Ann Microbiol (2012) 62:849–859
DOI 10.1007/s13213-011-0288-4

SHORT COMMUNICATION

Subdivision of the genus *Gluconacetobacter* Yamada, Hoshino and Ishikawa 1998: the proposal of *Komagatabacter* gen. nov., for strains accommodated to the *Gluconacetobacter xylinus* group in the α-*Proteobacteria*

Yuzo Yamada · Pattaraporn Yukphan · Huong Thi Lan Vu · Yuki Muramatsu · Duangjai Ochaikul · Yasuyoshi Nakagawa

Received: 8 February 2011 / Accepted: 20 May 2011 / Published online: 12 June 2011

Abstract The genus *Gluconacetobacter* is divided into two groups phylogenetically, phenotypically and ecologically: the *Gluconacetobacter liquefaciens* group and the *Gluconacetobacter xylinus* group. For the latter group, the genus *Komagatabacter* is newly introduced, and the type species of the new genus is designated as *Komagatabacter xylinus* (Brown 1886) comb. nov. Twelve species of the *Gluconacetobacter xylinus* group are transferred to the new genus as new combinations.

Keywords *Gluconacetobacter* · *Komagatabacter* gen. nov. · *Komagatabacter xylinus* comb. nov. · Acetic acid bacteria · *Acetobacteraceae*

Y. Yamada (✉) · P. Yukphan
BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology,
National Science and Technology Development Agency,
113 Thailand Science Park, Phaholyothin Road, Klong 1,
Klong Luang, Pathumthani 12120, Thailand
e-mail: yamada333@kch.biglobe.ne.jp

H. T. L. Vu
Department of Microbiology, Faculty of Biology,
University of Sciences, Vietnam National University–HCMC,
227 Nguyen Van Cu Street, Ward 4, District 5,
Hochiminh City, Vietnam

Y. Muramatsu · Y. Nakagawa
NITE Biological Resource Center,
National Institute of Technology and Evaluation,
2-5-8 Kazusa Kamatari,
Kisarazu 292–0818, Japan

D. Ochaikul
Department of Biology, Faculty of Science,
King Mongkut's Institute of Technology Ladkrabang,
Chalongkrung Road,
Ladkrabang, Bangkok 10520, Thailand

Y. Yamada
JICA Senior Overseas Volunteer,
Japan International Cooperation Agency,
Shibuya-ku, Tokyo 151–8558, Japan

Y. Yamada
Professor Emeritus, Shizuoka University,
Suruga-ku, Shizuoka 422–8529, Japan

Introduction

The genus *Gluconacetobacter* Yamada et al. 1998 (*Gluconoacetobacter* sic) was introduced by an elevation of the subgenus *Gluconacetobacter* (ex Asai 1935) Yamada and Kondo 1985, with the following five species, *Gluconacetobacter liquefaciens* (Asai 1935) Yamada et al. 1998 (the type species), *Gluconacetobacter xylinus* (Brown 1886) Yamada et al. 1998, *Gluconacetobacter hansenii* (Gosselé et al. 1983) Yamada et al. 1998, *Gluconacetobacter diazotrophicus* (Gillis et al. 1989) Yamada et al. 1998 and *Gluconacetobacter europaeus* (Sievers et al. 1992) Yamada et al. 1998 (Yamada et al. 1997, 1998).

Upon the proposal of the subgenus *Gluconacetobacter*, only two species, *Acetobacter* (*Gluconacetobacter*) *liquefaciens* (Asai 1935) Gosselé et al. 1983 (the type species) and *Acetobacter* (*Gluconacetobacter*) *xylinus* (Brown 1886) Yamada 1984 were accommodated to the subgenus (Yamada and Kondo 1985). However, Yamada and Kondo (1985) did not consider that the two species were similar to

each other chemotaxonomically and phenotypically (Asai et al. 1964; Leifson 1954; Yamada 1976, 1983; Yamada et al. 1969). Chemotaxonomically, for example, the former presented Q-10(Q-9), in which Q-9, a minor component of the respiratory quinone homologues, corresponded to Q-9, a major component of Q-9(Q-8) in strains of species of the genus *Acetobacter*, but the latter showed only Q-10, as found in strains of species of the genus *Gluconobacter*. Phenotypically, the former was motile with peritrichous flagella, but the latter was non-motile.

The species accommodated to the genus *Gluconacetobacter* are divided into two groups as mentioned above, namely the *Gluconacetobacter xylinus* group and the *Gluconacetobacter liquefaciens* group, on the basis of results obtained by 16S rRNA gene sequence analyses and by phenotypic characterization.

Yamada et al. (2000) calculated the 16S rRNA gene sequence similarities between the type strains of species belonging to *Gluconacetobacter* subclusters 1 and 2 to be 96.5–97.3%. Yamada (2000) transferred *Acetobacter oboediens* and *Acetobacter intermedius* to *Gluconacetobacter* subcluster 1, in which *G. xylinus*, *G. hansenii* and *G. europaeus* had been located, as *Gluconacetobacter oboediens* (Sokollek et al. 1998) Yamada 2000 and *Gluconacetobacter intermedius* (Boesch et al. 1998) Yamada 2000, but not to the *Gluconacetobacter* subcluster 2, in which *G. liquefaciens* and *G. diazotrophicus* had been located.

Dellaglio et al. (2005) reported that the two isolates that classified as *Gluconacetobacter swingsii* Dellaglio et al. 2005 and *Gluconacetobacter rhaeticus* Dellaglio et al. 2005 belonged phylogenetically to the genus *Gluconacetobacter*, in the *Gluconacetobacter xylinus* branch.

Lisdiyanti et al. (2006) described that the species of the genus *Gluconacetobacter* seemed likely to be divided into two subclusters phylogenetically, one consisting of *G. liquefaciens*, *Gluconacetobacter sacchari* Franke et al. 1999, *G. diazotrophicus*, *Gluconacetobacter azotocaptans* Fuentes-Ramírez et al. 2001 and *Gluconacetobacter johannae* Fuentes-Ramírez et al. 2001; and the other of *G. swingsii*, *G. europaeus*, *Gluconacetobacter nataicola* Lisdiyanti et al. 2006, *G. xylinus*, *G. oboediens*, *G. intermedius*, *G. rhaeticus*, *Gluconacetobacter saccharivorans* Lisdiyanti et al. 2006, *G. hansenii* and *Gluconacetobacter entanii* Schüller et al. 2000. The calculated pair-wise 16S rRNA gene sequence similarities were 95.7–97.6% between the two subclusters.

