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ORIGINAL ARTICLE

Acetobacter sacchari sp. nov., for a plant growth-promoting acetic acid bacterium isolated in Vietnam

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Abstract

Purpose Two bacterial strains, designated as isolates VTH-Ai14^T and VTH-Ai15, that have plant growth-promoting ability were isolated during the study on acetic acid bacteria diversity in Vietnam. The phylogenetic analysis based on 16S rRNA gene sequences showed that the two isolates were located closely to *Acetobacter nitrogenifigens* RG1^T but formed an independent cluster.

Methods The phylogenetic analysis based on 16S rRNA gene and three housekeeping genes' (*dna*K, *gro*EL, and *rpo*B) sequences were analyzed. The genomic DNA of the two isolates, VTH-Ai14^T and VTH-Ai15, *Acetobacter nitrogenifigens* RG1^T, the closest phylogenetic species, and *Acetobacter aceti* NBRC 14818^T were hybridized and calculated the %similarity. Then, phenotypic and chemotaxonomic characteristics were determined for species' description using the conventional method.

Results The 16S rRNA gene and concatenated of the three housekeeping genes phylogenetic analysis suggests that the two isolates were constituted in a species separated from *Acetobacter nitrogenifigens, Acetobacter aceti*, and *Acetobacter sicerae*. The two isolates VTH-Ai14^T and VTH-Ai15 showed 99.65% and 98.65% similarity of 16S rRNA gene when compared with *Acetobacter nitrogenifigens* and *Acetobacter aceti* and they were so different from *Acetobacter nitrogenifigens* RG1^T with 56.99 ± 3.6 and 68.15 ± 1.8% in DNA-DNA hybridization, when isolates VTH-Ai14^T and VTH-Ai15 were respectively labeled. Moreover, the two isolates were phenotypically distinguished from *Acetobacter nitrogenifigens* in growth in the presence of 0.35% acetic acid (*v*/*v*), on nitrogen-free LGI medium and D-mannitol, and in no ability to solubilize phosphate.

Conclusion Therefore, the two isolates, VTH-Ai14^T(= VTCC 910031^T= BCC 67843^T= TBRC 11175^T= NRIC 0977^T) and VTH-Ai15 (= VTCC 910032 = BCC 67844 = TBRC 11176 = NRIC 0978), can be assigned to an independent species within the genus *Acetobacter*, and the name of *Acetobacter sacchari* sp. nov. is proposed for the two isolates.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13213-019-01497-0) contains supplementary material, which is available to authorized users.

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Keywords *Acetobacter sacchari* sp. nov. · Acetic acid bacteria · Plant growth-promoting bacteria · Vietnam

Introduction

The genus *Acetobacter* is the largest in the acetous group of the family *Acetobacteraceae* from the viewpoint of generic circumscription and includes 28 validly published species at present (Ferrer et al. 2016; Komagata et al. 2014; Li et al. 2014; Malimas et al. 2017; Pitiwittayakul et al. 2016, 2015; Spitaels et al. 2014; Yamada 2016). The genus was divided into two groups, i.e., the *Acetobacter aceti* group and the *Acetobacter pasteurianus* group phylogenetically (Yamada and Yukphan 2008). The species of the genus were characterized by the oxidation of acetate and lactate, acetic acid production from ethanol, no production of 2,5-diketo-D-gluconic acid from D-glucose, and UQ-9 as major (Komagata et al. 2014; Malimas et al. 2017).

Acetobacter diazotrophicus was first reported for acetic acid bacteria capable of nitrogen fixation (Gillis et al. 1989). However, this species was later transferred to the genus *Gluconacetobacter* as *Gluconacetobacter diazotrophicus* (Yamada et al. 1997).

The acetic acid bacteria with plant growth-promoting characteristics were additionally reported in the genus *Acetobacter*. Muthukumarasamy et al. (2005) isolated some nitrogen-fixing acetic acid bacteria from wetland rice cultivated in India and identified them as *Acetobacter peroxydans*. The type strain of *Acetobacter peroxydans* (LMG 1635^T) was also proved to have the same characteristics as the isolates mentioned above (Muthukumarasamy et al. 2005; Pedraza 2008, 2016).

Acetobacter nitrogenifigens was the second species possessing a nitrogen-fixing ability and considered to be a plant growth-promoting bacterium as well (Dutta and Gachhui 2006; Pedraza 2008, 2016). The two nitrogen-fixing species are quite distance phylogenetically; the former was classified in the *A. pasteurianus* group, and the latter was in the *A. aceti* group.

Previously, the two *Acetobacter* strains, namely isolates VTH-Ai14 and VTH-Ai15, were isolated and phylogenetically located in the *A. aceti* group and closely related to *Acetobacter nitrogenifigens* (Vu et al. 2016b).

This paper describes *Acetobacter sacchari* sp. nov., as an additional plant growth-promoting species of the genus *Acetobacter*, for the two isolates that were isolated in Binh Phuoc Province, Vietnam, on January 28th, 2013.

Materials and methods

Two strains, designated as isolates VTH-Ai14 and VTH-Ai15, were isolated from the stems of sugar cane (*Saccharum* species) by an enrichment culture approach at pH 3.5 (Vu et al. 2013; Yamada et al. 1999). When microbial growth was seen in the medium, one loop of the culture was streaked onto an agar plate comprised of D-glucose, ethanol, peptone, yeast extract, and calcium carbonate to select acetic acid bacteria (Vu et al. 2013; Yamada et al. 1999). *Acetobacter nitrogenifigens* TBRC 15^T (= RG1^T) (Dutta and Gachhui 2006) and *Acetobacter aceti* NBRC 14818^T were used as reference strains.

