***Gluconobacter asaii* Mason and Claus 1989 is a junior subjective synonym of *Gluconobacter cerinus* Yamada and Akita 1984**

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Five strains received as *Gluconobacter cerinus* and *Gluconobacter asaii* were examined for DNA base composition, DNA–DNA similarity, 16S rRNA gene sequences and phenotypic characteristics, including acid production from ethanol, growth on L-arabitol and meso-ribitol and requirement for nicotinic acid. The five strains showed DNA base compositions ranging from 54 to 56 mol% G+C. *G. cerinus* IFO 3267^T and IAM 1832 and *G. asaii* IFO 3276^T and IFO 3275 showed high levels of DNA–DNA similarity (70–100%) between each other and low values of DNA–DNA similarity (16–35%) to *Gluconobacter frateurii* IFO 3264^T and *Gluconobacter oxydans* IFO 14819^T. *G. cerinus* IFO 3267^T and *G. asaii* IFO 3276^T were located at an identical position in a phylogenetic tree deduced from 16S rRNA gene sequences. Two *G. cerinus* strains and two *G. asaii* strains did not require nicotinic acid for growth and did not grow on L-arabitol or meso-ribitol. *G. cerinus* IAM 1832 did not produce acid and required nicotinic acid and/or other growth factors. *G. asaii* IFO 3265 showed a high degree of DNA–DNA similarity (97%) to *G. frateurii* IFO 3264^T and low similarity values (each 32%) to *G. cerinus* IFO 3267^T and *G. asaii* IFO 3276^T. This strain did not require nicotinic acid and grew well on L-arabitol and meso-ribitol. Therefore, *G. asaii* IFO 3265 was reclassified as *G. frateurii*. The results obtained revealed a synonymous relationship between *G. cerinus* and *G. asaii*. *G. asaii* is a junior subjective synonym of *G. cerinus* because *G. cerinus* has priority over *G. asaii*.

Keywords: *Gluconobacter cerinus*, *Gluconobacter asaii*, acetic acid bacteria, *Acetobacteraceae*, *Proteobacteria*

INTRODUCTION

The species *Gluconobacter cerinus* was first proposed by Asai & Shoda (1958) but was not included in the *Approved Lists of Bacterial Names* (Skerman *et al.*, 1980). This species was revived as the second species of the genus *Gluconobacter* on the basis of DNA base composition, DNA–DNA similarity and electrophoretic patterns of enzymes (Yamada & Akita, 1984a) and was subsequently validated (Yamada & Akita, 1984b). Strains assigned to *G. cerinus* were characterized by low values for DNA G+C content

(Yamada and Akita, 1984a) and were included in phenon A on the basis of numerical analysis of phenotypic features and in cluster A' on the basis of the electrophoretic protein pattern (Gosselé *et al.*, 1983). In contrast, strains assigned to *Gluconobacter oxydans*, the type species of the genus *Gluconobacter*, were characterized by high values for DNA G+C content (Yamada & Akita, 1984a), and were included in phenon B on the basis of numerical analysis of phenotypic features and in cluster B' on the basis of the electrophoretic protein pattern (Gosselé *et al.*, 1983).

Micales *et al.* (1985) reported the division of strains assigned to the genus *Gluconobacter* into three homology groups according to DNA–DNA similarity. Later, Mason & Claus (1989) introduced three species, *G. oxydans*, *Gluconobacter frateurii* and *Gluconobacter asaii*, respectively, for homology groups I, II and III of Micales *et al.* (1985). However, it is worth noting that

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The DDBJ accession numbers for the 16S rRNA gene sequences of *Gluconobacter cerinus* IFO 3267^T and IAM 1832 and *Gluconobacter asaii* IFO 3276^T are AB063286, AB063288 and AB063287.

G. asaii was described without data on DNA–DNA similarity between *G. asaii* IFO 3276^T and *G. cerinus* IFO 3267^T. These homology groups should be reclassified, since the DNA–DNA hybridization data reported by Micales *et al.* (1985) did not correspond to the modern concept of bacterial species recommended by Wayne *et al.* (1987). Recently, Yamada *et al.* (1999) and Tanaka *et al.* (1999) reported high values for DNA–DNA similarity between the type strains of *G. cerinus* and *G. asaii* and suggested a synonymous relationship between the two species. However, neither Yamada *et al.* (1999) nor Tanaka *et al.* (1999) mentioned which of the species has priority.