Yamada and Yukphan (2008) suggested that the *Gluconactobacter liquefaciens* group and the *Gluconacetobacter xylinus* group in the genus *Gluconacetobacter* can be distinguished from each other at the generic level from the phylogenetical, phenotypical and ecological points of view: (1) The calculated bootstrap value between the two groups in a 16S rRNA gene sequence phylogenetic tree derived from the neighbor-joining method was 70%. This indicated that the two groups were phylogenetically 'not so tightly coupled.' (2) The *Gluconacetobacter liquefaciens* group formed a large cluster along with the genera *Acidomonas*, *Kozakia*, *Neoasaia*, *Asaia*, *Swaminathania*, *Gluconobacter* and *Acetobacter*, rather than the cluster of the *Gluconacetobacter xylinus* group in a 16S rRNA gene sequence phylogenetic tree derived from the maximum parsimony method. The calculated bootstrap value was 74%. (3) A similar clustering was found in a 16S rRNA gene sequence phylogenetic tree derived from the maximum likelihood method. The calculated bootstrap value was 60%. Such a clustering was never found in acetic acid bacteria. For example, in the genus *Gluconobacter*, the calculated bootstrap values were 100% in the neighbor-joining method, 91% in the maximum parsimony method and 90% in the maximum likelihood method, in contrast to those of 70, 74 and 60% in the genus *Gluconacetobacter*. (4) Physiologically, strains of the *Gluconacetobacter liquefaciens* group produced 2,5-diketo-D-gluconate, γ-pyrone compounds and a water-soluble brown pigment, but strains of the *Gluconacetobacter xylinus* group did not. (5) Ecologically, the *Gluconacetobacter liquefaciens* group is plant-associated, namely strains of this group were isolated mostly from flowers, fruits, sugarcane, coffee plants and so on, but the *Gluconacetobacter xylinus* group is not necessarily plant-associated, namely, strains of this group were isolated mostly from fermented foods such as vinegar, nata de coco, tea fungus beverages and so on, in addition to the above-mentioned isolation sources.

This paper proposes *Komagatabacter* gen. nov., for strains of the species accommodated to the *Gluconacetobacter xylinus* group of the genus *Gluconacetobacter*, the family *Acetobacteraceae* Gillis and De Ley 1980.

Materials and methods

All the 16S rRNA gene sequences used in this study are present in the GenBank/EMBL/DDBJ databases. Multiple alignments of the cited DNA sequences were performed with the program Clustal X (version 1.8, Thompson et al. 1997). Alignment gaps and unidentified bases were eliminated. Distance matrices for the aligned sequences were calculated by the two-parameter method of Kimura (1980). Phylogenetic trees based on 16S rRNA gene sequences of 1,219 bases were constructed by the neighbor-joining method (Saitou and Nei 1987) and the maximum parsimony method (Felsenstein 1983), using the program MEGA (version 4.0, Tamura et al. 2007). In constructing a phylogenetic tree by the maximum likelihood method (Felsenstein 1981), the program PHYLIP (version 3.6, J. Felsenstein, University of Washington) was

used instead of the program MEGA (version 4.0). The robustness of individual branches was estimated by bootstrapping with 1,000 replications (Felsenstein 1985), and bootstrap values are not shown if below 50% in phylogenetic trees. The type strain of *Acidocella facilis* was used as an outgroup.

The type strain of *Gluconacetobacter kombuchae* of the *Gluconacetobacter xylinus* group was examined for morphology. The bacterial strain was grown at 20°C for 18–20 h on an agar plate, which contained 2.0% glucose w/v, 1.0% ethanol v/v, 0.5% peptone w/v, 0.2% yeast extract w/v, 0.7% calcium carbonate w/v and 2.0% agar w/v. Motility was tested by the hanging-drop method, and cells were negatively stained and observed under an electron microscope.

Phenotypic features were determined by the methods of Asai et al. (1964), Gosselé et al. (1983), Navarro and Komagata (1999), Lisdiyanti et al. (2002, 2006), Yamada et al. (1969, 1976, 2000) and Yukphan et al. (2005, 2008, 2009, 2011).

Results and discussion

In a phylogenetic tree based on 16S rRNA gene sequences derived from the neighbor-joining method (Fig. 1), the five species classified in the *Gluconacetobacter liquefaciens* group were divided into two subclusters, one comprising the two species, *G. liquefaciens* and *G. sacchari* and the other including the three species, *G. diazotrophicus*, *G. johannae* and *G. azotocaptans*. The two subclusters were connected to each other with a bootstrap value of 97%. The former was not characterized by a nitrogen fixation capability, but the latter was (Gillis et al. 1989; Fuentes-Ramírez et al. 2001). In the *Gluconacetobacter xylinus* group, the 12 species constituted a cluster in which species that strictly require acetic acid for growth, such as *G. europaeus* and *G. entanii*, and that produce biocellulose, such as *G. xylinus* and *G. nataicola*, were likely to be distributed randomly, and there did not appear to be any rule governing their distribution (Table 1). Between the clusters of the *Glconacetobacter liquefaciens* group and the *Glconacetobacter xylinus* group, the calculated bootstrap value was 66%.

In a phylogenetic tree based on 16S rRNA gene sequences derived from the maximum parsimony (MP) method (Fig. 2), the five species classified in the *Gluconacetobacter liquefaciens* group also grouped into two subclusters or subgroups. The cluster of the *Gluconacetobacter xylinus* group showed similar clustering, as found in the phylogenetic tree constructed by the neighbor-joining method. The calculated bootstrap value between the two clusters was 53%.

In a phylogenetic tree based on 16S rRNA gene sequences derived from the maximum likelihood (ML) method (Fig. 3), a similar clustering was found in both the *Gluconacetobacter liquefaciens* group and the *Gluconacetobacter xylinus* group. The calculated bootstrap value was 42% between the two clusters.

It was noted that the 19 species assigned to the genus *Acetobacter* represented a similar clustering to the genus *Gluconacetobacter* (Figs. 1–3). One included *Acetobacter aceti* (the type species), *Acetobacter estunensis* and so on, and the other included *Acetobacter pasteurianus*, *Acetobacter peroxydans* and so on. Between the two clusters, the calculated bootstrap values were 72, 60 and 71% in the phylogenetic trees derived from the neighbour-joining method, the MP method and the ML method, respectively.

In all the three phylogenetic trees based on 16S rRNA gene sequences, the species assigned to the *Gluconacetobacter xylinus* group were phylogenetically independent of the *Gluconacetobacter liquefaciens* group, as indicated previously (Yamada and Yukphan 2008).

Dutta and Gachhui (2007) reported that the type strain of *G. kombuchae* was motile with polar flagellation. Considering the morphology of *G. kombuchae* assigned to the *Gluconacetobacter xylinus* group, the type strain of the species was examined for motility and flagellation. The type strain of *G. kombuchae* (= LMG 23726^T) was non-motile, and no flagellation was found (data not shown). The results obtained differed from those of Dutta and Gachhui (2007). The morphological property of the type strain observed in this study was reasonable and consistent with those of strains of the species assigned to the *Gluconacetobacter xylinus* group (Table 1).

