Heliyon 10 (2024) e32825

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Endosaccharibacter trunci gen. nov., sp. nov. and *Rhizosaccharibacter radicis* gen. nov., sp. nov., two novel bacteria of the family *Acetobacteraceae* isolated from sugarcane

Nittaya Pitiwittayakul [a,*], Pattaraporn Yukphan [b,**], Piyanat Charoenyingcharoen [b], Somboon Tanasupawat [c]

[a] *Faculty of Agricultural Innovation and Technology, Rajamangala University of Technology Isan, Nakhon Ratchasima Campus, Nakhon Ratchasima 30000, Thailand*

[b] *Microbial Diversity and Utilization Research Team, Thailand Bioresource Research Center (TBRC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani 12120, Thailand*

[c] *Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand*

ARTICLE INFO

Keywords:
Acetobacteraceae
Endosaccharibacter
Rhizosaccharibacter
Sugarcane

ABSTRACT

Two novel endophytic bacterial strains, designated KSS8^T and KSS12^T, were isolated from the stems and roots of sugarcane, respectively, collected in Nakhon Ratchasima, Thailand. They were Gram-stain-negative, aerobic, and rod-shaped. The strain KSS8^T was a motile bacterium with a subpolar flagellum, while the strain KSS12^T was non-motile. Strains KSS8^T and KSS12^T were closely related to *Lichenicola cladoniae* PAMC 26569^T (97.3 and 95.6 %, respectively) and *Lichenicoccus roseus* KEBCLARHB70R^T (97.2 and 95.8 %, respectively) based on the similarity on their 16S rRNA gene sequence. This similarity corresponded to their phylogenomic positions within the evolutionary radiation of the family *Acetobacteraceae*. The average nucleotide identities and digital DNA-DNA hybridization values between the genome sequences of the two strains and other genera were significantly lower than the defined threshold values of 95–96 % and 70 %, respectively, which are used for the delineation of prokaryotic species. Both strains contained summed feature 8 ($C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*), $C_{16:0}$, $C_{19:0}$ cyclo ω8*c*, $C_{18:0}$, and $C_{18:1}$ 2OH as the predominant cellular fatty acids, but $C_{18:3}$ ω6*c* (6, 9, 12) were found only in strain KSS12^T. Based on phenotypic, chemotaxonomic, phylogenetic, and genomic analyses, these strains clearly represented two novel genera within the family *Acetobacteraceae*, for which the name *Endosaccharibacter* gen. nov., with the type species *Endosaccharibacter trunci* sp. nov. (type strain, KSS8^T = TBRC 14669^T = NBRC 115232^T = KCTC 92115^T = LMG 32414^T) and the name *Rhizosaccharibacter* gen. nov., with the type species *Rhizosaccharibacter radicis* sp. nov. (type strain, KSS12^T = TBRC 13066^T = NBRC 114898^T = KCTC 82433^T = LMG 32137^T) are proposed.

Abbreviations: AAB, Acetic Acid Bacteria; AAI, Average Amino Acid Identity; ANI, Average Nucleotide identity; dDDH, Digital DNA-DNA hybridization; GPY, Glucose–Peptone–Yeast extract; GYPG, Glucose–Yeast extract–Peptone–Glycerol; LGI, Liquid Glucose Ivo; MSAs, Multiple Sequence Alignments; POCP, Percentage of Conserved Proteins; TEM, Transmission Electron Microscopy; YPGD, Yeast extract-Peptone-Glycerol-Dextose.

* Corresponding author.
** Corresponding author.
E-mail addresses: nittaya.pi@rmuti.ac.th (N. Pitiwittayakul), pattaraporn@biotec.or.th (P. Yukphan).

https://doi.org/10.1016/j.heliyon.2024.e32825
Received 25 February 2024; Received in revised form 27 May 2024; Accepted 10 June 2024
Available online 10 June 2024

1. Introduction

Gram-negative bacterial genera belonging to the family *Acetobacteraceae* are taxonomically classified in the order *Rhodospirillales* of the class *Alphaproteobacteria*. The List of Prokaryotic Names with Standing in Nomenclature consists of 48 names that have been validated [1]. The genera are classified into two groups based on their application, ecology, and phylogeny. The first group, commonly referred to as the acetous group or acetic acid bacteria (AAB), have a common ability to convert ethanol to acetic acid, except for *Asaia* spp. [2]. This group consists of genera such as *Acetobacter*, *Gluconacetobacter*, *Gluconobacter*, *Asaia*, *Granulibacter*, and *Komagataeibacter*. The second group, referred to as the acidophilic group, consists of genera such as *Acidiphilium* and *Roseomonas* [2,3]. Recently, two novel bacterial genera, *Lichenicoccus* and *Lichenicola*, have been isolated from lichens. They were classified and proposed as members of the acetous group based on their similarity in the 16S rRNA gene sequence [4,5]. Several members of the acetous group are found in roots, root nodules, leaves, and other plant tissues or the rhizosphere of plants like sugarcane, alfalfa, rice, coffee, and the tropical flower *Michalia champaca* [6–16].

Endophytes are microbial symbionts that live inside plants for most of their life cycle without causing harm to their host plants [17]. Endophytic bacteria can directly benefit host plants through nutrient uptake and hormone regulation, as well as indirectly through various mechanisms such as hydrolytic enzymes, antibiotic provision, nutrient limitation, and boosting plant defenses against pests and diseases [18]. There have been reports associating sugarcanes with several endophytic bacteria that are advantageous for their growth [19,20]. In Nakhon Ratchasima, a province known for its numerous sugarcane farms, there are ample opportunities to isolate endophytic bacteria associated with sugarcane. In this study, two novel endophytic bacteria isolated from the stems and roots of sugarcane in Kham Sakaesaeng district, *Endosaccharibacter trunci* sp. nov. and *Rhizosaccharibacter radicis* sp. nov., were proposed as new members of the family *Acetobacteraceae* using a polyphasic approach.

2. Materials and methods

2.1. Isolation and related type strains

Sugarcane (*Saccharum officinarum*) samples (a nearly 6-month-old plant) were collected in June 2018 from eight districts in Nakhon Ratchasima province, Thailand. Three individual plants were collected at each site. The stem and root of sugarcane were chopped into small pieces (approximately 1 cm) and surface sterilized in 70 % ethanol for 3 min, 2.5 % fresh sodium hypochlorite solution for 5 min, and 70 % ethanol for 30 s, followed by 5 rinses in sterile distilled water. The sterilized stems and roots were ground with a sterile mortar and pestle prior to being inoculated in Liquid Glucose Ivo (LGI) broth (10 % sucrose w/v, 0.06 % KH_2PO_4 w/v, 0.02 % K_2HPO_4, 0.02 % $MgSO_4$, 0.002 % $CaCl_2$ w/v, 0.001 % $FeCl_3$ w/v, and 0.0002 % Na_2MoO_4 w/v). The cultures were streaked and purified on LGI agar after a 4-6-day incubation at 30 °C [13,16]. The pure cultures were cultivated on Yeast extract–Peptone–Glycerol–Dextrose (YPGD) medium (5 g of yeast extract, 5 g of peptone, 5 g of glycerol, and 5 g of glucose in 1 L of water) at 30 °C for 48 h and preserved in Glucose–Yeast extract–Peptone–Glycerol (GYPG) medium (10 g of D-glucose, 5 g of yeast extract, 10 g of peptone, and 10 g of glycerol, with or without 15 g agar in 1 L of water) containing 50 % glycerol at −80 °C. The strains KSS8^T (= TBRC 14669^T = NBRC 115232^T = KCTC 92115^T = LMG 32414^T) and KSS12^T (= TBRC 13066^T = NBRC 114898^T = KCTC 82433^T = LMG 32137^T) were compared with the type strains of *Lichenicola cladoniae* JCM 33604^T (=PAMC 26569^T), *Lichenicoccus roseus* KCTC 72321^T (=KEBCLARHB70R^T), *Nguyenibacter vanlangensis* TBRC 4639^T (= LMG 31431^T), *Acidomonas methanolica* TBRC 4990^T (= DSM 5432^T), *Gluconacetobacter liquefaciens* TBRC 378^T (= DSM 5603^T), and *Endobacter medicaginis* LMG 26838^T (= CECT 8088^T).

2.2. 16S rRNA gene phylogeny

The genomic DNA of the KSS8^T and KSS12^T were extracted by using a genomic isolation kit (Vivantis Technologies Sdn Bhd, Malaysia). The 16S rRNA gene sequence was amplified as previously reported [21]. The PCR product was purified using the GeneJet PCR purification Kit (ThermoFisher, USA), and then sequenced using primers 27F, 585F, and 1525R (First BASE Laboratories, Selangor, Malaysia). The 16S rRNA gene sequence of KSS8^T (1415 bp) and KSS12^T (1430 bp) were available under the accession number MW605159 and MW187776, respectively. The 16S rRNA gene pairwise sequence similarity of KSS8^T with sequences of KSS12^T and the type strains of related species available in the databases of GenBank/ENA/DDBJ and EzBioCloud (www.ezbiocloud.net/) [22–25] were compared. The phylogenetic analysis based on 16S rRNA gene sequences was performed using Molecular Evolutionary Genetics Analysis Version 11 (MEGA 11 version 11.0.13) software [26]. The multiple sequence alignments (MSAs) of the 16S rRNA gene sequences were performed using the MUSCLE algorithm [27]. All gaps and ambiguous nucleotides within the MSAs were completely removed. Phylogenetic trees of the 16S rRNA gene sequences were reconstructed using the neighbor-joining (NJ) [28], maximum-likelihood (ML) [29,30], and maximum parsimony (MP) [31] methods with 1000 bootstrap replicates [32]. Kimura's two-parameter correction model [33] was used to calculate the evolutionary distances and topologies.

