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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.

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Thirty-one *Acetobacter* strains obtained from culture collections and 45 *Acetobacter* strains isolated from Indonesian sources were investigated for their phenotypic characteristics, ubiquinone systems, DNA base compositions, and levels of DNA-DNA relatedness. Of 31 reference strains, six showed the presence of ubiquinone 10 (Q-10). These strains were eliminated from the genus *Acetobacter*. The other 25 reference strains and 45 Indonesian isolates were subjected to a systematic study and separated into 8 distinct groups on the basis of DNA-DNA relatedness. The known species, *Acetobacter aceti*, *A. pasteurianus*, and *A. peroxydans* are retained for three of these groups. New combinations, *A. orleanensis* (Henneberg 1906) comb. nov., *A. lovaniensis* (Frateur 1950) comb. nov., and *A. estunensis* (Carr 1958) comb. nov. are proposed for three other groups. Two new species, *A. indonesiensis* sp. nov. and *A. tropicalis* sp. nov. are proposed for the remaining two. No Indonesian isolates were identified as *A. aceti*, *A. estunensis*, and *A. peroxydans*. Phylogenetic analysis on the basis of 16S rDNA sequences was carried out for representative strains from each of the groups. This supported that the eight species belonged to the genus *Acetobacter*. Several strains previously assigned to the species of *A. aceti* and *A. pasteurianus* were scattered over the different species. It is evident that the value of DNA-DNA relatedness between strains comprising a new species should be determined for the establishment of the species. Thus current bacterial species without data of DNA-DNA relatedness should be reexamined for the stability of bacterial nomenclature.

Key Words——*Acetobacter aceti*; *Acetobacter estunensis*; *Acetobacter indonesiensis*; *Acetobacter lovaniensis*; *Acetobacter orleanensis*; *Acetobacter pasteurianus*; *Acetobacter peroxydans*; *Acetobacter tropicalis*

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Introduction

In Bergey's Manual of Determinative Bacteriology, 8th edition, the genus *Acetobacter* comprised 3 species, namely, *Acetobacter aceti*, *A. pasteurianus*,

and *A. peroxydans* (De Ley and Frateur, 1974). Furthermore, *A. aceti* consisted of 4 subspecies: *A. aceti* subsp. *aceti*, *A. aceti* subsp. *liquefaciens*, *A. aceti* subsp. *orleanensis*, and *A. aceti* subsp. *xylinum. A. pasteurianus* consisted of 5 subspecies: *A. pasteurianus* subsp. *pasteurianus*, *A. pasteurianus* subsp. *ascendens*, *A. pasteurianus* subsp. *estunensis*, *A. pasteurianus* subsp. *lovaniensis*, and *A. pasteurianus* subsp. *paradoxus.* All *Acetobacter* species and subspecies were validated on the Approved Lists of Bacterial Names (Skerman et al., 1980). Gosselé et al. (1983) studied 98 *Acetobacter* strains by numerical analysis on the basis of 177 phenotypic features and protein gel electrophoregrams. They rejected the previous subspecies concept and proposed four species instead, namely, *A. aceti*, *A. hansenii*, *A. liquefaciens*, and *A. pasteurianus.* These results were cited in Bergey's Manual of Systematic Bacteriology (De Ley et al., 1984). Yamada (1983) revived *A. xylinus* [*sic*] as an independent species of *Acetobacter* on the basis of quinone systems and DNA-DNA relatedness.

Yamada et al. (1997a, b) proposed the genus *Gluconoacetobacter* [*sic*] in acetic acid bacteria on the basis of ubiquinone systems and partial 16S rDNA sequences. As a result, *A. liquefaciens*, *A. hansenii*, and *A. xylinus* [*sic*] were transferred to *Gluconoacetobacter liquefaciens* [*sic*], *Gluconoacetobacter hansenii* [*sic*], and *Gluconoacetobacter xylinus* [*sic*], respectively.

Sokollek et al. (1998) proposed two new species of the genus *Acetobacter*: *A. oboediens* and *A. pomorum*, and Boesch et al. (1998) described a new species, *A. intermedius.* Yamada (2000) transferred *A. oboediens* and *A. intermedius* to the genus *Gluconacetobacter* based on the ubiquinone system and 16S rDNA sequences. Therefore three species are now in the genus *Acetobacter*, namely, *A. aceti* and *A. pasteurianus*, together with *A. pomorum.*

Some taxonomic problems, however, remained in the genus *Acetobacter*: (1) a broad range (9.7 mol%) of the G+C content of DNA in *A. pasteurianus*; (2) no comprehensive data of DNA-DNA relatedness between strains identified as *A. aceti* and those identified as *A. pasteurianus*; (3) no consideration on the taxonomic significance of ubiquinone systems (Gosselé et al., 1983).

Most acetic acid bacteria obtainable from culture collections were those isolated from materials in the temperate regions. Yamada et al. (1999) succeeded in isolating acetic acid bacteria from fruits, flowers, and traditional fermented foods collected in Indonesia, a representative tropical country. A total of 64 acetic acid bacteria were isolated by the enrichment culture approach. Of 64 isolates, 45 oxidized acetate and lactate and had Q-9 as the major ubiquinone. These isolates were regarded as *Acetobacter* strains.

This study deals with the reidentification of 31 *Acetobacter* strains obtained from culture collections, the identification of 45 *Acetobacter* strains isolated from Indonesian sources, and the establishment of improved systematics of the genus *Acetobacter.*

Materials and Methods

Bacterial strains. Strains obtained from culture collections are listed in Table 1. *A. aceti* IFO 14818^T was separated into two stable colonial types; they were labeled IFO 14818^{T}R for the rough type and IFO 14818^{T}S for the smooth type. Isolation sources of 45 *Acetobacter* isolates from Indonesia are presented in Table 2, and these strains are referred to as Indonesian isolates. *A. pomorum* was not used in this study because of the strict cultural requirements for growth of a strain of this species.

Phenotypic characterization. Cell form and cell size were determined for cells grown on a basal medium containing 1.0% glucose, 1.0% glycerol, 1.0% ethanol, 1.0% peptone, and 0.5% yeast extract. If necessary, 1.5% agar was added for the preparation of solid media. Otherwise stated, strains were cultivated at 30°C. Gram staining was carried out by the method of Hucker and Conn (1923). Motility was observed by the hanging-drop method, and flagellation was examined by the staining method of Toda (1928). The activity of catalase and oxidase was determined by the methods described by Navarro and Komagata (1999). Cellulose production was determined by boiling pellicles in 5.0% NaOH for 2 h. Real cellulose was confirmed when the pellicle did not dissolve after boiling (Navarro et al., 1999). Oxidation of ethanol to acetic acid was tested by the method of Frateur (1950) by using a medium containing 3% ethanol, 3% yeast extract, 2% $CaCO_3$, and 2% agar. Oxidation of acetate and lactate to CO_2 and H_2O was examined by using Leifson's method as described by Asai et al. (1964). The growth on glutamate agar, the formation of γ-pyrones from D-glucose and D-fructose, and the acid production from sugars and sugar alcohols were investigated by the methods described by Asai et al. (1964).

Table 1. List of strains obtained from culture collections.

Strain designation received as	Strain number	Source	Strain number and strain designation used by Gosselé et al. (1983)	DNA group in this study
A. aceti	AJ 2913	T. Asai (=Asai 3)		V
A. aceti	AJ 2914	T. Asai (=Asai 4)		V
A. aceti	IAM 1814	T. Asai (=A-15)	IAM 1814 *A. aceti*	I
A. aceti	IFO 3281	K. Kondo 58		I
A. aceti	IFO 3284	K. Kondo 47	IFO 3284 *A. pasteurianus*	V
A. aceti	IFO 3299	K. Kondo 63	IFO 3299 *A. pasteurianus*	II
A. aceti	IFO 14818^{T}R	Y. Yamada←NCIB 8621	NCIB 8621t1^{T} *A. aceti*	I
A. aceti	IFO 14818^{T}S	Y. Yamada←NCIB 8621	NCIB 8621t2^{T} *A. aceti*	I
A. aceti	IFO 14821	Y. Yamada←NCIB 6656		
A. aceti	LMG 1496	R. Vaughn (=De Ley 24)	LMG 24WR *A. aceti*	I
A. aceti	LMG 1535	J. Frateur (=Frateur CH31)	LMG CH31 *A. aceti*	I
A. pasteurianus	IFO 3170	ATCC 6438←NRRL B-468	NCIB 2224 *A. pasteurianus*	IV
A. pasteurianus	IFO 3189		NCIB 8089 *A. pasteurianus*	
A. pasteurianus	IFO 3223	ATCC 6033←A. J. Kluyver	NCIB 8088 *A. pasteurianus*	IV
A. pasteurianus	IFO 3296	K. Kondo 9	IFO 3296 *A. hansenii*	IV
A. pasteurianus	IFO 13751	J. De Ley←NCIB 8935←J. G. Carr	NCIB 8935 *A. pasteurianus*	VI
A. pasteurianus	IFO 13752	J. De Ley←NCIB 8622←W. Verhoeven	NCIB 8622 *A. pasteurianus*	IV
A. pasteurianus	IFO 13753	J. De Ley←NCIB 8620←W. Verhoeven	NCIB 8620 *A. pasteurianus*	V
A. pasteurianus	IFO 13754	J. De Ley←NCIB 9015←J. De Ley	NCIB 9015 *A. pasteurianus*	II
A. pasteurianus	IFO 13755	J. De Ley←NCIB 8618←W. Verhoeven	NCIB 8618 *A. pasteurianus*	III
A. pasteurianus	IFO 14814	Y. Yamada←NCIB 7029 ←NCTC 7029←T. K. Walker	NCIB 7029 *A. pasteurianus*	
A. pasteurianus	LMG 5	Technische Hogeschool Delft←J. Frateur	LMG 5 *A. pasteurianus*	I
A. pasteurianus	LMG 1262^{T}	LMD 22.1←M. Beijerinck	LMD 22.1 *A. pasteurianus*	II
A. pasteurianus	LMG 1571	LMD 39.2←K. Bernhauer	LMD 39.2 *A. pasteurianus*	VII
A. pasteurianus	LMG 1580	LMD 50.6←N. Wieringa-Wijsman ←LMD Student course	LMD 50.6 *A. pasteurianus*	VI
A. pasteurianus	LMG 1584	LMD 39.5←K. Bernhauer	LMD 39.5 *A. pasteurianus*	
A. pasteurianus	LMG 1588	LMD 39.6←K. Bernhauer	LMD 39.6 *A. pasteurianus*	VII
A. pasteurianus	LMG 1590	LMD 51.1←S. Soriano←W. Henneberg	LMD 51.1 *A. pasteurianus*	II
A. xylinus	LMG 1510	NCIB 613←NCTC 613 ←Thaysen←Jørgensen	NCIB 613 *A. pasteurianus*	
A. xylinus	LMG 1518	NCIB 5346←NCTC 5346←O. Verona	NCIB 5346 *A. pasteurianus*	
Acetobacter sp.	IFO 3248	K. Kondo 68	IFO 3248 *A. pasteurianus*	II
Other reference strains				
G. oxydans	IFO 14819^{T}			
G. frateurii	NRIC 0228^{T}			
G. cerinus	NRIC 0229^{T}			
Ga. liquefaciens	IFO 12388^{T}			
Ga. hansenii	LMG 1527^{T}			
Ga. xylinus	IFO 15237^{T}			
As. bogorensis	JCM 10569^{T}			