This study aims to re-examine the homogeneity of strains received as *G. cerinus* and those received as *G. asaii* on the basis of DNA–DNA similarity, 16S rRNA gene sequences and phenotypic characteristics and to describe the synonymous relationship between *G. cerinus* and *G. asaii* and the priority of *G. cerinus* over *G. asaii* in bacterial nomenclature.

METHODS

Bacterial strains. The five *Gluconobacter* strains studied were obtained from culture collections, as shown in Table 1. *G. cerinus* IAM 1832 was examined, since this strain was reported not to produce acetic acid from ethanol (Asai *et al.*, 1964; Gosslé *et al.*, 1983). An additional strain, IFO 3297, received as *Acetobacter* sp., was also used, since Micales *et al.* (1985) had reported two colony types for this strain and had identified both as *Gluconobacter* sp. *G. oxydans* IFO 14819^T and *G. frateurii* IFO 3264^T were used as reference strains. These bacterial strains were maintained on a basal medium composed of 2.0% D-glucose, 1.0% yeast extract, 0.7% CaCO₃ and 1.5% agar (all by weight).

Acid production from ethanol. Acid production from ethanol was tested by using the colour change of bromocresol purple in a test medium (Yamada *et al.*, 1976).

Oxidation of acetate and lactate. Testing of the oxidation of lactate and acetate to CO₂ and H₂O was carried out using a method described previously (Asai *et al.*, 1964; Yamada *et al.*, 1999, 2000; Katsura *et al.*, 2001).

Quinone homologues. Isoprenoid quinones were extracted from bacterial cells and purified by the method of Yamada *et al.* (1969). Ubiquinone isoprenologues were determined

quantitatively by reverse-phase HPLC (Tamaoka *et al.*, 1983).

DNA base composition and DNA–DNA similarity. Bacterial DNAs were extracted and purified by the modified method of Marmur (1961) (Saito & Miura, 1963). The DNA base composition was determined by reverse-phase HPLC (Tamaoka & Komagata, 1984). DNA–DNA similarity was determined by using a microdilution-well technique with photobiotin for labelling (Ezaki *et al.*, 1989). Isolated, single-stranded and labelled DNAs were hybridized with DNAs from test strains in 2 × SSC and 50% formamide at 46.9 or 49.0 °C for 2–3 h.

Phylogenetic analysis. The 16S rRNA gene sequences were determined as described previously (Yamada *et al.*, 2000; Katsura *et al.*, 2001). The sequence data obtained were aligned with the program CLUSTAL W version 1.8 (Thompson *et al.*, 1994). Distance matrices for the aligned sequences (1377 bases) were calculated by using Kimura's two-parameter method (Kimura, 1980) and a phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987). The robustness of individual branches was estimated by bootstrapping with 1000 replicates (Felsenstein, 1985).

Growth on pentitols. Growth on L-arabitol and meso-ribitol was tested by the use of a medium containing 0.2% pentitol (L-arabitol or meso-ribitol), 0.3% yeast extract (Difco), 0.1% (NH₄)₂SO₄, 0.01% K₂HPO₄, 0.09% KH₂PO₄, 0.025% MgSO₄ · 7H₂O and 0.0005% FeCl₃ · 6H₂O (all components added by weight).

Requirement for nicotinic acid. Growth of strains with and without nicotinic acid was tested by using a medium containing three carbon sources [0.5% (w/v) each for D-glucose, D-mannitol and glycerol], 0.5% vitamin-free Casamino acids (Difco) (w/v), 0.1% (w/v) (NH₄)₂SO₄, 100 mg K₂HPO₄, 900 mg KH₂PO₄, 250 mg MgSO₄ · 7H₂O and 5.0 mg FeCl₃ · 6H₂O l⁻¹ and vitamins. Vitamins were supplemented in the test media at the following final concentrations (mg l⁻¹) with or without 1.5 mg nicotinic acid l⁻¹: pyridoxal hydrochloride, 1.5; riboflavin, 1.5; thiamin, 1.0; pantothenic acid, 1.0; *p*-aminobenzoic acid, 1.0; biotin, 1.0. A loopful of cells grown on the basal medium was inoculated into the test medium containing nicotinic acid. The inoculated medium was incubated at 30 °C for 2 days with shaking and the culture broth was then inoculated into two media, with or without nicotinic acid. Growth was judged to be positive when the micro-organisms grew well after two passages with the medium lacking nicotinic acid.

