhizobium*, which is separate from the cluster of the genera *Rhizobium* and *Ciceribacter.* We propose re-classification of *Rhizobium ipomoeae*, *R. wuzhouense*, *R. rosettiformans* and *R. rhizophilum* into the novel genus *Peteryoungia*. The average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values of ADMK78^T were less than 82 and 81%, respectively, among all type strains included in the genus *Peteryoungia.* The strain ADMK78^T showed differences in physiological, phenotypic, and protein profiles estimated by MALDI-TOF MS to its closest relatives. Based on the phenotypic, chemotaxonomic properties, and phylogenetic analyses, the strain ADMK78^T represents a novel species, *Peteryoungia desertarenae* sp. nov. The type strain is ADMK78^T (= MCC 3400^T; KACC 21383^T; JCM 33657^T). We also proposed the reclassification of *Rhizobium daejeonense*, *R. naphthalenivorans* and *R. selenitireducens*, into the genus *Ciceribacter*, based on core gene phylogeny and AAI values.

Keywords Saline Desert · Phylogenomics · Polyphasic taxonomy · MALDI-TOF MS biotyping

Introductions

The family *Rhizobiaceae* Conn 1938, at present, consists of 16 genera summarized in the List of Prokaryotic Names with Standing in Nomenclature (www.bacterio.net) (Parte 2020). Most *Rhizobiaceae* genera like, *Rhizobium*, *Agrobacterium*, *Ensifer* (*Sinorhizobium*), *Allorhizobium*, *Liberibacter*, *Pararhizobium, Neorhizobium,* and *Shinella* are associated with plants. However, members of *Rhizobiaceae* have been reported from diverse ecosystems, including from aquatic and marine ecosystems (Peix et al. 2005; Cao et al. 2020). Members of the genera like *Georhizobium*, *Hoeflea*, *Lentilitoribacter*, *Martelella*, *Rhizobium,* and *Pseudorhizobium* have been reported from marine or aquatic environments (Peix et al. 2005; Rivas et al. 2005; Park et al. 2013; Kimes et al. 2015; Cao et al. 2020; Chaudhari et al. 2020). High-throughput amplicon sequence-based study suggested the dominance of the members of *Rhizobiaceae* in the brackish water Pangong Lake (Chaudhari et al. 2020).

Rhizobium is one of the main genera in the family *Rhizobiaceae*, and it was first proposed in 1889 (Frank 1889; Kuykendall et al. 2005). Presently, the genus *Rhizobium*

Communicated by Erko Stackebrandt.

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comprises of 91 validly published species (https://lpsn.dsmz.de/genus/rhizobium). The majority of *Rhizobium* species are known for their symbiotic fixation of nitrogen within the root nodules of leguminous plants (Lindström et al. 2010, 2015). However, non-symbiotic and free-living members of *Rhizobium* have been found in various niches viz. soils, including the rhizosphere, bioreactor, lake water, arsenic-rich groundwater, and beach sand (Quan et al. 2005; Panday et al. 2011; Ramana et al. 2013; Sheu et al. 2016; Li et al. 2017a, b; Mohapatra et al. 2017). Recently, multiple new genus names such as *Allorhizobium*, *Pararhizobium*, *Neorhizobium,* and *Pseudorhizobium* have been proposed by dissecting *Rhizobium* (Mousavi et al. 2014; Mousavi et al. 2015; Kimes et al. 2015; Hördt et al. 2020).

Rann of Kachchh is reputed to be the largest salt desert in the world and is a transitional area between marine and terrestrial ecosystems (Pandit et al. 2015). The region experiences diagonal fluctuations of temperature, in summers it reaches up to 50 °C and drops down below zero during winters. Due to the hot and hypersaline environment, there is a vast possibility of isolating novel halophilic and halotolerant microorganisms with high economic and industrial potential (Ruginescu et al. 2020). During the investigations on bacterial diversity of the saline desert soil collected from the Rann of Kachchh, Gujarat, India, the strain ADMK78^T was isolated with 16S rRNA gene sequence similarity less than 98.7%. The present study aimed to demonstrate the taxonomic position of strain ADMK78^T through a polyphasic and genomic analysis.

Materials and methods

Isolation of bacterium

A bacterial strain ADMK78^T was isolated on Zobell Marine Agar following the serial dilution of the saline desert soil (23.7337° N, 69.8597° E) collected from the Rann of Kachchh, Gujarat, India. The newly isolated strain ADMK78^T was maintained on Zobell Marine Agar at 37 °C, and preserved at − 80 °C as a suspension in 20% (v/v) glycerol and by lyophilization with 20% (w/v) skimmed milk.

16S RNA phylogeny

High-quality genomic DNA was extracted from the strain following the manual bacterial genomic DNA isolation protocol using CTAB (Minas et al. 2011). The 16S rRNA gene sequence was amplified using universal primers (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′and 1492R: 5′-TACGGCTACCTTGTTACGACTT-3′) according to the methods described by Gulati et al (2008), and the amplified product was purified and subjected to DNA sequencing using the ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing kit on a 3730xl Genetic Analyzer (Applied BioSystems, Thermo Scientific, USA). The similarity search for the 16S rRNA gene sequence of strain ADMK78^T was performed against the type strains of prokaryotic species in the EzBioCloud's valid species database (Yoon et al. 2017). The 16S rRNA gene sequence of strain ADMK78^T was also used as queries to closely related gene sequences using the NCBI BLASTn tool against the non-redundant nucleotide database (Altschul et al. 1990). Multiple alignments of sequences of strain ADMK78^T and its nearest neighbours retrieved from EzBioCloud's server and NCBI GenBank were performed using ClustalW (Larkin et al 2007). Phylogenetic analysis of the 16S rRNA was performed using the neighbour-joining, maximum-parsimony, and maximum-likelihood algorithms in the MEGA software (version 10.2.1) (Kumar et al. 2018). Bootstrap values were determined based on 1000 replications (Efron et al. 1996). The newly generated 16S rRNA gene sequence was deposited with NCBI GenBank under accession MK942856.