The 16S rRNA gene sequences of the two isolates were sequenced, as described previously (Vu et al. 2013). Sequenced were 1,419–1,420 bases for the two isolates. Multiple sequence alignments were made with MUSCLE (Edgar 2004). Sequence gaps and ambiguous bases were excluded. A phylogenetic tree based on 16S rRNA gene sequences of 1,275 bases was constructed by the maximum likelihood method based on DNA substitution model selected under the Bayesian Information Criterion (Kumar et al. 2016) using the program MEGA7 version 5.05 (Kumar et al. 2016). In the phylogenetic tree constructed, the type strains of *Gluconobacter oxydans*, *Saccharibacter floricola*, *Neokomagataea tanensis*, and *Swingsia samuiensis* were used as outgroups. The confidence values of individual branches were calculated by the use of bootstrap analysis of Felsenstein (Felsenstein 1985).

Calculation of sequence similarity levels was calculated using the EzBioCloud server by pairwise sequence alignment, in which all gaps were not considered (Kim et al. 2014; Kim et al. 2012; Tindall et al. 2010; Yoon et al. 2017).

Extraction and isolation of chromosomal DNAs were made by the use of the modified method of Marmur (Ezaki et al. 1983; Marmur 1961; Saito and Miura 1963). DNA base composition was determined by the method of Tamaoka and Komagata (Tamaoka and Komagata 1984). DNA-DNA hybridization was done with five wells for each reciprocal reactions (e.g., A × B and B × A) by the photobiotin-labeling method using microplate wells, as described by Ezaki and coauthors (Ezaki et al. 1989). Percent similarities in DNA-DNA hybridization were determined colorimetrically (Verlander 1992). The color density was measured at A_{450} on a Synergy™ HTX Multi-Mode Microplate Reader (BioTek Instruments Inc., USA). Isolated, single-stranded, and labeled DNA was hybridized with DNAs from test strains in 2 × SSC containing 50% formamide at 48 °C. The highest and the lowest values were excluded, and the mean of the remaining three values was taken as a similarity value and calculation of standard derivation.

The housekeeping genes *dna*K (encoding the heat shock 70 kDa protein), *gro*EL (encoding a 60-kDa chaperonin), and *rpo*B (encoding the DNA-directed RNA polymerase subunit beta) of the two isolates, VTH-Ai14^T and VTH-Ai15, were partially sequenced (Cleenwerck et al. 2010; Li et al. 2014; Pitiwittayakul et al. 2015). The phylogenetic position based on the concatenated sequences of these housekeeping genes was compared with the type strains of the genus *Acetobacter*. The respectively concatenated sequences of 528, 579, and

573 bp of partial *dna*K, *gro*EL, and *rpo*B were used for constructed the phylogenetic analysis. Accession numbers of *dna*K, *gro*EL, and *rpo*B sequence of the type strains are according to the previous study (Cleenwerck et al. 2010; Li et al. 2014; Pitiwittayakul et al. 2015).

Whole-cell fatty acid methyl esters (FAME) of the two isolates, VTH-Ai14^T and VTH-Ai15, and type strains of *Acetobacter nitrogenifigens* TBRC 15^T were extracted and analyzed as described by Vu et al. (2016a) after grown on GECA medium for 48 h at 28 °C under aerobic conditions.

Additionally, the two isolates, VTH-Ai14^T and VTH-Ai15, and *Acetobacter nitrogenifigens* TBRC 15^T were subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) after grown in glucose-yeast extracted-peptone medium consisting of 2.0% glucose, 1.2% peptone, and 0.3% yeast extracted at 30 °C for 18 h with shaking at 200 rpm. The samples were prepared using the standard extraction method as described by Matsuda et al. (2012). Then, applied to a MicroFlex LT mass spectrometer (Bruker Daltonik), and the results were analyzed by MALDI Biotyper 4.1 software (Bruker Daltonik). *Escherichia coli* DH5α was used as a quality control as recommended by the manufacturer on each experiment.

Phenotypic characteristics were determined by the conventional methods (Asai et al. 1964; Gosselé et al. 1980; Kersters et al. 2006; Lisdiyanti et al. 2000; Swings et al. 1992; Yamada et al. 1999, 1976; Yukphan et al. 2011). A major isoprenoid quinone was extracted and quantitatively analyze by HPLC (Komagata and Suzuki 1988; Tamaoka et al. 1983; Yamada et al. 1969).

Results

In a phylogenetic tree deduced from the maximum likelihood method, isolates VTH-Ai14^T and VTH-Ai15 formed a cluster, which was connected to the cluster of *Acetobacter nitrogenifigens* RG1^T with the bootstrap value of 100% (Fig. 1). The resulting cluster was then connected to a cluster containing *Acetobacter aceti* NBRC 14818 ^{T} and *Acetobacter sicerae* LMG 1531^T with the bootstrap value of 67%. The pairwise sequence similarities of isolate VTH-Ai14^T were 100, 99.65, 98.65, 98.09, 97.87, 97.87, 97.59, 96.59, and 96.31% respectively to isolate VTH-Ai15, *Acetobacter nitrogenifigens* RG1^T, *Acetobacter aceti* NBRC 14818^T, *Acetobacter musti* Bo7^T, *Acetobacter estunensis* NBRC 13751^T, *Acetobacter oeni* B13^T, *Acetobacter sicerae* LMG 1531^T, *Acetobacter peroxydans* NBRC 13755^T, and *Acetobacter pasteurianus* LMD 22.1^T. The phylogenetic data obtained suggested that the two isolates constitute a species separate from either *Acetobacter nitrogenifigens* or *Acetobacter aceti*.