Abbreviations: *A.*, *Acetobacter*; *G.*, *Gluconobacter*; *Ga.*, *Gluconacetobacter*; *As.*, *Asaia*; ^{T}, Type strain; AJ, Central Research Laboratories, Ajinomoto Company Inc., Kawasaki, Japan; ATCC, American Type Culture Collection, Manassas, VA, U.S.A.; IAM, IAM Culture Collection, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Wako, Saitama, Japan; LMD, Laboratorium voor Microbiologie, Technische Universiteit, Delft, The Netherlands; LMG, BCCM^{TM}/LMG Bacteria Collection Laboratorium Microbiologie, Universiteit Gent, Belgium; NCIB, The National Collections of Industrial and Marine Bacteria, Aberdeen, Scotland, U.K.; NCTC, National Collection of Type Cultures, London, U.K.; NRIC, NODAI Research Institute Culture Collection, Tokyo University of Agriculture, Tokyo, Japan; NRRL, Agricultural Research Service Culture Collection, Northern Regional Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Peoria, IL, U.S.A.

Ketogenesis from glycerol was detected by the method described by Carr (1968), and the production of 2-keto-, 5-keto-, and 2,5-diketo-D-gluconates from D-glucose was determined by the method of Swings et al. (1992). Growth at different temperatures was determined on a basal medium.

Growth on YPM broth and agar medium. Growth on D-mannitol was determined by using YPM medium. This medium contained 2.5% D-mannitol, 0.3% peptone, and 0.5% yeast extract. If necessary, 2.0% agar was added for the preparation of solid media (Asai et al., 1964). Medium without D-mannitol was used as a control. Growth on stationary cultures in liquid medium was monitored by measuring the absorbance at 660 nm. Transfers of cultures were carried out three times to avoid carryover of the first culture.

Utilization of ammoniacal nitrogen. Utilization of ammoniacal nitrogen was determined by using the Hoyer-Frateur medium (De Ley and Frateur, 1974) and Frateur's modified Hoyer medium (De Ley et al., 1984). The Hoyer-Frateur medium was composed of 3.0% ethanol, 3.0% D-glucose or 3% D-mannitol, 0.1% $(NH_4)_2SO_4$, 0.01% K_2HPO_4, 0.09% KH_2PO_4, 0.025% $MgSO_4 \cdot 7H_2O$, and 0.0005% $FeCl_3$. The Frateur's modified Hoyer medium corresponded with the Hoyer-Frateur medium supplemented with vitamins: 0.0001% D-biotin, 0.0001% Ca-pantothenate, 0.0001% thiamine, 0.0001% folic acid, 0.0001% *p*-aminobenzoic acid, 0.0001% vitamin B_{12}, 0.00015% pyridoxal-HCl, 0.00015% niacin, and 0.00015% riboflavin. The medium without a carbon source was used as a control. Cultures were incubated at 30°C under stationary and shaking conditions. In shaking conditions, growth was measured by absorbance at 660 nm by using an L-tube. Transfers of cultures were carried out three times to avoid carryover of the first cultures.

Quinone analysis. Quinones were extracted by the method of Yamada et al. (1969) and analyzed quantitatively by HPLC with a Nova-Pak C18, 3.9×150 mm (Nihon Waters Corporation, Tokyo, Japan) (Komagata and Suzuki, 1987).

Determination of DNA base composition. DNAs were isolated by the method of Saito and Miura (1963). DNA base composition was determined by the method of Tamaoka and Komagata (1984).

DNA-DNA relatedness. DNA-DNA relatedness was determined by the fluorometric DNA-DNA hybridization in microdilution wells described by Ezaki et al. (1989).

Sequencing of 16S rDNA. All methods used for PCR amplification of the 16S rDNA and primers were those of Kawasaki et al. (1993) and Yamada et al. (2000). The 16S rDNA was amplified by PCR with the following two primers: 20F (5′-GATTTTGATCCTGGCTCAG-3′, positions 9 through 27) and 1500R (5′-GTTACCTTGTTACGACTT-3′, positions 1509 through 1492). The numbering of positions was based on the *Escherichia coli* numbering system (accession number V00348, Brosius et al., 1981). Purified PCR products were sequenced directly by using an ABI PRISM™ Bigdye Terminator Cycle Sequencing Ready Reaction Kit and ABI PRISM™ model 310 Genetic Analyzer. The following six primers were used: 20F, 1500R, 520F (5′-CAGCAGCCGCGGTAATAC-3′, positions 519 through 536), 520R (5′-GTATTACCGCGGCTGCTG-3′, positions 536 through 519), 920F (5′-AAACTCAAATGAATTGACGG-3′, positions 907 through 926), and 920R (5′-CCGTCAATTCATTTGAGTTT-3′, positions 926 through 907).

Phylogenetic analysis. The multiple alignments were performed by the program Clustal W (ver. 1.6) (Thompson et al., 1994). The distance matrixes for the aligned sequences were calculated by the two-parameter method of Kimura (1980). The neighbor-joining method was used for constructing a phylogenetic tree (Saitou and Nei, 1987). The comparison of the sequence data obtained was made on 1,403 bases. The robustness for individual branches were estimated by bootstrapping with 1,000 replicates (Felsenstein, 1985). The type strains of the species and the accession numbers of the nucleotide sequences were cited from databases and are presented in Fig. 1. The accession numbers of the 16S rDNA sequences of eight strains determined in this study and submitted to the DDBJ and GenBank are as follows: AB032349 (IFO 13751^T), AB032350 (IFO 13752^T), AB032351 (IFO 13753^T), AB032352 (IFO 13755^T), AB032353 (LMG 1571), AB032354 (Ni-6b=NRIC 0312^T), AB032355 (26F-1=NRIC 0321), and AB032356 (5H-1=NRIC 0313^T).

Results

Oxidation of acetate and lactate and ubiquinone systems

Most *Acetobacter* strains obtained from culture collections and all Indonesian isolates oxidized acetate and lactate and had Q-9 as the major ubiquinone, as

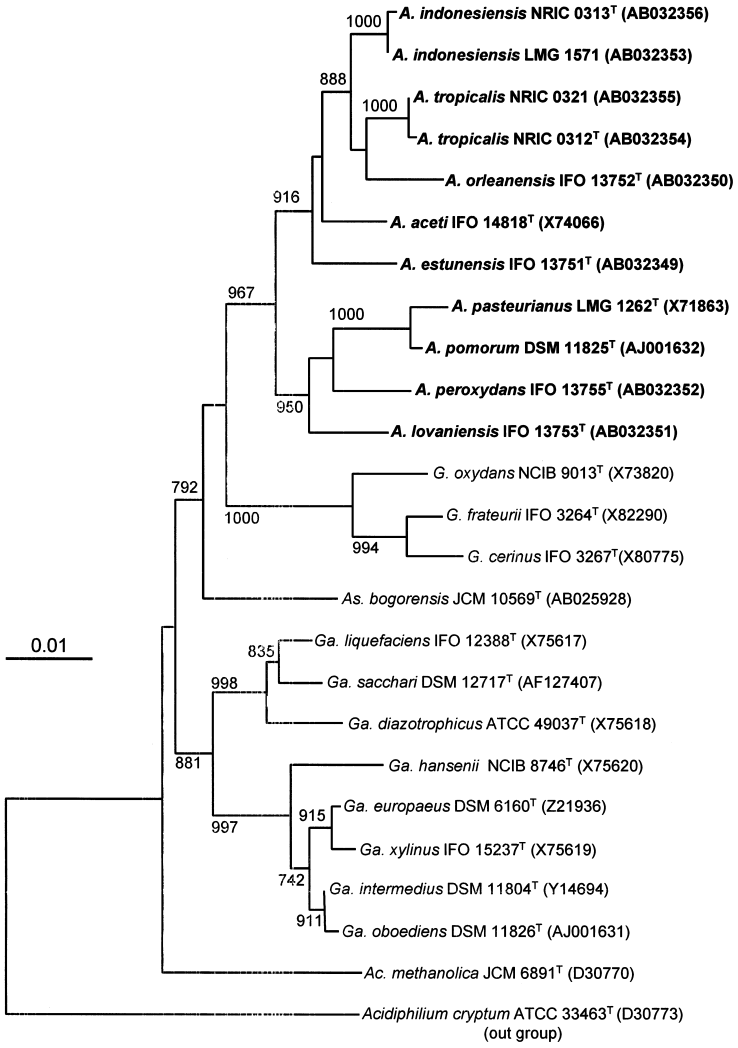

Fig. 1. Phylogenic relationships of *Acetobacter* strains based on the sequences of 16S rDNA.