Whole genome sequencing and core-genes based phylogeny

The strain was grown on Zobell Marine liquid medium incubated at 28 °C for 32 h and genomic DNA was harvested following the JGI protocol version 3 for bacterial genomic DNA isolation using CTAB (Minas et al. 2011). Genome sequencing was performed using a hybrid approach of two platforms, first on an Illumina MiSeq platform with 2 × 250 bp v2 chemistry, followed by sequencing with Oxford Nanopore Technology (ONT) on a minION platform. The Nanopore reads were assembled using Canu v. 2.0 with default settings (Koren et al. 2017). The overlaps between the ends of circular contigs were identified using NUCmer v. 3.1 (Kurtz et al. 2004) and removed using a custom Perl script. Two rounds of polishing were performed using the paired-end Illumina reads. In each round, the Illumina reads were mapped to the genome assembly using bowtie2 v. 2.3.4.1 with default parameters, followed by polishing using Pilon v. 1.23 with default settings (Langmead and Salzberg 2012; Walker et al. 2014). Whole-genome sequences were annotated using the RAST web server (http://rast.nmpdr.org/rast.cgi) (Aziz et al. 2008). The biosynthetic gene clusters (BGCs) for various secondary metabolites, were identified by using an online genome mining pipeline antiSMASH 5.0 (Blin et al. 2019). The complete genome sequence of strain ADMK78^T was deposited with NCBI under the accession number CP058350- CP058352.

Genome sequences from species of the different genera of the family *Rhizobiaceae* were downloaded in FASTA format from the NCBI database (www.ncbi.nlm.nih.gov). To find genome sequences of closely related strains, gene

sequence of *rpoB* gene of strain ADMK78^T was used as a search query against RefSeq select proteins database of *Rhizobiaceae* group (taxid:356). A total of 197 genomes were used for core-gene phylogeny (Table S1). The core gene-based phylogenetic analysis was carried out using the UBCG pipeline from the concatenated sequences of 92 core genes extracted by UBCG, and a maximum-likelihood phylogenetic tree was inferred using RAxML version 8.2.8 with the GTRGAMMA model and 100 bootstrap replications (Stamatakis et al. 2014; Na et al. 2018). The core-gene phylogenetic tree was displayed using iTOL (Letunic and Bork 2019). The Average Nucleotide Identity (ANI) was determined between strain ADMK78^T and 81 strains of the *Rhizobiaceae* family using FastANI (Jain et al. 2018) with the default settings (kmer = 16, fragment length = 3000, minimum shared fraction = 0.2). Digital DDH values and confidence intervals were calculated using the recommended settings of the GGDC 2.1 (Meier-Kolthoff et al. 2013). The percentage of conserved proteins (POCP) was calculated based on the previously described approach (Qin et al. 2014). The pairwise amino acid identity (AAI) was calculated using the ComparM software package (https://github.com/dparks1134/CompareM). The results of ANI and AAI were displayed as a heatmap using HeatMap Illustrator of TBtools (Chen et al. 2020). The AAI values were plotted for the strain ADMK78^T and type species of each genera of family *Rhizobiaceae* using ggplot function in R.

Physiology and chemotaxonomy

The type strains of *Rhizobium ipomoeae* LMG 27163^T and *Ciceribacter lividus* DSM 25528^T were obtained from the BCCM/LMG Bacteria Collection, Belgium (LMG) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) respectively. Both type strains were used as reference strains and evaluated together under identical experimental conditions to those for strain ADMK78^T.

Morphological, physiological, and biochemical tests for strain ADMK78^T were performed on Zobell Marine Agar plates incubated under aerobic conditions. Gram-staining (K001, Himedia, India) was used following the manufacturer's instructions. The hanging drop technique was used to check the motility (Tittsler and Sandholzer 1936). Scanning electron microscopy was performed to observe cell morphology as described in Rahi et al. (2017). Oxidase disc (DD018, Himedia, India) was used for testing oxidase activity, and catalase activity was determined by bubble formation in a 3% (v/v) H_2O_2 solution. Growth at different temperatures (4, 10, 15, 20, 28, 37, 45 and 55 °C), NaCl concentrations [0–2% (w/v) at 0.5% intervals] and pH values (4.0–11.0 at 1.0 pH unit intervals) was examined after incubation in Zobell Marine broth for 7 days in automated microbial growth analyzer (Bioscreen C, OY Growth Curves, Finland). The initial pH of the inoculation broth was adjusted using 1 M HCl and 1 M NaOH. Biochemical characteristics, enzyme activities, and oxidation/or reduction of carbon sources were performed using the API 20E and API ZYM systems (07584D and 25,200, bioMérieux, France) and Biolog GN III system (OmniLog, Biolog, USA) following manufacturer's instructions.

For analysis of chemotaxonomic features, the strain was grown on Zobell Marine Agar, while *Rhizobium ipomoeae* LMG 27163^T and *Ciceribacter lividus* DSM 25528^T were grown on TSA, and incubated at 28 °C, and cell biomass was harvested after 24 h. Preparation and analysis of fatty acid methyl esters were performed as described by Sasser (2001) using the Microbial Identification System (MIDI) and the Microbial Identification software package (Sherlock version 6.1; MIDI database, TSBA6). Whole-cell proteins were extracted using ethanol/formic acid after 24 h growth on TSA, to generate the Mean Spectral Profile (MSP). The proteins ranging from 2 to 20 KDa were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) autoflex speed (Bruker Daltonik GmbH, Germany) with Biotyper 3.0 database (Kurli et al. 2018). A total of 27 replicate spectra were used to generate a MALDI-TOF MS mean spectra profile (MSP) of strain ADMK78^T, which was compared with MSPs of the reference strains *Rhizobium ipomoeae* shin9-1^T and *Ciceribacter lividus* MSSRFBL1^T generated during this study following the same procedure (Kurli et al. 2018).

Results and discussion

16S RNA phylogeny

Strain ADMK78^T showed the highest similarity to *Rhizobium wuzhouense* W44^T (98.7%), followed by *Rhizobium ipomoeae* LMG 27163^T (97.9%) in the search against type strain database of EzBioCloud. The sequence search in the NCBI nucleotide database resulted in more than 99% sequence similarity for two sequences. The first one was from an *alpha-proteobacterium* (EU770254.1) associated with *Microcystis aeruginosa* culture, and the second one was from *Ciceribacter* sp. strain AIY3W (MH463946.2) isolated from low salinity lakes on the Tibetan plateau.