The DNA G+C contents of isolates VTH-Ai14^T and VTH-Ai15 were 59.9 and 59.9 mol%, respectively (Table 1). The calculated values were so different from that of *A. nitrogenifigens* RG1^T (64.1 mol%). The DNA-DNA similarities when the isolate VTH-Ai14^T was reciprocally hybridized with VTH-Ai15 were high level at 100.00 ± 7.9% and 73.10 ± 9.3%. The DNA-DNA similarities of isolates VTH-Ai14^T and VTH-Ai15 with the labeled *Acetobacter nitrogenifigens* TBRC 15^T were 61.39 ± 8.0% and 44.21 ± 6.9%. While the isolates VTH-Ai14^T and VTH-Ai15 were labeled, the DNA-DNA similarities with *Acetobacter nitrogenifigens* TBRC 15^T were 56.99 ± 3.6% and 68.15 ± 1.8%, respectively. All the reciprocal DNA-DNA similarity between VTH-Ai14^T and VTH-Ai15 with *Acetobacter aceti* NBRC 14818^T was between 4.16 ± 0.4% and 15.80 ± 2.1%. The concatenated sequences of the three housekeeping genes (1,676 bp) were constructed with MEGA7 using the maximum likelihood model. The DNA substitution GTR+G+I was selected under the Bayesian Information Criterion (Kumar et al. 2016). The isolates VTH-Ai14^T and VTH-Ai15 were grouped together and separated from *Acetobacter nitrogenifigens* TBRC 15^T as same as the topologies of the phylogenetic tree based on the 16S rRNA gene (Fig. 2).

The major cellular fatty acid of the isolates VTH-Ai14^T, VTH-Ai15, and *Acetobacter nitrogenifigens* TBRC 15^T was C18:1ω7c at 55.94%, 57.04%, and 57.94%, respectively (Table 2).

The MALDI-TOF MS profiles of the isolate VTH-Ai14^T, VTH-Ai15, and *Acetobacter nitrogenifigens* TBRC 15^T were compared to each other and expressed by identification scores. The isolates VTH-Ai14^T and VTH-Ai15 showed identification scores of 2.66 and 2.67, when comparing with VTH-Ai15 and VTH-Ai14^T, respectively. While the identification score of the isolates VTH-Ai14^T and VTH-Ai15 when comparing with *Acetobacter nitrogenifigens* TBRC 15^T was only 1.88 and 1.92. The two isolates were discriminated from *Acetobacter nitrogenifigens* TBRC 15^T according to the manufacturer's recommended log score identification criteria (Matsuda et al. 2012). All the results obtained suggested that the isolates are genetically separated from either *Acetobacter nitrogenifigens* or *Acetobacter aceti.*

The phenotypic characteristics of isolates VTH-Ai14^T and VTH-Ai15 were given in the species description of *Acetobacter sacchari* sp. nov. (Table 1).

Discussion

Acetobacter nitrogenifigens was first reported as a nitrogen-fixing bacterium equipped with polar flagellation and with a high G+C content of DNA (64.1 mol% G + C). In general, the G+C contents of DNA are ranged from 53.5 to 60.7 mol% in the genus *Acetobacter* (Komagata et al. 2014; Malimas et al.

