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 3267T and IAM 1832 and G. asaii IFO 3276T 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 3264T and Gluconobacter oxydans IFO 14819T. G. cerinus IFO 3267T and G. asaii IFO 3276T 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 3264T and low similarity values (each 32%) to G. cerinus IFO 3267T and G. asaii IFO 3276T. 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
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; Gossele et al., 1983). An additional strain, IFO 3297, received as *Acetobacter* sp., was also used, since Micales et al. (1985) had reported a colony type 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% d-glucose, 1% yeast extract, 0.7% CaCO$_3$ 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 CO$_2$ and H$_2$O 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 48-9°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 using the medium containing 0.2% pentitol (L-arabitol or meso-ribitol), 0.3% yeast extract (Difco), 0.1% (NH$_4$)$_2$SO$_4$, 0.01% K$_2$HPO$_4$, 0.09% KH$_2$PO$_4$, 0.025% MgSO$_4$ . 7H$_2$O and 0.005% FeCl$_3$. 6H$_2$O (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) (NH$_4$)$_2$SO$_4$, 100 mg K$_2$HPO$_4$, 900 mg KH$_2$PO$_4$, 250 mg MgSO$_4$. 7H$_2$O and 50 mg FeCl$_3$. 6H$_2$O 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; panthothenic 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Received as:</th>
<th>Used by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This study</td>
<td>Gosselé et al. (1983)</td>
</tr>
<tr>
<td>IFO 3267^T</td>
<td>G. cerinus</td>
<td>+</td>
</tr>
<tr>
<td>IAM 1832</td>
<td>G. cerinus</td>
<td>+</td>
</tr>
<tr>
<td>IFO 3276^T</td>
<td>G. asaii</td>
<td>+</td>
</tr>
<tr>
<td>IFO 3275</td>
<td>G. asaii</td>
<td>+</td>
</tr>
<tr>
<td>IFO 3265</td>
<td>G. asaii</td>
<td>+</td>
</tr>
<tr>
<td>IFO 3264^T</td>
<td>G. frateurii</td>
<td>+</td>
</tr>
<tr>
<td>IFO 14819^T</td>
<td>G. oxydans</td>
<td>+</td>
</tr>
<tr>
<td>IFO 3297</td>
<td><em>Acetobacter</em> sp.</td>
<td>+</td>
</tr>
</tbody>
</table>
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 *Acidiphilium cryptum* (accession no. AB063287) and those reported by *IFO 3276* G (1995). Discrepancies were found in the sequence of strains differed from those in the tree of Sievers et al. (1995). Discrepancies were found in the sequence of

Four other bases, at positions 580, 589, 629 and 645, 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, 33463T was used as an outgroup. Numerals at nodes indicate bootstrap values derived from 1000 replications (Felsenstein, 1985).

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

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

(values for DNA–DNA similarity among *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$^T$.

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Received as:</th>
<th>DNA G + C content (mol%)</th>
<th>Relative binding (%) to DNA from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1. IFO 3267$^T$</td>
<td><em>G. cerinus</em></td>
<td>56</td>
<td>100</td>
</tr>
<tr>
<td>2. IAM 1832</td>
<td><em>G. cerinus</em></td>
<td>55</td>
<td>83</td>
</tr>
<tr>
<td>3. IFO 3276$^T$</td>
<td><em>G. asaii</em></td>
<td>55</td>
<td>77</td>
</tr>
<tr>
<td>4. IFO 3275</td>
<td><em>G. asaii</em></td>
<td>54</td>
<td>70</td>
</tr>
<tr>
<td>5. IFO 3265</td>
<td><em>G. asaii</em></td>
<td>56</td>
<td>32</td>
</tr>
<tr>
<td>6. IFO 3264$^T$</td>
<td><em>G. frateurii</em></td>
<td>55</td>
<td>31</td>
</tr>
<tr>
<td>7. IFO 14819$^T$</td>
<td><em>G. oxydans</em></td>
<td>60</td>
<td>17</td>
</tr>
</tbody>
</table>

NT, Not tested.

**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).

**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-arabinitol nor on meso-riboitol, but *G. asaii* IFO 3265 and *G. frateurii* IFO 3264$^T$ grew well on these pentitols (Table 3).


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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Received as:</th>
<th>Growth on:</th>
<th>Growth without nicotinic acid</th>
<th>Oxidation of:</th>
<th>Major quinone</th>
<th>Identified as:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>meso-Ribitol</td>
<td>1-Arabitol</td>
<td>Acetate</td>
<td>Lactate</td>
<td>Q-10</td>
</tr>
<tr>
<td>IFO 3267T</td>
<td>G. cerinus</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IAM 1832</td>
<td>G. cerinus</td>
<td>–</td>
<td>–</td>
<td>–*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IFO 3276T</td>
<td>G. asaii</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IFO 3275</td>
<td>G. asaii</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IFO 3265</td>
<td>G. frateurii</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IFO 3264T</td>
<td>G. frateurii</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IFO 14819T</td>
<td>G. oxydans</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IFO 3297†</td>
<td>Acetobacter sp.</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

* Other growth factors are required by this strain.  
† Data from Yamada & Akita (1984a).  
‡ Mason & Claus (1989) classified strain IFO 3297 as G. asaii.  
NT, Not tested.

Requirement for nicotinic acid

G. cerinus IFO 3267T, G. asaii IFO 3276T, IFO 3275 and IFO 3265 and G. frateurii IFO 3264T did not require nicotinic acid for growth, whereas G. oxydans IFO 14819T 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 Gossele 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 noted 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 Gossele 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 3267T and G. asaii IFO 3276T 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 3267T. 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 3267T 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 3267T.

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 Gossele 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<sup>T</sup>*. *G. asaii IFO 3276<sup>T</sup>* 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 3275<sup>a</sup> in homology group I (= *G. oxydans*) and strain IFO 3275<sup>b</sup> 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 3297<sup>a</sup> was placed in homology group III (= *G. asaii*) and strain IFO 3297<sup>b</sup> 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<sup>T</sup> and grew well on L-arabitol and meso-ribitol, as did *G. frateurii* IFO 3264<sup>T</sup>. 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*


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<sup>T</sup>.

**ACKNOWLEDGEMENTS**

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