Phylogeny-based on 16S rRNA gene sequences placed the strain ADMK78^T branch along with closely related sequences of an alphaproteobacterium and *Ciceribacter* sp. AIY3W in the cluster of *Ciceribacter* species (Fig. S1). The 16S rRNA gene phylogeny for only type strains placed strain ADMK78^T along with *Rhizobium wuzhouense* W44^T, though the bootstrap value was below 50%, and its phylogenetic position was not consistent across trees obtained with the maximum likelihood, maximum parsimony, and

neighbour-joining methods (Fig. 1). The 16S rRNA gene phylogeny also revealed the scattered branching of *Rhizobium* species. Several species of *Rhizobium* were distantly placed from the core clade that contained the type species of the genus making it a non-monophyletic group (Hördt et al. 2020).

Genome features

The genome of ADMK78^T had a size of 4,342,374 bp, which is smaller than the genome size of symbiotic members of *Rhizobium* and in the range of the sizes of the non-symbiotic strains of *Rhizobium* (Table 1). It consisted of a circular chromosome of 3,590,542 bp and two circular plasmids of 708,533 and 43,299 bp. The overall genome sequencing coverage for the strain ADMK78^T was 147.5x, with an N50 value of 3,590,542 bp. The genome sequence quality of strain ADMK78^T was as per the genome standards proposed by Chun et al (2018), and the detailed genome features are provided in Table 1. The genome of strain ADMK78^T contains 4377 protein-coding sequences (CDS), of which 64 genes were assigned to the stress response functions, including heat and cold shock, hyperosmotic stress, and protection from reactive oxygen.

Fig. 1 Phylogenetic tree based on 16S rRNA gene sequences of strain ADMK78^T and type strains of members of the family *Rhizobiaceae* inferred by using the Maximum Likelihood method and Kimura 2-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Empty circles indicate branches of the tree that were also recovered using the neighbour-joining method, and black filled circles indicate that all three methods recovered the corresponding nodes. Bar, 0.1 substitutions per nucleotide position

Table 1 Standard genome features of strain ADMK78^T and related type strains of the family *Rhizobiaceae*

Strain (GenBank accession numbers)	Genome size (Mbp)	Total contigs (nos.)	DNA G+C content (mol%)	N50 (kb)	Genome coverage	Genome ANI with ADMK78^T (%)	dDDH relatedness with ADMK78^T (%)	Difference in G+C content (%)
ADMK78^T (CP058350-CP058352)	4.31	03	58.6	3590	147x	100.0	100	0
Peteryoungia wuzhouense W44^T (NZ_QJRY01000001)	4.93	32	61.6	493	277x	81.3	22.0	3.0
Peteryoungia rosettiformans W3^T (NZ_STGU00000000)	4.98	86	61.7	288	200x	81.9	22.0	3.03
"*Rhizobium glycinendophyticum*" CL12^T (NZ_VFYP00000000)	4.84	16	61.1	2995	206x	81.2	21.8	2.45
Peteryoungia ipomoeae shin9-1 ^{T} (NZ_STGV00000000)	4.39	23	60.0	535	200x	81.0	21.6	1.78
Peteryoungia rhizophilum 7209-2 ^{T} (GCA_004912145)	5.24	20	61.2	971	200x	81.3	21.7	2.57
Ciceribacter selenitireducens ATCC BAA-1503 ^{T} (GCA_000518785)	4.98	29	63.5	372	–	79.9	20.7	4.88
Ciceribacter naphthalenivorans NBRC 107585^T (GCA_007992095)	4.95	139	61.2	151	112x	79.2	20.2	2.56
Ciceribacter daejeonense CCBAU10050^T (GCA_011045155)	5.11	25	60.5	726	200x	79.6	20.2	1.87
"*Mycoplana subbaraonis*" JC85^T (NZ_OBQD00000000)	6.58	91	63.1	255	153x	78.8	20.7	4.44
Ciceribacter lividus DSM 25528^T (NZ_QPIX00000000)	4.52	37	63.2	302	226x	79.2	20.2	4.58
Ciceribacter thiooxidans F43b^T (GCF_014126615)	5.04	02	62.8	3661	129x	79.3	20.7	4.17
Ciceribacter ferrooxidans F8825^T (GCA_004137355)	5.09	429	63.3	438	168x	79.2	16.6	4.65
Rhizobium endolithicum JC140^T (GCA_902153245)	4.18	62	62.7	206	64x	79.0	20.2	4.11
Rhizobium leguminosarum USDA 2370^T (GCA_002008365)	7.81	72	60.6	432	60x	78.50	19.9	1.95
Allorhizobium undicola ATCC 700741^T (GCA_000621665)	4.13	49	59.3	170	–	78.7	21.0	0.7
Agrobacterium nepotum 39/7 ^{T} (GCF_000949865)	5.32	79	59.1	219	88.7x	78.5	20.4	0.5
Pararhizobium giardinii H152^T (GCF_000379605)	6.81	190	60.7	256	–	78.7	20.1	2.05
Neorhizobium alkalisoli DSM 21826^T (GCA_002968635)	5.86	68	60.3	390	30x	78.8	20.1	1.69
Mycoplana dimorpha DSM 7138^T (GCA_003046475)	4.59	22	64.5	406	208x	78.7	21.5	5.91
Ensifer saheli LMG 7837^T (GCF_001651875)	5.99	102	63.6	115	100x	78.6	20.6	4.99
Mesorhizobium loti DSM 2626^T (GCA_003148495)	7.45	52	62.4	607	139x	78.4	19.9	3.73

We could not find any nitrogen-fixation and nodulation genes in the genome of strain ADMK78^T. Four secondary metabolite regions were identified in the main chromosome, while two in the plasmid pPRADMK78_01, using anti-SMASH pipeline (Table S5). The putative secondary metabolite clusters include bacteriocin, terpene, homoserine lactone cluster, beta-lactone-containing protease inhibitor and TfuA-related RiPPs. Members of the genus *Rhizobium* have been shown to produce antimicrobial compounds like bacteriocins (Oresnik et al. 1999), and quorum-sensing molecules, including homoserine lactones (He et al. 2003).