The scale bar represents 1 nucleotide substitution per 100 nucleotides. Numerals indicate the bootstrap value derived from 1,000 replications. Abbreviations of the generic names: *A.*, *Acetobacter*; *G.*, *Gluconobacter*; *Ga.*, *Gluconacetobacter*; *As.*, *Asaia*; *Ac.*, *Acidomonas.*

shown in Table 2. *A. aceti* IFO 14821, *A. pasteurianus* IFO 3189, IFO 14814, LMG 1510, LMG 1518, and LMG 1584 were eliminated from further study and transferred to other genera in the acetic acid bacteria because they had Q-10 as the major ubiquinone. The genus *Acetobacter* has been characterized by the presence of Q-9 and had an ability to oxidize acetate and lactate (Yamada et al., 1969, 1997a, b). *Gluconacetobacter hansenii* (=*A. hansenii*) IFO 3296 was included in this study because it showed the presence of Q-9 and was identified as an *Acetobacter* strain (Navarro et al., 1999).

DNA base composition

DNA base composition of *Acetobacter* strains studied was distributed from 51.8 to 60.3 mol% G+C content, as shown in Table 3.

DNA-DNA relatedness

When 14 strains, including two strains of *A. aceti*, 10 strains of *A. pasteurianus*, and two Indonesian isolates were used as probes, 70 strains tested were separated into eight groups on the basis of DNA-DNA relatedness, as shown in Table 3. The groups were labeled Groups I to VIII.

Table 2. Isolation sources, oxidation of acetate and lactate, and ubiquinone systems of *Acetobacter* strains tested.

Strain No.	Species/Source	Oxidation of		Quinone (%)			DNA group in this study
		Acetate	Lactate	Q-8	Q-9	Q-10	
AJ 2913	*A. aceti*	+	+	6.9	**88.5**	4.6	V
AJ 2914	*A. aceti*	+	+	13.7	**81.9**	4.4	V
IAM 1814	*A. aceti*	+	+		**100.0**		I
IFO 3281	*A. aceti*	+	+	5.4	**88.6**	6.0	I
IFO 3284	*A. aceti*	+	+	7.2	**89.5**	3.3	V
IFO 3299	*A. aceti*	+	+		**100.0**		II
IFO 14818^{T}R	*A. aceti*	+	+	13.4	**86.6**		I
IFO 14818^{T}S	*A. aceti*	+	+	13.3	**83.6**	3.1	I
LMG 1496	*A. aceti*	+	+	15.2	**83.2**	1.6	I
LMG 1535	*A. aceti*	+	+	7.6	**88.4**	4.0	I
IFO 3170	*A. pasteurianus*	+	+	4.6	**90.3**	5.1	IV
IFO 3223	*A. pasteurianus*	+	+	10.3	**87.1**	2.6	IV
IFO 3296	*A. hansenii*	+	+	3.8	**88.2**	8.0	IV
IFO 13751	*A. pasteurianus*	+	+	10.8	**89.2**		VI
IFO 13752	*A. pasteurianus*	+	+	14.2	**81.0**	4.8	IV
IFO 13753	*A. pasteurianus*	+	+	8.6	**84.9**	6.5	V
IFO 13754	*A. pasteurianus*	+	+	7.0	**87.1**	5.9	II
IFO 13755	*A. pasteurianus*	+	+	15.9	**84.1**		III
LMG 5	*A. pasteurianus*	+	+	17.8	**79.2**	3.0	I
LMG 1262^{T}	*A. pasteurianus*	+	+	12.3	**87.7**		II
LMG 1571	*A. pasteurianus*	+	+	7.9	**88.3**	3.8	VII
LMG 1580	*A. pasteurianus*	+	+	9.1	**84.5**	6.4	VI
LMG 1588	*A. pasteurianus*	+	+	16.5	**81.5**	2.0	VII
LMG 1590	*A. pasteurianus*	+	+		**54.7**	45.3	II
IFO 3248	*A. pasteurianus*	+	+	1.0	**98.4**	0.6	II
Ha-2	palm seed	+	+	11.1	**88.9**		V
Ni-6	coconut juice	+	+		**100.0**		V
Ni-6b	coconut juice	+	+		**100.0**		VIII
He-2	young coconut	+	+		**100.0**		VIII
He-3	young coconut	+	+	9.0	**91.0**		VIII
He-6	young coconut	+	+	9.1	**90.9**		VIII
O-3	coconut	+	+	4.1	**90.0**	5.9	VII
Ka-1	banana	+	+	4.8	**89.2**	6.0	VII
1G-1	palm vinegar	+	+	8.6	**91.0**	0.4	II
1G-2	palm vinegar	+	+		**100.0**		II
2G-1	palm vinegar	+	+	13.0	**87.0**		II
2G-2	palm vinegar	+	+		**100.0**		II
5G-1	nata de coco	+	+	2.1	**97.9**		V
5G-2	nata de coco	+	+	5.9	**87.9**	6.2	IV
8G-3	moromi soya	+	+	11.1	**88.9**		V
10G-1	palm wine	+	+	5.9	**87.8**	6.3	V
11G-2	markisa	+	+	10.5	**86.6**	2.9	V
12G-2	pickled fruits	+	+	7.7	**89.8**	2.5	II
13G-1	tape cassava	+	+	11.0	**87.2**	1.8	II
20G-1	nata de coco	+	+	6.6	**89.5**	3.9	V
21G-1	nata de coco	+	+	11.0	**85.6**	3.4	IV
21G-2	nata de coco	+	+	14.0	**84.5**	1.5	IV
24G-1	pickle	+	+	5.5	**89.8**	4.7	V

Table 2. Continued.

Strain No.	Species/Source	Oxidation of		Quinone (%)			DNA group in this study
		Acetate	Lactate	Q-8	Q-9	Q-10	
26G-1	guava	+	+	12.0	**85.2**	2.8	IV
3F-1	flower	+	+	12.0	**88.0**		VII
8F-1	flower	+	+		**100.0**		VII
9F-1	fruit	+	+	8.8	**91.2**		VII
9F-2	fruit	+	+	4.6	**87.0**	8.4	VII
12F-1	flower	+	+	9.1	**88.0**	2.9	VII
12F-2	flower	+	+		**100.0**		VII
14F-1	fruit	+	+	6.5	**93.5**		V
16F-1	fruit	+	+	10.0	**90.0**		VII
23F-1	fruit	+	+	4.1	**90.0**	5.9	V
24F-1	fruit	+	+	7.1	**90.1**	2.8	VII
25F-1	papaya	+	+		**94.3**	5.7	VII
26F-1	lime	+	+	9.1	**86.5**	4.4	VIII
26F-2	lime	+	+	7.1	**89.2**	3.7	VIII
3H-1	starfruit	+	+	14.9	**82.8**	2.3	V
5H-1	zirzak	+	+		**86.0**	14.0	VII
5H-2	zirzak	+	+		**100.0**		VII
6H-1	sapodilla plum	+	+	9.0	**91.0**		IV
6H-2	sapodilla plum	+	+	7.7	**92.3**		IV
Mg-1	mango	+	+	7.1	**89.4**	3.5	VII
Mg-2	mango	+	+		**100.0**		VII
6G-1A	water of nata	+	+	9.1	**90.9**		II
IFO 14821	*A. aceti*	+	+	0.8	3.9	**95.3**	
IFO 14814	*A. pasteurianus*	+	+		3.0	**97.0**	
LMG 1510	*A. pasteurianus*	+	+			**100.0**	
LMG 1518	*A. pasteurianus*	+	+	0.6	6.4	**93.0**	
LMG 1584	*A. pasteurianus*	+	+		7.4	**92.6**	
IFO 3189	*A. pasteurianus*	−	−		9.5	**90.5**	

Abbreviations: ^{T}, Type strain; +, positive; −, negative; *A.*, *Acetobacter.*

Comparison of 16S rDNA sequences

One representative strain from each of Groups III to VI and two representative strains from each of Groups VII and VIII were determined for their 16S rDNA sequences. These sequences were compared with those of three other *Acetobacter* species and 12 other species in the family *Acetobacteraceae.* All eight strains were placed in the *Acetobacter* cluster (967 bootstrap value), as shown in Fig. 1. LMG 1571 and NRIC 0313 were placed in the same cluster (1,000 bootstrap value) and correlated with high levels of DNA relatedness. NRIC 0312 and NRIC 0321 joined together (1,000 bootstrap value).

Phenotypic characteristics

Phenotypic characteristics of the strains studied are shown in Table 4. All *Acetobacter* strains studied were Gram-negative rods measuring from 0.4 to 1.0 by 1.5 to 2.0 μm. More than half the strains tested were motile by peritrichous flagella. They were catalase-positive except for IFO 13754 and IFO 13755, and they were oxidase-negative. They oxidized ethanol to acetic acid. None of the *Acetobacter* strains produced cellulose in the medium. IFO 13751 produced a mucous pellicle that dissolved after boiling in 5% NaOH. All *Acetobacter* strains grew scantily on glutamate agar.