As described above, the *Gluconacetobacter liquefaciens* group and the *Gluconacetobacter xylinus* group were differentiated phylogenetically from each other at the generic level. Morphologically, the former was motile with peritrichous flagella, and the latter was non-motile without any flagellation. Physiologically, the former produced a water soluble-brown pigment when grown on glucose/yeast extract/calcium carbonate medium while the latter did not. Biochemically, the former produced 2,5-diketo-D-gluconate and γ-pyrone compounds from D-glucose, but the latter did not. Ecologically, the former was plant-associated, but the latter was not necessarily plant-associated. These phylogenetic, phenotypic and ecological characteristics are enough to separate the *Gluconacetobacter xylinus* group from the *Gluconacetobacter liquefaciens* group at the generic level (Table 1) (Yamada and Yukphan 2008).

Furthermore, Cleenwerck et al. (2010) described that the genus *Gluconacetobacter* should not remain as a single genus on the basis of multilocus sequence analyses of the three housekeeping genes of *dna*K, *gro*EL and *rpo*B, as suggested by Yamada and Yukphan (2008). However, they stated that some of the above-mentioned phenotypic features were of little use for the differentiation of acetic

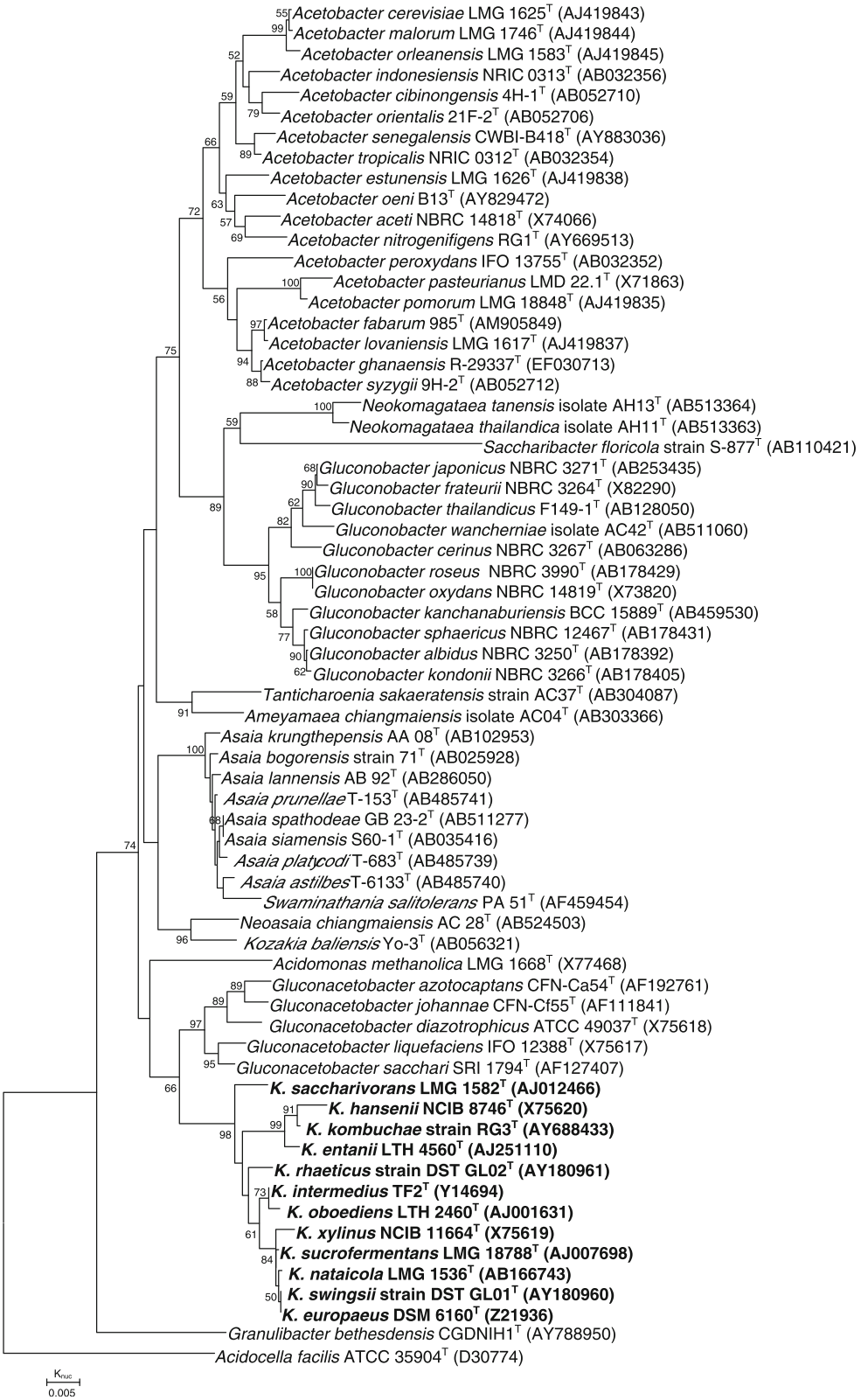

Fig. 1 Phylogenetic relationships of the genera *Gluconacetobacter* and *Komagatabacter*. A phylogenetic tree based on 16S rRNA gene sequences was constructed by the neighbor-joining method. The type strain of *Acidocella facilis* was used as an outgroup. Numerals at nodes indicate bootstrap values (%) derived from 1,000 replications. *K. Komagatabacter*

acid bacteria and that, for this reason, other differentiating features should be looked for before splitting the genus. Our opinion is, however, quite different from theirs.

The above-mentioned phenotypic features are useful for differentiating the *Gluconacetobacter xylinus* group from the *Gluconacetobacter liquefaciens* group. In fact, one can

Table 1 Differential characteristics of the genera *Gluconacetobacter* and *Komagatabacter*. *Gluconacetobacter* strains: 1 *Gluconacetobacter liquefaciens* NBRC 12388^T (Navarro and Komagata 1999), 2 *G. diazotrophicus* LMG 7603^T (Gillis et al. 1989), 3 *G. sacchari* strain SRI 1794^T (Franke et al. 1999), 4 *G. johannae* strain CFN-Cf55^T (Fuentes-Ramírez et al. 2001), 5 *G. azotocaptans* strain CFN-Ca54^T (Fuentes-Ramírez et al. 2001); *Komagatabacter* strains: 6 *Komagatabacter xylinus* JCM 7644^T (Navarro and Komagata 1999), 7 *K. hansenii* NBRC 14820^T (Lisdiyanti et al. 2006), 8 *K. europaeus* strain DES 11^T (Sievers et al. 1992), 9 *K. oboediens* LTH 2460^T (Sokollek et al. 1998), 10 *K. intermedius* strain TF2^T (Boesch et al. 1998), 11 *K. entanii* strain LTH 4560^T (Schüller et al. 2000), 12 *K. swingsii* strain DST GL01^T (Dellaglio et al. 2005), 13 *K. rhaeticus* strain DST GLO2^T (Dellaglio et al. 2005), 14 *K. saccharivorans* LMG 1582^T (Lisdiyanti et al. 2006), 15 *K. nataicola* LMG 1536^T (Lisdiyanti et al. 2006), 16 *K. kombuchae* strain RG3^T (Dutta and Gachhui 2007), 17 *K. sucrofermentans* strain BPR 2001^T (Toyosaki et al. 1995; Cleenwerck et al. 2010)