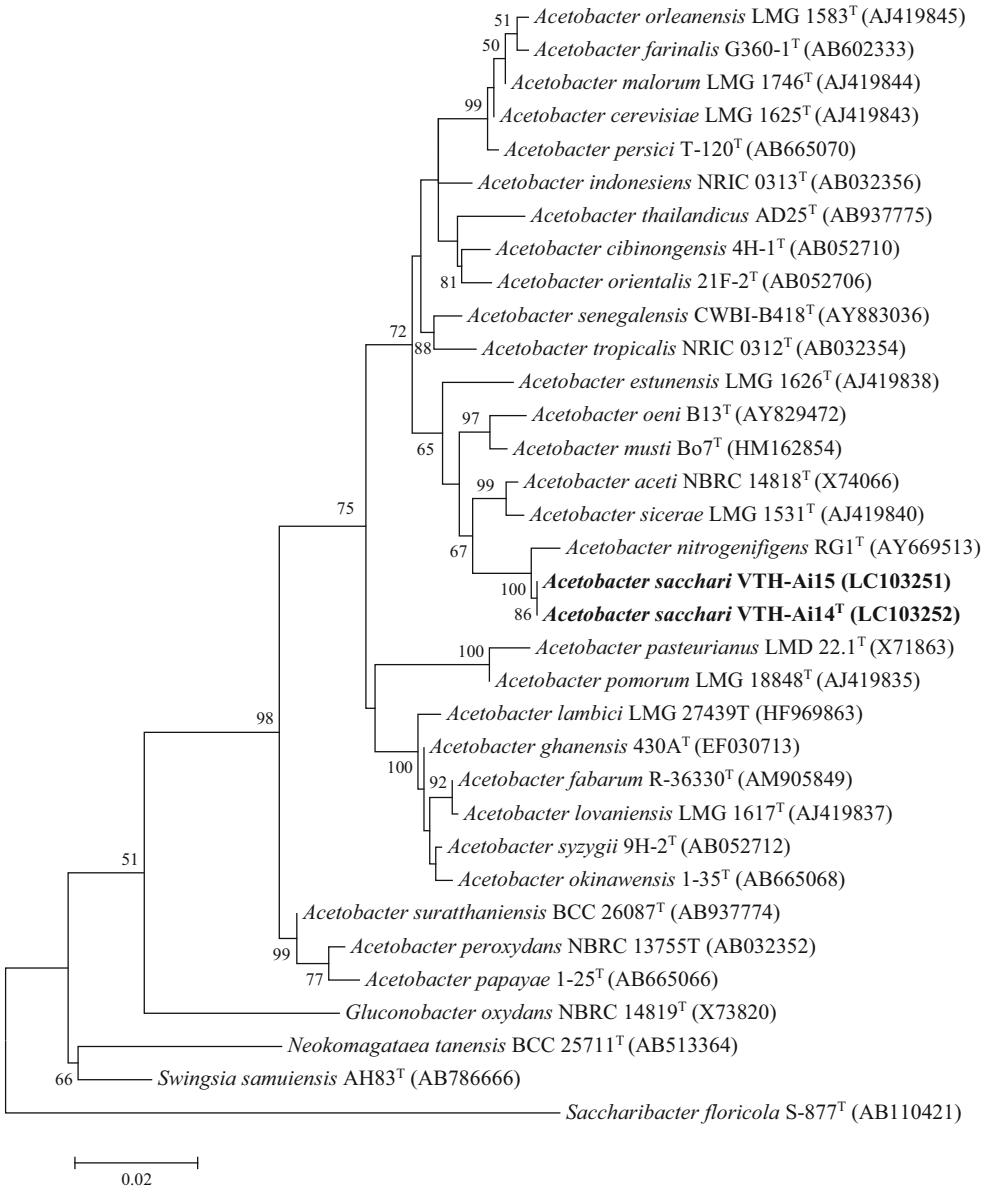

Fig. 1 A phylogenetic relationship of isolates VTH-Ai14^T and VTH-Ai15. The phylogenetic tree based on 16S rRNA gene sequences was constructed by the maximum likelihood method. The type strains of *Gluconobacter oxydans*, *Saccharibacter floricola*, *Neokomagataea tanensis*, and *Swingsia samuiensis* were used as outgroups. The numerals at the nodes of the respective branches indicate bootstrap values (%) derived from 1,000 replications. Bar, 0.02% sequence divergence

2017). In addition, the peritrichous flagellation appears to be widely distributed in the genus (Komagata et al. 2014; Malimas et al. 2017). The present authors' estimation of 59.7 mol% G+C and observation of peritrichous flagellation in *Acetobacter nitrogenifigens* TBRC 15^T may be reasonable (Table 1).

The result of fatty acid composition showed that C18:1ω7c is a major fatty acid and the data were consistent with those reported for the validly published species of the genus *Acetobacter* reported by Ferrer et al. (2016). Although, the composition of minor fatty acid was slightly different and cannot discriminate the two isolates from the closest known species (Li et al. 2014; Spitaels et al. 2014), but the result of the two isolates is different from the other genera of acetic acid bacteria (Li et al. 2015; Vu et al. 2013; Yukphan et al. 2011).

The two isolates were phenotypically distinguished from *Acetobacter nitrogenifigens* in growth in the presence of 0.35% acetic acid (*v*/*v*), nitrogen-free LGI medium, and D-mannitol and in no ability to solubilize phosphate and from *Acetobacter aceti* in growth in the presence of 0.35% (*v*/*v*) acetic acid, nitrogen-free LGI medium, and D-mannitol and in the absence of phosphate solubility and production of 2-keto-D-gluconic acid (Komagata et al. 2014; Malimas et al. 2017). In addition, they were genetically and physiologically discriminated by DNA-DNA similarities obtained from the reciprocal DNA-DNA hybridization, MALDI-TOF MS profiles of the isolate, and the concatenated sequences of the three housekeeping genes from the type strain of *Acetobacter nitrogenifigens* (Ferrer et al. 2016; Li et al. 2014).

Table 1 Differential characteristics of *Acetobacter sacchari* sp. nov., for isolates VTH-Ai14^T and VTH-Ai15, from the phylogenetically closest species of the genus *Acetobacter*. (1) *A. sacchari* isolate VTH-Ai14^T; (2) *A. sacchari* isolate VTH-Ai15; (3) *A. nitrogenifigens* RG1^T; (4) *A. aceti* NBRC 14818^T; (5) *A. sicerae* LMG 1531^T; (6) *A. estunensis* NBRC 13751^T; (7) *A. oeni* B13^T; +, positive; −, negative; *w*, weakly positive; *vw*, very weakly positive; *per*, peritrichous; *nd*, not determined