2.3. Genome sequencing and analysis

DNA libraries of strains KSS8^T and KSS12^T were paired-end sequenced (2x150 bp) on the Illumina HiSeq X-ten sequencer. Raw read quality was checked using FASTQC software. Adaptors and poor-quality reads were removed using Trim Galore. The high-quality bases (average base-quality score; q score of ≥30) were *de novo* assembled using the Unicycler v0.4.4. The genome annotation was carried

out by the RASTtk annotation service in PATRIC [34]. The GenBank accession numbers for the whole genome sequences of strain KSS8^T and KSS12^T are JAMSKV000000000 and JAMZEJ000000000, respectively. The genome sequences of strains KSS8^T, KSS12^T, and the closest *Acetobacteraceae* genera that were most similar to these strains in terms of 16S rRNA similarity were evaluated for average nucleotide identity (ANIb) computation using JspeciesWS (http://jspecies.ribohost.com/jspeciesws) [35], and the digital DNA-DNA hybridization (dDDH) inferred from the sequence identity-based genome BLAST distance phylogeny (GBDP) formula d_4 by using the Type (Strain) Genome Server (https://tygs.dsmz.de/) [36,37]. The average amino acid identity (AAI) values were calculated by the orthologous ANI algorithm [38]. The percentage of conserved proteins (POCP) was analyzed by Protologger, a dedicated Galaxy-based website (www.protologger.de) [39]. The consensus phylogenetic tree was generated based on data derived from a multi-locus alignment of core genes in the strains KSS8^T and KSS12^T with related species in the NCBI Assembly database using the automated multi-locus species tree (autoMLST) (https://automlst.ziemertlab.com) [40]. Orthovenn2 was used to compare orthologous gene clusters between KSS8^T, KSS12^T and closely related type strains [41].

2.4. Phenotypic characteristics and chemotaxonomy

The strains KSS8^T and KSS12^T were cultured on rich Glucose–Peptone–Yeast extract (GPY) agar (25 g of glucose, 5 g of peptone, 3 g of yeast extract, 15 g of agar in 1 L of water) and incubated at 30 °C for 48 h to observe colony morphology. Cell morphology and Gram-staining were determined by a light microscope (x1000; E−100, Nikon) [42]. Motility was investigated in GPY semi-solid agar medium (agar 0.5 %) at 28 °C for 7 days and confirmed in GPY broth incubated for 24 h at 28 °C using the hanging-drop method and then observed under a light microscope. The cell size and flagellation were examined by transmission electron microscopy (TEM; HT7700, Hitachi). In the case of TEM, cells were negatively stained with 1.0 % uranyl acetate on a carbon-coated copper grid [43,44] and operated at 80 kV with magnifications of×4000 and ×6000. The oxidation of acetate and lactate was indicated by the observation of color change from yellow to blue in a liquid medium [45,46]. To determine the production of 2-keto-, 5-keto-, and 2,5-diketo-gluconates from D-glucose, the bacterial strains were grown in a medium (3 % glucose, 0.3 % yeast extract) for 3 and 6 days. Then the culture broth was analyzed by thin-layer chromatography [47]. Growth was measured on an agar medium in the presence of 30 % (w/v) glucose, 0.5 % (w/v) glutamate, and 2.5 % (w/v) mannitol [46]. The utilization and acid formation of the different carbon sources were investigated, as previously reported [45,46]. In brief, the tests were performed in a medium containing 0.5 % (w/v) yeast extract, 1 % (w/v) carbon source, and 0.2 % (w/v) bromocresol purple and kept at 30 °C for 7 days, except for the strains *Lichenicola cladoniae* JCM 33604^T and *Lichenicoccus roseus* KCTC 72321^T, which were performed at 15 °C for 21 d. The turbidity was used to determine growth, and the color shift of the medium from purple to yellow was used to indicate acid production [45,46]. The temperature range for growth was tested in GPY broth at 5, 15, 25, 30, 37, and 40 °C for 7 days. The pH range for growth at pH 3–8.5 (at 0.5 pH unit intervals) was tested in GPY broth, which was adjusted to the desired pH with hydrochloric acid or sodium hydroxide.

For the respiratory quinone analysis, strains KSS8^T and KSS12^T were cultured on GPY medium at 30 °C for 72 h. Respiratory quinones were extracted from collected cells, purified [48], and identified using HPLC (Waters Alliance 1690). For the total fatty acid analyses, the strains KSS8^T, KSS12^T, and reference strains were grown on GPY at 30 °C for 3 days, except for the strains *Lichenicola cladoniae* JCM 33604^T and *Lichenicoccus roseus* KCTC 72321^T (GPY adjusted pH 5.5), which were grown at 15 °C for 21 days and 25 °C for 8 days, respectively. The collected cells were then extracted according to the MIDI standard protocol (Sherlock Microbial Identification System, version 6.4) and identified via the TSBA database (version 6.21) [49].

2.5. Characterization of plant growth promoting activities

To investigate the ammonia production, the strains KSS8^T and KSS12^T were grown in peptone water at 30 °C for 72 h. The development of a yellow to brown color in the supernatant reacting with Nessler's reagent indicated that the ammonia production test was positive [50]. Moreover, strains KSS8^T and KSS12^T were individually cultivated in YPGD supplemented with 1 μg of L-tryptophan at 30 °C for 72 h under shaking conditions for the screening of indole-3-acetic acid (IAA) production. Salkowski's reagent-reacting supernatant was measured spectrophotometrically at 530 nm in comparison to the standard indole-3-acetic acid solution [51]. To examine the ability of these two strains to solubilize P and Zn, their cultured broth was dropped on Pikovskaya agar medium supplemented with tricalcium phosphate and on Tris-mineral salts media supplemented with insoluble zinc compounds and incubated for 7 days at 30 °C. The solubilizing capacity was demonstrated by a hollow zone around the bacterial colony. The solubilization index (SI) was calculated by dividing the sum of the colony diameter and the diameter of the halo zone by the diameter of the colony [52,53]. The development of an orange halo zone on the chrome-azurol S (CAS) medium, where siderophore production was evaluated, verified siderophore production [54].

3. Results and discussion

3.1. Isolation of endophytic bacteria from sugarcane

The surface-sterilized stems and roots of sugarcane were used for isolating bacterial endophytes. In total, 127 endophytic bacterial isolates were obtained from sugarcane in eight districts in Nakhon Ratchasima using LGI media. Of these, 79 isolates were obtained from roots, and 48 were obtained from stems (data not shown). Out of 118 isolates, the majority were Gram-negative strains, while the remaining were Gram-positive. The predominant endophytic bacteria isolated from sugarcanes belonged to the phylum *Pseudomonadota,* specifically *Acetobacteraceae*, including species, such as *N. vanlangensis*, *Acidomonas methanolica*, *Asaia bogorensis*,

Table 1
Genome characteristics and pairwise sequence similarities (%) of the 16S rRNA gene sequences of the strains KSS8^T, KSS12^T, and the closely related species. The draft genome sequences of the strains KSS8^T and KSS12^T were determined in this study. The genome sequences of other species were retrieved from the GenBank database.

Species	Strain	DNA G+C content (mol%)	16S rRNA gene similarity (%)		ANIb value (%)		dDDH value (%)		AAI value (%)	
			KSS8^T	KSS12^T	KSS8^T	KSS12^T	KSS8^T	KSS12^T	KSS8^T	KSS12^T
Endosaccharibacter trunci	KSS8^T	67.1	100	96.2	100	74.2	100	20.9	100	66.1
Rhizosaccharibacter radicis	KSS12^T	70.0	96.2	100	74.1	100	20.9	100	66.1	100
Lichenicola cladoniae	PAMC 26569^T	64.6	97.3	95.6	74.2	73.8	19.9	20.1	67.5	66.0
Lichenicoccus roseus	KEBCLARHB70R^T	67.8	97.2	95.8	72.9	73.4	19.6	19.6	63.7	64.1
Gluconacetobacter aggeris	T6203-4-1a^T	65.2	96.6	95.1	71.4	71.6	19.9	20.3	60.2	60.3
Ameyamaea chiangmaiensis	AC04^T	64.7	96.4	94.2	70.3	71.0	19.6	21.5	60.0	60.8
Komagataeibacter xylinus	LMG 1515^T	62.3	96.2	93.8	70.2	70.5	19.4	19.4	58.6	58.5
Nguyenibacter vanlangensis	LMG 31431^T	65.8	96.1	94.7	73.0	72.6	22.3	21.4	61.3	61.3
Gluconacetobacter liquefaciens	DSM 5603^T	64.4	96.0	95.4	71.1	71.4	19.5	20.0	60.0	60.5
Acidomonas methanolica	DSM 5432^T	64.7	95.9	95.2	70.2	70.1	18.9	19.4	57.5	57.7
Asaia bogorensis	NBRC 16594^T	59.8	95.8	94.4	68.5	68.4	20.7	22.5	56.7	57.3
Endobacter medicaginis	CECT 8088^T	67.6	95.1	94.9	71.3	71.8	19.7	20.2	60.7	61.0
Acetobacter aceti	NBRC 14818^T	57.0	94.8	93.1	69.0	69.1	19.3	19.6	57.6	57.4
Granulibacter bethesdensis	CGDNIH1^T	59.1	94.6	94.5	68.7	68.9	20.2	22.0	57.4	57.7
Commensalibacter intestini	A911^T	36.8	92.5	91.2	63.7	63.6	40.8	44.7	52.9	52.8