The core-gene based phylogenetic analysis placed strain ADMK78^T as an independent branch with *Rhizobium ipomoeae* shin9-1 ^{T} as the closest neighbour and followed by *Rhizobium wuzhouense* W44^T, *R. rosettiformans* W3^T, and recently described *Rhizobium rhizophilum* 7209-2^T (Gao et al. 2020) and "*R. glycinendophyticum*" CL12^T (Wang et al. 2020) (Fig. 2). Strain ADMK78^T was placed distantly from the species of *Ciceribacter*, and *Rhizobium naphthalenivorans*, *R. selenitireducens* and *R. daejeonense*. The core-gene phylogeny also exhibited that strain ADMK78^T is distant from the members of *Allorhizobium*, *Agrobacterium* and *Rhizobium* (Fig. 2). The clade consisting of the strain ADMK78^T along with *Rhizobium ipomoeae*, "*R. glycinendophyticum*", *R. wuzhouense*, *R. rosettiformans*, *R. rhizophilum* and 21 non-type strains represents a new genus. Previous studies, proposed four new genera by dissecting the genus *Rhizobium*, based on the phylogeny of housekeeping genes (de Lajudie et al. 1998, Kathiravan et al. 2013; Mousavi et al. 2015; Mousavi et al. 2015). However, the Genome Taxonomy Database (GTDB) a resource that provides a comprehensive genome-based taxonomy of all prokaryotes, placed all members of *Ciceribacter* and the putative new genus clade containing ADMK78^T under *Allorhizbium* (Parks et al 2020). Phylogenomic analysis has been considered as a key tool in defining genera (Espariz et al. 2016; Chun et al. 2018), the core-gene phylogeny exhibited clear delineation of all genera of *Rhizobiaceae* (Fig. 2). The core-gene phylogeny also

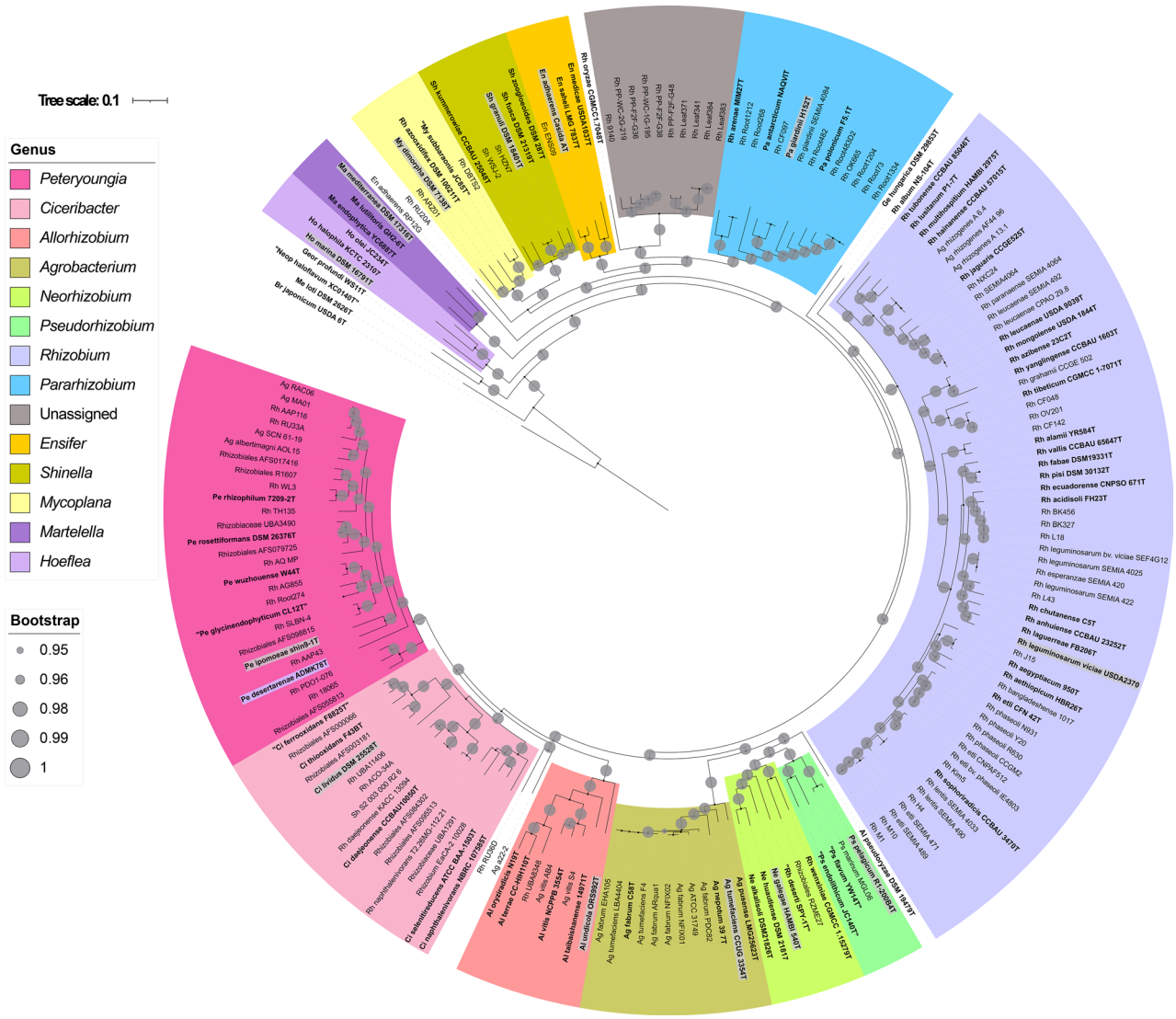

Fig. 2 Phylogenetic tree inferred by the UBCG phylogenomics pipeline using the concatenated alignment of 92 core genes, of strain ADMK78^T and members of the family *Rhizobiaceae*. Grey circles are indicating percentage of bootstrap values at branching points. Bar, 0.1 substitution per position

grouped *Rhizobium daejeonense*, *R. naphthalenivorans* and *R. selenitireducens* along with members of genus *Ciceribacter*. The possibility of transferring *Rhizobium naphthalenivorans* and *R. selenitireducens* in the genus *Ciceribacter* has already been discussed (Hördt et al. 2020). However, a formal proposal was not made, citing the low resolution and difficulties to infer any taxonomic conclusions (Hördt et al. 2020).

Although the core-gene phylogeny provided an excellent measure to group together the phylogenetically related members, the taxonomy reorganization also requires a set of criteria to define the diversity within genera. The genus boundary has been proposed at 50% POCP (Qin et al. 2014). The POCP value inter-generic comparisons were more than 50% for the genera belonging to the *Rhizobiaceae* family (Table S2). Hence, this universal cut-off of 50% POCP was not applied to the *Rhizobiaceae* family. Several studies have reported the POCP cut-off failure for genus-level circumscription for the members of *Bacillaceae*, *Burkholderiaceae*, *Neisseriaceae,* and *Rhodobacteraceae* (Aliyu et al. 2016; Li et al. 2017a, b; Lopes-Santos et al. 2017; Wirth and Whitman 2018). These studies confirmed that a single cut-off value of POCP is unlikely to be a universal threshold for delimiting prokaryotic genera.