Characteristic	1	2	3[a]	4[b]	5[c]	6[b]	7[d]
Flagellation	per	per	per[f,g]	nd	per	nd	per
Ketogenesis from glycerol	+	+	+	+	nd	–	nd
Production of ketogluconate from D-glucose							
5-Keto-D-gluconate	+	+	+[e]	+	+	–	+
2-Keto-D-gluconate	–	–	–[e]	+	+	+	–
Production of levan-like polysaccharide from							
D-Glucose	+	+	+[f]	–[f]	nd	nd	nd
D-Fructose	+	+	+[f]	–[f]	nd	nd	nd
D-Mannitol	+	+	+[f]	–[f]	nd	nd	nd
Glycerol	+	+	+[f]	–[f]	nd	nd	nd
Growth on ammoniac nitrogen with ethanol	+	+	+	+[c]	+	+[c]	–
Growth in presence of 10% ethanol (*v*/*v*)	w	w	+	–[c]	–	–[c]	+
Growth in presence of 30% D-glucose (*w*/*v*)	–	–	+	–[c]	+	–[c]	–
Growth in presence of 0.35% acetic (*w*/*v*)	+	+	w[f]	w[f]	nd	nd	nd
Growth on							
D-Glucose	+	+	w[f]	vw[f]	nd	nd	nd
D-Fructose	w	w	+	–[c]	+	+[c]	+[c]
D-Sorbitol	–	–	–[c]	–[c]	+	+[c]	–[c]
D-Mannitol	+	+	–[f]	–[f]	nd	nd	nd
Ethanol	+	+	w[f]	+[f]	nd	nd	–
2-Propanol	w	+	vw[f]	vw[f]	nd	nd	nd
Acid production from							
D-Arabinose	–	–	+[c]	w	w	–	+[c]
Ethanol[h]	+[h]	+[h]	+	+[h]	nd	+	nd
Glycerol	vw	vw	–[f]	–	nd	–	nd
1-Propanol[h]	+	+	+[f]	+[h]	nd	nd	nd
Solubilization of							
$Ca_3(PO4_4)_2$ on Pikovskaya agar medium	–	–	vw[f]	vw[f]	nd	nd	nd
ZnO on LGI agar medium	+	+	+	nd	nd	nd	nd
Production phytohormon IAA with the presence of L-tryptophan in LGI medium	+	+	+	nd	nd	nd	nd
Growth on nitrogen-free LGI medium	+	+	w[f]	–[a]	nd	–[a]	–[a]
γ-Pyrone production from							
D-Fructose	–	–	–[f]	–	nd	–	nd
D-Glucose	vw	vw	+	–	nd	–	nd
Ubiquinone system	Q-9	Q-9	Q-9	Q-9	Q-9	Q-9	nd
DNA G + C content (mol%)	59.9	59.9	59.7[f,g]	57.2	58.3	59.3	58.1

[a] Dutta and Gachhui (2006)

[b] Lisdiyanti et al. (2000)

[c] Li et al. (2014)

[d] Silva et al. (2006)

[e] Spitaels et al. (2014)

[f] The present work

[g] According to the original authors of the species, flagellation is polar and DNA G+C content is 64.1 mol% (Dutta and Gachhui 2006)

[h] Ethanol (1%, *v*/*v*) was completely oxidized to carbon dioxide and water for 9, 9, and 5 days respectively in *A. sacchari* isolate VTH-Ai14^T, *A. sacchari* isolate VTH-Ai15, and *A. aceti* NBRC 14818^T, and 1-propanol (1%, *v*/*v*) was done for 10 days in *A. aceti* NBRC 14818^T