Characteristic[a]	*Gluconacetobacter*					*Komagatabacter*											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Flagellation	*per*	*per*	*per*	*per*	*per*	*no*	*no*	*no*	*no*	*no*	*no*	*no*	*no*	*no*	*no*	*no*[b]	*no*
Oxidation of:																	
Acetate	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Lactate	+	+	+	+	+	+	+	+	*nd*	*nd*	−	*nd*	*nd*	+	+	+	+
Growth without acetic acid	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+
Growth on:[c]																	
Glutamate agar	+	+	+	*nd*	*nd*	+	+	+	*nd*	+	*nd*	*nd*	*nd*	+	+	*nd*	+
Mannitol agar	+	+	+	*nd*	*nd*	+	+	+	*nd*	+	*nd*	*nd*	*nd*	+	+	*nd*	+
Production of acetic acid from ethanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Production of a water-soluble brown pigment	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Production of dihydroxyacetone from glycerol	+	+	−	*nd*	*nd*	+	+	*nd*	+	*nd*	*nd*	*nd*	*nd*	+	+	*nd*	+
Cellulose production	−	−	−	−	−	+	−	−	−	−	−	+	+	−	+	+	+
Production of γ-pyrone compound	+	+[b]	+[b]	+[b]	+[b]	−	−	−	−	−	−	−	−	−	−	−	−
Assimilation of ammoniac nitrogen on:[d]																	
Mannitol	+	+	+	−	−	*nd*	+	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	+	+	*nd*	*nd*
Ethanol	−[b,e]	+	+	+	+	*nd*	−	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	−	−	*nd*	*nd*
Production of:																	
2-Keto-D-gluconate	+	+	+	+[b]	+[b]	+	+	+	+	−	−	+	+	+	+	−	+
5-Keto-D-gluconate	+	−	+	+[b]	+[b]	+	+	+	−	−	−	+	+	−	+	+	−
2,5-Diketo-D-gluconate	+	+	+	+[b]	+[b]	−	−	−	−	−	−	−	−	−	−	−	−
DNA G+C content (mol%)	64.5[f]	61	65	58.0	64.0	61.8[g]	59.0	56.2-57.3	59.9	61.6	58	61.7	63.4	61	62	55.8	62.3

[a] *per* peritrchous, *no* none, + positive, − negative, *nd* not determined; a major ubiquinone was Q-10 in all strains tested

[b] Present study

[c] Navarro and Komagata 1999; Lisdiyanti et al. 2006

[d] Lisdiyanti et al. 2006

[e] Lisdiyanti et al. 2000

[f] Yamada et al. 1981

[g] Tanaka et al. 2000

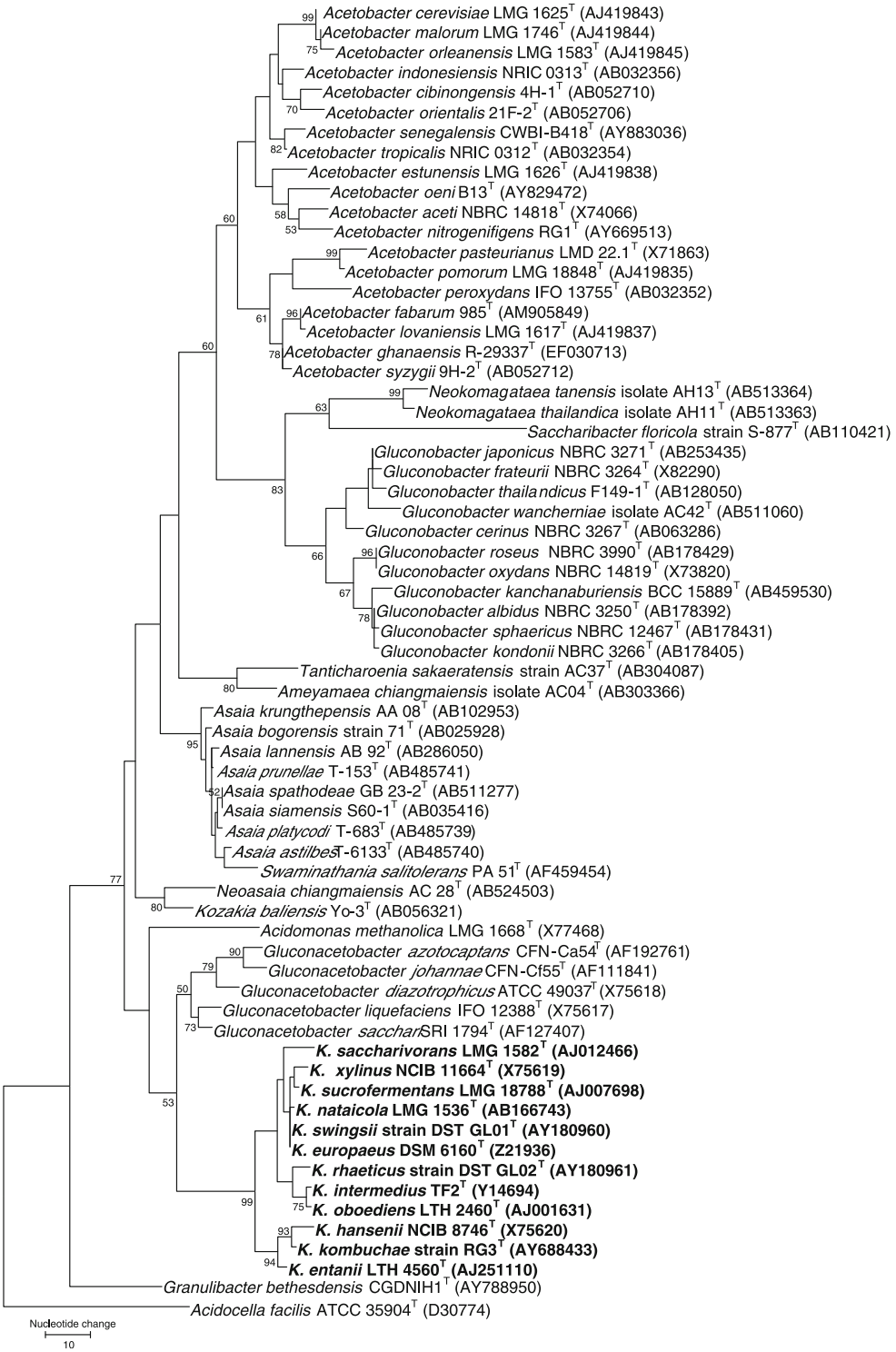

Fig. 2 Phylogenetic relationships of the genera *Gluconacetobacter* and *Komagatabacter*. A phylogenetic tree based on 16S rRNA gene sequences was constructed by the maximum parsimony (MP) method. The type strain of *Acidocella facilis* was used as an outgroup. The phylogenetic relationships were represented by a consensus tree of two most parsimonious trees. There were a total of 1,219 positions (bases) in the final dataset, of which 129 were parsimony informative. Consistency index=0.441, retention index=0.795, rescaled consistency index=0.351, homoplasy index=0.559. Numerals at nodes indicate bootstrap values (%) derived from 1,000 replications.
K. Komagatabacter