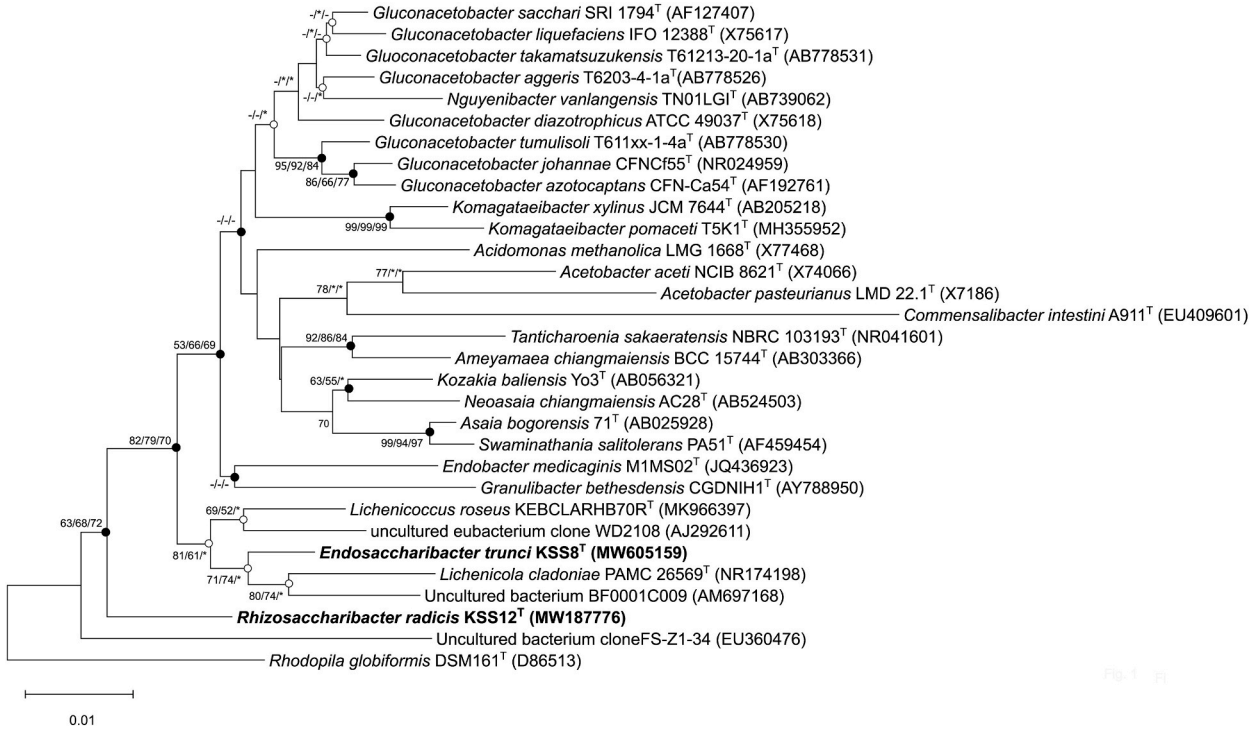

Fig. 1. The neighbor-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of *Endosaccharibacter* gen. nov., *Rhizosaccharibacter* gen. nov., and related taxa. *Rhodopila globiformis* DSM 161^T was used as an outgroup. Closed circles indicate that the corresponding nodes were also recovered in both trees generated with the maximum-likelihood (ML) and the maximum-parsimony (MP) algorithms, open circles indicate the nodes were recovered in the ML tree. Percentage bootstrap values $\geq$ 50 % (1000 bootstrap replicates) are shown for NJ, ML and MP at branch nodes. Asterisks indicate that the nodes do not appear in the ML or MP trees. Bar, 0.01 represents substitutions per nucleotide position.

Tanticharoenia aidae [16,55], *Asaia spathodeae*, *Asaia siamensis,* and *Neoasaia chiangmaiensis* (data not shown). In this study, the strain KSS8^T was isolated from the stems of sugarcane, while the strain KSS12^T was isolated from the roots of sugarcane, both in Kham Sakaesaeng district. These two strains exhibited smooth yellow colonies on LGI agar. The green LGI agar plates turned yellow due to the acid produced by these two bacteria. Further characterization involved genotypic, biochemical, and physiological tests.

3.2. Genotypic characterization

The KSS8^T displayed the highest 16S rRNA similarity to *Lichenicola cladoniae* PAMC 26569^T (97.3 %), *Lichenicoccus roseus* KEBCLARHB70R^T (97.2 %), *Gluconacetobacter aggeris* T6203-4-1a^T (96.6 %), *N. vanlangensis* LMG 31431^T (96.1 %), *Acidomonas methanolica* DSM 5432^T (95.9 %), and *Asaia bogorensis* NBRC 16594^T (95.8 %) (Table 1). The percentage similarities of KSS12^T to KSS8^T, *Lichenicoccus roseus* KEBCLARHB70R^T, and *Lichenicola cladoniae* PAMC 26569^T were 96.2, 95.8, and 95.6 %, respectively. These similarities were much below the recommended cut-off value (98.65 %) proposed for bacterial species delineation [24]. Therefore, the two strains, KSS8^T and KSS12^T, were potentially novel species within the family *Acetobacteraceae*.

In the phylogenetic trees based on 16S rRNA gene sequences generated using the three approaches, the strain KSS8^T formed a clade that was separate from a cluster of *Lichenicoccus roseus* KEBCLARHB70R^T and a cluster of *Lichenicola cladoniae* PAMC 26569^T, in addition to a group of closely related uncultured clones (Fig. 1). The strain KSS12^T formed a distinct phylogenetic lineage with the KSS8^T and the closely related relatives, *Lichenicoccus roseus* KEBCLARHB70R^T and *Lichenicola cladoniae* PAMC 26569^T.

Based on the 16S rRNA gene sequence and phylogenetic tree analysis, the strains KSS8^T and KSS12^T were considered candidates for two novel species closely related to *Lichenicola cladoniae* PAMC 26569^T and *Lichenicoccus roseus* KEBCLARHB70R^T within the family *Acetobacteraceae*.

3.3. Genomic features and comparative genomics

Strains KSS8^T and KSS12^T had genome sizes of 3,718,282 bp (number of contigs, 43; N50 value, 209,382) and 3,954,055 bp (number of contigs, 24; N50 value, 439,367) with genomic G+C content of 67.1 and 70.0 mol%, respectively. The ANIb values among strains KSS8^T, KSS12^T, and other related type strains of *Acetobacteraceae* ranged from 63.6 % to 74.2 % (Table 1), significantly below the ANI cut-off value (95–96 %) used to distinguish bacterial species [56]. Furthermore, strains KSS8^T and KSS12^T showed digital