Table 3. DNA base compositions and levels of DNA-DNA relatedness of *Acetobacter* strains tested.

DNA group	Strain No.	G+C mol%	Levels of DNA relatedness with strain													
			IFO 14818^{T}R	LMG 1262^{T}	IFO 13754	LMG 1590	IFO 13755	IFO 13752	IFO 3296	IFO 13753	AJ 2914	IFO 13751	5H-1	LMG 1571	LMG 1588	Ni-6b
I	IFO 14818^{T}R	57.2	100	18	35	20	10	34	34	24	16	54	22	27	34	21
	IFO 14818^{T}S	56.7	94	17	6	17	15			18	9					
	IAM 1814	56.4	103	18		21	29				32					
	IFO 3281	56.2	84	10			13	29	37			44				
	LMG 5	57.1	73	27			38				32					
	LMG 1496	56.8	124	3												
	LMG 1535	56.4	106	7			15					45				
II	LMG 1262^{T}	52.7	17	100	73	84	17	44	49	10	29	28	13	31		39
	IFO 3248	52.9	25	92							36					
	IFO 3299	52.6	18	81	73	76	4			39	37	36				
	IFO 13754	52.9	13	92	100	83	18	20	31	50	51	42		28		
	LMG 1590	52.9	19	89	78	100	22			45	38					
	1G-1	51.8	15	85							34	27				
	1G-2	52	13	85							45					
	2G-1	52.4		97				42	42	11		38				
	2G-2	51.9	13	83							45	31				
	12G-2	52.6	10	114				31	25		44					
	13G-1	52.2		88				44	32		44					
	6G-1A	52.1		91							18			39		
III	IFO 13755	60.3	25	37	46	25	100	32	39	30	29	31	28	26		24
IV	IFO 13752	56.5	23	36	54		37	100	73	42	35	28	34	42	34	37
	IFO 3170	58.0	26	39				90	89	28	35			32	19	47
	IFO 3223	58.1		45				98	85		34	26		27	17	
	IFO 3296	57.3	33	44	60	10	44	82	100	39	31	35		46	20	51
	5G-2	58.7	26				19	76			38	34			25	30
	21G-1	58.1	11	43				80	70		45	32				33
	21G-2	57.5	38	28			49	76	87		44	22				
	26G-1	58.1	12	45				77	95		47	24		59		
	6H-1	57.4	34	26				74	94			19				
	6H-2	57.9	40	20			42	73	71			20				
V	IFO 13753	58.6	25	23	51		44	35	45	100	90	31	35	27		35
	AJ 2913	57.3		55						89	96					
	AJ 2914	58.7	16	42	34	34	23	48	24	90	100	35	37	25	15	
	IFO 3284	58.0	15	53		25	38				79	18		14		13
	Ha-2	58.6	18	53				48	40	95	105		13	29		31
	Ni-6	58.6	10	47				6	42	88	93			22		26
	5G-1	58.8	16	23			39		31	92	84	32		17		20
	8G-3	58.9	8	39				40	34	92	84	33		24		19
	10G-1	58.3	18	24			39	39	33	98	79	16		18		26
	11G-2	57.8	32	46			34				92	34				
	20G-1	57.1	22	46				40	43	98	100			47		46

Table 3. Continued.

DNA group	Strain No.	G+C mol%	Levels of DNA relatedness with strain														
			IFO 14818^{T}R	LMG 1262^{T}	IFO 13754	LMG 1590	IFO 13755	IFO 13752	IFO 3296	IFO 13753	AJ 2914	IFO 13751	5H-1	LMG 1571	LMG 1588	Ni-6b
V	24G-1	58	28	45						78	98	32		12		14
	14F-1	57.7	14	55							78					
	23F-1	57.7	10	44							82					
	3H-1	58.1	31	52				39	43	71	87			15		15
VI	IFO 13751	59.3	35	14	47	28	39	24	39	38	29	100	23	20		34
	LMG 1580	59.7	56	43			31	45	49	31	51	103				
VII	5H-1	53.7	23	19			17	39	34	16	18	26	100	91	90	40
	LMG 1571	54.4	24	24			13	46	57	23	32	24	80	100	92	42
	LMG 1588	55.1	23	19			13	27	38	30	25		86	92	100	53
	O-3	55	24	50				50	46		37	31		97	98	
	Ka-1	54.8	27				29	51	56		7	23	91	92	92	
	3F-1	53.5	17	18				42	49		53	10	93	90	109	
	8F-1	53.3	15	15			28		33		10	18	87	75		
	9F-1	53.3	19	21			15	40	14		37	10	104	99		
	9F-2	53.6	11	14			36		12		19	23	99			
	12F-1	54	12				29	47	49		11	17	109	83		34
	12F-2	54.5	22	16					35			23	92			
	16F-1	53.9	23	10			14	49	41		2	15	92	78		
	24F-1	53.6	11	12					34		29	18	80			
	25F-1	54.5		28					34		26	16	100	97	89	
	5H-2	53.9		14			25				36	18	95	94		
	Mg-1	53.7	9	34			29		41			38	95	90	89	
	Mg-2	53.7		34			11		47		42	18	87	99	93	
VIII	Ni-6b	55.9	17	25			22	29	34	48	32	31	37	46		100
	He-2	56.6	12	28			15	31	37		24	25		42	49	99
	He-3	55.6	17	46			37	56	25		35	24		33	23	88
	He-6	55.9	16	53			13	45	30		37	44		28		103
	26F-1	55.6	25	52			21	52	26		32	34		30		89
	26F-2	55.2	22	10			18	51	26		38	27		30		85

Abbreviation: ^{T}, Type strain.

Acid production from D-glucose, D-mannose, D-galactose, D-xylose, and L-arabinose was variable among the *Acetobacter* strains, and no acids were produced from D-fructose, L-sorbose, D-arabinose, L-rhamnose, D-mannitol, D-sorbitol, glycerol, trehalose, sucrose, maltose, lactose, melibiose, raffinose, and starch.

All *Acetobacter* strains produced D-gluconate from D-glucose except for IFO 13754, IFO 13755, and LMG 1590. The members of Group I produced both 2- and 5-keto-D-gluconates from D-glucose, but the members of Groups IV, VI, VII, and VIII produced only 2-keto-D-gluconate. The members of Groups II, III, and V produced neither 2-keto-D-gluconate nor 5-keto-D-gluconate. *Acetobacter* strains produced neither 2,5-diketo-D-gluconate from D-glucose nor γ-pyrones from D-glucose and D-fructose. Ketogenesis from glycerol occurred in all members of Group I and in strain IFO

Table 4. Phenotypic characteristics of *Acetobacter* strains tested.

DNA group	Strain No.	Catalase	Motility	Ketogenesis from glycerol	Production of			Acid production from					
					D-Gluconate	2-Ketogluconate	5-Ketogluconate	D-Glucose	D-Mannose	D-Galactose	D-Arabinose	L-Arabinose	D-Xylose
I	IFO 14818^{T}R	+	−	+	+	+	+	+	+	+	w	+	+
	IFO 14818^{T}S	+	+	+	+	+	+	+	+	+	w	+	+
	IAM 1814	+	−	+	+	+	+	+	+	+	−	+	+
	IFO 3281	+	+	+	+	+	+	+	+	+	−	+	+
	LMG 5	+	−	+	+	+	+	+	+	+	−	+	+
	LMG 1496	+	−	+	+	+	+	+	+	+	w	+	+
	LMG 1535	+	−	+	+	+	+	+	+	+	w	+	+
II	LMG 1262^{T}	+	+	−	+	−	−	+	−	−	−	−	−
	IFO 3248	+	−	−	+	−	−	+	−	−	−	−	−
	IFO 3299	+	+	−	+	−	−	+	−	−	−	−	−
	IFO 13754	−	+	−	−	−	−	−	−	−	−	−	−
	LMG 1590	+	−	−	−	−	−	−	−	−	−	−	−
	1G-1	+	+	−	+	−	−	+	−	+	−	−	+
	1G-2	+	+	−	+	−	−	+	−	+	−	−	+
	2G-1	+	+	−	+	−	−	+	+	+	−	+	+
	2G-2	+	+	−	+	−	−	+	−	+	−	−	+
	12G-2	+	−	−	+	−	−	+	+	+	−	+	+
	13G-1	+	−	−	+	−	−	+	−	−	−	−	+
	6G-1A	+	−	−	+	−	−	+	+	−	−	+	+
III	IFO 13755^{T}	−	+	−	−	−	−	−	−	−	−	−	−
IV	IFO 13752^{T}	+	+	+	+	+	−	+	+	−	−	−	+
	IFO 3170	+	+	−	+	+	−	+	−	−	−	−	−
	IFO 3223	+	+	−	+	+	−	+	−	−	−	−	−
	IFO 3296	+	+	−	+	+	−	+	+	+	−	+	+
	5G-2	+	−	−	+	+	−	+	+	+	−	+	+
	21G-1	+	+	−	+	+	−	+	−	−	−	−	−
	21G-2	+	−	−	+	+	−	+	+	w	−	+	+
	26G-1	+	−	−	+	+	−	+	+	w	−	+	+
	6H-1	+	−	−	+	+	−	+	w	+	−	+	+
	6H-2	+	−	−	+	+	−	+	+	+	−	+	w
V	IFO 13753^{T}	+	+	−	+	−	−	+	−	−	−	−	−
	AJ 2913	+	+	−	+	−	−	+	−	−	−	−	−
	AJ 2914	+	+	−	+	−	−	+	−	−	−	−	−
	IFO 3284	+	+	−	+	−	−	+	−	−	−	−	−
	Ha-2	+	+	−	+	−	−	+	+	−	−	−	+
	Ni-6	+	+	−	+	−	−	+	+	+	−	+	+

Table 4. Continued.