Table 1. Correspondence of strains used in this study and other studies

Strain	Received as:	Used by:			
		This study	Gosslé <i>et al.</i> (1983)	Yamada & Akita (1984a)	Micales <i>et al.</i> (1985)
IFO 3267 ^T	<i>G. cerinus</i>	+	+	+	—
IAM 1832	<i>G. cerinus</i>	+	+	+	—
IFO 3276 ^T	<i>G. asaii</i>	+	+	+	+
IFO 3275	<i>G. asaii</i>	+	—	+	+
IFO 3265	<i>G. asaii</i>	+	+	+	+
IFO 3264 ^T	<i>G. frateurii</i>	+	+	+	+
IFO 14819 ^T	<i>G. oxydans</i>	+	+	+	+
IFO 3297	<i>Acetobacter</i> sp.	+	—	—	+

RESULTS AND DISCUSSION

Phylogenetic analysis

The sequences of *G. cerinus* IFO 3267^T and *G. asaii* IFO 3276^T were identical to each other in this study and *G. cerinus* IFO 3267^T and IAM 1832 and *G. asaii* IFO 3276^T were located at an identical position in the phylogenetic tree in Fig. 1. The positions of the type strains differed from those in the tree of Sievers *et al.* (1995). Discrepancies were found in the sequence of *G. asaii* IFO 3276^T between the data from this study (accession no. AB063287) and those reported by Sievers *et al.* (1995) (accession no. X80165). These were assumed to be due to the misreading of 17 bases, corresponding to 1.2% of the complete sequence of 16S rRNA. Thirteen bases, at positions 65, 110, 318, 371, 403, 537, 618, 634, 948, 1069, 1083, 1092 and 1372 (*Escherichia coli* numbering system, accession no. V00348; Brosius *et al.*, 1981), in the sequence of

Fig. 1. Phylogenetic relationships of *Gluconobacter* species, based on 16S rRNA gene sequences. *Acidiphilium cryptum* ATCC 33463^T was used as an outgroup. Numerals at nodes indicate bootstrap values derived from 1000 replications (Felsenstein, 1985).

G. asaii (accession no. X80165) should be read respectively as C, C, G, A, C, G, C, C, C, C, T, A and T. The bases at the above positions are commonly found in the type strains of the type species of the genera *Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, *Acidomonas* and *Asaia* along with the type strain of *Acidiphilium cryptum* (α -Proteobacteria) (respectively accession nos X74066, X73820, X75617, D30770, AB025928 and D30773). However, the 13 bases at the above-mentioned positions were read by Sievers *et al.* (1995) as T, T, A, T, G, C, G, G, G, G, C, G and C. Four other bases, at positions 580, 589, 629 and 645, were read as C, T, A and C in this study. In contrast, Sievers *et al.* (1995) recognized them as G, G, G and G.

Quinone homologues

All the strains received as *Gluconobacter* species had Q-10 as the major quinone and *Acetobacter* sp. IFO 3297 had Q-9 (Table 3).

DNA base compositions and DNA–DNA similarity

The G + C contents of the DNA of the two *G. cerinus* strains and three *G. asaii* strains ranged from 54 to 56 mol % (Table 2).

Values for DNA–DNA similarity ranged from 70 to 100 % among four strains received as *G. cerinus* and *G. asaii* when DNAs of *G. cerinus* IFO 3267^T and *G. asaii* IFO 3276^T were used as probes (Table 2). *G. asaii* IFO 3265 showed a high degree of DNA–DNA similarity (97%) to *G. frateurii* IFO 3264^T and low similarity (each 32 %) to *G. cerinus* IFO 3267^T and *G. asaii* IFO 3276^T.