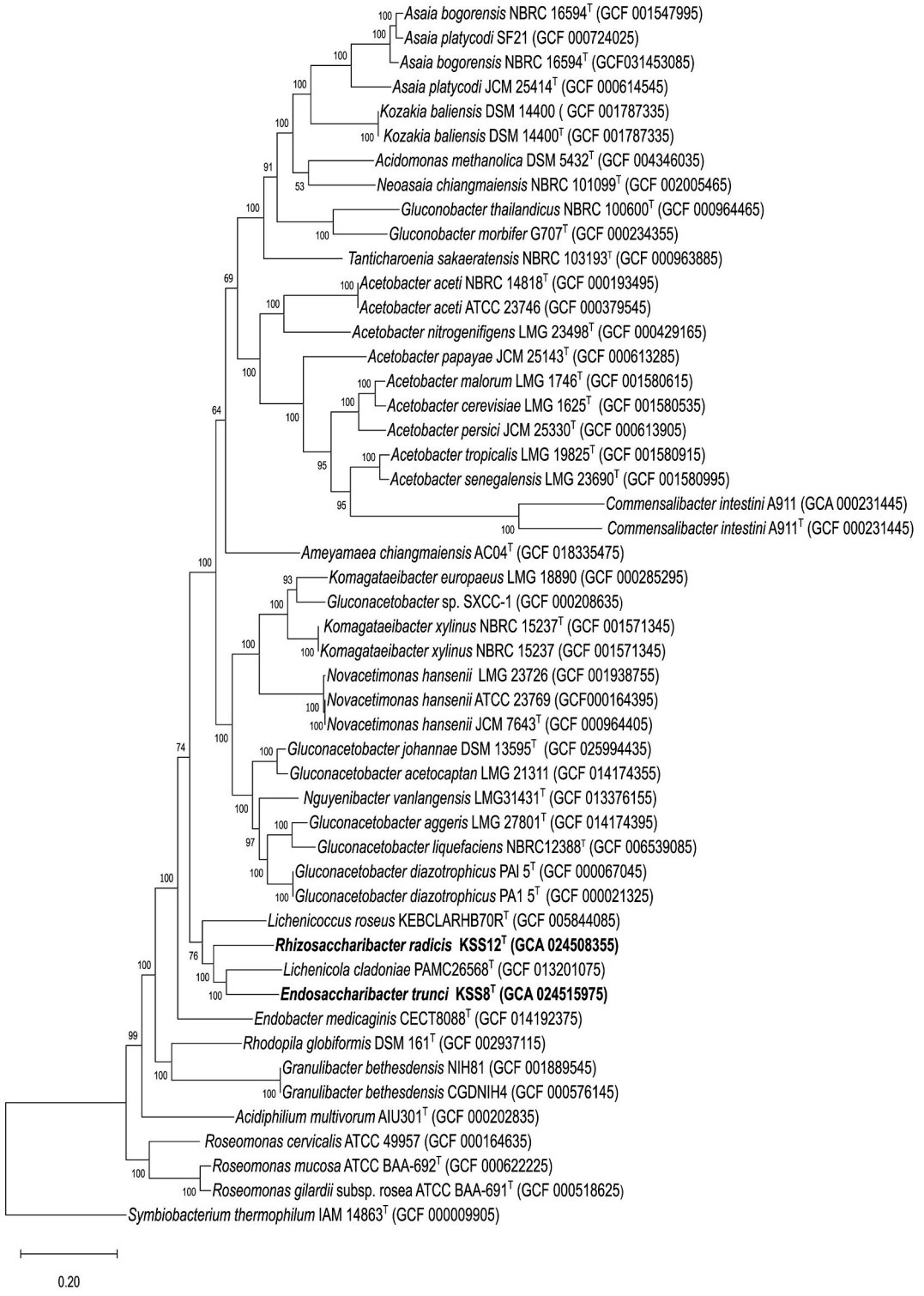

Fig. 2. Maximum-likelihood phylogenetic tree based on the core genome indicating the phylogenetic positions of strains KSS8^T and KSS12^T with the related species. The scale bar indicates 0.2 substitutions per nucleotide position. *Symbiobacterium thermophilum* IAM 14863^T was used as an outgroup. GenBank accession numbers are listed for each sequence in parentheses.

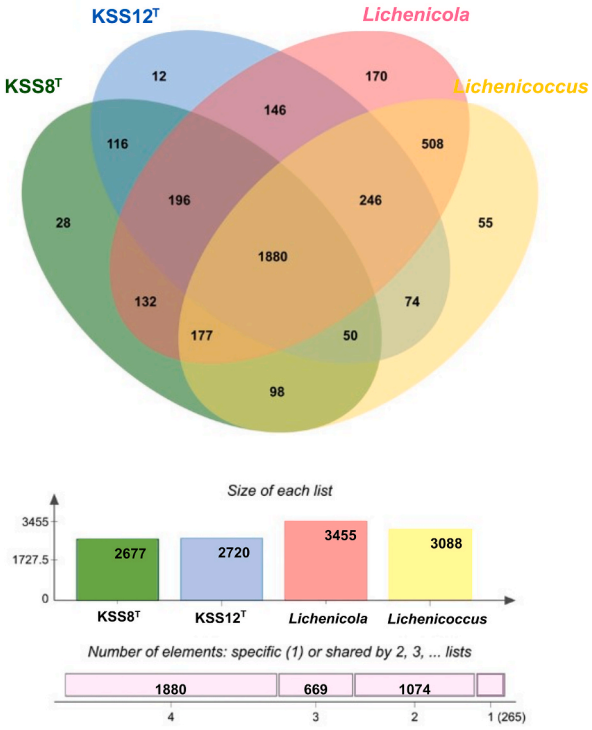

Fig. 3. Venn diagram illustrating the distribution of shared orthologous clusters among strains KSS8^T, KSS12^T, and the closely related genera, *Lichenicola cladoniae* PAMC 26569^T and *Lichenicoccus roseus* KEBCLARHB70R^T. The numbers in the diagram indicate overlapped conserved genes or non-overlapped unique genes in each species.

DNA-DNA hybridization (dDDH) values of 19.3–44.7 % when compared with the closest genera, which was significantly less than the 70 % dDDH threshold level as novel species within an extant genus, therefore classifying them as the first species of two novel genera [57,58]. AAI values between strains KSS8^T, KSS12^T, and the most closely related type strains of *Acetobacteraceae* ranged from 52.8 % to 67.5 % (Table 1), which were within the previously reported range of genus-level pairwise differences (65–72 %) [59]. POCP comparison conducted via Protologger revealed that strains KSS8^T and KSS12^T had the highest similarity (>51 %) to the type strains within the family *Acetobacteraceae*: *Gluconacetobacter diazotrophicus* Pal5^T, *Tanticharoenia sakaeratensis* NBRC 103193^T, *Asaia bogorensis* NBRC 16594^T, and *Kozakia baliensis* DSM 14400^T. According to the genome-based phylogenetic tree (Fig. 2), strain KSS12^T formed a distinct phylogenetic clade with the cluster of strain KSS8^T and *Lichenicola cladoniae* PAMC 26569^T with a high bootstrap value, confirming the topology determined by 16S rRNA gene sequences. The genome tree was in accordance with the highest ANI and AAI values of strains KSS8^T to KSS12^T and *Lichenicola cladoniae* PAMC 26569^T, which were 73.8–74.2 % and 66.1–67.5, respectively.

The comparative analysis of the presence and absence of genes in KSS8^T, KSS12^T, and the closest related genera, *Lichenicola cladoniae* PAMC 26569^T and *Lichenicoccus roseus* KEBCLARHB70R^T, revealed that the core shared by the four genera accounted for 1880 core genome orthologs (Fig. 3). In terms of the strain-specific genome, 28 and 12 genes were unique for strains KSS8^T and KSS12^T, respectively. The orthologous groups specific to KSS8^T were related to the glutathione metabolic process, sequence-specific DNA binding, haloacetate dehalogenase activity, phthalate catabolic process, fatty acid elongation, siderophore transport, oxidoreductase activity, etc. (Supplementary Table S1, available in the online version of this article). The orthologous groups specific to KSS12^T were associated with processes such as transferase activity, xenobiotic catabolic process, and DNA transposition. The strain KSS8^T shared 116 orthologous genes with strain KSS12^T and 132 genes with *Lichenicola cladoniae* PAMC 26569^T, both of which were higher than the shared genes with *Lichenicoccus roseus* KEBCLARHB70R^T. This pattern aligns with the topology of the phylogenetic tree based on the core genome.

3.4. Phenotypic and chemotaxonomic characteristics

After incubation at 30 °C for 48 h, KSS8^T exhibited pink-colored circular colonies with smooth surfaces, raised elevations, and entire margins on GPY agar, while those of KSS12^T were light-pink-colored circular colonies with smooth surfaces, convex elevations, and entire margins. The cells of strains KSS8^T and KSS12^T were Gram-stain-negative rods, similar to most other acetous taxa. Strain KSS8^T was motile through a single subpolar flagellum (Supplementary Fig. S1, available in the online version of this article), which was similar to the type strains of *Endobacter* [12] and *Lichenicoccus* [5] but different from the other closely related genera that are motile by peritrichous or polar flagella and the non-motile strains of KSS12^T and *Lichenicola* [4]. The strains KSS8^T and KSS12^T showed

Table 2
Characteristics differentiating *Endosaccharibacter trunci* KSS8^T and *Rhizosaccharibacter radicis* KSS12^T from their closely related species in the family *Acetobacteraceae* Strain: 1, KSS8^T; 2, KSS12^T; 3, *Lichenicola cladoniae* JCM 33604^T; 4, *Lichenicoccus roseus* KCTC 72321^T; 5, *Nguyenibacter vanlangensis* TBRC 4639^T; 6, *Gluconacetobacter liquefaciens* TBRC 378^T; 7, *Acidomonas methanolica* TBRC 4990^T; 8, *Endobacter medicaginis* LMG 26838^T. +, Positive; w, weakly positive; -, negative.

Characteristic	1	2	3[a]	4[a]	5[a]	6[a]	7[a]	8[a]
Cell shape	Rod-shaped	Rod-shaped	Rod-shaped	Coccoid	Rod-shaped	Rod-shaped	Rod-shaped	Coccoid to rod-shaped
Flagellation	Subpolar	Non-motile	Non-motile	Subpolar	Peritrichous	Peritrichous	Polar or non-motile	Subpolar
Colony color	Pink	Light pink	Pink	Pink to salmon-pink	Creamy to brownish	Beige to light brownish	White to light yellow	White
Optimum temperature for growth (°C)	25–30	25–30	15	10–15	30	28	30–32	28
Growth at 5 °C	–	–	+	+	–	–	–	–
Growth at 15 °C	+	+	+	+	–	–	–	–
Growth at 37 °C	+	+	–	–	+	–	+	+
Optimum pH (range) for growth	4.5–5.0	4.0–4.5	5.5–6.5	4.5–5.5	4.5–6.5	5.4–6.3	2.0–5.5	5.0–7.0
Utilization of								
Methanol	–	–	+	–	–	+	+	w
Mannose	–	+	+	–	+	+	+	+
D-Sorbitol	w	+	+	+	+	+	–	–
D-Mannitol	+	+	+	–	+	+	–	+
Propane 1,2 diol	–	–	+	–	–	–	–	+
Acid formation from								
D-Mannose	–	–	+	–	+	+	+	+
Melibiose	–	w	+	–	+	–	–	–
D-Ribose	–	–	+	–	+	w	w	w
Glycerol	–	–	–	–	–	+	w	w
Sucrose	+	+	–	–	+	–	–	–
Maltose	–	–	–	–	+	–	–	–
Ethanol	+	–	–	–	+	+	+	+
Soluble starch	–	–	w	–	–	–	–	w
Raffinose	–	–	–	–	+	–	–	–
DNA G+C content (%)	67.1	70.0	64.6	67.8	65.8	64.4	64.7	67.6

[a] Cell shape, flagellation, colony color, temperature, and pH for growth of strains 3, 4, 5, 6, 7 and 8 were obtained from Noh et al. [4], Pankratov et al. [5], Vu et al. [13], Yamada et al. [68], Yamashita et al. [69], Sievers and Swings [70], and Ramírez-Bahena et al. [12].