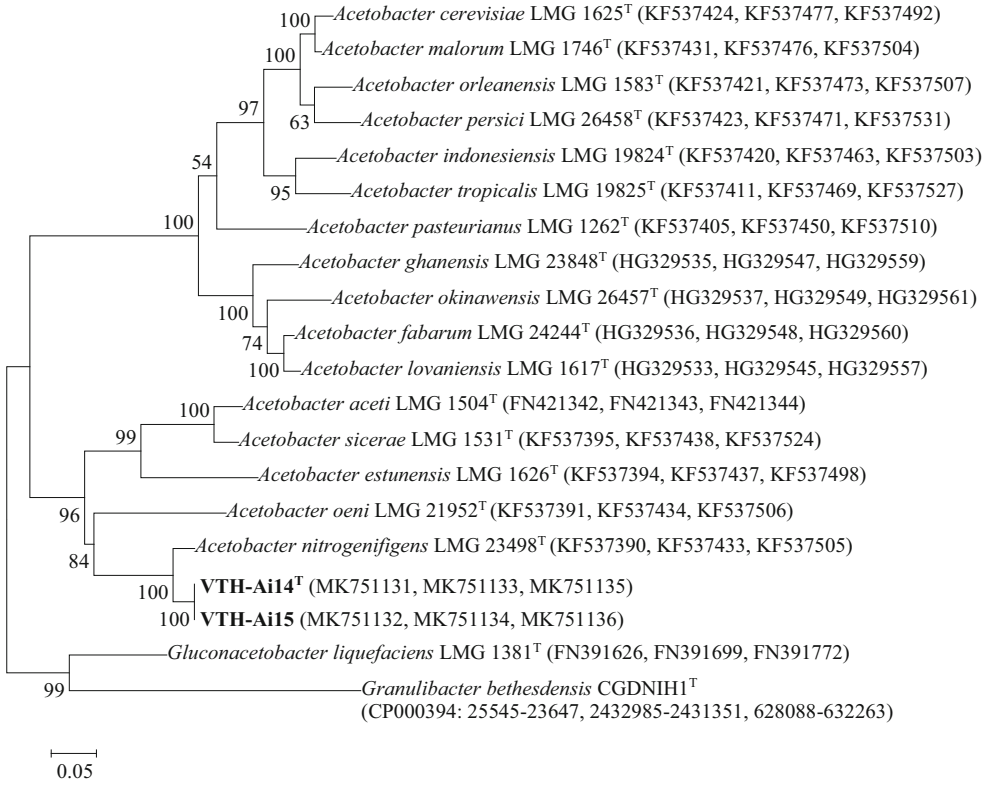

Fig. 2 Maximum likelihood tree based on the concatenated sequence (1,676 bp) of *dna*K (528 bp), *gro*EL (579 bp), and *rpo*B (573 bp) showing the phylogenetic position of isolates VTH-Ai14^T and VTH-Ai15 within the genus *Acetobacter*. The type strains of *Gluconacetobacter liquefaciens* and *Granulibacter bethesdensis* were used as outgroup. Numbers at branching points are percentage bootstrap values based on 1,000 replications. Bar, 0.05% sequence divergence

As described above, the two isolates VTH-Ai14^T and VTH-Ai15 were separated phenotypically, genetically, and physiologically from either *Acetobacter nitrogenifigens* or *Acetobacter aceti*, the phylogenetically closest species (Komagata et al. 2014; Malimas et al. 2017). The two isolates can therefore be assigned to an independent species within the genus *Acetobacter*, and the name of *Acetobacter sacchari* sp. nov. is introduced for the two isolates (Table 1).

Table 2 Cellular fatty acid contents (%) of *Acetobacter sacchari* sp. nov., for isolates VTH-Ai14^T and VTH-Ai15, and the phylogenetically closest species of the genus *Acetobacter*. (1) *A. sacchari* isolate VTH-Ai14^T; (2) *A. sacchari* isolate VTH-Ai15; (3) *A. nitrogenifigens* RG1^T; (4) *A. aceti* NBRC 14818^T; (5) *A. sicerae* LMG 1531^T

Fatty acid	1	2	3	4[a]	5[a]
$C_{12:0}$	0.1	0.1	0.1	0.1	–
Summed feature 2*	1.88	1.78	2.06	3.5	3.2
Summed feature 3	0.44	0.39	0.42	0.3	–
$C_{17:0}$	0.1	0.1	–	–	–
$C_{18:1}$ 2-OH	2.34	2.31	2.18	0.3	–
$C_{14:0}$	1.77	1.64	1.5	0.5	1.2
$C_{14:0}$ 2-OH	5.66	5.39	5.72	–	8.8
$C_{16:0}$	16.63	16.86	14.62	12.0	9.2
$C_{16:0}$ 2-OH	10.37	9.89	10.73	21.0	5.8
$C_{16:0}$ 3-OH	1.67	1.65	1.47	2.9	3.7
$C_{18:0}$	1.03	0.91	0.76	0.6	1.2
$C_{18:1}$ ω7c	55.94	57.04	57.94	50.9	59.6*
$C_{18:0}$ 3-OH	0.68	0.69	0.61	2.6	1.9
$C_{19:0}$ cyclo ω8c	0.68	0.48	0.94	1.7	3.6

[a] Ferrer et al. (2016)

Description of *Acetobacter sacchari* sp. nov.

Acetobacter sacchari (sac'cha,ri. L. gen. *sacchari*; L. neut. n. *Saccharum* sugar cane, from which the two isolates were isolated).

Gram-negative short rods and motile with peritrichous flagella, measuring 0.4–0.5 × 0.6–1.5 μm. Colonies are entire, smooth, transparent, glistening, and creamy to slightly light pink. Catalase is positive, and oxidase is negative. Grows on LGI medium. Oxidize acetate and lactate. Produces acetic acid from ethanol. Does not grow in the presence of 30% D-glucose (*w*/*v*) or 1% potassium nitrate (*w*/*v*) but in the presence of 0.35% acetic acid (*v*/*v*). Does not hydrolyze starch and casein. Produces 5-keto-D-gluconate but not 2-keto-D-gluconate and 2,5-diketo-D-gluconate from D-glucose. Produces levan-like polysaccharide in the presence of 3.5% and 5% D-glucose (*w*/*v*), D-fructose, and glycerol. Produces dihydroxyacetone from glycerol. Very weak production of γ-pyrone compounds from D-glucose is shown.

Acid is produced from L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, glycerol very weakly, 1-propanol, and ethanol, but not from D-arabinose, D-fructose, L-sorbose, L-rhamnose, D-mannitol, D-sorbitol, dulcitol, *myo*-inositol, maltose, lactose, melibiose, sucrose, raffinose, 2-propanol,

and methanol. Grows on L-arabinose weakly, D-galactose very weakly, D-glucose, D-mannose very weakly, D-fructose weakly, D-mannitol, glycerol, 1-propanol weakly (VTH-Ai15 grows), 2-propanol weakly, but not on D-arabinose, L-arabinose, D-xylose, L-rhamnose, L-sorbose, D-sorbitol, dulcitol, *myo*-inositol, maltose, sucrose, raffinose, and methanol.

Growth occurs at 20–37 °C, and no growth is found at 40 °C. Optimal growth temperature is at 25–33 °C. Optimal growth pH is from 3.0 to 8.0, and growth occurs at pH 2.5–8.0. Optimum growth is from 0 to 0.5% NaCl, and no growth at 2.0% NaCl.

