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Asaia bogorensis gen. nov., sp. nov., an unusual acetic acid bacterium in the α-*Proteobacteria*

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Eight Gram-negative, aerobic, rod-shaped and peritrichously flagellated strains were isolated from flowers of the orchid tree (*Bauhinia purpurea*) and of plumbago (*Plumbago auriculata*), and from fermented glutinous rice, all collected in Indonesia. The enrichment culture approach for acetic acid bacteria was employed, involving use of sorbitol medium at pH 3·5. All isolates grew well at pH 3·0 and 30 °C. They did not oxidize ethanol to acetic acid except for one strain that oxidized ethanol weakly, and 0·35% acetic acid inhibited their growth completely. However, they oxidized acetate and lactate to carbon dioxide and water. The isolates grew well on mannitol agar and on glutamate agar, and assimilated ammonium sulfate for growth on vitamin-free glucose medium. The isolates produced acid from D-glucose, D-fructose, L-sorbose, dulcitol and glycerol. The quinone system was Q-10. DNA base composition ranged from 59·3 to 61·0 mol% G+C. Studies of DNA relatedness showed that the isolates constitute a single species. Phylogenetic analysis based on their 16S rRNA gene sequences indicated that the isolates are located in the acetic acid bacteria lineage, but distant from the genera *Acetobacter*, *Gluconobacter*, *Acidomonas* and *Gluconacetobacter*. On the basis of the above characteristics, the name *Asaia bogorensis* gen. nov., sp. nov. is proposed for these isolates. The type strain is isolate 71^T (= NRIC 0311^T = JCM 10569^T).

Keywords: *Asaia bogorensis*, *Proteobacteria*, acetic acid bacteria

INTRODUCTION

During the course of a taxonomic study of acetic acid bacteria, eight interesting bacterial strains were isolated from flowers of the orchid tree (*Bauhinia purpurea*) and of plumbago (*Plumbago auriculata*), and from fermented glutinous rice, all collected in Indonesia. The isolates had unusual characteristics compared with those of known acetic acid bacteria and showed no or a scanty production of acetic acid from ethanol and a complete inhibition of growth by 0·35% acetic acid. However, the strains grew on medium adjusted to pH 3·0 with hydrochloric acid, and oxidized acetate and lactate to carbon dioxide and water. In addition, on the basis of 16S rRNA gene sequences, the isolates showed a phylogenetic location in the acetic acid bacteria lineage, but distant from the genera *Acetobacter*, *Gluconobacter*, *Acidomonas* and *Gluconacetobacter*. On the basis of these characteristics, the isolates could be included in the category of acetic acid bacteria.

This paper deals with the characterization of the above isolates, for which the name *Asaia bogorensis* gen. nov., sp. nov. is proposed. The type strain is isolate 71^T (= NRIC 0311^T = JCM 10569^T).

The DDBJ accession numbers for the 16S rRNA gene sequences of isolates 71^T, 86, 87 and 90 are AB025928, AB025929, AB025930 and AB025931, respectively.

METHODS

Isolation and cultivation of acetic acid bacteria. The enrichment culture approach was employed for the isolation of acetic acid bacteria; the enrichment medium contained 2·0% D-sorbitol, 0·5% peptone, 0·3% yeast extract and 100 p.p.m. cycloheximide, and was adjusted to pH 3·5 with hydrochloric acid. This enrichment medium differed from those of previous studies (Yamada *et al.*, 1976, 1999) in that there was no acetic acid supplementation. Flowers of the orchid

Table 1. Strain designations, isolation sources and morphological characteristics

All isolates were rod-shaped.

Isolate*	Source†	NRIC no.‡	Size (μm)	Flagellation	Colony colour
57	Bunga bauhinia	0314	0·6–0·8 × 1·0–1·2	None	Pink
64	Bunga bauhinia	0315	0·8–1·0 × 1·0–2·0	None	Pink
71^T	Bunga bauhinia	0311^T	0·8–1·0 × 1·0–1·5	Peritrichous	Pink
78	Bunga ceraka biru	0316	0·6–0·8 × 1·0–1·2	Peritrichous	Pink
86	Bunga bauhinia	0317	0·4–0·6 × 1·0–1·2	None	Pink
87	Bunga ceraka biru	0318	0·8–1·0 × 1·0–2·0	Peritrichous	Pink
90	Bunga ceraka biru	0319	0·6–0·8 × 0·8–1·0	Peritrichous	Yellowish white
168	Tape ketan	0320	0·6–0·8 × 0·8–1·0	Peritrichous	Pink

* Isolates 57, 64, 71^T, 78, 86, 87 and 90 were from samples obtained in Bogor, Indonesia, and isolate 168 from a sample obtained in Yogyakarta, Indonesia.