Growth on pentitols

G. cerinus IFO 3267^T and IAM 1832, *G. asaii* IFO 3276^T and IFO 3275 and *G. oxydans* IFO 14819^T grew neither on L-arabitol nor on meso-ribitol, but *G. asaii* IFO 3265 and *G. frateurii* IFO 3264^T grew well on these pentitols (Table 3).

Table 2. DNA base compositions and values for DNA–DNA similarity among *G. cerinus* and *G. asaii* strains

Strain	Received as:	DNA G + C content (mol %)	Relative binding (%) to DNA from:			
			1	3	6	7
1. IFO 3267 ^T	<i>G. cerinus</i>	56	100	87	33	27
2. IAM 1832	<i>G. cerinus</i>	55	83	NT	35	19
3. IFO 3276 ^T	<i>G. asaii</i>	55	77	100	27	16
4. IFO 3275	<i>G. asaii</i>	54	70	86	26	18
5. IFO 3265	<i>G. asaii</i>	56	32	32	97	35
6. IFO 3264 ^T	<i>G. frateurii</i>	55	31	36	100	29
7. IFO 14819 ^T	<i>G. oxydans</i>	60	17	10	22	100

NT, Not tested.

Table 3. Phenotypic characteristics of *G. cerinus* and *G. asaii* strains

Strain	Received as:	Growth on:		Growth without nicotinic acid	Oxidation of:		Major quinone	Identified as:
		<i>meso</i> -Ribitol	L-Arabitol		Acetate	Lactate		
IFO 3267 ^T	<i>G. cerinus</i>	—	—	+	—	—	Q-10	<i>G. cerinus</i>
IAM 1832	<i>G. cerinus</i>	—	—	—*	—	—	Q-10†	<i>G. cerinus</i>
IFO 3276 ^T	<i>G. asaii</i>	—	—	+	—	—	Q-10	<i>G. cerinus</i>
IFO 3275	<i>G. asaii</i>	—	—	+	—	—	Q-10†	<i>G. cerinus</i>
IFO 3265	<i>G. asaii</i>	+	+	+	—	—	Q-10†	<i>G. frateurii</i>
IFO 3264 ^T	<i>G. frateurii</i>	+	+	+	—	—	Q-10	<i>G. frateurii</i>
IFO 14819 ^T	<i>G. oxydans</i>	—	—	—	—	—	Q-10	<i>G. oxydans</i>
IFO 3297‡	<i>Acetobacter</i> sp.	NT	NT	NT	—	+	Q-9	<i>Acetobacter</i> sp.

* Other growth factors are required by this strain.

† Data from Yamada & Akita (1984a).

‡ Mason & Claus (1989) classified strain IFO 3297b as *G. asaii*.

NT, Not tested.

Requirement for nicotinic acid

G. cerinus IFO 3267^T, *G. asaii* IFO 3276^T, IFO 3275 and IFO 3265 and *G. frateurii* IFO 3264^T did not require nicotinic acid for growth, whereas *G. oxydans* IFO 14819^T required nicotinic acid (Table 3). *G. cerinus* IAM 1832 did not grow with nicotinic acid and was assumed to require other growth factors, as suggested by Gosselé *et al.* (1983). The requirement for nicotinic acid is employed for the differentiation of members of *G. oxydans* from those of other species in the genus *Gluconobacter* (Yamada & Akita, 1984a; Mason & Claus, 1989). However, an exception should be recognized in the case of *G. cerinus* IAM 1832, which did not grow with nicotinic acid.

Acid production from ethanol

Strain IAM 1832 did not produce acid from ethanol, as reported by Asai *et al.* (1964) and Gosselé *et al.* (1983).

Oxidation of acetate and lactate

None of the *Gluconobacter* strains tested oxidized either acetate or lactate. *Acetobacter* sp. IFO 3297 oxidized lactate but not acetate (Table 3).

Synonymous relationship between *G. cerinus* and *G. asaii*

As mentioned above, the type strains of *G. cerinus* and *G. asaii* had high values of DNA–DNA similarity with each other. However, Sievers *et al.* (1995) reported that the type strains of *G. cerinus* and *G. asaii* were not located close to each other, and a significant difference was found in their phylogenetic trees. Accordingly, a question arose as to whether the locations of the two species are correct. Therefore, the sequences of *G. cerinus* IFO 3267^T and *G. asaii* IFO 3276^T were reinvestigated in this study. As mentioned above, the 16S rRNA sequences of these strains were identical.