Table 3
Cellular fatty acid profiles of the strains KSS8^T, KSS12^T, and the closely related species. Strain: 1, KSS8^T; 2, KSS12^T; 3, *Lichenicola cladoniae* JCM 33604^T; 4, *Lichenicoccus roseus* KCTC 72321^T; 5, *Nguyenibacter vanlangensis* LMG 31431^T; 6, *Gluconacetobacter liquefaciens* DSM 5603^T; 7, *Acidomonas methanolica* DSM 5432^T; 8, *Endobacter medicaginis* CECT 8088^T. All data from the present study. Values are represented as the percentage of total cellular fatty acids; the major components (>5 %) are shown in bold. Fatty acids that represented <1.0 % of the total are not shown. -, not detected.

Fatty acid	1	2	3	4	5	6	7	8
Saturated straight chain:								
$C_{14:0}$	–	0.4	–	–	0.9	4.6	0.3	0.1
$C_{16:0}$	**13.9**	**9.9**	2.2	4.7	**11.2**	**9.0**	**8.4**	**8.6**
$C_{17:0}$	–	0.4	–	–	0.1	0.6	0.4	1.4
$C_{18:0}$	**9.0**	**7.9**	1.3	**5.3**	3.5	2.2	2.5	4.7
Unsaturated:								
$C_{18:3}$ ω6c (6, 9, 12)	–	**6.1**	1.4	–	–	–	–	–
Hydroxy:								
$C_{14:0}$ 2OH	–	–	–	–	1.0	3.6	1.1	–
$C_{16:0}$ 2OH	2.8	2.0	2.7	2.2	**6.8**	**6.1**	**6.1**	2.7
$C_{16:0}$ 3OH	2.7	2.2	1.6	2.3	2.9	2.4	2.1	3.4
iso-$C_{17:0}$ 3OH	2.9	1.2	**6.1**	0.8	1.9	–	–	–
$C_{18.0}$ 3OH	3.0	2.2	2.2	3.7	1.8	1.4	2.1	2.8
$C_{18:1}$ 2OH	**7.4**	**9.6**	**9.6**	**17.6**	0.3	0.6	**7.7**	**15.4**
Cyclopropane:								
$C_{19:0}$ cyclo ω8c	**10.7**	**10.7**	**7.4**	1.0	4.3	**5.8**	4.3	3.4
Summed features:[a]								
2	2.4	2.1	2.23	2.7	1.6	1.5	2.4	3.0
8	**40.7**	**40.6**	**47.5**	**55.4**	**59.0**	**58.6**	**57.5**	**48.9**

[a] Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 2 comprises $C_{12:0}$ aldehyde and/or unknown 10.9525; summed feature 3 comprises $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c; summed feature 8 comprises $C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c.

comparative results of oxidizing lactate and acetate to carbon dioxide and water, in contrast to the related genera, *Nguyenibacter* and *Lichenicoccus,* which were able to oxidize only acetate and not both, respectively [5]. The inability to grow on 30 % glucose distinguished strains KSS8^T and KSS12^T from the genera *Nguyenibacter* and *Acidomonas.* All three types of 2-keto-D, 5-keto-D, and 2,5-dike-to-D-gluconates were produced from D-glucose by strain KSS8^T and were different from other related genera in this study. However, strain KSS12^T could not produce the three types of D-gluconates from glucose. The strain KSS8^T exhibited the ability to produce acid from ethanol similarly to the genera *Nguyenibacter*, *Gluconacetobacter*, *Acidomonas*, and *Endobacter*, while this was not the case for the strain KSS12^T and the genera *Lichenicola* and *Lichenicoccus*. In contrast to the closest genera *Lichenicoccus* and *Lichenicola*, which are psychrophiles or psychrotolerant, the strain KSS8^T and KSS12^T grew at 15–37 °C (optimally at 25–30 °C) [4,5]. The characteristics that differentiate strains KSS8^T, KSS12^T from the type strains of the closely related species, *Lichenicola cladoniae* PAMC 26569^T, *Lichenicoccus roseus* KEBCLARHB70R^T, *N. vanlangensis* LMG 31431^T, *Acidomonas methanolica* DSM 5432^T, *Gluconacetobacter liquefaciens* DSM 5603^T, and *E. medicaginis* CECT 8088^T are shown in Table 2.

The respiratory quinone of the strains KSS8^T and KSS12^T was ubiquinone Q-10. The predominant cellular fatty acids (>5 %) in strains KSS8^T and KSS12^T were summed as feature 8 ($C_{18:1}$ ω6*c* and/or $C_{18:1}$ ω7*c*), $C_{16:0}$, $C_{19:0}$ cyclo ω8*c*, $C_{18:0}$, and $C_{18:1}$ 2OH. Additionally, $C_{18:3}$ ω6*c* (6, 9, 12) was observed in strain KSS12^T exclusively (Table 3). The dominant fatty acid in the neighboring type strains *N. vanlangensis* LMG 31431^T, *Acidomonas methanolica* DSM 5432^T, and *Gluconacetobacter liquefaciens* DSM 5603^T was $C_{16:0}$ 2OH, unlike in strains KSS8^T and KSS12^T. Strains KSS8^T and KSS12^T exhibited a major fatty acid, $C_{16:0}$, similar to all tested-type strains except *Lichenicola cladoniae* PAMC 26569^T and *Lichenicoccus roseus* KEBCLARHB70R^T. A major fatty acid $C_{18:0}$ was observed only in strains KSS8^T and KSS12^T, including the type strains of *Lichenicoccus roseus* KEBCLARHB70R^T.

3.5. Plant growth promoting activities

In this study, two strains, KSS8^T and KSS12^T, could produce indole-3-acetic acid in the range of 21.0 and 17.5 μg/mL, respectively. These two strains could not solubilize tricalcium phosphate. Only strain KSS8^T demonstrated the ability to dissolve zinc phosphate (SI = 3.03) (Supplementary Fig. S2), whereas it was unable to dissolve zinc oxide or zinc carbonate. On the other hand, strain KSS12^T could not solubilize any forms of inorganic zinc sources used in this study. Several organic acids, including keto-D-glutarate, 2, and 2,5-keto-derivatives, have been reported to be related to zinc solubilizing mechanisms [60]. These results were consistent with the ability of strain KSS8^T to produce 2-keto-D, 5-keto, and 2,5-diketo-D-gluconate in contrast to KSS12^T. Moreover, these two strains could not fix nitrogen as shown in the negative results of NH_3 production. Only strain KSS12^T possessed the ability to produce siderophores (Supplementary Fig. S2). The genome analysis using RASTtk identified genes related to the plant-beneficial properties of these two strains (Supplementary Table S2). The tryptophan synthase genes, such as *trpABCDEFG*, and genes involved in synthesizing indole-3-acetic acid (IAA) were observed in the genomes of KSS8^T and KSS12^T. Moreover, the KSS8^T and KSS12^T genomes contained the *Pho* regulon (*PhoR-PhoB*, *PhoC*, *PhoU*), the phosphate ABC transporter complex (*PstSCAB*), as well as the *ppx*, *ppa*, *ppk*, and *gcd* genes related to inorganic phosphate solubilization [61]. In the Nif system, only *nifU*, which is involved in the formation of metal-loclusters of nitrogenase as well as in the maturation of other FeS proteins, was found in both genomes. Iron-sulfur (FeS) clusters are crucial cofactors for a wide range of proteins involved in biological functions, including nitrogen fixation, respiration, DNA repair, and gene regulation [62]. In order to produce FeS clusters, Nif proteins are also found in organisms that do not fix nitrogen [63]. The presence of the gene encoding the identical protein polyketide synthase, associated with siderophore biosynthesis [64], only in the KSS12^T genome aligns with the result, confirming that only strain KSS12^T possessed the ability to produce siderophores. Analysis of the KSS8^T genome revealed putative genes encoding siderophore interacting proteins, TonB-dependent receptors, and Fe-ABC transport system (*pitADC*), but not for siderophore biosynthesis. Siderophore-interacting proteins (SIPS), such as YqjH from *Escherichia coli* and ViuB from *Vibrio cholerae*, are commonly associated with iron-dependent induction and siderophore utilization by ferric reductase activity [65]. Three cytoplasmic membrane proteins (TonB, ExbB, and ExbD) must be present for siderophore uptake across the outer membrane, and they must be able to associate a proton gradient with siderophore transport [66]. Many plant-associated endophytic bacteria have been categorized as plant growth-promoting bacteria because they possess mechanisms, such as phytohormone production, nitrogen fixation, mineral solubilization, and siderophore synthesis. It has been noted that certain bacteria from the family *Acetobacteraceae* have relationships with plants, exhibiting the ability to enhance plant growth [67].