† Bunga bauhinia, flower of the orchid tree (*Bauhinia purpurea*); bunga ceraka biru, flower of plumbago (*Plumbago auriculata*); tape ketan, fermented glutinous rice.

‡ NRIC, NODAI Culture Collection Center, Tokyo University of Agriculture, Tokyo, Japan.

tree and plumbago, and fermented glutinous rice were used as isolation sources. Isolation material was incubated in 7·0 ml of the enrichment culture medium. When microbial growth occurred, the micro-organisms were streaked on a $CaCO_3$ agar plate containing 2·0 % D-glucose, 0·5 % ethanol, 0·8 % yeast extract, 0·7 % $CaCO_3$ and 1·2 % agar. Colonies capable of causing clearing of the $CaCO_3$ were selected and purified for further study. Isolates were maintained on agar slants of AG medium containing 0·1 % D-glucose, 1·5 % glycerol, 0·5 % peptone, 0·5 % yeast extract, 0·2 % malt extract, 0·7 % $CaCO_3$ and 1·5 % agar.

Bacterial strains. Strain designations and their isolation sources are shown in Table 1. *Acetobacter aceti* IFO 14818^T, *Gluconobacter oxydans* IFO 14819^T, *Gluconobacter cerinus* NRIC 0229^T (= IFO 3267^T) and *Gluconacetobacter liquefaciens* IFO 12388^T were used as reference strains.

Morphological, biochemical and physiological characterization. Morphological, biochemical and physiological characteristics were examined by the methods reported previously (Asai *et al*., 1964; Yamada *et al*., 1976, 1999). The presence of catalase was tested by adding a few drops of 3·0 % hydrogen peroxide solution to the bacterial colonies. Production of 2-keto-D-gluconate, 5-keto-D-gluconate and 2,5-diketo-D-gluconate was investigated by TLC (Swings *et al*., 1992). Assimilation of ammoniacal nitrogen was examined by testing growth of the isolates at 30 °C for 5 d on a medium containing 3·0 % D-glucose, 0·1 % $(NH_4)_2SO_4$, 0·01 % K_2HPO_4, 0·09 % KH_2PO_4, 0·025 % $MgSO_4.7H_20$ and 0·0005 % $FeCl_3.6H_20$, adjusting the pH to both 4·0 and 6·8. Growth was also tested in media containing various concentrations of acetic acid up to 0·35 % at 30 °C for 5 d; AG medium without $CaCO_3$ and agar was used as a basal medium. Acid production from sugars and sugar alcohols was tested by the method of Asai *et al*. (1964).

Determination of ubiquinone isoprenologues. Ubiquinone isoprenologues were determined by the use of reversed-phase paper chromatography (Yamada *et al*., 1969). The ubiquinones were quantitatively determined using reversed-phase HPLC (Tamaoka *et al*., 1983). Standard preparations of Q-10, Q-9 and Q-8 were prepared from cells of *Gluconobacter cerinus* NRIC 0229^T, *A. aceti* IFO 14818^T and *Frateuria aurantia* IFO 3245^T, respectively (Yamada *et al*., 1969).

Determination of DNA base composition and DNA relatedness. DNAs for the determination of DNA base composition and DNA relatedness were extracted and purified by modification of the methods of Marmur (1961) and Ezaki *et al*. (1983). DNA base composition was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). DNA relatedness was determined by the fluorometric DNA–DNA hybridization method in microdilution wells described by Ezaki *et al*. (1989).