Several studies have indicated that AAI represents a powerful tool for genome-based taxonomy assignments. The AAI values to delimit genera typically vary between 60–80% and do not exceed 85% (Luo et al. 2014). The AAI values calculated for the *Rhizobiaceae* family members indicate a cut-off at 75% delimits all its genera (Figs. 3, 4). All the strains grouped under the new genus clade in the core-gene phylogeny (Fig. 2), were clustered together in AAI plots above 75% cut-off value (Fig. 3). Therefore we adopted this cut-off for defining the genus boundaries, which led us to propose a new genus *Peteryoungia* by including four *Rhizobium* species, *R. ipomoeae*, *R. rosettiformans*, *R. wuzhouense* and *R. rhizophilum*, and the strain ADMK78^T. In addition to this, the core-gene phylogeny (Fig. 2) and pairwise AAI values (Figs. 3, 4) provided sufficient evidence to reclassify three *Rhizobium* species, namely *R. daejeonense*, *R. naphthalenivorans* and *R. selenitireducens,* into the genus *Ciceribacter*.

The genomic DNA G + C content of strain ADMK78^T was 58.6 mol%, which is slightly lower than its closely

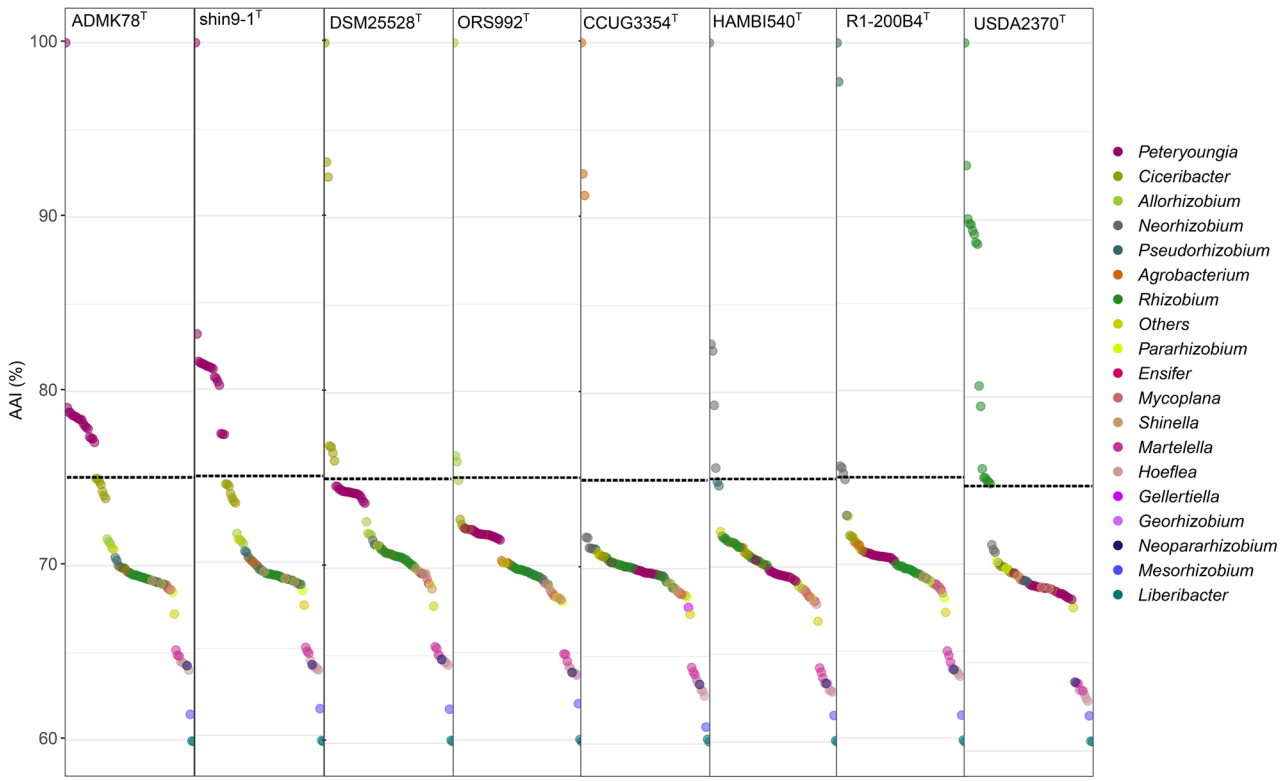

Fig. 3 Average amino acid identity (AAI) plots using the type species of different genera of the family *Rhizobiaceae*. *Peteryoungia desertarenae* ADMK78^T, *Peteryoungia ipomoeae* shin9-1^T, *Ciceribacter lividus* MSSRFBL1^T, *Allorhizobium undicola* ORS992^T, *Agrobacterium tumefaciens* CCBAU3354^T, *Neorhizobium galegae* HAMBI 540^T, *Pseudorhizobium pelagicum* R1-200B4^T and *Rhizobium leguminosarum* USDA 2370^T