exactly classify or identify such an acetic acid bacterium, without any problem and without any confusion, by the use of the phenotypic features mentioned above, as has already been done by Lisdiyanti et al. (2002), Loganathan and Nair (2004), Jojima et al. (2004) and Greenberg et al. (2006) (see Yamada and Yukphan 2008).

For example, a certain isolate can be assigned to or classified into the genus *Gluconacetobacter*, specifically to the *Gluconacetobacter liquefaciens* group but not to the *Gluconacetobacter xylinus* group, when it shows the oxidation of acetate and lactate and produces a characteristic water-soluble brown pigment (Table 1). The isolate

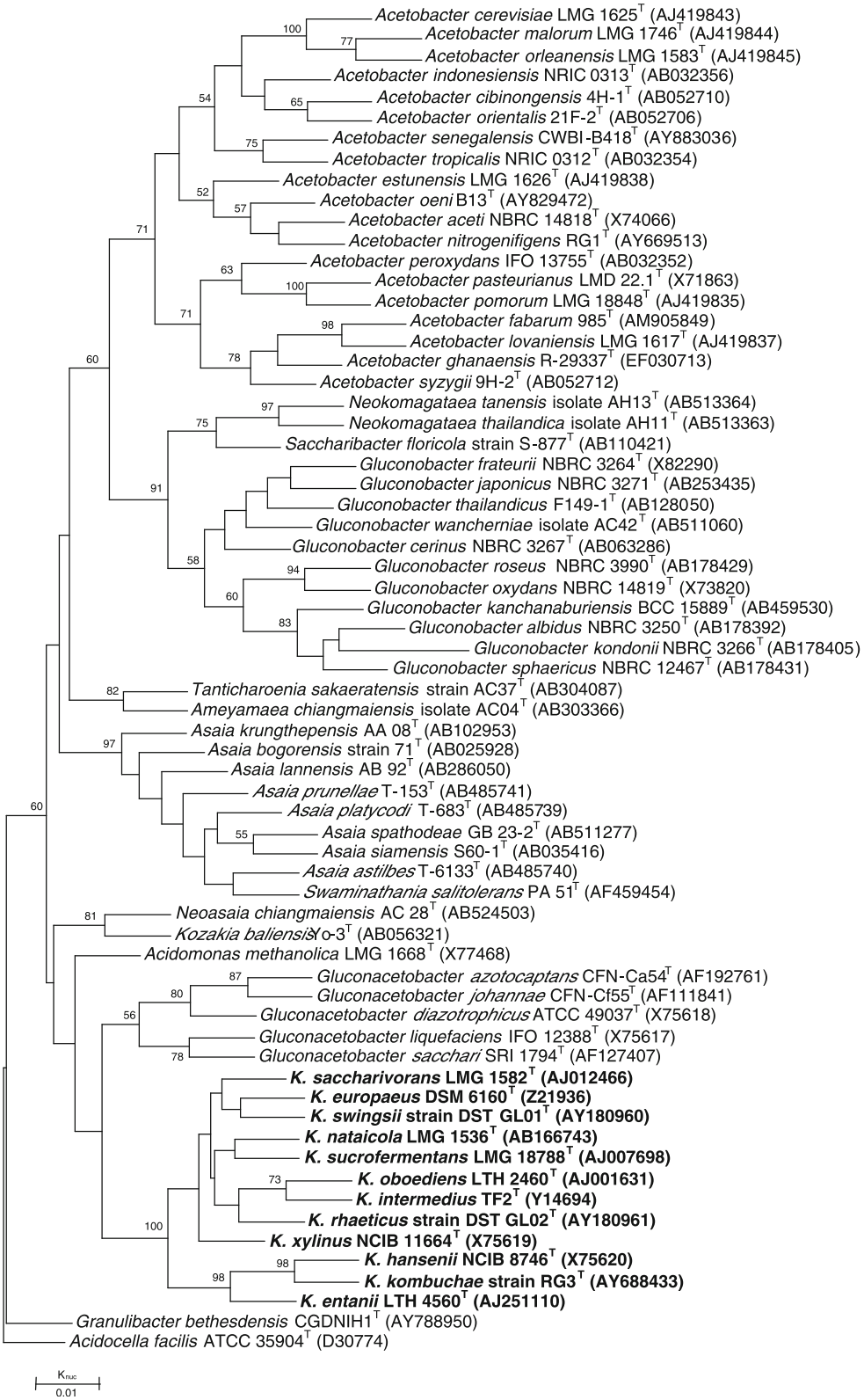

Fig. 3 Phylogenetic relationships of the genera *Gluconacetobacter* and *Komagatabacter*. A phylogenetic tree based on 16S rRNA gene sequences was constructed by the maximum likelihood (ML) method. The type strain of *Acidocella facilis* was used as an outgroup. Numerals at nodes indicate bootstrap values (%) derived from 1,000 replications. *K. Komagatabacter*

also can be differentiated from some strains of the genus *Gluconobacter* and strains of the genus *Tanticharoenia*, all of which have no oxidation capability of acetate and lactate, and also from strains of the genus *Swaminathania* that have weak acetate and lactate oxidation, although all of them produce the water-soluble brown pigment (Yukphan et al. 2011). In contrast, an isolate that is non-motile and does not produce the water-soluble brown pigment can, in general,

be assigned to or classified into the *Gluconacetobacter xylinus* group of the genus *Gluconacetobacter*, when it shows oxidation of acetate and lactate and has Q-10 as a major isoprenoid quinone (Table 1). The isolate also can be differentiated from strains of the genus *Acetobacter* that have Q-9 and the quite intense ability to oxidize acetate and lactate to carbon dioxide and water, from strains of the genus *Acidomonas* that grow on methanol by showing no growth on methanol, from strains of the genera *Asaia*, *Kozakia* and *Granulibacter* that show weak acetate oxidation, from strains of the genus *Ameyamaea* that are motile with polar flagella and weak oxidation of lactate and from the genera *Gluconobacter*, *Neoasaia*, *Saccharibacter* and *Neokomagataea* that have no oxidation of acetate and lactate (Yamada and Yukphan 2008; Yukphan et al. 2008, 2009, 2011). Finally, the systematic assignments or the taxonomic positions of the isolate based on routine identification methods can be confirmed phylogenetically, for example, by constructing a phylogenetic tree based on 16S rRNA gene sequences.