Based on the results obtained from phenotypic, chemotaxonomic, and genomic analyses, strains KSS8^T and KSS12^T were distinguished from the type strains of the genera of the family *Acetobacteraceae*. The genus *Endosaccharibacter trunci* gen. nov., sp. nov., and *Rhizosaccharibacter radicis* gen. nov., sp. nov., are proposed as two new members of the family *Acetobacteraceae*.

4. Description of novel taxa

4.1. Description of Endosaccharibacter gen. nov.

Endosaccharibacter (En.do.sac.cha.ri.bac'ter. Gr. pref. *endo*-, within; N.L. neut. n. *Saccharum* the generic name of the sugarcane; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Endosaccharibacter*, a rod isolated from the stem of sugarcane).

Cells are Gram-stain-negative, rod-shaped, aerobic, and motile with subpolar flagellum. Cells are catalase-positive but oxidase-negative. Capable of producing 2-keto-D, 5-keto-D, and 2,5-diketo-D-gluconate from D-glucose. Oxidizes acetate and lactate. The dominant cellular fatty acids consist of summed feature 8 ($C_{18:1}$ ω6*c* and/or $C_{18:1}\omega$7*c*), $C_{16:0}$, $C_{19:0}$ cyclo ω8*c*, $C_{18:0}$, and $C_{18:1}$ 2OH including summed feature 2 ($C_{14:0}$ 3OH and isoI-$C_{16:1}$). The major respiratory ubiquinone is Q10. The genomic DNA G+C content of the type strain calculated from the genome sequence is 67.1 %. The type species is *Endosaccharibacter trunci.*

4.2. Description of Endosaccharibacter trunci sp. nov.

Endosaccharibacter trunci sp. nov. (trun'ci. L. gen. n. *trunci*, of a stem, of a trunk, referring to the stem of sugarcane that was the source of the type strain).

Cells are Gram-strain-negative, aerobic, rod-shaped (approximately 0.8–1.0 × 1.9–2.6 μm) and motile by means of subpolar flagellum. Colonies on GPY medium are pink-colored circular colonies with smooth surfaces, raised elevations and entire margins after incubation at 30 °C for 48 h. Growth occurs at 15–37 °C (optimum, 25–30 °C) and at pH 3.0–8.0 (optimum, pH 4.5–5.0). Grows on 0.5 % (w/v) glutamate and 2.5 % (w/v) mannitol medium but not on 30 % (w/v) D-glucose. Oxidation occurs in acetate (weakly) and lactate. 2-keto-D, 5-keto-D and 2,5-diketo-D gluconates are formed from D-glucose. Catalase is positive and oxidase is negative. Acid is produced from D-glucose, D-galactose, sucrose, ethanol, L-arabinose (weakly), and D-xylose (weakly). Growth is observed on D-glucose, D-mannose, D-galactose, D-fructose, L-sorbose, D-xylose, L-arabinose, L-rhamnose, D-mannitol, D-sorbitol, Dulcitol, m-erythritol, glycerol, melibiose, sucrose, raffinose, D-ribose, xylitol, L-arabinitol, inositol, and D-arabitol. The major cellular fatty acids (>5 %) are composed of summed feature 8 ($C_{18:1}$ $\omega 6c$ and/or $C_{18:1}$ $\omega 7c$), $C_{16:0}$, $C_{19:0}$ cyclo $\omega 8c$, $C_{18:0}$, and $C_{18:1}$ 2OH. The major respiratory ubiquinone is Q10. The DNA G+C content of the type strain is 67.1 % based on the complete genome sequence.

The type strain, KSS8^T (= TBRC 14669^T = NBRC 115232^T = KCTC 92115^T = LMG 32414^T), was isolated from the stems of sugarcane (*Saccharum officinarum* L.), collected from Kham Sakaesaeng, Nakhon Ratchasima Province, Thailand.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the whole genome sequence of strain KSS8^T are MW605159 and JAMSKV000000000, respectively.

4.3. Description of Rhizosaccharibacter gen. nov.

Rhizosaccharibacter (Rhi.zo.sac.cha.ri.bac'ter. Gr. fem. n. *rhiza* a root; N.L. neut. n. *Saccharum* the generic name of the sugarcane; N. L. masc. n. *bacter* a rod; N.L. masc. n. *Rhizosaccharibacter* a rod from sugarcane root).

Cells are Gram-stain-negative, rod-shaped, aerobic, and non-motile. Cells are catalase-positive but oxidase-negative. Does not produce 2-keto-D, 5-keto-D, and 2,5-diketo-D-gluconate from D-glucose. Oxidizes acetate and lactate. The dominant cellular fatty acids consist of summed feature 8 ($C_{18:1}$ $\omega 6c$ and/or $C_{18:1}$ $\omega 7c$), $C_{19:0}$ cyclo $\omega 8c$, $C_{16:0}$, $C_{18:1}$ 2OH, $C_{18:0}$ and $C_{18:3}$ $\omega 6c$ (6, 9, 12) including summed feature 2 ($C_{14:0}$ 3OH and isoI-$C_{16:1}$). The major respiratory ubiquinone is Q10. The genomic DNA G+C content of the type strain calculated from the genome sequence is 70.0 %. The type species is *Rhizosaccharibacter radicis.*

4.4. Description of Rhizosaccharibacter radicis sp. nov.

Rhizosaccharibacter radicis sp. nov. (ra'di.cis. L. gen. fem. n. *radicis*, of a root referring to the root of sugarcane that was the source of the type strain).

Cells are Gram-staining-negative, non-motile, aerobic, and rod-shaped (approximately 1.0–1.4 × 1.6–2.5 μm). Colonies on GPY medium are light-pink-colored circular colonies with smooth surfaces, convex elevations, and entire margins after incubation at 30 °C for 48 h. Growth occurs at 15–37 °C (optimum, 25 °C) and at pH 3.5–8.5 (optimum, pH 4–4.5). Growth is observed on 0.5 % (w/v) glutamate and 2.5 % (w/v) mannitol medium but not on 30 % (w/v) D-glucose. Oxidizes acetate and lactate to carbon dioxide and water. Incapable of producing 2-keto, 5-keto, and 2,5-diketo-D-gluconic acid from glucose. Glucose, mannose, D-galactose, D-fructose, L-sorbose, D-xylose, L-arabinose, D-mannitol, D-sorbitol, ducitol, glycerol, melibiose, sucrose, raffinose, D-ribose, inositol, xylitol, L-arabinitol, and D-arabitol can be used as carbon sources. Acid is produced from D-glucose, D-galactose, sucrose, L-arabinose (weakly), and melibiose (weakly). The genomic DNA G+C content of the type strain is 70.0 % based on the complete genome sequence.

The type strain, KSS12^T (= TBRC 13066^T = NBRC 114898^T = KCTC 82433^T = LMG 32137^T), was isolated from the roots of sugarcane (*Saccharum officinarum* L.), collected from Kham Sakaesaeng, Nakhon Ratchasima Province, Thailand.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the whole genome sequence of strain KSS12^T are MW187776 and JAMZEJ000000000, respectively.

Funding information

This work was supported financially by the Thailand Research Fund and the Office of the Higher Education Commission, Thailand (Grant no. MRG6180036).

Ethical statement

This article does not contain any studies with animals performed by any of the authors.

Data availability statement

Data associated with this study has been deposited at GenBank database under the accession number MW605159 and MW187776 for 16S rRNA sequence and JAMSKV000000000 and JAMZEJ000000000, for whole genome sequences.

CRediT authorship contribution statement

Nittaya Pitiwittayakul: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pattaraporn Yukphan:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Piyanat Charoenyingcharoen:** Software, Methodology. **Somboon Tanasupawat:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to Ms. Martha Maloi Eromine for the English editing of our manuscript and Professor Aharon Oren, The Hebrew University of Jerusalem, Jerusalem, Israel for the etymologies. We would like to thank National Biobank of Thailand, National Science and Technology Development Agency (NSTDA) for the fatty acid analytical instrument accessibility.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32825.