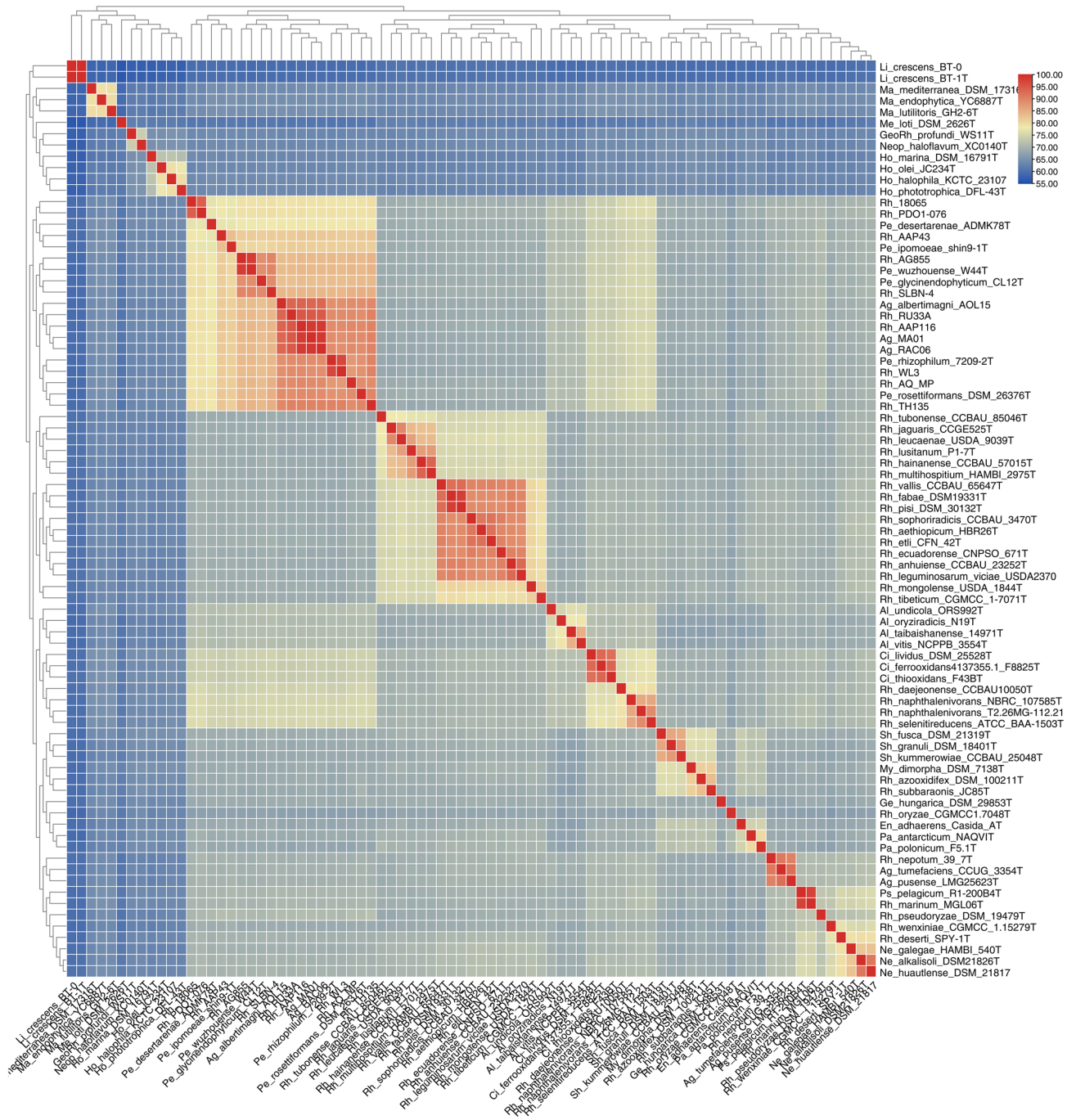

Fig. 4 Pairwise AAI values between the strains ADMK78^T and members of the family *Rhizobiaceae*. The clustering at row and columns was done using Euclidean distance

related species included in the newly proposed genus *Peteryoungia* (ranging between 60.0–61.7 mol%) (Table 1). The ANI and dDDH relatedness values of ADMK78^T with all species reclassified under the genus *Peteryoungia* are lower than (Table 1; Fig. S2), the proposed species boundaries of 95–96% for ANI and 70% for dDDH (Chun et al. 2018; Wang et al. 2016). This supported our claim that the strain ADMK78^T is a putative novel species of this newly proposed genus.

Physiology and chemotaxonomy

The colonies of strain ADMK78^T, were circular and translucent on Zobell Marine Agar (Fig. S4). Microscopic

observation revealed that strain ADMK78^T is a rod-shaped bacterium with cell size ranging from 0.3–0.5 × 1.5–2 µm, Gram-stain-negative (Fig. S3), and non-motile. It is interesting to note that all the species included in the newly proposed genus *Peteryoungia* are able to tolerate at least 2% of NaCl (Table 1). Details on the phenotypic characteristics, including results of carbon source utilization and chemical sensitivity in Biolog GENIII plates (Table S5), and enzyme activity in API-Zym strips (Table S6), and the differences with respect to the closely-related phylogenetic neighboring species of *Peteryoungia* and *Ciceribacter,* are shown in Table 2.

The primary fatty acids detected in strain ADMK78^T were $C_{18:1}$ *ω7c* and $C_{18:0}$ (Table S3). Small proportions of $C_{16:0}$, $C_{18:1}$ *ω7c* 11-methyl, $C_{18:0}$ 3-OH, $C_{20:1}$ *ω7c*, and summed feature 2 ($C_{12:0}$ aldehyde) were also detected for strain ADMK78^T. The strain ADMK78^T exhibits higher proportions of $C_{18:0}$, which was present in relatively lower amounts in the other species of *Peteryoungia* (Table S3). Contrary to this, a similar proportion of $C_{18:0}$ was detected for *Ciceribacter lividus* MSSRFBL1^T. However, strain ADMK78^T and all other members of *Peteryoungia* do not possess C19:0 cyclo ω8c, which is the key fatty acid for *Ciceribacter lividus*. The mean spectra profile of strain ADMK78^T has 33 unique peaks in comparison to the closely related taxa out of a total of 70 peaks (Table S4), which attributed to the discrimination of ADMK78^T from the closely related species.

In conclusion, the core-gene phylogeny and pairwise AAI analyses showed a separate clade for five *Rhizobium* species with strain ADMK78^T, which indicated their membership to a novel genus. Based on these results, we propose a new genus *Peteryoungia* in the family *Rhizobiaceae* by reclassifying five *Rhizobium* species. The genotypic and phenotypic data generated for the strain ADMK78^T revealed that the strain represents a novel species in the newly proposed genus *Peteryoungia*, for which the name *Peteryoungia desertarenae* sp. nov. is proposed. In addition to this, we also propose the transfer of three rhizobium species into *Ciceribacter*.

Description of *Peteryoungia gen.* nov.

(Pe.ter.young'i.a. N.L. fem. n. *Peteryoungia*, named to honour Prof J. Peter W. Young, who contributed extensively towards the taxonomy and genomics of the family *Rhizobiaceae*).

Cells are Gram-stain-negative, aerobic, rod- to oval-shaped, and non-motile. Moderately halophilic. Positive for oxidase and catalase. All members can utilize α-d-Lactose. The major fatty acid (> 10% of total fatty acids) is $C_{18:1}$ *ω7c*. The DNA G + C content is 58.6–61.7 mol %. A member of the family *Rhizobiaceae*, class *Alphaproteobacteria* according to 16S rRNA gene sequence analysis and core-gene phylogeny. The type species for the genus is *Peteryoungia ipomoeae*.