Yamada & Akita (1984a) revived *G. cerinus*, with the type strain IFO 3267^T. Micales *et al.* (1985) reported three homology groups in the genus *Gluconobacter*, but they did not mention the DNA–DNA similarity between *G. cerinus* IFO 3267^T and each of members of homology group III. Four years later, Mason & Claus (1989) introduced a novel species, *G. asaii*, for homology group III of Micales *et al.* (1985). However, they did not determine DNA–DNA similarity between the type strains of *G. asaii* and *G. cerinus*. Mason & Claus (1989) should have determined the DNA–DNA similarity between the type strains of *G. asaii* and *G. cerinus* because the type strain of *G. cerinus* was available at that time. Furthermore, Mason & Claus (1989) did not present growth data on L-arabitol and *meso*-ribitol for *G. cerinus* IFO 3267^T.

From the viewpoint of modern bacterial systematics (Wayne *et al.*, 1987), bacterial species established without data on DNA–DNA similarity between the type strains of species in that genus should be re-examined. The present study clearly demonstrates that all of the strains received as *G. asaii*, except for strain IFO 3265, were reidentified as *G. cerinus* on the basis of DNA–DNA similarity, 16S rRNA gene sequence and phenotypic characteristics. It is clear from the results obtained in this study that *G. cerinus* and *G. asaii* are synonymous. *G. cerinus* Yamada and Akita 1984 has priority over *G. asaii* Mason and Claus 1989, and *G. asaii* is therefore a junior subjective synonym of *G. cerinus*.

G. cerinus IAM 1832

Asai *et al.* (1964) and Gosselé *et al.* (1983) reported that this strain did not produce acid from ethanol. This was confirmed in this study. Interestingly, the nutritional requirements of this strain were found to be rather complex compared with other *G. cerinus* strains.

Segregation of colony types

G. asaii IFO 3276^T. *G. asaii* IFO 3276^T showed two colony types in this study, a large type and a small type, but the two colony types were identical in having high values for DNA–DNA similarity (data not shown). The same result was obtained by Mason & Claus (1989).

G. asaii IFO 3275. Micales *et al.* (1985) reported two colony types for *G. asaii* IFO 3275. Furthermore, they placed strain IFO 3275a in homology group I (= *G. oxydans*) and strain IFO 3275b in homology group III (= *G. asaii*) (Micales *et al.*, 1985; Mason & Claus, 1989). In this study, only a single colony type was recognized for strain IFO 3275 received from the Institute for Fermentation (IFO), Osaka, Japan, and this strain was identified as *G. cerinus* on the basis of DNA G+C content, DNA–DNA similarity and growth on pentitols.

Acetobacter sp. IFO 3297. Micales *et al.* (1985) recognized two colony types for *Acetobacter* sp. IFO 3297, and strains with different colony types were identified as *Gluconobacter* species. Strain IFO 3297a was placed in homology group III (= *G. asaii*) and strain IFO 3297b was placed in homology group I (= *G. oxydans*) (Micales *et al.*, 1985; Mason & Claus, 1989). In this study, a single colony type was found for *Acetobacter* sp. IFO 3297 received from the IFO, and the strain was reidentified as an *Acetobacter* species on the basis of the presence of Q-9 and the oxidation of lactate. This strain was in fact located within a cluster composed of *Acetobacter pasteurianus* and *Acetobacter pomorum* in a phylogenetic tree based on the partial 16S rRNA gene sequence (positions 62–611, 494 bases; data not shown). It is interesting to note that this strain oxidized lactate but not acetate. We tried to obtain the same strains employed by Micales *et al.* (1985) and Mason & Claus (1989), but their strains were not available.