DNA group	Strain No.	Catalase	Motility	Ketogenesis from glycerol	Production of			Acid production from					
					D-Gluconate	2-Ketogluconate	5-Ketogluconate	D-Glucose	D-Mannose	D-Galactose	D-Arabinose	L-Arabinose	D-Xylose
V	5G-1	+	+	−	+	−	−	+	+	+	−	+	+
	8G-3	+	+	−	+	−	−	+	−	−	−	−	−
	10G-1	+	+	−	+	−	−	+	+	+	−	+	+
	11G-2	+	−	−	+	−	−	+	w	w	−	w	w
	20G-1	+	−	−	+	−	−	+	w	w	−	w	w
	24G-1	+	+	−	+	−	−	+	+	+	−	+	+
	14F-1	+	+	−	+	−	−	+	w	w	−	w	w
	23F-1	+	+	−	+	−	−	+	w	w	−	w	w
	3H-1	+	+	−	+	−	−	+	+	+	−	+	+
VI	IFO 13751^T	+	+	−	+	+	−	+	+	−	−	−	+
	LMG 1580	+	+	−	+	+	−	+	+	−	−	−	+
VII	5H-1 (NRIC 0313^T)	+	−	−	+	+	−	+	+	+	−	+	+
	LMG 1571	+	+	−	+	+	−	+	−	−	−	−	−
	LMG 1588	+	+	−	+	+	−	+	−	−	−	−	−
	O-3	+	+	−	+	+	−	+	+	+	−	+	+
	Ka-1	+	+	−	+	+	−	+	+	+	−	+	+
	3F-1	+	−	−	+	+	−	+	w	w	−	w	w
	8F-1	+	−	−	+	+	−	+	w	w	−	w	w
	9F-1	+	−	−	+	+	−	+	+	+	−	+	+
	9F-2	+	−	−	+	+	−	+	+	+	−	+	+
	12F-1	+	−	−	+	+	−	+	w	w	−	w	w
	12F-2	+	−	−	+	+	−	+	w	w	−	w	w
	16F-1	+	−	−	+	+	−	+	+	+	−	+	+
	24F-1	+	−	−	+	+	−	+	w	w	−	w	w
	25F-1	+	+	−	+	+	−	+	w	w	−	w	w
	5H-2	+	−	−	+	+	−	+	+	+	−	+	+
	Mg-1	+	−	−	+	+	−	+	w	w	−	w	w
	Mg-2	+	−	−	+	+	−	+	w	w	−	w	w
VIII	Ni-6b (NRIC 0312^T)	+	+	−	+	+	−	+	+	+	−	+	+
	He-2	+	+	−	+	+	−	+	w	w	−	w	w
	He-3	+	+	−	+	+	−	+	w	w	−	w	w
	He-6	+	+	−	+	+	−	+	+	+	−	+	+
	26F-2 (NRIC 0321)	+	−	−	+	+	−	+	w	w	−	w	w
	26F-2	+	−	−	+	+	−	+	w	w	−	w	w

Abbreviations: ^{T}, Type strain; +, positive; w, weak; −, negative.

All strains were Gram-negative rods, positive for oxidase, oxidation of ethanol, and acid production from ethanol, and negative for production of 2,5-diketogluconate from D-glucose and acid production from D-fructose, D-sorbitol, D-mannitol, and glycerol.

13752 of Group IV. All *Acetobacter* strains grew well at 37°C except for members of Group I.

Growth on YPM broth and agar medium

All members of the *Acetobacter* groups scarcely grew on YPM broth and agar, and they also scarcely grew without D-mannitol. When the growth of cultures on YPM broth was determined by absorbance at 660 nm, no significant differences (A_{660}=0.1–0.2) were found between the growth with and without D-mannitol for *Acetobacter* strains. In contrast, *Gluconobacter oxydans* IFO 14819^T, *Gluconobacter frateurii* NRIC 0228^T, *Gluconobacter cerinus* NRIC 0229^T, *Gluconacetobacter liquefaciens* IFO 12388^T, *Gluconacetobacter hansenii* LMG 1527^T, *Gluconacetobacter xylinus* IFO 15327^T, and *Asaia bogorensis* JCM 10569^T grew well in YPM broth (A_{660}=0.4–1.65) and on YPM agar, but they scarcely grew without D-mannitol.

Utilization of ammoniacal nitrogen

A few *Acetobacter* strains tended to show scanty growth in the Hoyer-Frateur medium and the Frateur's modified Hoyer medium containing ethanol or D-glucose as a carbon source under both stationary and shaking conditions. Furthermore, no *Acetobacter* strains grew in the Hoyer-Frateur medium and the Frateur's modified Hoyer medium containing D-mannitol as a carbon source. In contrast, under the same conditions *Gluconacetobacter liquefaciens* IFO 12388^T, *Gluconacetobacter hansenii* LMG 1527^T, *Gluconacetobacter xylinus* IFO 15327^T, and *Asaia bogorensis* JCM 10569^T grew in the Hoyer-Frateur medium and the Frateur's modified Hoyer medium containing D-glucose or D-mannitol, but they did not grow at the expense of ethanol. Growth of *Gluconobacter oxydans* IFO 14819^T, *Gluconobacter frateurii* NRIC 0228^T, and *Gluconobacter cerinus* NRIC 0229^T was enhanced in the Frateur's modified Hoyer medium containing D-glucose or D-mannitol. These strains did not grow at the expense of ethanol. Figure 2 shows the growth of eight representative strains tested on the Hoyer-Frateur medium and the Frateur's modified Hoyer medium containing ethanol, D-glucose, or D-mannitol.

Discussion

Phylogenetic analysis based on 16S rDNA sequences from each of the eight groups based on DNA-DNA relatedness reveals that the above groups are the members of the genus *Acetobacter* and phylogenetically distinct from other genera in the family *Acetobacteraceae.* The levels of 16S rDNA sequence similarity among *Acetobacter* strains were more than 95.8%, and the levels of 16S rDNA sequence similarity between *Acetobacter* strains and those of other genera were less than 96.6%.

DNA-DNA relatedness provides clear evidence that the *Acetobacter* strains studied are separated into eight distinct groups on the basis of DNA-DNA relatedness. These groups showed good agreement with the concept of bacterial species; that is, a well-defined species shows 70% or greater DNA-DNA relatedness (Wayne et al., 1987). Moreover, the range of G+C mol% of each group was ±2 mol%.

Of the eight groups, two are proposed as new species, three as new combinations, and the remaining three are the established species, *A. aceti*, *A. pasteurianus*, and *A. peroxydans.* Strains previously assigned to *A. aceti* and *A. pasteurianus*, were scattered over the different species. Differential characteristics of the species in the genus *Acetobacter* are given in Table 5.

The genus *Acetobacter* is separated from other genera in the family *Acetobacteraceae* by differences in ubiquinone systems and by the oxidation of acetate and lactate (Yamada et al., 1997a, b). Furthermore, members of the genus *Acetobacter* do not produce γ-pyrones from either D-glucose or D-fructose, not do they produce acid from D-fructose, D-mannitol, D-sorbitol, and glycerol. The range of the G+C content obtained for this genus is 51.8 to 60.3 mol% and narrower than that (51 to 65 mol%, T_m) reported previously by Gosselé et al. (1983).

Asai et al. (1964) first suggested the usefulness of YPM agar for the differentiation of acetic acid bacteria. Yamada et al. (1997a, b) reported that no growth of the genus *Acetobacter* on YPM agar could be used for separating it from the genus *Gluconobacter* or partly from the genus *Gluconacetobacter.* This was rejected, however, by Boesch et al. (1998) because YPM medium is recommended to cultivate *Acetobacter* and *Gluconobacter* strains according to the BCCM Catalogue (Janssens et al., 1998). Recently, Franke et al. (1999) reported the growth of *Acetobacter* strains on YPM agar. According to this study, no significant differences were noted between the growth of *Acetobacter* strains tested on YPM broth with and without D-manni-