Description of *Peteryoungia ipomoeae comb. nov.*

Peteryoungia ipomoeae (i.po.moe′ae. N.L. gen. n. *ipomoeae*, of the water convolvulus *Ipomoea*; pertaining to the isolation of the type strain from a water convolvulus field).

Basonym: *Rhizobium ipomoeae* (Sheu et al. 2016).

The description is the same as for *R. ipomoeae* (Sheu et al. 2016). Phylogenetic analysis of the core-genes and pairwise provided strong evidence for the placement of this species in the novel genus *Peteryoungia*. The type strain is shin9-1 ^{T} (= LMG 27163 ^{T} = KCTC 32148 ^{T}).

Description of *Peteryoungia desertarenae sp. nov.*

(de.sert.a.re'nae. L. neut. n. *desertum* desert; L. fem. n. *arena* sand; N.L. gen. n. *desertarenae* of desert sand).

Cells are Gram-negative, straight rods with round ends (0.3–0.5 × 1.5–2 µm), and non-motile. Colonies grown on Zobell Marine Agar are 1–3 mm in diameter, circular, raised with an entire margin, and translucent opacity. The optimal temperature for growth is 28 °C and the optimal pH is 7.0. Growth occurs in the absence of NaCl with up to 2% tolerance in Zobell Marine broth. It is oxidase and catalase positive. The strain showed positive results in Biolog GN III analyses for utilization of D-maltose, D-trehalose, D-cellobiose, D-gentiobiose, sucrose, D-turanose, α- D-lactose, D-melibiose, β-methyl-d-glucoside, D-salicin, *N*-acetyl- D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, α-D-glucose, D-mannose, D-fructose, D-galactose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, glycerol, D-glucose-6-phosphate, D-fructose-6-phosphate, D-aspartic acid, Glycyl-L-proline, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, pectin, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, glucuronamide, mucic acid, D-saccharic acid, p-hydroxy-phenylacetic acid, D-lactic acid methyl easter, L-lactic acid, citric acid, α-keto-glutaric acid, D,L-malic acid, bromo-succinic acid, Tween 40, γ-amino-butyric acid, α-hydroxy-butyric acid, α-hydroxy-D,L butyric acid, α-keto-butyric acid, acetoacetic acid, propionic acid, acetic acid, formic acid, sodium lactate, tetrazolium violet and blue, nalidixic acid, lithium chloride (Table S3). Positive results in API ZYM strips for leucine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α-glucosidase, *N*-acetyl-β-glucosaminidase activities (Table S4). $C_{18:0}$ and $C_{18:1}$ *ω7c* are the predominant cellular fatty acids. The DNA G + C content of the type strain is 58.6 mol%.

The type strain ADMK78^T (= MCC 3400^T; KACC 21383^T; JCM 33657^T) was isolated from saline desert sand

Table 2 Differentiating characteristics of strain ADMK78^T in comparison to its closely related phylogenetic neighbours

Characteristics	1	2	3[a]	4[a]	5[ab]	6[c]	7
Isolation source	Saline soil (Desert)	Field	Rhizosphere of rape	Groundwater	Roots of *Oryza officinalis*	Roots of *Glycine max*	Rhizosphere of chickpea
Colony colour	Cream	Cream	Milky	Cream	Cream	Cream	Bluish black
pH range for growth	4.0–11.0 (7.0)	7.0–9.0 (7.0)	6.0–8.0 (7.0)	5.0–9.0 (7.0)	5.0–8.0 (7.0)	5.0–9.5 (7.0)	6.0–8.5 (7.0)
Temperature range for growth (°C) (optimum)	10–45 (28)	10–45 (30)	15–37 (30)	25–40 (28)	15–40 (30)	10–42 (30)	10–45 (28)
NaCl range for growth (%) (optimum)	0–2.0 (1.5)	0–3.0 (1.5)	0–4.0	0–3.0 (1.0)	0–2.0 (0.5)	0–4.5 (2.0)	0.5–1.5 (1.0)
D-Maltose	+	+	−	+	+	ND	+
D-Cellobiose	+	+	−	+	+	ND	+
D-Gentiobiose	+	+	−	−	+	ND	+
D-Raffinose	−	−	−	−	+	ND	+
α-D-Lactose	+	+	+	+	+	+	+
D-Fucose	+	+	−	−	+	+	+
L-Rhamnose	+	+	−	+	+	ND	+
Sodium lactate 1%	+	+	−	−	+	ND	+
D-Glucose-6-PO_4	+	W	+	−	+	ND	+
D-Fructose-6-PO_4	+	+	−	−	+	ND	+
Rifamycin sv	-	+	+	−	+	ND	+
Gelatin	-	−	−	+	−	ND	−
Glycyl-L-Proline	+	+	−	−	+	ND	+
L-Alanine	+	+	−	−	+	ND	+
L-Arginine	+	+	−	+	+	+	+
L-Aspartic Acid	+	+	−	−	+	ND	+
L-Histidine	+	−	−	+	+	ND	−
Mucic acid	+	+	−	+	−	ND	−
Quinic acid	−	+	+	+	−	ND	+
L-Lactic Acid	+	+	−	−	+	ND	+
Citric Acid	+	+	−	+	+	ND	+
D-Malic Acid	+	+	−	−	+	+	−
L-Malic Acid	+	+	+	−	+		−
Bromo-Succinic Acid	+	+	−	−	+	ND	−
Nalidixic Acid	+	+	−	−	+	ND	+
Lithium Chloride	+	W	−	−	−	ND	+
Potassium Tellurite	−	+	+	+	−	ND	+
Tween 40	+	−	−	+	−	−	−
γ-Amino-Butryric Acid	+	+	−	−	+	ND	+
Acetoacetic Acid	+	−	+	+	+	ND	+
Propionic Acid	+	+	−	−	+	ND	+
Alkaline phosphatase	−	+	+	+	+	ND	+
Valine acrylamidase	−	−	+	+	+	ND	−
Acid phosphatase	−	−	+	W	+	+	+
α-glucosidase	+	+	+	−	W	ND	+

Strain 1, ADMK78^T; 2, Peteryoungia ipomoeae shin9-1^T; 3, Peteryoungia rhizophilum 7209-2^T; 4, Peteryoungia rosettiformans CCM 7583^T; 5, Peteryoungia wuzhouense W44^T; 6, "Rhizobium glycinendophyticum" CL12^T; 7, Ciceribacter lividus MSSRFBL1^T

[a]Data from Gao et al. 2020

[b]Data from Yuan et al. 2018

[c]data from Wang et al. 2020

+ positive, −, negative, *w* weak activity, *ND* no data

collected from the Kutch District of Gujarat, India. The GenBank sequence accession number of the genome sequence is CP058350-CP058352, and the 16S rRNA gene sequence of strain ADMK78^T is MK942856.