To date, only five genera of acetic acid bacteria include more than one species, namely *Acetobacter* Beijerinck 1898, *Gluconobacter* Asai 1935, *Gluconacetobacter*, *Asaia* Yamada et al. 2000 and *Neokomagataea* Yukphan et al. 2011. Others are monotypic genera and seem likely to be composed of rare microorganisms in distribution. When acetic acid bacteria, which are oxidative but not fermentative and grow at pH 3.5, are isolated from the natural environments, one will encounter mostly acetic acid bacteria that are classified into either the genus *Acetobacter*, *Gluconobacter* or *Asaia*. To discriminate these acetic acid bacteria at the generic level, the acetate and lactate oxidation test is useful (Asai et al. 1964; Yamada and Yukphan 2008). For example, in isolates to be assigned to the genus *Acetobacter*, which is characterized by Q-9 as a major quinone homologue, a deep blue color will appear fast and clearly. Isolates to be assigned to the genus *Gluconobacter* will generally show a clear yellow color. In the case of isolates to be assigned to the genus *Asaia*, which is characterized by little production of acetic acid from ethanol, color change will be very slow. In isolates to be assigned to the genus *Gluconacetobacter*, the color change to blue will be less vigorous compared to isolates to be assigned to the genus *Acetobacter*.

To divide isolates already assigned to the genus *Gluconacetobacter* into two groups, namely the *Gluconacetobacter liquefaciens* group and the *Gluconacetobacter xylinus* group, the characteristic brown-pigment production can be utilized. The former produces a water-soluble brown pigment, but the latter does not. It is noteworthy that, in our experience, there have been no exceptions to this routine identification method. Thus, one can identify a large number of isolates in a short period.

The 19 species assigned to the genus *Acetobacter* to date represent two clusters with low bootstrap values at the branching points in the phylogenetic trees (Figs.1–3) (Yamada and Yukphan 2008). At the present time, however, the species of the two clusters cannot be differentiated at the generic level, since there are likely to be no decisive phenotypic features to discriminate the two clusters or the two groups taxonomically, differing from the genus *Gluconacetobacter*.

The phylogenetic and phenotypic characteristics obtained above are enough to separate the *Gluconacetobacter xylinus* group at the generic level from the *Gluconacetobacter liquefaciens* group, and the species of the former group can appropriately be classified into a separate new genus. The name of the genus is *Komagatabacter* gen. nov.

Description of *Komagatabacter* gen. nov.

Komagatabacter (Ko.ma.ga.ta.bac'ter. N. L. masc. n. *bacter* from Gr. neut. n. *baktron* rod; N. L. masc. n. *Komagatabacter* Komagata rod, derived from Dr. Kazuo Komagata, Professor Emeritus, The University of Tokyo, Bunkyo-ku, Tokyo, Japan, who contributed to the bacterial systematics, especially of acetic acid bacteria).

Gram-negative rods and non-motile, measuring 0.5–0.8 × 1.0–3.0 μm. Colonies are white–creamy and smooth with entire margin or rough. Production of cellulose is positive or negative. Does not produce a water-soluble brown pigment on glucose/yeast extract/calcium carbonate medium. Produces acetic acid from ethanol. Growth is positive in the presence of 0.35% acetic acid v/v. In some strains, acetic acid is strictly required for growth. Oxidizes acetate and lactate to carbon dioxide and water. In some strains, acetate and lactate are not oxidized. In general, grows on glutamate agar and mannitol agar. Ammoniac nitrogen is generally assimilated on D-mannitol. Production of dihydroxyacetone from glycerol is positive or negative. Produces 2-keto-D-gluconate or 5-keto-D-gluconate from D-glucose, but 2,5-diketo-D-gluconate is not produced. γ-Pyrone compounds are not produced. In some strains, ketogluconates are not produced. Acid is produced from D-glucose, D-galactose, D-xylose, L-arabinose or ethanol, but not from D-fructose, L-sorbose, D-mannitol, D-sorbitol, maltose or lactose. Grows on D-glucose, D-fructose or D-mannitol, but not on lactose. A major isoprenoid quinone is Q-10. DNA base composition is 55.8–63.4 mol%G+C with a range of 7.6 mol%. The type species is *Komagatabacter xylinus* (Brown 1886) comb. nov.

Since the new genus was introduced, the following species should be transferred to the genus *Komagatabacter*.

Komagatabacter xylinus (Brown 1886) comb. nov.

Synonym: *Gluconacetobacter xylinus* (Brown 1886) Yamada, Hoshino and Ishikawa, Biosci Biotechnol Biochem 61: 1250, 1997 (Validation list no. 64, Int J Syst Bacterial 48:

327, 1998); *Acetobacter xylinus* (Brown 1886) Yamada, J Gen Appl Microbiol 29: 419, 1983 (Validation list no. 14, Int J Syst Bacteriol 34: 270, 1984)

Basonym: *Acetobacter aceti* subsp. *xylinus* (*xylinum* sic) (Brown 1886) De Ley and Frateur 1974 (Approved lists, Int J Syst Bacteriol 30: 239, 1980). The type strain is NCIMB 11664^T.

Komagatabacter hansenii (Gosselé, Swings, Kersters, Pauwels and De Ley 1983) comb. nov.

Synonym: *Guconacetobacter hansenii* Gosselé, Swings, Kersters, Pauwels and De Ley 1983) Yamada, Hoshino and Ishikawa, Biosci Biotechnol Biochem 61: 1250, 1997 (Validation list no. 64, Int J Syst Bacteriol 48: 327, 1998)

Basonym: *Acetobacter hansenii* Gosselé, Swings, Kersters, Pauwels and De Ley, Syst Appl Microbiol 4: 366, 1983 (Validation list no. 12, Int J Syst Bacterial 33: 896, 1983). The type strain is NCIMB 8746^T.

Komagatabacter europaeus (Sievers, Sellmer and Teuber 1992) comb. nov.

Synonym: *Gluconacetobacter europaeus* (Sievers, Sellmer and Teuber 1992) Yamada, Hoshino and Ishikawa, Biosci Biotechnol Biochem 61: 1250, 1997 (Validation list no. 64, Int J Syst Bacterial 48: 327, 1998)

Basonym: *Acetobacter europaeus* Sievers, Sellmer and Teuber, Syst Appl Microbiol 15: 391, 1992 (Validaton list no. 43, Int J Syst bacterial 42: 656, 1992). The type strain is DSM 6160^T.