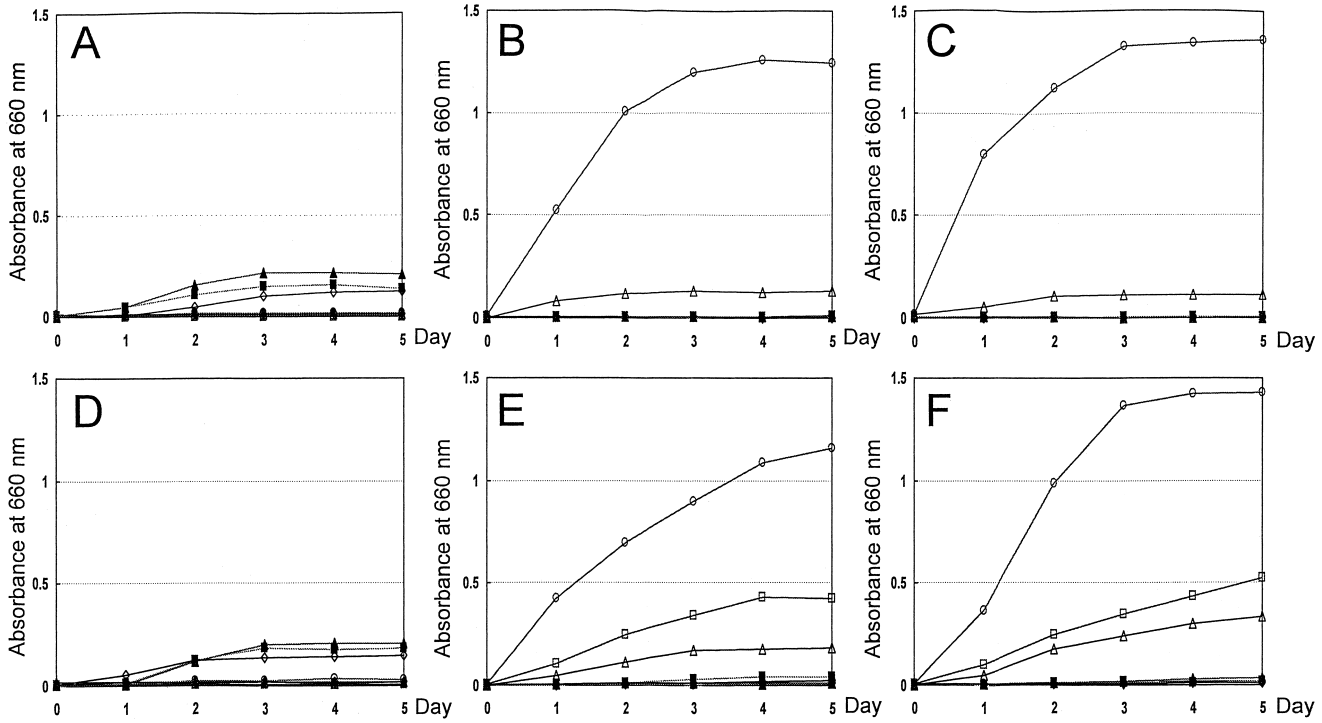

Fig. 2. Growth of eight representative strains of acetic acid bacteria on Hoyer-Frateur medium (upper) containing ethanol (A), D-glucose (B), and D-mannitol (C) as a carbon source, and on Frateur's modified Hoyer medium (below) containing ethanol (D), D-glucose (E), and D-mannitol (F) as a carbon source.

Abbreviations of the generic names: See Fig. 1. ◆ *A. aceti* IFO 14818^T; ■ *A. pasteurianus* LMG 1262^T; ▲ *A. peroxydans* IFO 13755^T; ● *A. lovaniensis* IFO 13753^T; ◇ *A. estunensis* IFO 13751^T; □ *G. oxydans* IFO 14819^T; △ *Ga. liquefaciens* IFO 12388^T; ○ *As. bogorensis* JCM 10569^T.

tol when the growth of cultures was measured by absorbance at 660 nm. A scanty growth of *Acetobacter* strains on YPM broth would be ascribed to the use of yeast-extract and peptone as carbon sources. This false positive growth could result in misinterpretation of the growth on D-mannitol as mentioned above. In contrast, strains tested in the genera *Gluconobacter*, *Gluconacetobacter*, and *Asaia* grew remarkably well on YPM broth and agar with D-mannitol, but scantily without D-mannitol. Therefore it is concluded that *Acetobacter* strains are unable to utilize D-mannitol as a carbon source.

De Ley and Frateur (1974), Gosselé et al. (1983), and De Ley et al. (1984) reported that some strains in the genus *Acetobacter* utilized ammoniacal nitrogen on the Hoyer-Frateur medium containing ethanol. Furthermore, Gosselé et al. (1983) and De Ley et al. (1984) got the same results by using the Frateur's modified Hoyer medium containing ethanol, D-glucose, or D-mannitol as a carbon source. The growth of *Acetobacter* strains on both media in the presence of ethanol or D-glucose, however, was indefinable compared with those of the strains of the genera *Gluconobacter*, *Gluconacetobacter*, and *Asaia* (Fig. 2). The *Acetobacter* strains were also unable to use ammoniacal nitrogen when D-mannitol was used as a carbon source, even supplemented with vitamins. Consequently, we concluded that the test of the use of ammoniacal nitrogen on the Hoyer-Frateur medium and on the Frateur's modified Hoyer medium is of no value for the classification and identification of the genus *Acetobacter.*

Approximately 20% of *Acetobacter* strains obtained from culture collections were misidentified. IFO 3189 should be transferred to the genus *Gluconobacter* because it did not oxidize acetate and lactate and had Q-10 as the major ubiquinone. This strain also produced γ-pyrones only from D-fructose, dihydroxyacetone from glycerol, and 2- and 5-keto-D-gluconates from D-glucose, which are the distinctive characteristics of the genus *Gluconobacter.* IFO 14821, IFO 14814, LMG 1510, LMG 1518, and LMG 1584 should be transferred to the genus *Gluconacetobacter* because they oxidized acetate and lactate and had Q-10 as the

Table 5. Differential characteristics of *Acetobacter* species.

Features	*A. aceti*	*A. pasteurianus*	*A. peroxydans*	*A. orleanensis*	*A. lovaniensis*	*A. estunensis*	*A. indonesiensis*	*A. tropicalis*	*A. pomorum*[a]
Catalase	+	d	−	+	+	+	+	+	+
Ketogenesis from glycerol	+	−	−	d	−	−	−	−	+
Production of ketogluconates from D-glucose									
2-Keto-D-gluconate	+	−	−	+	−	+	+	+	−
5-Keto-D-gluconate	+	−	−	−	−	−	−	−	−
Acid production from:									
D-Glucose	+	d	−	+	+	+	+	+	ND
D-Mannose	+	−	−	d	d	+	d	+	ND
D-Galactose	+	d	−	d	d	−	d	+	ND
L-Arabinose	+	d	−	d	d	−	d	+	ND
D-Xylose	+	d	−	d	d	+	d	+	ND
Ubiquinone system	Q-9	Q-9	Q-9	Q-9	Q-9	Q-9	Q-9	Q-9	Q-9
G+C content (mol%)	56.2–57.2	51.8–52.9	60.3	56.5–58.7	57.1–58.9	59.3–59.7	53.3–55.1	55.2–56.6	50.5

Abbreviations: +, 90% or more of strains are positive; d, 11–89% of strains are positive; −, 90% or more of strains are negative; ND, not determined.
[a] Data were cited from Sokollek et al. (1998).

major ubiquinone. IFO 14814, IFO 14821, LMG 1510, and LMG 1518 produced cellulose in liquid medium. Conversely, *Gluconacetobacter hansenii* (=*A. hansenii*) IFO 3296 should be transferred to *A. orleanensis* because it oxidized acetate and lactate, had Q-9 as the major ubiquinone, and showed a high level of DNA-DNA relatedness with the type strain of *A. orleanensis.*

Until now, the species of the genus *Acetobacter* were determined mainly on the basis of phenotypic characteristics. Shimwell (1957) and Asai et al. (1964) failed to differentiate the species of this genus on the basis of phenotypic characteristics, including morphological, cultural, and biochemical characteristics. Gosselé et al. (1983) classified 98 strains of the genus *Acetobacter* on the basis of the phenetic classification and whole protein profiles. However, the heterogeneity of the species of this genus has appeared in the light of DNA-DNA relatedness and 16S rDNA sequences. Therefore it is recommended in this study that the genus *Acetobacter* should be classified and identified on the basis of both genotypic and phenotypic characteristics. Before the establishment of a species, DNA-DNA relatedness should be determined between strains comprising the species. This ensures the stability of bacterial nomenclature.

As mentioned above, *Acetobacter* strains studied were separated into the following eight groups based on DNA-DNA relatedness and phylogenetic relationships. Each group is regarded as a species.

Group I: *Acetobacter aceti* (Pasteur 1864) Beijerinck 1898

This group includes *A. aceti* IFO 14818^{T}R, IFO 14818^{T}S, IAM 1814, IFO 3281, LMG 1496, and LMG 1535, and *A. pasteurianus* LMG 5. The name *A. aceti* (Pasteur 1864) Beijerinck 1898 should be retained for this group because the type strain of *A. aceti* (IFO 14818^{T}R and IFO 14818^{T}S) is included in this group. These two strains with the different colonial types had the same phenotypic and genotypic characteristics. Rough colony-forming IFO 14818^{T}R is recommended to be the representative strain of *A. aceti* because a rough colonial type is more stable than a smooth colonial type. Moreover, rough colony-forming strains are capable of forming film, which was reported to be typical of *A. aceti* (Shimwell, 1957). LMG 1496 and LMG 1535 formed rough colonies, and IAM 1814, IFO 3281, and LMG 5 showed smooth colonies.

A. aceti is clearly distinguished from other species in

the genus *Acetobacter* by the following phenotypic characteristics: Production of dihydroxyacetone from glycerol and production of D-gluconate and 2- and 5-keto-D-gluconates from D-glucose. The range of the G+C content of DNA is 56.2 to 57.2 mol% and is narrower than that (55.9 to 59.5 mol%) reported by Gosselé et al. (1983). The level of 16S rDNA sequence similarity is in a range of 96.5 to 98.5% between the type strain of *A. aceti* and the type strains of other *Acetobacter* species.

LMG 5 was once transferred from *A. aceti* to *A. pasteurianus* by Gosselé et al. (1983) because it lacked the ability to produce dihydroxyacetone from glycerol and 5-keto-D-gluconate from D-glucose. In this study, however, this strain produced the above two substances and showed a high level of DNA-DNA relatedness (73%) with the type strain of *A. aceti* and a low level (27%) with the type strain of *A. pasteurianus.* Thus LMG 5 should be reidentified as *A. aceti.* Interestingly, no Indonesian isolates were identified as *A. aceti.*

Group II: *Acetobacter pasteurianus* (Hansen 1879) Beijerinck and Folpmers 1916

This group includes *A. pasteurianus* LMG 1262^T, IFO 3248, IFO 3299, IFO 13754, and LMG 1590, and 7 Indonesian isolates. *A. pasteurianus* (Hansen 1879) Beijerinck and Folpmers 1916 should be retained for this group because the type strain of *A. pasteurianus* LMG 1262^T is included in this group.