16S rRNA gene sequence. Gene fragments specific for the 16S-rRNA-coding regions of the isolates were amplified by PCR with the following two primers: 20F (5′-GAGTTTG-ATCCTGGCTCAG-3′, positions 9–27) and 1500R (5′-GTTACCTTGTTACGACTT-3′, positions 1509–1492) [the numbering of positions was based on the *Escherichia coli* numbering system (accession no. V00348, Brosius *et al*., 1981)], as described by Kawasaki *et al*. (1993). Amplified 16S rRNA genes were sequenced directly using an ABI PRIMS Bigdye Terminator Cycle Sequencing Ready Reaction kit and ABI PRIMS model 310 Genetic Analyzer. The following six primers were used: 20F, 1500R, 520F (5′-CAGCAGC-CGCGGTAATAC-3′, positions 519–536), 520R (5′-GTA-TTACCGCGGCTGCTG-3′, positions 536–519), 920F (5′-AAACTCAAATGAATTGACGG-3′, positions 907–926) and 920R (5′-CCGTCAATTCATTTGAGTTT-3′, positions 926–907).

Phylogenetic analysis. Multiple alignments were performed by the program CLUSTAL W (version 1.6) (Thompson *et al*., 1994). Distance matrices for the aligned sequences were calculated by using the two-parameter method of Kimura (1980). The neighbour-joining method was used for constructing a phylogenetic tree (Saitou & Nei, 1987). Since two kinds of bases (T/C) were detected at positions 91 and 139, the bases in these positions were excluded for constructing the phylogenetic tree. The comparison of the sequence data obtained was therefore made on 1402 bases. The robustness of individual branches was estimated by bootstrapping with

1000 replicates (Felsenstein, 1985). The species, type strains and accession numbers of the nucleotide sequences taken from databases are presented in Fig. 1. Percentage similarities among acetic acid bacteria, including a new isolate, were calculated in pairs of the sequences of the 1402 bases.

RESULTS

Morphological, biochemical and physiological characteristics

Cells of all the isolates were Gram-negative, strictly aerobic and rod-shaped, measuring from 0·4 to 1·0 by 0·8 to 2·0 μm (Table 1). They had peritrichous flagella when motile. Colonies were pink–yellowish white, shiny, smooth, raised and with an entire margin on AG agar plates. All new isolates grew on glutamate agar and mannitol agar, but did not grow at the expense of methanol. They did not produce a water-soluble brown pigment on a glucose-yeast extract-$CaCO_3$ medium. The isolates oxidized acetate and lactate to carbon dioxide and water. Seven of the eight isolates did not produce acetic acid from ethanol and one (isolate 86) poorly produced the acid. Acetic acid at 0·3% allowed scanty growth but at 0·35% inhibited growth completely. Production of dihydroxyacetone from glycerol was generally weak but the intensity depended on the isolate. The isolates produced 2-keto-D-gluconate and 5-keto-D-gluconate, but not 2,5-diketo-D-gluconate. They produced γ-pyrone compounds from D-fructose but not from D-glucose. Good growth occurred at the expense of D-glucose and ammonium sulfate as sole sources of carbon and nitrogen on vitamin-free medium. Catalase was produced. Good growth occurred at pH 3·0 and 30 °C. All the isolates produced acid from D-glucose, D-mannose, D-fructose, L-sorbose, D-xylose, L-arabinose, D-ribose, dulcitol, *myo*-inositol, ribitol, D-arabitol, xylitol, *meso*-erythritol, glycerol, melibiose and sucrose, but not from lactose. Production of acid from D-mannitol and D-sorbitol differed among strains.

Quinone systems

The major quinone of all the isolates was Q-10. The quinone system was composed of 93–100% Q-10 and 0–7% Q-9. Q-8 was not found.

DNA base composition and DNA relatedness

The DNA base composition of the isolates ranged from 59·3 to 61·0 mol% G+C (Table 2). Levels of DNA relatedness were from 64 to 107% when the DNAs from isolates 71^T, 86 and 87 were used as probes. By contrast, *A. aceti* IFO 14818^T, *Gluconobacter oxydans* IFO 14819^T and *Gluconacetobacter liquefaciens* IFO 12388^T showed very low levels of DNA relatedness to the isolates from 5 to 10% (Table 2). The DNA relatedness indicated that all the isolates constitute a single species.