A major isoprenoid quinone is Q-9. DNA G+C contents is 59.9 mol%. Major fatty acid composition is C18:1ω7c. The type strain is VTH-Ai14^T (= VTCC 910031^T = BCC 67843^T = TBRC 11175^T = NRIC 0977^T), which was isolated from the stem of sugar cane (*Saccharum* species) collected in Thuận Phú, Đồng Phú, Bình Phước (GPS location is 11.59, 106.85), Vietnam, and whose DNA G+C content is 59.9 mol%.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

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

Cleenwerck I, De Vos P, De Vuyst L (2010) Phylogeny and differentiation of species of the genus *Gluconacetobacter* and related taxa based on multilocus sequence analyses of housekeeping genes and reclassification of *Acetobacter xylinus* subsp. *sucrofermentans* as *Gluconacetobacter sucrofermentans* (Toyosaki et al. 1996) sp. nov., comb. nov. Int J Syst Evol Microbiol 60:2277–2283. https://doi.org/10.1099/ijs.0.018465-0

Dutta D, Gachhui R (2006) Novel nitrogen-fixing *Acetobacter nitrogenifigens* sp. nov., isolated from Kombucha tea. Int J Syst Evol Microbiol 56:1899–1903. https://doi.org/10.1099/ijs.0.64101-0

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

Ezaki T, Yamamoto N, Ninomiya K, Suzuki S, Yabuuchi E (1983) Transfer of *Peptococcus indolicus, Peptococcus asaccharolyticus, Peptococcus prevotii,* and *Peptococcus magnus to the genus Peptostreptococcus* and proposal of *Peptostreptococcus tetradius* sp. nov. Int J Syst Evol Microbiol 33:683–698. https://doi.org/10.1099/00207713-33-4-683

Ezaki T, Hashimoto Y, Yabuuchi E (1989) Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Evol Microbiol 39:224–229. https://doi.org/10.1099/00207713-39-3-224

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

Ferrer S, Manes-Lazaro R, Benavent-Gil Y, Yepez A, Pardo I (2016) *Acetobacter musti* sp. nov., isolated from Bobal grape must. Int J Syst Evol Microbiol 66:957–961. https://doi.org/10.1099/ijsem.0.000818

Gillis M et al (1989) *Acetobacter diazotrophicus* sp. nov., a nitrogen-fixing acetic acid bacterium associated with sugarcane. Int J Syst Evol Microbiol 39:361–364. https://doi.org/10.1099/00207713-39-3-361

Gosselé F, Swings J, De Ley J (1980) 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. Zentralbl Bakteriol 1:178–181. https://doi.org/10.1016/S0172-5564(80)80039-X

Kersters K, Lisdiyanti P, Komagata K, Swings J (2006) The family *Acetobacteraceae*: the genera *Acetobacter, Acidomonas, Asaia, Gluconacetobacter, Gluconobacter,* and *Kozakia*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The Prokaryotes. Springer, New York, pp 163–200. https://doi.org/10.1007/0-387-30745-1_9

Kim OS et al (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721. https://doi.org/10.1099/ijs.0.038075-0

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

Komagata K, Suzuki K-I (1988) Lipid and cell-wall analysis in bacterial systematics. In: Colwell RR, Grigorova R (eds) Methods in microbiology, vol 19. Academic, Cambridge, pp 161–207. https://doi.org/10.1016/S0580-9517(08)70410-0

Komagata K, Iino T, Yamada Y (2014) The family *Acetobacteraceae*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes: *Alphaproteobacteria* and *Betaproteobacteria.* Springer, Berlin Heidelberg, pp 3–78. https://doi.org/10.1007/978-3-642-30197-1_396

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Li L et al (2014) *Acetobacter sicerae* sp. nov., isolated from cider and kefir, and identification of species of the genus *Acetobacter by dnaK, groEL* and *rpoB* sequence analysis. Int J Syst Evol Microbiol 64:2407–2415. https://doi.org/10.1099/ijs.0.058354-0

Li L et al (2015) *Bombella intestini* gen. nov., sp. nov., an acetic acid bacterium isolated from bumble bee crop. Int J Syst Evol Microbiol 65:267–273. https://doi.org/10.1099/ijs.0.068049-0

Lisdiyanti P, Kawasaki H, Seki T, Yamada Y, Uchimura T, Komagata K (2000) Systematic study of the genus *Acetobacter* with descriptions of *Acetobacter indonesiensis* sp. nov., *Acetobacter tropicalis* sp. nov., *Acetobacter orleanensis* (Henneberg 1906) comb. nov., *Acetobacter lovaniensis* (Frateur 1950) comb. nov., and *Acetobacter estunensis* (Carr 1958) comb. nov. J Gen Appl Microbiol 46:147–165. https://doi.org/10.2323/jgam.46.147

Malimas T, Thi Lan Vu H, Muramatsu Y, Yukphan P, Tanasupawat S, Yamada Y (2017) Systematics of acetic acid bacteria. In: Acetic Acid Bacteria. Food Biology Series. CRC Press, Boca Raton, pp 3–43. https://doi.org/10.1201/9781315153490-3

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J Mol Biol 3:208–218. https://doi.org/10.1016/s0022-2836(61)80047-8

Matsuda N, Matsuda M, Notake S, Yokokawa H, Kawamura Y, Hiramatsu K, Kikuchi K (2012) Evaluation of a simple protein extraction method for species identification of clinically relevant

staphylococci by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J Clin Microbiol 50:3862–3866. https://doi.org/10.1128/jcm.01512-12