None of the strains in this species produces dihydroxyacetone from glycerol, and 2- and 5-keto-D-gluconates from D-glucose. The range of the G+C content of DNA is 51.8 to 52.9 mol%. This range was narrower than that (52.8 to 62.5 mol%) reported by Gosselé et al. (1983). The level of 16S rDNA sequence similarity is in a range of 95.8 to 99.4% between the type strain of *A. pasteurianus* and the type strains of other *Acetobacter* species.

Interestingly, this species contains the former type strain of *A. pasteurianus* subsp. *paradoxus* IFO 13754 and the former type strain of *A. pasteurianus* subsp. *ascendens* LMG 1590. Both strains showed a high level of DNA-DNA relatedness (>74%) with the type strain of *A. pasteurianus* LMG 1262^T.

Seven Indonesian isolates were obtained from Indonesian traditional fermented foods such as palm vinegar, tape cassava, and pickles. No isolates were obtained from fruits and flowers in Indonesia.

Group III: *Acetobacter peroxydans* Visser't Hooft 1925

This group consists only of *A. pasteurianus* IFO 13755. The name *A. peroxydans* should be retained for this group because the former type strain of *A. peroxydans* IFO 13755^T is included in it. The type strain of this species is catalase-negative; it oxidizes ethanol, acetate, and lactate but does not produce acid from almost all carbon sources tested. The G+C content of DNA of this strain is 60.3 mol%. The level of 16S rDNA sequence similarity is in a range of 96.6 to 98.0% between the type strain of *A. peroxydans* and the type strains of other *Acetobacter* species. Gosselé et al. (1983) regarded this species as a subjective synonym of *A. pasteurianus.* However, this species is clearly independent from other *Acetobacter* species on the basis of DNA-DNA relatedness and 16S rDNA sequences. The type strain of this species, IFO 13755^T (=ATCC 12874^T=NCIB 8618^T=LMG 1635^T), was isolated from ditch water by Struyck, A. (Janssens et al., 1998). No Indonesian isolates were found in this group.

Group IV: *Acetobacter orleanensis* (Henneberg 1906) comb. nov.

This group includes *A. pasteurianus* IFO 13752, IFO 3170, and IFO 3223, *Gluconacetobacter hansenii* (=*A. hansenii*) IFO 3296, and 6 Indonesian isolates. According to Rule 34a and Rule 50a of the Bacteriological Code (Lapage et al., 1992), *A. aceti* subsp. *orleanensis* De Ley and Frateur (1974) should be elevated to *Acetobacter orleanensis* as a new combination because the former type strain of *A. aceti* subsp. *orleanensis* IFO 13752 is included in this group. "*A. orleanensis*" was established by Henneberg in 1906 and had been regarded as *A. aceti* subsp. *orleanensis* by De Ley and Frateur (1974). Gosselé et al. (1983) rejected *A. aceti* subsp. *orleanensis* and transferred the type strain of this subspecies to *A. pasteurianus* and three strains of it to *A. hansenii* (=*Gluconacetobacter hansenii*). It was found in this study that *A. orleanensis* could be clearly differentiated from *Gluconacetobacter hansenii* by ubiquinone systems. The level of 16S rDNA sequence similarity is in a range of 95.9 to 98.7% between the type strain of *A. orleanensis* and the type strains of other *Acetobacter* species.

Six Indonesian isolates were obtained from fruits (guava and sapodilla) and fermented foods (nata de coco). No isolates were found in flowers in Indonesia.

Description

Basonym: *Acetobacter aceti* subsp. *orleanensis* (Henneberg 1906) De Ley and Frateur 1974[AL].

The phenotypic characteristics and quinone systems are shown in Tables 2 and 4. Differential characteristics from other *Acetobacter* species are shown in Table 5. The range of the G+C content of DNA of this species is 56.5 to 58.7 mol%. The type strain is IFO 13752[T] (=ATCC 12876[T]=NCIB 8622[T]=LMG 1583[T]), of which the G+C content is 56.5 mol%. This strain was isolated from beer by Frateur in 1927 (Janssens et al., 1998).

Group V: *Acetobacter lovaniensis* (Frateur 1950) comb. nov.

This group includes *A. pasteurianus* IFO 13753 and IFO 3284, *A. aceti* AJ 2913 and AJ 2914, and 11 Indonesian isolates. According to Rule 34a and Rule 50a of the Bacteriological Code (Lapage et al., 1992), *A. pasteurianus* subsp. *lovaniensis* De Ley and Frateur 1974 should be elevated to *Acetobacter lovaniensis* as a new combination because the former type strain of *A. pasteurianus* subsp. *lovaniensis* IFO 13753 is included in this group. "*A. lovaniensis*" was established by Frateur in 1950 and had been regarded as *A. pasteurianus* subsp. *lovaniensis* by De Ley and Frateur (1974). Gosselé et al. (1983) rejected *A. pasteurianus* subsp. *lovaniensis* on the basis of phenotypic characteristics and transferred two strains of this subspecies to *A. pasteurianus.*

A. lovaniensis has phenotypic characteristics similar to *A. pasteurianus.* All strains of this species, however, are clearly distinguished from *A. pasteurianus* strains by DNA base composition and DNA-DNA relatedness. The range of the G+C content of DNA of *A. lovaniensis* is 57.1 to 58.9 mol% and higher than that (51.8 to 52.9 mol%) of *A. pasteurianus.* The level of 16S rDNA sequence similarity is in a range of 96.9 to 97.9% between the type strain of *A. lovaniensis* and the type strains of other *Acetobacter* species.

Eleven Indonesian strains were obtained from fruits (palm seed and starfruit) and fermented foods (nata de coco, moromi soya, palm wine, and pickle). No isolates were obtained from flowers in Indonesia.

Description

Basonym: *Acetobacter pasteurianus* subsp. *lovaniensis* (Frateur 1950) De Ley and Frateur 1974[AL].

The phenotypic characteristics and the quinone system are shown in Tables 2 and 4. Differential characteristics from other *Acetobacter* species are shown in Table 5. The range of the G+C content of DNA of this species is 57.1 to 58.9 mol%. The type strain is IFO 13753[T] (=ATCC 12875[T]=NCIB 8620[T]=LMG 1579[T]), of which the G+C content is 58.6 mol%. This strain was isolated from sewage on soil by Frateur in 1929 (Janssens et al., 1998).

Group VI: *Acetobacter estunensis* (Carr 1958) comb. nov.

This group consists of two *A. pasteurianus* strains, IFO 13751 (the former type strain of *A. pasteurianus* subsp. *estunensis*) and LMG 1580. According to Rule 34a and Rule 50a of the Bacteriological Code (Lapage et al., 1992), *Acetobacter pasteurianus* subsp. *estunensis* De Ley and Frateur 1974 should be elevated to *Acetobacter estunensis* as a new combination because the type strain of *A. pasteurianus* subsp. *estunensis* IFO 13751 is included in this group. "*A. estunensis*" was established by Carr in 1958 with emphasis of the production of cellulose. It was found in this study, however, that the pellicles produced by IFO 13751 were not real cellulose because they dissolved after being boiled in 5% NaOH. The level of 16S rDNA sequence similarity is in a range of 96.7 to 98.4% between the type strain of *A. estunensis* and the type strains of other *Acetobacter* species. No Indonesian isolates were found in this group.

Description

Basonym: *Acetobacter pasteurianus* subsp. *estunensis* (Carr 1958) De Ley and Frateur 1974[AL].

The phenotypic characteristics and quinone systems are shown in Tables 2 and 4. Differential characteristics from other *Acetobacter* species are shown in Table 5. The range of the G+C content of DNA is 59.3 to 59.7 mol%. The type strain is IFO 13751[T] (=ATCC 23753[T]=NCIB 8935[T]=LMG 1626[T]), of which the G+C content is 59.7 mol% (this study). This strain was isolated from cider by Carr (Janssens et al., 1998).

Group VII: *Acetobacter indonesiensis* sp. nov.

This group includes *A. pasteurianus* strains LMG 1571 and LMG 1588 and 15 Indonesian isolates. No type strains of species or subspecies in the genus *Acetobacter* are included in this group. Therefore a new species *Acetobacter indonesiensis* sp. nov. is proposed for this group. Fifteen isolates were obtained from Indonesian fruits (banana, papaya, zirzak, and mango) and flowers.

Description

Acetobacter indonesiensis (in.do.ne.si.en'sis. M. L. adj. indonesiensis, referring to Indonesia where most strains studied were isolated). Cells are Gram-negative rods, measuring 0.8 to 1.0 by 1.8 to 2.0 μm, occurring singly, in pairs or in chains. Colonies are circular, convex, glistening, and nonpigmented. No growth on D-mannitol. Positive for catalase activity, oxidation of ethanol to acetic acid, oxidation of acetate and lactate to carbon dioxide and water, production of D-gluconate and 2-keto-D-gluconate from D-glucose, and acid production from D-glucose. Acid production from L-arabinose, D-xylose, D-galactose, and D-mannose is variable from strain to strain. Negative for oxidase activity, formation of γ-pyrones from both D-glucose and D-fructose, and ketogenesis from glycerol. The major quinone is Q-9. The range of the G+C content of DNA is 53.3 to 55.1 mol%. The level of 16S rDNA sequence similarity is in a range of 96.7 to 98.9% between the type strain of *A. indonesiensis* and the type strains of other *Acetobacter* species. Found in fruits and flowers. The type strain is 5H-1^T, of which the G+C content is 53.7 mol%. This strain was isolated from fruit of zirzak (*Annona muricata*). The type strain has been deposited at the Culture Collection Center, Tokyo University of Agriculture, Tokyo, Japan, with the accession number NRIC 0313^T, and at the Japan Collection of Microorganisms with the accession number JCM 10948 and the Institute for Fermentation, Osaka, Japan, with the accession number IFO 16471^T.