Phylogenetic analysis based on 16S rRNA gene sequences

Isolates 71^T, 86, 87 and 90 were subjected to phylogenetic analysis based on 16S rRNA gene sequences. Base differences were found only at two positions, 989

Table 2. DNA base composition and DNA relatedness

Strain or species	DNA base composition (mol% G+C)	Relative binding (%) of DNA from:					
		71^T	86	87	IFO 14818^T	IFO 14819^T	IFO 12388^T
Isolate							
57	61·0	70	97	64	12	18	17
64	60·5	76	100	80	11	22	16
71^T	60·2	100	75	91	11	22	17
78	59·3	86	69	95	NT	26	NT
86	59·7	68	100	69	8	20	12
87	59·3	87	77	100	17	24	16
90	59·5	86	69	107	19	25	15
168	59·4	93	64	107	NT	NT	NT
Acetobacter aceti IFO 14818^T	58·3	5	5	5	100	24	19
Gluconobacter oxydans IFO 14819^T	61·7	10	9	7	11	100	22
Gluconacetobacter liquefaciens IFO 12388^T	64·9	9	8	6	17	23	100

NT, Not tested.

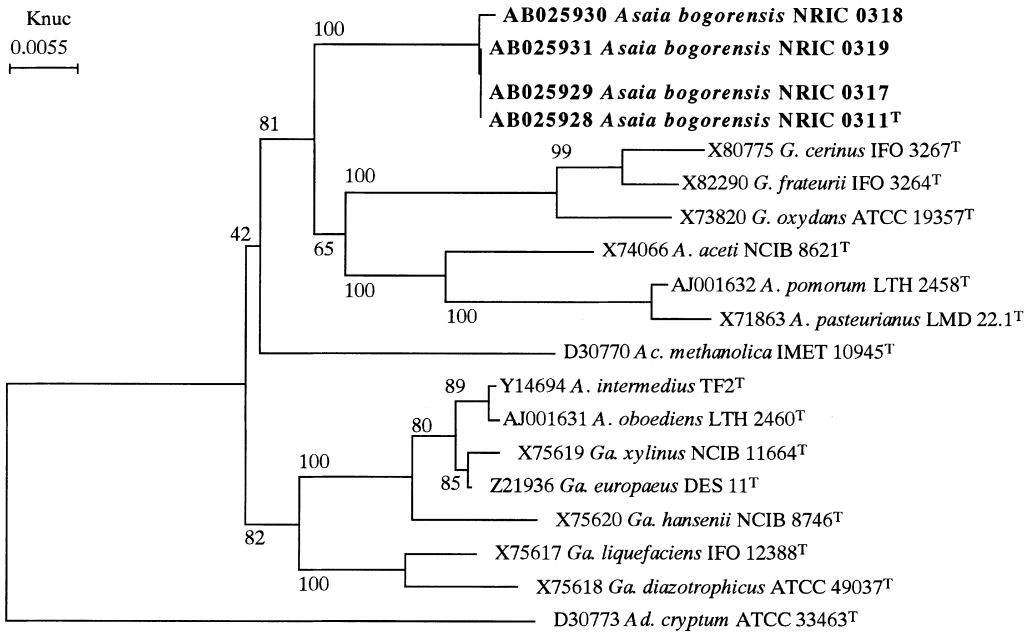

Fig. 1. Phylogenetic relationships of isolates to the genera *Acetobacter*, *Gluconobacter*, *Acidomonas* and *Gluconacetobacter* based on 16S rRNA gene sequences. *Acidiphilium cryptum* ATCC 33463^T was used as an outgroup. Numerals indicate the bootstrap percentages derived from 1000 samples. Abbreviations: *A.*, *Acetobacter*; *Ac.*, *Acidomonas*; *Ad.*, *Acidiphilium*; *As.*, *Asaia*; *G.*, *Gluconobacter*; and *Ga.*, *Gluconacetobacter*.

Table 3. Calculated percentage similarities among an isolate and strains of acetic acid bacteria based on 16S rRNA gene sequences

The accession numbers of the nucleotide sequences are shown in Fig. 1; 1402 bases were compared.