Description of *Peteryoungia rosettiformans comb.* nov.

Peteryoungia rosettiformans (ro.set.ti.for′mans. N.L. fem. n. *rosetta* (from L. fem. n. *rosa rose*) rosette; L. pres. part. *formans* forming; N.L. part. adj. *rosettiformans* rosette-forming, referring to the ability of the organism to form rosette-shaped structures).

Basonym: *Rhizobium rosettiformans* (Kaur et al. 2011).

The description is the same as for *R. rosettiformans* (Kaur et al. 2011). Phylogenetic analysis of the core-genes and pairwise provided strong evidence for the placement of this species in the novel genus *Peteryoungia*. The type strain is W3^T (=CCM 7583^T=MTCC 9454^T).

Description of *Peteryoungia wuzhouensis comb.* nov.

Peteryoungia wuzhouensis (wu.zhou.en'sis. N.L. fem. n. *wuzhouensis* pertaining to Wuzhou, a city in China, where the type strain was isolated).

Basonym: *Rhizobium wuzhouensis*

Homotypic synonym: *Rhizobium wuzhouense* (Yuan et al. 2018).

The description is the same as for *R. wuzhouense* (Yuan et al. 2018). Phylogenetic analysis of the core-genes and pairwise provided strong evidence for the placement of this species in the novel genus *Peteryoungia*. The type strain is W44^T (=CCTCC AB 2017179^T=GDMCC 1.1257^T=KCTC 62194^T).

Description of *Peteryoungia rhizophila comb.* nov.

Peteryoungia rhizophila (rhi.zo'phi.la. Gr. fem. n. *rhiza* root; Gr. masc. adj. *philos* loving; N.L. fem. adj. *rhizophila* root-loving).

Basonym: *Rhizobium rhizophila*

Homotypic synonym: *Rhizobium rhizophilum* (Gao et al. 2020).

The description is the same as for *R. rhizophilum* (Gao et al. 2020). Phylogenetic analysis of the core-genes and pairwise provided strong evidence for the placement of this species in the novel genus *Peteryoungia*. The type strain is 7209-2^T (=CGMCC 1.15691^T=DSM 103161^T).

Emended description of the genus *Ciceribacter* Kathiravan et al. 2013

The description is the same as given by Kathiravan et al. (2013) with the following amendments. Colonies vary in colour from bluish-black to semi-translucent. A few species can fix di-nitrogen. Catalase-positive and oxidase-negative. The major fatty acid is $C_{18:1}\omega7c$, and the DNA G+C content ranges between 60.5–63.5 mol %. The type species is *Ciceribacter lividus*.

Description of *Ciceribacter daejeonensis comb.* nov.

Ciceribacter daejeonensis (dae.jeon.en'sis. N.L. masc. adj. *daejeonensis* pertaining to Daejeon, a city in Korea, where the type strain was isolated).

Basonym: *Rhizobium daejeonensis*

Homotypic synonym: *Rhizobium daejeonense* (Quan et al. 2005).

The description is the same as for *R. daejeonense* (Quan et al. 2005). Phylogenetic analysis of the core-genes and pairwise provided strong evidence for the placement of this species in the novel genus *Ciceribacter*. The type strain is L61^T (=KCTC 12121^T=IAM 15042^T=CCBAU 10050^T).

Description of *Ciceribacter selenitireducens comb.* nov.

Ciceribacter selenitireducens (se.le.ni.ti.re.du'cens N.L. n. *selenis -itis*, selenite; L. pres. part. *reducens*, converting to a different state; N.L. part. adj. *selenitireducens*, selenite reducing, referring to the organism's ability to reduce the selenium oxyanion selenite to elemental selenium).

Basonym: *Rhizobium selenitireducens* (Hunter et al. 2007).

The description is the same as for *R. selenitireducens* (Hunter et al. 2007). Phylogenetic analysis of the core-genes and pairwise provided strong evidence for the placement of this species in the novel genus *Ciceribacter*. The type strain is B1^T (=ATCC BAA-1503^T=LMG 24075^T=NRRL B-41997^T).

Description of *Ciceribacter naphthalenivorans comb.* nov.

Ciceribacter naphthalenivorans (naph.tha.le.ni.vo'rans. N.L. neut. n. *napthalenum*, naphthalene; L. pres. part. *vorans*, devouring; N.L. part. adj. *naphthalenivorans*, naphthalene-devouring).

Basonym: *Rhizobium naphthalenivorans* (Kaiya et al. 2012).

The description is the same as for *R naphthalenivorans* (Kaiya et al. 2012). Phylogenetic analysis of the core-genes

and pairwise provided strong evidence for the placement of this species in the novel genus *Ciceribacter*. The type strain is TSY03b^T (= KCTC 23252^T = NBRC 107585^T).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02349-9.

Acknowledgements The authors thank Prof. Aharon Oren, Department of Plant and Environmental Sciences, The Hebrew University of Jerusalem, Israel, and Prof. Bernhard Schink, Department of Biology, University of Konstanz, Germany, for their expert suggestions concerning the correct species name, species epithet and Latin etymology. Authors also thank Prof J Peter W Young, University of York, to grant us permission to propose new genus *Peteryoungia*. The authors acknowledge the financial support of the Department of Biotechnology (BT/Coord. II/01/03/2016).

Author's contributions MK: methodology, formal analysis, validation; AH and AP: methodology, formal analysis; AN and KJ methodology, isolation; DM and YS funding acquisition; PR conceptualization, methodology, data curation, formal analysis, supervision, and writing.

Funding Financial support by the Department of Biotechnology (BT/Coord. II/01/03/2016) and (BT/PR8218/BCE/8/1044/2013).

Data availability The GenBank accession number for the 16S rRNA gene sequence of strain ADMK78^T is MK942856. The draft genome sequence has been deposited in GenBank under the accession number CP058350-CP058352.

Declarations

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

Ethical approval The experiments reported in this manuscript did not involve human participants and/or animals.

Consent for publication The manuscript is submitted with the consent of all authors.

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