References

[1] A.C. Parte, J. Sardá Carbasse, J.P. Meier-Kolthoff, L.C. Reimer, M. Göker, List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ, Int. J. Syst. Evol. Microbiol. 70 (2020) 5607–5612, https://doi.org/10.1099/ijsem.0.004332.
[2] J. Guzman, A. Vilcinskas, Genome analysis suggests the bacterial family *Acetobacteraceae* is a source of undiscovered specialized metabolites, Antonie Leeuwenhoek 115 (1) (2022) 41–58, https://doi.org/10.1007/s10482-021-01676-7.
[3] K. Komagata, T. Iino, Y. Yamada, The family *Acetobacteraceae*, in: E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, F. Thompson (Eds.), The Prokaryotes: Alphaproteobacteria and Betaproteobacteria, Springer, Berlin, Germany, 2014, pp. 3–78, https://doi.org/10.1007/978-3-642-30197-1.
[4] H.J. Noh, S.C. Shin, Y. Park, A. Choi, K. Baek, *Lichenicola cladoniae* gen. nov., sp. nov., a member of the family *Acetobacteraceae* isolated from an Antarctic lichen, Int. J. Syst. Evol. Microbiol. 70 (2020) 5918–5925, https://doi.org/10.1099/ijsem.0.004495.
[5] T.A. Pankratov, D.S. Grouzdev, E.O. Patutina, T.V. Kolganova, J.J. Berestovskaya, A.A. Ashikhmin, *Lichenicoccus roseus* gen. nov., sp. nov., the first bacteriochlorophyll a-containing, psychrophilic and acidophilic *Acetobacteraceae* bacteriobiont of lichen *Cladonia* species, Int. J. Syst. Evol. Microbiol. 70 (2020) 4591–4601, https://doi.org/10.1099/ijsem.0.004318.
[6] V.A. Cavalcante, J. Döbereiner, A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane, Plant Soil 108 (1988) 23–31, https://doi.org/10.1007/BF02370096.
[7] L.E. Fuentes-Ramírez, R. Bustillos-Cristales, A. Tapia-Hernández, T. Jiménez-Salgado, E.T. Wang, E. Martínez-Romero, J. Caballero-Mellado, Novel nitrogen-fixing acetic acid bacteria, *Gluconacetobacter johannae* sp. nov. and *Gluconacetobacter azotocaptans* sp. nov., associated with coffee plants, Int. J. Syst. Evol. Microbiol. 51 (2001) 1305–1314, https://doi.org/10.1099/00207713-51-4-1305.
[8] P. Loganathan, S. Nair, *Swaminathania salitolerans* gen. nov., sp. nov., a salt-tolerant, nitrogen-fixing and phosphate-solubilizing bacterium from wild rice (*Porteresia coarctata Tateoka*), Int. J. Syst. Evol. Microbiol. 54 (2004) 1185–1190, https://doi.org/10.1099/ijs.0.02817-0.
[9] R. Muthukumarasamy, I. Cleenwerck, G. Revathi, M. Vadivelu, D. Janssens, B. Hoste, K.D. Gum, C.Y. Son, T. Sa, J. Caballero-Mellado, Natural association of *Gluconacetobacter diazotrophicus* and diazotrophic *Acetobacter peroxydans* with wetland rice, Syst. Appl. Microbiol. 28 (3) (2005) 277–286, https://doi.org/10.1016/j.syapm.2005.01.006.
[10] P. Yukphan, T. Malimas, Y. Muramatsu, M. Takahashi, M. Kaneyasum, S. Tanasupawat, Y. Nakagawa, K.I. Suzuki, W. Potacharoen, Y. Yamada, *Tanticharoenia sakaratensis* gen. nov., sp. nov., a new osmotolerant acetic acid bacterium in the *α-Proteobacteria*, Biosci. Biotechnol. Biochem. 72 (3) (2008) 672–676, https://doi.org/10.1271/bbb.70319.
[11] N. Samaddar, A. Paul, S. Chakravorty, W. Chakraborty, J. Mukherjee, D. Chowdhuri, R. Gachhui, Nitrogen fixation in *Asaia* sp. (family Acetobacteraceae), Curr. Microbiol. 63 (2011) 226–231, https://doi.org/10.1007/s00284-011-9968-3.
[12] M.H. Ramírez-Bahena, C. Tejedor, I. Martín, E. Velázquez, A. Peix, *Endobacter medicaginis* gen. nov., isolated from alfalfa nodules in an acidic soil, Int. J. Syst. Evol. Microbiol. 63 (2013) 1760–1765, https://doi.org/10.1099/ijs.0.041368-0.
[13] H.T.L. Vu, P. Yukphan, W. Chaipitakchonlatarn, T. Malimas, Y. Muramatsu, U.T.T. Bui, S. Tanasupawat, K.C. Duong, Y. Nakagawa, H.T. Pham, Y. Yamada, *Nguyenibacter vanlangensis* gen. nov., sp. nov., an unusual acetic acid bacterium in the *α Proteobacteria*, J. Gen. Appl. Microbiol. 59 (2013) 153–166, https://doi.org/10.2323/jgam.59.2_153.
[14] H.T.L. Vu, T. Malimas, W. Chaipitakchonlatarn, V.T.T. Bui, P. Yukphan, U.T.T. Bui, Y. Muramatsu, J. Sitdhipol, S. Tanasupawat, K.C. Duong, Y. Nakagawa, H. T. Pham, Y. Yamada, *Tanticharoenia aidae* sp. nov., for acetic acid bacteria isolated in Vietnam, Ann. Microbiol. 66 (2016) 417–423, https://doi.org/10.1007/s13213-015-1124-z.
[15] H.T.L. Vu, P. Yukphan, V.T.T. Bui, P. Charoenyingcharoen, S. Malimas, *Acetobacter sacchari* sp. nov., for a plant growth-promoting acetic acid bacterium isolated in Vietnam, Ann. Microbiol. 69 (2019) 1155–1163, https://doi.org/10.1007/s13213-019-01497-0.
[16] N. Pitiwittayakul, D. Wongsorn, S. Tanasupawat, Characterization of plant growth-promoting endophytic bacteria from sugarcane and their antagonistic activity against *Fusarium moniliforme*, Trop. Life Sci. Res. 32 (2) (2021) 97–118, https://doi.org/10.21315/tlsr2021.32.3.6.
[17] S.L. Kandel, P.M. Joubert, S.L. Doty, Bacterial endophyte colonization and distribution within plants, Microorganisms 5 (2017) 77, https://doi.org/10.3390/microorganisms5040077.
[18] I. Afzal, Z.K. Shinwari, S. Sikandar, S. Shahzad, Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants, Microbiol. Res. 221 (2019) 36–49, https://doi.org/10.1016/j.micres.2019.02.001.

[19] Y.X. Xing, C.Y. Wei, Y. Mo, L.T. Yang, S.L. Huang, Y.R. Li, Nitrogen-fixing and plant growth-promoting ability of two endophytic bacterial strains isolated from sugarcane stalks, Sugar Tech 18 (2016) 373–379, https://doi.org/10.1007/s12355-015-0397-7.

[20] A.P.D. da Silveira, R.P.F. Iório, F.C.C. Marcos, A.O. Fernandes, S.A.C.D. de Souza, E.E. Kuramae, M.A.P. Cipriano, Exploitation of new endophytic bacteria and their ability to promote sugarcane growth and nitrogen nutrition, Antonie Leeuwenhoek 112 (2019) 283–295, https://doi.org/10.1007/s10482-018-1157-y.

[21] A. Seearunruangchai, S. Tanasupawat, S. Keeratipibut, C. Thawai, T. Itoh, Y. Yamada, Identification of acetic acid bacteria isolated from fruits and related materials collected in Thailand, J. Gen. Appl. Microbiol. 50 (2004) 47–53, https://doi.org/10.2323/jgam.50.47.

[22] B.J. Tindall, R. Rosselló-Móra, H.J. Busse, W. Ludwig, P. Kämpfer, Notes on the characterization of prokaryote strains for taxonomic purposes, Int. J. Syst. Evol. Microbiol. 60 (2010) 249–266, https://doi.org/10.1099/ijs.0.016949-0.

[23] O.S. Kim, Y.J. Cho, K. Lee, S.H. Yoon, M. Kim, H. Na, S.C. Park, Y.S. Jeon, J.H. Lee, H. Yi, S. Won, J. Chun, Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species, Int. J. Syst. Evol. Microbiol. 62 (2012) 716–721, https://doi.org/10.1099/ijs.0.038075-0.

[24] M. Kim, H.S. Oh, S.C. Park, J. Chun, Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes, Int. J. Syst. Evol. Microbiol. 64 (2014) 346–351, https://doi.org/10.1099/ijs.0.059774-0.

[25] S.H. Yoon, S.M. Ha, S. Kwon, J. Lim, Y. Kim, Introducing EzBioClound: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies, Int. J. Syst. Evol. Microbiol. 67 (5) (2017) 1613–1617, https://doi.org/10.1099/ijsem.0.001755.

[26] K. Tamura, G. Stecher, S. Kumar, Mega11: Molecular evolutionary genetics analysis version 11, Mol. Biol. Evol. 38 (2021) 3022–3027, https://doi.org/10.1093/molbev/msab120.

[27] R.C. Edgar, MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Res. 32 (2004) 1792–1797, https://doi.org/10.1093/nar/gkh340.

[28] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 14 (1987) 406–425, https://doi.org/10.1093/oxfordjournals.molbev.a040454.

[29] J. Felsenstein, Evolutionary trees from DNA sequences: a maximum likelihood approach, J. Mol. Evol. 17 (1981) 368–376, https://doi.org/10.1007/BF01734359.

[30] K. Tamura, M. Nei, Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, Mol. Biol. Evol. 10 (1993) 512–526, https://doi.org/10.1093/oxfordjournals.molbev.a040023.

[31] W.M. Fitch, Toward defining the course of evolution: minimum change for a specific tree topology, Syst. Zool. 20 (1971) 406, https://doi.org/10.1093/sysbio/20.4.406.

[32] J. Felsenstein, Confidence limits on phylogenies: an approach using the bootstrap, Evolution 39 (1985) 783–791, https://doi.org/10.2307/2408678.

[33] M. Kimura, A simple method of estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences, J. Mol. Evol. 16 (1980) 111–120, https://doi.org/10.1007/BF01731581.

[34] T. Brettin, J.J. Davis, T. Disz, R.A. Edwards, S. Gerdes, G.J. Olsen, R. Olsen, R. Overbeek, B. Parrello, G.D. Pusch, M. Shukla, J.A. Thomason, R. Stevens, V. Vonstein, A.R. Wattam, F. Xia, RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes, Sci. Rep. 5 (2015) 8365, https://doi.org/10.1038/srep08365.

[35] M. Richter, R. Rosselló-Móra, F. Oliver Glöckner, J. Peplies, JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison, Bioinformatics 32 (2016) 929–931, https://doi.org/10.1093/bioinformatics/btv681.