Muthukumarasamy R et al (2005) Natural association of *Gluconacetobacter diazotrophicus* and diazotrophic *Acetobacter peroxydans* with wetland rice. Syst Appl Microbiol 28:277–286. https://doi.org/10.1016/j.syapm.2005.01.006

Pedraza RO (2008) Recent advances in nitrogen-fixing acetic acid bacteria. Int J Food Microbiol 125:25–35. https://doi.org/10.1016/j.ijfoodmicro.2007.11.079

Pedraza RO (2016) Acetic acid bacteria as plant growth promoters. In: Matsushita K, Toyama H, Tonouchi N, Okamoto-Kainuma A (eds) Acetic acid bacteria: ecology and physiology. Springer Japan, Tokyo, pp 101–120. https://doi.org/10.1007/978-4-431-55933-7_4

Pitiwittayakul N, Yukphan P, Chaipitakchonlatarn W, Yamada Y, Theeragool G (2015) *Acetobacter thailandicus* sp. nov., for a strain isolated in Thailand. Ann Microbiol 65:1855–1863. https://doi.org/10.1007/s13213-014-1024-7

Pitiwittayakul N et al (2016) *Acetobacter suratthanensis* sp. nov., an acetic acid bacterium isolated in Thailand. Ann Microbiol 66:1157–1166. https://doi.org/10.1007/s13213-016-1200-z

Saito H, Miura KI (1963) Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim Biophys Acta 72:619–629. https://doi.org/10.1016/0926-6550(63)90386-4

Silva LR, Cleenwerck I, Rivas R, Swings J, Trujillo ME, Willems A, Velazquez E (2006) *Acetobacter oeni* sp. nov., isolated from spoiled red wine. Int J Syst Evol Microbiol 56:21–24. https://doi.org/10.1099/ijs.0.46000-0

Spitaels F et al (2014) *Acetobacter lambici* sp. nov., isolated from fermenting lambic beer. Int J Syst Evol Microbiol 64:1083–1089. https://doi.org/10.1099/ijs.0.057315-0

Swings J, Monique G, Karel K (1992) Phenotypic identification of acetic acid bacteria. Appl Environ Microbiol 29:103–110

Tamaoka J, Komagata K (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol Lett 25:125–128

Tamaoka J, Katayama-Fujimura Y, Kuraishi H (1983) Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. J Appl Bacteriol 54:31–36. https://doi.org/10.1111/j.1365-2672.1983.tb01297.x

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

Verlander PC (1992) Detection of horseradish peroxidase by colorimetry. In: Kricka LJ (ed) Nonisotopic DNA probe techniques. Academic Press, Boston, pp 185–201. https://doi.org/10.1016/B978-0-12-426296-6.50012-5

Vu HTL et al (2013) *Nguyenibacter vanlangensis* gen. nov., sp. nov., an unusual acetic acid bacterium in the α-*Proteobacteria*. J Gen Appl Microbiol 59:153–166. https://doi.org/10.2323/jgam.59.2_153

Vu HTL et al (2016a) *Tanticharoenia aidae* sp. nov., for acetic acid bacteria isolated in Vietnam. Ann Microbiol 66:417–423. https://doi.org/10.1007/s13213-015-1124-z

Vu HTL, Yukphan P, Muramatsu Y, Thao DTP, Tanaka N, Ho PT, Yamada Y (2016b) The microbial diversity of acetic acid bacteria in the south of Vietnam. Vietnam J Biotechnol 14:397–408

Yamada Y (2016) Systematics of acetic acid bacteria. In: Kazunobu Matsushita HT, Tonouchi N, Okamoto-Kainuma A (eds) Acetic acid bacteria: ecology and physiology. Springer, Tokyo, pp 1–50. https://doi.org/10.1007/978-4-431-55933-7

Yamada Y, Yukphan P (2008) Genera and species in acetic acid bacteria. Int J Food Microbiol 125:15–24. https://doi.org/10.1016/j.ijfoodmicro.2007.11.077

Yamada Y, Aida K, Ocirc UT (1969) Enzymatic studies on the oxidation of sugar and sugar alcohol. V. Ubiquinone of acetic acid bacteria and its relation to classification of *Gluconobacter* and *Acetobacter,* especially of the so-called intermediate strains. J Gen Appl Microbiol 15:181–196. https://doi.org/10.2323/jgam.15.181

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

Yamada Y, Hoshino K, Ishikawa T (1997) The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA: the elevation of the subgenus *Gluconoacetobacter* to the generic level. Biosci Biotechnol Biochem 61:1244–1251. https://doi.org/10.1271/bbb.61.1244

Yamada Y, Hosono R, Lisdyanti P, Widyastuti Y, Saono S, Uchimura T, Komagata K (1999) Identification of acetic acid bacteria isolated from Indonesian sources, especially of isolates classified in the genus *Gluconobacter*. J Gen Appl Microbiol 45:23–28. https://doi.org/10.2323/jgam.45.23

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

Yukphan P et al (2011) *Neokomagataea* gen. nov., with descriptions of *Neokomagataea thailandica* sp. nov. and *Neokomagataea tanensis* sp. nov., osmotolerant acetic acid bacteria of the alpha-*Proteobacteria*. Biosci Biotechnol Biochem 75:419–426

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