	Species*	Strain no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*As. bogorensis*	NRIC 0311^T															
2	*Ga. europaeus*	DES 11^T	96·4														
3	*Ga. xylinus*	NCIB 11664^T	96·2	99·7													
4	*A. oboediens*	LTH 2460^T	96·6	99·6	99·3												
5	*A. intermedius*	TF2^T	96·7	99·6	99·3	99·9											
6	*Ga. hansenii*	NCIB 8746^T	95·5	98·6	98·4	98·3	98·3										
7	*Ga. diazotrophicus*	ATCC 49037^T	96·4	96·9	96·8	96·5	96·7	96·9									
8	*Ga. liquefaciens*	IFO 12388^T	96·7	97·3	97·2	97	97·2	96·9	98·6								
9	*Ac. methanolica*	IMET 10945^T	96	95·8	95·7	95·7	95·9	95·3	95·7	96·3							
10	*A. pasteurianus*	LMD 22·1^T	95·3	94·7	94·4	95	95·2	94·1	95	95·4	94·7						
11	*A. pomorum*	LTH 2458^T	95·7	94·9	94·6	95·2	95·3	94·2	95·2	95·6	94·9	99·4					
12	*A. aceti*	NCIB 8621^T	96·5	95·4	95·3	95·6	95·7	94·9	95·8	95·9	94·9	96·7	97·3				
13	*G. oxydans*	ATCC 19357^T	96	94·9	94·7	95·2	95·3	94·4	94·5	94·4	95·1	94·8	95·3	95·9			
14	*G. frateurii*	IFO 3264^T	96·2	94·8	94·7	95·1	95·2	94·1	94·8	94·7	95	94·6	95·1	95·3	98·1		
15	*G. cerinus*	IFO 3267^T	96·3	94·4	94·3	94·6	94·7	93·9	94·6	94·5	94·4	94·2	94·7	95·4	98·1	98·9	
16	*Ad. cryptum*	ATCC 33463^T	92·4	92·5	92·4	92·7	92·8	91·9	92·2	92·4	92·1	90·7	91·2	91·8	91·7	91·2	90·9

* Abbreviations: *A.*, *Acetobacter*; *Ac.*, *Acidomonas*; *Ad.*, *Acidiphilium*; *As.*, *Asaia*; *G.*, *Gluconobacter*; *Ga.*, *Gluconacetobacter*.

and 1274: A and A, respectively, in isolate 87, but G and G in isolates 71^T, 86 and 90. As shown in Fig. 1, the four isolates showed 99·9–100·0 % rDNA similarity to each other and constituted a single cluster in the 16S rRNA phylogenetic tree. This cluster was distant from those of members of the genera *Acetobacter*, *Gluconobacter*, *Acidomonas* and *Gluconacetobacter*, though the isolates were included in a broad cluster together with the above genera. Calculated rDNA similarities between isolate 71^T (= NRIC 0311^T) and each of the

type strains of *A. aceti*, *Gluconobacter oxydans*, *Acidomonas methanolica* and *Gluconacetobacter liquefaciens* were 96·5, 96·0, 96·0 and 96·7 %, respectively. Details of data are presented in Table 3. These results indicated that all the isolates should be separated from the genera *Acetobacter*, *Gluconobacter*, *Acidomonas* and *Gluconacetobacter*.

DISCUSSION

Acetic acid bacteria are classified into four genera: *Acetobacter*, *Gluconobacter*, *Acidomonas* and *Gluconacetobacter* (Yamada *et al.*, 1997a, b). 16S rRNA gene sequence analysis indicates that the isolates characterized in this study are phylogenetically distinguished from sublineages of the genera *Gluconobacter*, *Acetobacter* and *Acidomonas* on the basis of high bootstrap values as shown in Fig. 1. In addition, the genus *Gluconacetobacter* sublineage is distinct from the above sublineages. The isolates constitute a single cluster with 100 % bootstrap value. This warrants the inclusion of the new isolates as members of a new genus of acetic acid bacteria. The new isolates and the members of the genera *Acetobacter*, *Gluconobacter*, *Acidomonas* and *Gluconacetobacter* join phylogenetically into a broad rRNA cluster.