[36] J.P. Meier-Kolthoff, M. Göker, TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy, Nat. Commun. 10 (2019) 2182, https://doi.org/10.1038/s41467-019-10210-3.

[37] J.P. Meier-Kolthoff, J.S. Carbasse, R.L. Peinado-Olarte, M. Göker, TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes, Nucleic Acids Res. 50 (2022) D801–D807, https://doi.org/10.1093/nar/gkab902.

[38] L.M. Rodriguez-R, K.T. Konstantinidis, Bypassing cultivation to identify bacterial species, Microbe 9 (2014) 111–118, https://doi.org/10.1128/microbe.9.111.1.

[39] T.C.A. Hitch, T. Riedel, A. Oren, J. Overmann, T.D. Lawley, T. Clavel, Automated analysis of genomic sequences facilitates high-throughput and comprehensive description of bacteria, ISME Commun 1 (2021) 16, https://doi.org/10.1038/s43705-021-00017-z.

[40] M. Alanjary, K. Steinke, N. Ziemert, AutoMLST, An automated web server for generating multi-locus species trees highlighting natural product potential, Nucleic Acids Res. 47 (2019) W276–W282, https://doi.org/10.1093/nar/gkz282.

[41] L. Xu, Z. Dong, L. Fang, Y. Luo, Z. Wei, H. Guo, G. Zhang, Y.Q. Gu, D. Coleman-Derr, Q. Xia, Y. Wang, OrthoVenn2: a web server for whole-genome comparison and annotation of orthologous clusters across multiple species, Nucleic Acids Res. 47 (2019) W52–W58, https://doi.org/10.1093/nar/gkz333.

[42] G.J. Hucker, H.J. Conn, Methods of gram staining, Tech Bull N Y St Agric, Exp Stn Tech Bull 93 (1923) 3–37, doi:hdl.handle.net/1813/30787.

[43] J. Dijkstra, C.P. De Jager, Practical Plant Virology: Protocols and Exercises, Springer Verlag, Berlin, 1988.

[44] J. Kommanee, S. Tanasupawat, P. Yukphan, T. Malimas, Y. Muramatsu, Y. Nakagawa, Y. Yamada, *Gluconobacter nephelii* sp. nov., an acetic acid bacterium in the class *Alphaproteobacteria*, Int. J. Syst. Evol. Microbiol. 61 (2011) 2117–2122, https://doi.org/10.1099/ijs.0.026385-0.

[45] T. Asai, H. Iizuka, K. Komagata, The flagellation and taxonomy of genera *Gluconobacter* and *Acetobacter* with reference to the existence of intermediate strains, J. Gen. Appl. Microbiol. 10 (1964) 95–126, https://doi.org/10.2323/jgam.10.95.

[46] Y. Yamada, Y. Okada, K. Kondo, Isolation and characterization of "polarly flagellated intermediate strains" in acetic acid bacteria, J. Gen. Appl. Microbiol. 22 (1976) 237–245, https://doi.org/10.2323/jgam.22.237.

[47] F. Gosselé, J. Swings, J. De Ley, A rapid, simple and simultaneous detection of 2-keto-, 5-keto-and 2,5-diketogluconic acids by thin-layer chromatography in culture media of acetic acid bacteria, Zentralblatt für Bakteriologie: I Abt Originale C: Allgemeine, angewandte und ökologische Mikrobiologie 1 (1980) 178–181, https://doi.org/10.1016/S0172-5564(80)80039-X.

[48] K. Komagata, K.I. Suzuki, Lipid and cell-wall analysis in bacterial systematics, in: R.R. Colwell, R. Grigorova (Eds.), Methods in Microbiology, vol. 19, Academic Press, 1988, pp. 161–207.

[49] M. Sasser (Ed.), Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids Technical Note# 1012001, MIDI Inc, DE, USA, 1990. Newark.

[50] J.C. Cappuccino, N. Sherman (Eds.), Microbiology: A Laboratory Manual, sixth ed., Benjamin/Cummings Publishing Company, New York, 2001.

[51] E. Glickmann, Y. Dessaux, A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria, Appl. Environ. Microbiol. 61 (1995) 793–796, https://doi.org/10.1128/aem.61.2.793-796.1995.

[52] A. Pande, P. Pandey, S. Mehra, M. Singh, S. Kaushik, Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize, J. Genet. Eng. Biotechnol. 15 (2017) 379–391, https://doi.org/10.1016/j.jgeb.2017.06.005.

[53] A. Gandhi, G. Muralidharan, Assessment of zinc solubilizing potentiality of *Acinetobacter* sp. isolated from rice rhizosphere, Eur. J. Soil Biol. 76 (2016) 1–8, https://doi.org/10.1016/j.ejsobi.2016.06.006.

[54] B. Schwyn, J.B. Neilands, Universal assay for the detection and determination of siderophores, Anal. Biochem. 160 (1987) 47–56, https://doi.org/10.1016/0003-2697(87)90612-9.

[55] N. Pitiwittayakul, P. Yukphan, S. Tanasupawat, Genome sequence of *Acidomonas methanolica* CPK24 isolated from sugarcane with plant growth-promoting traits, Agr. Nat. Resour. 56 (2022) 1197–1206, https://doi.org/10.34044/j.anres.2022.56.6.14.

[56] M. Richter, R. Rosselló-Móra, Shifting the genomic gold standard for the prokaryotic species definition, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 19126–19131, https://doi.org/10.1073/pnas.0906412106.

[57] J. Goris, K.T. Konstantinidis, J.A. Klappenbach, T. Coenye, P. Vandamme, J.M. Tiedje, DNA-DNA hybridization values and their relationship to whole-genome sequence similarities, Int. J. Syst. Evol. Microbiol. 57 (2007) 81–91, https://doi.org/10.1099/ijs.0.64483-0.

[58] Q.L. Qin, B.B. Xie, X.Y. Zhang, X.L. Chen, B.C. Zhou, A proposed genus boundary for the prokaryotes based on genomic insights, J. Bacteriol. 196 (2014) 2210–2215, https://doi.org/10.1128/JB.01688-14.

[59] R.A. Barco, G.M. Garrity, J.J. Scott, J.P. Amend, K.H. Nealson, D. Emerson, A genus definition for *Bacteria* and *Archaea* based on a standard genome relatedness index, mBio 11 (2020) e02475, https://doi.org/10.1128/mbio.02475-19, 19.

[60] R. Nitu, K. Rajinder, K. Sukhminderjit, Zinc solubilizing bacteria to augment soil fertility- A comprehensive review, Int. J. Agric. Sci. Vet. Med. 8 (2020) 38–44.
[61] X. Wu, C. Rensing, D. Han, K.Q. Xiao, Y. Dai, Z. Tang, W. Liesack, J. Peng, Z. Cui, F. Zhang, Genome-resolved metagenomics reveals distinct phosphorus acquisition strategies between soil microbiomes, mSystems 7 (1) (2022) e0110721, https://doi.org/10.1128/msystems.01107-21.
[62] Q. Li, Identification of genes involved in Fe-S cluster biosynthesis of nitrogenase in *Paenibacillus polymyxa* WLY78, Int. J. Mol. Sci. 22 (2021) 3771, https://doi.org/10.3390/ijms22073771.
[63] D.M. Hwang, A. Dempsey, K.T. Tan, C.C. Liew, A modular domain of NifU, a nitrogen fixation cluster protein, is highly conserved in evolution, J. Mol. Evol. 43 (5) (1996) 536–540, https://doi.org/10.1007/BF02337525.
[64] A.M. Timofeeva, M.R. Galyamova, S.E. Sedykh, Bacterial siderophores: classification, biosynthesis, perspectives of use in agriculture, Plants 11 (2022) 3065, https://doi.org/10.3390/plants11223065.
[65] M. Miethke, J. Hou, M.A. Marahiel, The siderophore-interacting protein YqjH acts a ferric reductase in different iron assimilation pathways of *Escherichia coli*, Biochemistry 50 (2011) 10951–10964, https://doi.org/10.1021/bi201517h.
[66] A.D. Ferguson, J. Deisenhofer, TonB-dependent receptors-structural perspectives, Biochim. Biophys. Acta 1565 (2002) 318–332, https://doi.org/10.1016/s0005-2736(02)00578-3.
[67] R.O. Pedraza, Acetic acid bacteria as plant growth promoters, in: K. Matsushita, H. Toyama, N. Tonouchi, A. Okamoto-Kainuma (Eds.), Acetic Acid Bacteria, Springer, Tokyo, 2016, https://doi.org/10.1007/978-4-431-55933-7_4.
[68] Y. Yamada, K.-I. Hoshino, T. Ishikawa, The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA: the elevation of the subgenus *Gluconacetobacter* to the generic level, Biosci. Biotech. Bichem. 61 (8) (1997) 1244–1251, https://doi.org/10.1271/bbb.61.1244.
[69] S.-I. Yamashita, T. Uchimura, K. Komagata, Emendation of the genus *Acidomonas* Urakami, Tamaoka, Suzuki and Komagata 1989, Int. J. Syst. Evol. Microbiol. 54 (2004) 865–870, https://doi.org/10.1099/ijs.0.02946-0.
[70] M. Sievers, J. Swings, *Acidomonas* Urakami, Tamaoka, Suzuki and Komagata 1989, 54VP, in: D.J. Brenner, N.R. Krieg, J.T. Stanley (Eds.), Bergey's Manual of Systematic Bacteriology, Springer, New York, 2015, pp. 1–5.