Reclassification of *G. asaii* IFO 3265

Micales *et al.* (1985) placed *G. asaii* IFO 3265 in homology group III, and Mason & Claus (1989) classified it as *G. asaii*. However, this strain showed a very high value for DNA–DNA similarity (97%) with *G. frateurii* IFO 3264^T and grew well on L-arabitol and meso-ribitol, as did *G. frateurii* IFO 3264^T. The data obtained in this study indicate that *G. asaii* IFO 3265 should be reclassified as *G. frateurii*.

Emended description of *Gluconobacter cerinus* Yamada and Akita 1984

Junior subjective synonym: *Gluconobacter asaii* Mason and Claus 1989.

The description of *Gluconobacter cerinus* is based on the original description (Yamada & Akita, 1984a), with the additional information that there is no growth on L-arabitol or meso-ribitol. The type strain of *G. cerinus* is IFO 3267^T.

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REFERENCES

- Asai, T. & Shoda, K. (1958). The taxonomy of *Acetobacter* and allied oxidative bacteria. *J Gen Appl Microbiol* **4**, 289–311.
- 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.
- Brosius, J., Dull, T. J., Sleeter, D. D. & Noller, H. F. (1981). Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* **148**, 107–127.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gosselé, F., Swings, J., Kersters, K. & De Ley, J. (1983). Numerical analysis of phenotypic features and protein gel electropherograms of *Gluconobacter* Asai 1935 emend. mut. char. Asai, Iizuka, and Komagata 1964. *Int J Syst Bacteriol* **33**, 65–81.
- Katsura, K., Kawasaki, H., Potacharoen, W., Saono, S., Seki, T., Yamada, Y., Uchimura, T. & Komagata, K. (2001). *Asaia siamensis* sp. nov., an acetic acid bacterium in the α -Proteobacteria. *Int J Syst Evol Microbiol* **51**, 559–563.
- 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.
- Marmur, J. (1961). A procedure for isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.
- Mason, L. M. & Claus, G. W. (1989). Phenotypic characteristics correlated with deoxyribonucleic acid sequence similarities for three species of *Gluconobacter*: *G. oxydans* (Henneberg 1897) De Ley 1961, *G. frateurii* sp. nov., and *G. asaii* sp. nov. *Int J Syst Bacteriol* **39**, 174–184.
- Micales, B. K., Johnson, J. L. & Claus, G. W. (1985). Deoxyribonucleic acid homologies among organisms in the genus *Gluconobacter*. *Int J Syst Bacteriol* **35**, 79–85.
- Saito, H. & Miura, K. (1963). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim Biophys Acta* **72**, 619–629.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sievers, M., Gaberthüel, C., Boesch, C., Ludwig, W. & Teuber, M. (1995). Phylogenetic position of *Gluconobacter* species as a coherent cluster separated from all *Acetobacter* species on the basis of 16S ribosomal RNA sequences. *FEMS Microbiol Lett* **126**, 123–126.
- Skerman, V. B. D., McGowan, V. & Sneath, P. H. A. (editors) (1980). Approved lists of bacterial names. *Int J Syst Bacteriol* **30**, 225–420.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed phase high performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tamaoka, J., Katayama-Fujimura, Y. & Kuraishi, H. (1983). Analysis of bacterial menaquinone mixtures by high-performance liquid chromatography. *J Appl Bacteriol* **54**, 31–36.
- Tanaka, M., Murakami, S., Shinke, R. & Aoki, K. (1999). Reclassification of the strains with low G+C contents of DNA belonging to the genus *Gluconobacter* Asai 1935 (*Acetobacteraceae*). *Biosci Biotechnol Biochem* **63**, 989–992.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity progressive multiple sequence alignment

through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Yamada, Y. & Akita, M. (1984a). An electrophoretic comparison of enzymes in strains of *Gluconobacter* species. *J Gen Appl Microbiol* **30**, 115–126.

Yamada, Y. & Akita, M. (1984b). *Gluconobacter cerinus*. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 16. *Int J Syst Bacteriol* **34**, 503–504.

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 *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., Hosono, R., Lisdiyanti, P., Widyastuti, Y., Saono, S., Uchimura, T. & Komagata, K. (1999). Identification of acetic acid bacteria isolated from Indonesian sources, especially of isolates classified in the genus *Gluconobacter*. *J Gen Appl Microbiol* **45**, 23–28.

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.