Komagatabacter oboediens (Sokollek, Hertel and Hammes 1998) comb. nov.

Synonym: *Gluconacetobacter oboediens* (Sokollek, Hertel and Hammes 1998) Yamada, Int J. Syst Evol Microbiol 50: 226, 2000

Basonym: *Acetobacter oboediens* Sokollek, Hertel and Hammes, Int J Syst Bacterial 48: 939, 1998. The type strain is DSM 11826^T.

Komagatabacter intermedius (Boesch, Trček, Sievers and Teuber 1998) comb. nov.

Synonym: *Gluconacetobacter intermedius* (Boesch, Trček, Sievers and Teuber 1998) Yamada, Int J. Syst Evol Microbiol 50: 226, 2000

Basonym: *Acetobacter intermedius* Boesch, Trček, Sievers and Teuber, Syst Appl Microbiol 21: 228, 1998 (Validation list no. 67, Int J Syst Bacteriol 48: 1083, 1998). The type strain is DSM 11804^T. According to Lisdiyanti et al. (2006), this species is a later heterotypic synonym of *Gluconacetobacter oboediens*.

Komagatabacter entanii (Schüller, Hertel and Hammes 2000) comb. nov.

Basonym: *Gluconacetobacter entanii* Schüller Hertel and Hammes, Int J Syst Evol Microbiol 50: 2019, 2000. The type strain is DSM 13536^T.

Komagatabacter swingsii (Dellaglio, Cleenwerck, Felis, Engelbeen, Janssens and Marzotto 2005) comb. nov.

Basonym: *Gluconacetobacter swingsii* Dellaglio, Cleenwerck, Felis, Engelbeen, Janssens and Marzotto, Int J Syst Evol Microbiol 55: 2368, 2005. The type strain is LMG 22125^T.

Komagatabacter rhaeticus (Dellaglio, Cleenwerck, Felis, Engelbeen, Janssens and Marzotto 2005) comb. nov.

Basonym: *Gluconacetobacter rhaeticus* Dellaglio, Cleenwerck, Felis, Engelbeen, Janssens and Marzotto, Int J Syst Evol Microbiol 55: 2369, 2005. The type strain is LMG 22126^T.

Komagatabacter saccharivorans (Lisdiyanti, Navarro, Uchimura and Komagata 2006) comb. nov.

Basonym: *Gluconacetobacter saccharivorans* Lisdiyanti, Navarro, Uchimura and Komagata, Int J Syst Evol Microbiol 56: 2108, 2006. The type strain is LMG 1582^T.

Komagatabacter nataicola (Lisdiyanti, Navarro, Uchimura and Komagata 2006) comb. nov.

Basonym: *Gluconacetobacter nataicola* Lisdiyanti, Navarro, Uchimura and Komagata, Int J Syst Evol Microbiol 56: 2109, 2006. The type strain is LMG 1536^T.

Komagatabacter kombuchae (Dutta and Gachhui 2007) comb. nov.

Basonym: *Gluconacetobacter kombuchae* Dutta and Gachhui, Int J Syst Evol Microbiol 57: 356, 2007. The type strain is LMG 23726^T. According to Cleenwerck et al. (2009), this species is a later heterotypic synonym of *Gluconacetobacter hansenii*.

Komagatabacter sucrofermentans (Toyosaki, Kojima, Tsuchida, Hoshino, Yamada and Yoshinaga 1996) comb. nov.

Synonym: *Gluconacetobacter sucrofermentans* (Toyosaki, Kojima, Tsuchida, Hoshino, Yamada and Yoshinaga 1996) Cleenwerck, Vos and Vuyst, Int J Syst Evol Microbiol 60: 2282, 2010

Basonym: *Acetobacter xylinus* (Brown 1886) Yamada 1984 subsp. *sucrofermentans* Toyosaki, Kojima, Tsuchida, Hoshino, Yamada and Yoshinaga, J Gen Appl Microbiol 41:312, 1995 (Validation list no. 58, Int J Syst Bacteriol 46: 836, 1996). The type strain is JCM 9730^T.

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

Boesch C, Trček J, Sievers M, Teuber M (1998) *Acetobacter intermedius* sp. nov. Syst Appl Microbiol 21:220–229

Cleenwerck I, De Wachter M, González Á, De Vuyst L, De Vos P (2009) Differentiation of species of the family *Acetobacteraceae* by AFLP DNA fingerprinting: *Gluconacetobacter kombuchae* is a later heterotypic synonym of *Gluconacetobacter hansenii*. Int J Syst Evol Microbiol 59:1771–1786

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. *sucroferementans* as *Gluconacetobacter sucrofermentans* (Toyosaki et al. 1996) sp. nov., comb. nov. Int J Syst Evol Microbiol 60:2277–2283

Dellaglio F, Cleenwerck I, Felis GE, Engelbeen K, Janssens D, Marzotto M (2005) Description of *Gluconacetobacter swingsii* sp. nov. and *Gluconacetobacter rhaeticus* sp. nov., isolated from Italian apple fruit. Int J Syst Evol Microbiol 55:2365–2370

Dutta D, Gachhui R (2007) Nitrogen-fixing and cellulose-producing *Gluconacetobacter kombuchae* sp. nov., isolated from Kombucha tea. Int J Syst Evol Microbiol 57:353–357

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1983) Parsimony in systematics: biological and statistical issues. Annu Rev Ecol Syst 14:313–333

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Franke IH, Fegan M, Hayward C, Leonard G, Stackebrandt E, Sly LI (1999) Description of *Gluconacetobacter sacchari* sp. nov., a new species of acetic acid bacterium isolated from the leaf sheath of sugar cane and from the pink sugar-cane mealy bug. Int J Syst Bacteriol 49:1681–1693

Fuentes-Ramírez LE, Bustillos-Cristales R, Tapia-Hernández A, Jiménes-Salgado T, Wang ET, Martínez-Romero E, Caballero-Mellado J (2001) Novel nitrogen-fixing acetic acid bacteria, *Gluconacetobacter johannae* sp. nov. and *Gluconacetobacter azotocaptans* sp. nov., associated with coffee plants. Int J Syst Evol Microbiol 51:1305–1314

Gillis M, Kersters K, Hoste B, Janssens D, Kroppenstedt RM, Stephan MP, Teixeira KRS, Döbereiner J, De Ley J (1989) *Acetobacter diazotrophicus* sp. nov., a nitrogen-fixing acetic acid bacterium associated with sugarcane. Int J Syst Bacteriol 39:361–364

Gosselé F, Swings J, Kersters K, Pauwels P, De Ley J (1983) Numerical analysis of phenotypic features and protein gel electrophoregrams of a wide variety of *Acetobacter* strains. Proposal for the improvement of the taxonomy of the genus *Acetobacter* Beijerinck 1898, 215. Syst Appl Microbiol 4:338–368