Group VIII: *Acetobacter tropicalis* sp. nov.

This group includes six Indonesian isolates and is differentiated from other groups in the genus *Acetobacter.* No type strains of species and subspecies in the genus *Acetobacter* are found in this group. Therefore a new species *Acetobacter tropicalis* sp. nov. is proposed for this group. It is noted that *A. orleanensis*, *A. indonesiensis*, and *A. tropicalis* were differentiated only by genotypic characteristics because they have phenotypic characteristics similar to one another.

Description

Acetobacter tropicalis (tro.pi.ca'lis. M. L. adj. tropicalis referring to the tropical region where the strains were first isolated). Cells are Gram-negative rods measuring 0.5 to 0.7 by 1.8 to 2.0 μm and occurring singly, in pairs, or in chains. Colonies are circular, convex, glistening, and nonpigmented. No growth on mannitol. Positive for catalase activity, oxidation of ethanol to acetic acid, oxidation of acetate and lactate to carbon dioxide and water, and production of D-gluconate and 2-keto-D-gluconate from D-glucose, and acid from D-glucose. Acid production from L-arabinose, D-xylose, D-galactose, and D-mannose is variable from strain to strain. Negative for oxidase activity, formation of γ-pyrones from both D-glucose and D-fructose, and ketogenesis from glycerol. The major quinone is Q-9. The range of the G+C content of DNA of this species is 55.2 to 56.6 mol%. The level of 16S rDNA sequence similarity is in a range of 96.5 to 98.9% between the type strain of *A. tropicalis* and the type strains of other *Acetobacter species.* The type strain is Ni-6b^T, of which the G+C content is 55.9 mol%. This strain was isolated from coconut (*Coccos nucifera*). The type strain has been deposited at the Culture Collection Center, Tokyo University of Agriculture, Tokyo, Japan, with the accession number NRIC 0312^T, and at the Japan Collection of Microorganisms with the accession number JCM 10947 and the Institute for Fermentation, Osaka, Japan, with the accession number IFO 16470^T.

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References

Asai, T., Iizuka, H., and 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.

Beijerinck, M. W. (1898) Ueber die Arten der Essigbakterien. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg.*, Abt. II, **4**, 209–216.

Beijerinck, M. W. and Folpmers, T. (1916) Formation of pyruvic acid from malic acid by microbes. *Verslag. gewone Vergad. Akad. Amst.*, **18**, 1198–1200 (Cited from Iterson, van G. Jr., den Dooren de Jong, L. E., and Kluyver, A. J., 1983).

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

Brosius, J., Dull, T. J., Sleeter, D. D., and Noller, H. F. (1981) Gene organization and primary structure of a ribosomal

RNA operon from *Escherichia coli. J. Mol. Biol.*, **148**, 107–127.

Carr, J. G. (1958) *Acetobacter estunense* nov. spec. An addition to Frateur's ten basic species. *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **24**, 157–160.

Carr, J. G. (1968) Methods for identifying acetic acid bacteria. *In* Identification Methods for Microbiologists. The Society for Applied Bacteriology Technical Series No. 2, Part B, ed. by Gibbs, B. M. and Shapton, D. A., Academic Press, London, pp. 1–8.

De Ley, J. and Frateur, J. (1974) Genus *Acetobacter* Beijerinck, 1898. *In* Bergey's Manual of Determinative Bacteriology, 8th ed., ed. by Buchanan, R. E. and Gibbons, N. E., The Williams & Wilkins Co., Baltimore, pp. 276–278.

De Ley, J., Swings, J., and Gosselé, F. (1984) Genus I. *Acetobacter* Beijerinck 1989, 215^{AL}. *In* Bergey's Manual of Systematic Bacteriology, Vol. 1, ed. by Krieg, N. R. and Holt, J. G., The Williams & Wilkins Co., Baltimore, pp. 268–274.

Ezaki, T., Hashimoto, Y., and 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. Bacteriol.*, **39**, 224–229.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using bootstrap. *Evolution*, **39**, 783–791.

Franke, I. H., Fegan, M., Hayward, C., Leonard, G., Stackebrandt, E., and Sly, L. I. (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.

Frateur, J. (1950) Essai sur la systématique des *Acetobacter*s. *Cellule*, **53**, 287–392 (Cited from De Ley and Frateur, 1974).

Gosselé, F., Swings, J., Kersters, K., Pauwels, P., and 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.

Hansen, E. C. (1879) *Mycoderma aceti* (Kütz) Pasteur et *Myc. pasteurianus. Compte Rendu des travaux du Laboratoire Carlsberg*, **1**, 96–100 (Cited from De Ley and Frateur, 1974).

Henneberg, W. (1906) Zur Kenntnis der Schnellessig und Weinessigbakteriën. *Deut. Essigindustrie*, **10**, 106–108 (Cited from De Ley and Frateur, 1974).

Hucker, G. J. and Conn, H. J. (1923) Methods of Gram staining. N. Y. S. Agric. Exp. Sta., Tech. Bull., No. 93, 3–37.

Iterson, van G., Jr., den Dooren de Jong, L. E., and Kluyver, A. J. (1983) Martinus Willem Beijerinck, His Live and His Work (Reprint), Science Tech. Inc., Madison, WI, pp. 132–134, and 178.

Janssens, D., Vereecke, C., Vanhonacker, K., Kindt, G., Vermeulen, M.-P., and Kersters, K. (ed.) (1998) BCCM™/LMG Catalogue of Cultures 1998, Universiteit Gent (RUG) Laboratorium voor Microbiologie, Gent, Belgium, pp. 39–40, pp. 421–422.

Kawasaki, H., Hoshino, Y., Hirata, A., and Yamasato, K. (1993) Is intracytoplasmic membrane structure a generic criterion? It is not parallel to phylogenetic interrelationships among photosynthetic purple non-sulfur bacteria. *Arch. Microbiol.*, **160**, 358–362.

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.

Komagata, K. and Suzuki, K. (1987) Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol.*, **19**, 162–207.

Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R., and Clark, W. A. (ed.). Sneath, P. H. A. editor for 1992 edition (1992) International Code of Nomenclature of Bacteria, 1990 Revision, Published for the International Union of Microbiological Societies by the American Society for Microbiology, Washington, D. C.

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

Navarro, R. R., Uchimura, T., and Komagata, K. (1999) Taxonomic heterogeneity of strains comprising *Gluconacetobacter hansenii. J. Gen. Appl. Microbiol.*, **45**, 295–300.

Pasteur, L. (1864) Mémoire sur la fermentation acétique. *Ann. Sci. Éc. norm. sup.*, Paris, **1**, 113–158 (Cited from De Ley and Frateur, 1974).

Saito, H. and Miura, K. (1963) Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta*, **72**, 619–629.

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

Shimwell, J. L. (1957) A pattern of evolution in the genus *Acetobacter. J. Inst. Brew.*, **63**, 45–56.

Skerman, V. B. D., MacGowan, V., and Sneath, H. A. (1980) Approved lists of bacterial names. *Int. J. Syst. Bacteriol.*, **30**, 225–420.

Sokollek, S. J., Hertel, C., and Hammes, W. P. (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.

Swings, J., Gillis, M., and Kersters, K. (1992) Phenotypic identification of acetic acid bacteria. *In* Identification Methods in Applied and Environmental Microbiology. The Society for Applied Bacteriology Technical Series No. 29, ed. by Board, R. G., Jones, D., and Skinner, F. A., Blackwell Scientific Publications, Oxford, pp. 103–110.

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

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids. Res.*, **22**, 4673–4680.

Toda, T. (1928) A flagellar staining. Nihon Ijishinpo, No. 283, p. 113 (in Japanese).

Visser't Hooft, F. (1925) Biochemische onderzoekingen over het geslacht *Acetobacter*. Diss. Tech. U., Delft, Meinema, pp. 1–129 (Cited from De Ley and Frateur, 1974).

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E., Stackebrandt, E., Starr, M. P., and Trüper, H. G. (1987) Report of the ad hoc committee on reconciliation of approaches to bacteria systematics. *Int. J. Syst. Bacteriol.*, **37**, 463–464.

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., Aida, K., and 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 *Gluconobacter* and *Acetobacter*, especially of the so-called intermediate strains. *J. Gen. Appl. Microbiol.*, **15**, 186–196.

Yamada, Y., Hoshino, K., and Ishikawa, T. (1997a) 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. Biotech. Biochem.*, **61**, 1244–1251.

Yamada, Y., Hoshino, K., and Ishikawa, T. (1997b) The phylogeny of acetic acid bacteria based on the partial sequences of 16S ribosomal RNA. *Bull. Fac. Agric. Shizuoka Univ.*, **47**, 37–44.

Yamada, Y., Hosono, R., Lisdiyanti, P., Widyastuti, Y., Saono, S., Uchimura, T., and 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.

Yamada, Y., Katsura, K., Kawasaki, H., Widyastuti, Y., Saono, S., Seki, T., Uchimura, T., and 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.