Phylogenetic trees of acetic acid bacteria reported by Sievers *et al.* (1994a, b), Yamada *et al.* (1997a, b), Boesch *et al.* (1998) and Sokollek *et al.* (1998) seem to be similar to one another. Yamada *et al.* (1997a) proposed *Gluconoacetobacter* (sic) and the reuse of *Acidomonas* on the basis of 16S rRNA partial sequences. Boesch *et al.* (1998) objected to the use of both these generic names because Yamada *et al.* (1997a) used partial sequences for the proposals, citing a paper of Stackebrandt & Goebel (1994). However, in this study we constructed a phylogenetic tree of acetic acid bacteria on the basis of full 16S rRNA sequences. Trees based on both partial and full sequences are quite similar. The objection of Boesch *et al.* (1998) is therefore invalid, and the proposals of Yamada *et al.* (1997a) dealing with the establishment of the genus *Gluconacetobacter* and the reuse of the genus *Acidomonas* are acceptable.

Interestingly, *Acetobacter intermedius* (Boesch *et al.*, 1998) and *Acetobacter oboediens* (Sokollek *et al.*, 1998) are included in the sublineage of the genus *Gluconacetobacter*. Taxonomic positions of these species will be reported elsewhere. The genus *Acetobacter* is differentiated from the genera *Gluconobacter*, *Acidomonas* and *Gluconacetobacter* on the basis of the quinone system, that is, the genus *Acetobacter* has Q-9 and the rest have Q-10 (Yamada *et al.*, 1969, 1997a).

According to the report of the Ad Hoc committee on Reconciliation of Approaches to Bacterial Systematics (Wayne *et al.*, 1987), a bacterial species is recommended to include strains with approximately 70 % or greater DNA–DNA relatedness. The level of DNA relatedness between isolates 86 and 168 is 64 %, and between isolates 87 and 57 is 64 %. However, the eight new isolates showed very low levels of DNA relatedness to each of the type strains of *A. aceti*, *Gluconobacter oxydans* and *Gluconacetobacter liquefaciens* as shown in Table 2. Furthermore, 16S rRNA gene sequence similarities among the isolates are more than 99·9 %. Therefore, the isolates are closely related to each other according to the definition of the bacterial species reported by Stackebrandt & Goebel (1994). Considering the above recommendation and comment, the isolates are included in a single species.

The eight new isolates have peritrichous flagella as found in members of the genera *Acetobacter* and *Gluconacetobacter*, but not in the genus *Gluconobacter*, which has polar flagellation.

The isolates oxidize acetate and lactate to carbon dioxide and water, as found in members of the genera *Acetobacter* and *Gluconacetobacter*, but not in the genus *Gluconobacter*. The isolates do not utilize methanol as a sole source of carbon. This indicates a clear difference from the genus *Acidomonas*.

Most of the isolates do not oxidize ethanol to acetic acid but an exceptional strain was found. They grow well even at pH 3·0, but cannot grow in medium containing 0·35 % acetic acid. This characteristic is useful for differentiation of the isolates from members of the genera *Acetobacter*, *Gluconobacter* and *Gluconacetobacter*.

The isolates produce acid from D-glucose, D-fructose and L-sorbose, as found in members of the genus *Gluconobacter*. In addition, the isolates can be distinguished from members of the genera *Acetobacter*, *Gluconobacter* and *Gluconacetobacter* by the production of acid from dulcitol. The isolates show vigorous growth on vitamin-free medium containing D-glucose and ammonium sulfate. This indicates a lack of requirement for any growth factors.

In a previous paper, Yamada *et al.* (1999) reported the isolation of 64 strains of acetic acid bacteria by the use of glucose-ethanol-acetic acid medium for enrichment at pH 3·5 from Indonesian sources. These isolates consisted of 45 strains of the genus *Acetobacter*, 11 strains of the genus *Gluconobacter* and 8 strains of the genus *Gluconacetobacter*. When sorbitol medium was used for enrichment adjusted to pH 3·5 with hydrochloric acid, members of the new species were isolated. This shows the importance of minor modifications in the composition of the enrichment culture medium for the isolation of new taxa.

In conclusion, the isolates have unique characteristics compared with known acetic acid bacteria and constitute a new phylogenetic sublineage separate from the sublineages containing the genera *Acetobacter*, *Gluconobacter*, *Acidomonas* and *Gluconacetobacter*. Consequently, these new isolates deserve to belong to a new genus for which we propose the name *Asaia* in honour of Dr Toshinobu Asai who was Professor Emeritus of the University of Tokyo, Japan, and

Table 4. Differential characteristics of the genus *Asaia* and other acetic acid bacteria genera

w, Weak.