Greenberg DE, Porcella SF, Stock F, Wong A, Conville PS, Murray PR, Holland SM, Zelazny AM (2006) *Granulibacter bethesdensis* gen. nov., sp. nov., a distinctive pathogenic acetic acid bacterium in the family *Acetobacteraceae*. Int J Syst Evol Microbiol 56:2609–2616

Jojima Y, Mihara Y, Suzuki S, Yokozeki K, Yamanaka S, Fudou R (2004) *Saccharibacter floricola* gen. nov., sp. nov., a novel osmophilic acetic acid bacterium isolated from pollen. Int J Syst Evol Microbiol 54:2263–2267

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Leifson E (1954) The flagellation and taxonomy of species of *Acetobacter*. Antonie Van Leeuwenhoek 20:102–110

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

Lisdiyanti P, Kawasaki H, Widyastuti Y, Saono S, Seki T, Yamada Y, Uchimura T, Komagata K (2002) *Kozakia baliensis* gen. nov., sp. nov., a novel acetic acid bacterium in the α-*Proteobacteria*. Int J Syst Evol Microbiol 52:813–818

Lisdiyanti P, Navarro RR, Uchimura T, Komagata K (2006) Reclassification of *Gluconacetobacter hansenii* strains and proposals of *Gluconacetobacter saccharivorans* sp. nov. and *Gluconacetobacter nataicola* sp. nov. Int J Syst Evol Microbiol 56:2101–2111

Loganathan P, Nair S (2004) *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 54:1185–1190

Navarro RR, Komagata K (1999) Differentiation of *Gluconacetobcter liquefaciens* and *Gluconacetobacter xylinus* on the basis of DNA base composition, DNA relatedness and oxidation products from glucose. J Gen Appl Microbiol 45:7–15

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Schüller G, Hertel C, Hammes WP (2000) *Gluconacetobacter entanii* sp. nov., isolated from submerged high-acid industrial vinegar fermentations. Int J Syst Evol Microbiol 50:2013–2020

Sievers M, Sellmer S, Teuber M (1992) *Acetobacter europaeus* sp. nov., a main component of industrial vinegar fermenters in central Europe. Syst Appl Microbiol 15:386–392

Sokollek SJ, Hertel C, Hammes WP (1998) Description of *Acetobacter oboediens* sp. nov. and *Acetobacter pomorum* sp. nov., two new species isolated from industrial vinegar fermentations. Int J Syst Bacteriol 48:935–940

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Tanaka M, Murakami S, Shinke R, Aoki K (2000) Genetic characteristics of cellulose-forming acetic acid bacteria identified phenotypically as *Gluconacetobacter xylinus*. Biosci Biotechnol Biochem 64:757–760

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Toyosaki H, Kojima Y, Tsuchida T, Hoshino K, Yamada Y, Yoshinaga F (1995) The characterization of an acetic acid bacterium useful for producing bacterial cellulose in agitation cultures: the proposal of *Acetobacter xylinum* subsp. *sucrofermentans* subsp. nov. J Gen Appl Microbiol 41:307–314

Yamada Y (1976) Characterization of *Acetobacter xylinum* by ubiquinone system. J Gen Appl Microbiol 22:285–292

Yamada Y (1983) *Acetobacter xylinus* sp. nov., nom. rev., for the cellulose-forming and cellulose-less, acetate-oxidizing acetic acid bacteria with the Q-10 system. J Gen Appl Microbiol 29:417–420

Yamada Y (2000) Transfer of *Acetobacter oboediens* Sokollek et al. 1998 and *Acetobacter intermedius* Boesch et al. 1998 to the genus *Gluconacetobacter* as *Gluconacetobacter oboediens* comb. nov. and *Gluconacetobacter intermedius* comb. nov. Int J Syst Evol Microbiol 50:2225–2227

Yamada Y, Kondo K (1985) *Gluconoacetobacter*, a new subgenus comprising the acetate-oxidizing acetic acid bacteria with ubiquinone-10 in the genus *Acetobacter*. J Gen Appl Microbiol 30:297–303

Yamada Y, Yukphan P (2008) Genera and species in acetic acid bacteria. Int J Food Microbiol 125:15–24

Yamada Y, Aida K, Uemura T (1969) Enzymatic studies on the oxidation of sugar and sugar alcohol. V. Ubiquinone of acetic acid bacteria and its relation to classification of genera *Gluconobacter* and *Acetobacter*, especially of the so-called intermediate strains. J Gen Appl Microbiol 15:186–196

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

Yamada Y, Ishikawa T, Yamashita M, Tahara Y, Yamasato K, Kaneko T (1981) Deoxyribonucleic acid base composition and deoxyribonucleic acid homology in acetic acid bacteria, especially in the polarly flagellated intermediate strains. J Gen Appl Microbiol 27:465–475

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

Yamada Y, Hoshino K, Ishikawa T (1998) Validation list no. 64. Validation of publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 48:327–328

Yamada Y, Katsura K, Kawasaki H, Widyastuti Y, Saono S, Seki T, Uchimura T, Komagata K (2000) *Asaia bogorensis* gen. nov., sp. nov., an unusual acetic acid bacterium in the α-*Proteobacteria*. Int J Syst Evol Microbiol 50:823–829

Yukphan P, Malimas T, Potacharoen W, Tanasupawat S, Tanticharoen M, Yamada Y (2005) *Neoasaia chiangmaiensis* gen. nov., sp. nov., a novel osmotolerant acetic acid bacterium in the α-*Proteobacteria*. J Gen Appl Microbiol 51:301–311

Yukphan P, Malimas T, Muramatsu Y, Takahashi M, Kaneyasu M, Tanasupawat S, Nakagawa Y, Suzuki K, Potacharoen W, Yamada Y (2008) *Tanticharoenia sakaeratensis* gen. nov., sp. nov., a new osmotolerant acetic acid bacterium in the α-*Proteobacteria*. Biosci Biotechnol Biochem 72:672–676

Yukphan P, Malimas T, Muramatsu Y, Takahashi M, Kaneyasu M, Potacharoen W, Tanasupawat S, Nakagawa Y, Hamana K, Tahara Y, Suzuki K, Tanticharoen M, Yamada Y (2009) *Ameyamaea chiangmaiensis* gen. nov., sp. nov., an acetic acid bacterium in the α-*Proteobacteria*. Biosci Biotechnol Biochem 73:2156–2162

Yukphan P, Malimas T, Muramatsu Y, Potacharoen W, Tanasupawat S, Nakagawa Y, Tanticharoen M, Yamada Y (2011) *Neokomagataea* gen. nov., with descriptions of *Neokomagataea thailandica* sp. nov. and *Neokomagataea tanensis* sp. nov., osmotolerant acetic acid bacteria of the α-*Proteobacteria*. Biosci Biotechnol Biochem 75:419–426