Character	*Asaia*	*Acetobacter*	*Gluconobacter*	*Acidomonas**	*Gluconacetobacter*
Flagellation	Peritrichous or none	Peritrichous or none	Polar or none	None	Peritrichous or none
Oxidation of:					
Acetate	+	+	−	+	+or−
Lactate	+	+	−	−	+or−
Growth on methanol	−	−†	−	+	−
Assimilation of ammonium sulfate on glucose medium	+	w+	−	−	w+
Growth on:					
Glutamate agar	+	+or−	−	−	+or−
Mannitol agar	+	− or +	+	−	+or−
Dihydroxyacetone from glycerol	− or w+	− or w+	+	−	+ or −
Acetic acid production on ethanol-$CaCO_3$ agar	− or w+	+	+	+	+
Growth on acetic acid-containing medium‡	−	+	+	+	+
Acid production from:					
D-Mannitol	+or−	− or w+	+	−	+or−
D-Sorbitol	+or−	−	+	−	−
Dulcitol	+	−	−	−	−
Glycerol	+	−	+	−	+
Ethanol	− or w+	+	+	+	+
DNA base composition (mol% G+C)	59−61	53−63	54−63	63−66	55−66
Major ubiquinone	Q-10	Q-9	Q-10	Q-10	Q-10

* Data from Urakami *et al.* (1989).

† *Acetobacter pomorum* was reported to assimilate methanol weakly (Sokollek *et al.*, 1998).

‡ Containing 0·35% acetic acid.

contributed a great deal to the systematics of acetic acid bacteria. The type species is *Asaia bogorensis* sp. nov.

Differential characteristics of the five genera are shown in Table 4.

Description of *Asaia* gen. nov.

Asaia (A.sa′i.a. M.L. fem. n. *Asaia* derived from Toshinobu Asai, a Japanese bacteriologist who contributed to the systematics of acetic acid bacteria).

Gram-negative, aerobic and rod-shaped. Peritrichously flagellated when motile. Oxidizes acetate and lactate to carbon dioxide and water. Does not produce acetic acid from ethanol (exceptionally, one strain produced acid weakly). Growth is inhibited by 0·35% acetic acid. No growth on methanol. Assimilates ammonium sulfate for growth on vitamin-free glucose medium. Grows on glutamate agar and mannitol agar. Produces 2-keto-D-gluconate and 5-keto-D-gluconate, but not 2,5-diketo-D-gluconate from D-glucose. Produces acid from D-glucose, D-mannose, D-fructose, L-sorbose and dulcitol. The major quinone is Q-10. DNA base composition ranges from 59·3 to 61·0 mol% G+C. The type species is *Asaia bogorensis* sp. nov.

Description of *Asaia bogorensis* sp. nov.

Asaia bogorensis (bo.go.r′en.sis. M.L. adj. *bogorensis* derived from Bogor, Java, Indonesia, where most of the strains were isolated).

Major characteristics are the same as those described in the generic description. Gram-negative, strictly aerobic and rod-shaped organisms measuring 0·4 to 1·0 by 0·8 to 2·0 μm. Colonies are pink–yellowish white, shiny, smooth, raised and with an entire margin on AG agar plates. Grows at pH 3·0 and 30 °C. No water-soluble brown pigment is produced. Production of dihydroxyacetone from glycerol is weak; the intensity depends on the strain. Acid is produced from D-glucose, D-mannose, D-galactose, D-fructose, L-sorbose, D-xylose, ribitol, *meso*-erythritol, glycerol and melibiose, but not from lactose. Production of acid from D-mannitol and D-sorbitol is different between strains. The major quinone is Q-10. The DNA base composition of the type strain is 60·2 mol% G+C.

The type strain is isolate 71^T, from bunga bauhinia (the flower of the orchid tree, *Bauhinia purpurea*) in Bogor, Indonesia. The type strain has been deposited at the NODAI Culture Collection Center (NRIC), Tokyo University of Agriculture, Tokyo, Japan, under the accession number NRIC 0311^T, and the Japan Collection of Microorganisms (JCM), Wako, Saitama, Japan, under the accession number JCM 10569^T.

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