Gluconacetobacter tumulicola sp. nov. and *Gluconacetobacter asukensis* sp. nov., isolated from the stone chamber interior of the Kitora Tumulus

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Six Gram-negative, rod-shaped, non-spore-forming bacterial strains were isolated from small holes on plaster walls of the stone chamber interior of the Kitora Tumulus in Asuka village, Nara Prefecture, Japan. These were investigated by means of a polyphasic approach. All the isolates were strictly aerobic and motile by peritrichous flagella. Phylogenetic trees generated based on 16S rRNA gene sequences identified two novel lineages (comprising five isolates and one isolate, respectively) within the genus *Gluconacetobacter*. The isolates were characterized by having Q-10 as the major ubiquinone system and C_{18:1}ω7c (58.7–63.1 % of the total) as the predominant fatty acid. DNA–DNA hybridization experiments endorsed the species rank for the two lineages, for which the names *Gluconacetobacter tumulicola* sp. nov. (type strain K5929-2-1b^T=JCM 17774^T=NCIMB 14760^T) and *Gluconacetobacter asukensis* sp. nov. (type strain K8617-1-1b^T=JCM 17772^T=NCIMB 14759^T) are proposed.

The Kitora Tumulus, which is located in Asuka-mura (Asuka village), Nara Prefecture, Japan, was built between the late 7th and early 8th centuries. It is well known to the people of Japan as a Special Historic Site with polychrome mural paintings. It was discovered in November 1983 and excavated in January/February 2004. At the time of its discovery, the wall paintings were beautiful and vivid. However, cracks on the plaster were obvious and some paintings were loosely held to the rock. The Agency for Cultural Affairs, Japan, decided to relocate the paintings in September 2004; all the paintings on the sidewalls and the star charts on the ceiling were relocated to a controlled environment by the end of 2008. During the relocation, the growth of micro-organisms on the surface of the wall paintings became obvious (Kigawa *et al.*, 2005). In early 2004, white moulds occurred on the surface of the murals. In early 2005, small colonies of viscous gel appeared on

some parts of the inside walls. The formation of biofilms was subsequently confirmed on the surface of the plaster walls of the mural paintings. After the summer of 2005, micro-organism contamination of the stone chamber increased, with gelatinous or viscous gel materials partly covering the plaster wall surface. These biofilms were mainly composed of bacteria, moulds and yeasts. In particular, biofilms had impregnated the surface of the plaster around the ceiling. A few small holes on the plaster walls were confirmed in the stone chamber interior in September 2005 (photos 14 and 15 in Kigawa *et al.*, 2006). These small holes were filled with black gel or viscous materials and increased in size with time (Kigawa *et al.*, 2006).

Initially, common, aerobic micro-organisms were the target for isolation. We isolated a range of micro-organisms, which included moulds, yeasts and bacteria, from viscous gel samples and made preliminary identifications (Kigawa *et al.*, 2006, 2009; Sugiyama *et al.*, 2009).

Among the bacterial isolates, we obtained six isolates (K5929-2-1b^T, K8617-1-1b^T, K5929-1-1b, K8617-3-4b, K8617-7-3b and K5929-3-1b) of acetic acid bacteria that

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains K5929-2-1b^T and K8617-1-1b^T are AB627116 and AB627120, respectively.

One supplementary figure and two supplementary tables are available with the online version of this paper.

were phylogenetically and phenotypically related to known species of the genus *Gluconacetobacter*. Navarro & Komagata (1999) and Yamada & Yukphan (2008) have shown that *Gluconacetobacter* could be divided into two groups, i.e. the *Gluconacetobacter liquefaciens* group (Asai, 1935) – including the type species of the genus – and the *Gluconacetobacter xylinus* group (Brown, 1886; Yamada *et al.*, 1998), on the basis of DNA G + C content, quinone system and 16S rRNA gene sequence divergence.

In this study, we describe six isolates of acetic acid bacteria that can be assigned to two novel lineages in the *G. liquefaciens* group within the genus *Gluconacetobacter*. Implications for the biodeterioration of mural paintings and plaster by the strains described will be discussed elsewhere by R. Kigawa and others.

The six acetic acid bacterial strains were isolated on nutrient agar (CM3; Oxoid) or on medium for isolation of acetic acid bacteria (Yamada, 1986), which consisted of 10 g glucose, 5 ml ethanol, 5 g yeast extract, 3 g peptone, 5 g CaCO₃ and 15 g agar in 900 ml distilled water and 10 % (v/v) potato extract (pH unadjusted). After incubation for 3 days or more at 30 °C, acetic acid bacteria were selected from the plates, where they dissolved the calcium carbonate (CaCO₃), resulting in clear zones around the colonies. These isolates were subsequently maintained on GYC agar plates (De Ley *et al.*, 1984) comprising (per litre distilled water): 5 g glucose, 1 g yeast extract, 3 g CaCO₃ and 2.5 g agar (pH unadjusted). *G. liquefaciens* NBRC 12388^T, *Gluconacetobacter diazotrophicus* DSM 5601^T, *Gluconacetobacter sacchari* DSM 12717^T, *Gluconacetobacter johannae* DSM 13595^T and *Gluconacetobacter azotocaptans* DSM 13594^T were used as reference strains. Details of all strains used in this study are given in Table S1, available in IJSEM Online. The reference strains were cultured according to the recommendations of the respective suppliers.

Genomic DNA was extracted by using the InstaGene Matrix kit (Bio-Rad) according to the manufacturer's instructions. PCR was achieved with PrimeSTAR HS DNA Polymerase (TakaraBio). Thermal cycling was performed by using a GeneAmp PCR System 9600 (Applied Biosystems). Sequencing reactions were performed with a BigDye Terminator v 3.1 Cycle Sequencing kit (Applied Biosystems). Primers used included 9F, 339F, 785F, 1099F, 536R, 802R, 1242R and 1510R (Nakagawa *et al.*, 2002; Uchino *et al.*, 1997). Sequences were determined by using an ABI 3100 DNA sequencer (Applied Biosystems). The respective 16S rRNA gene sequences determined were compared with those retrieved from GenBank/EMBL/DBJ by BLAST searches (Altschul *et al.*, 1997). The sequences of the isolated strains were aligned with those of closely related species from these databases by using CLUSTAL W (Thompson *et al.*, 1994) and were edited by eye with the BioEdit program (Hall, 1999) to exclude ambiguous positions and alignment gaps from the analysis. Levels of sequence similarity were calculated and a neighbour-joining

evolutionary distance tree (Saitou & Nei, 1987) was reconstructed by using MEGA ver3.1 (Kumar *et al.*, 2004).

The 16S rRNA gene sequences of strains K5929-2-1b^T and K8617-1-1b^T determined (1453 bp) were compared with those retrieved from public databases. The sequences of strains K5929-2-1b^T, K5929-1-1b, K8617-3-4b, K8617-7-3b and K5929-3-1b were related most closely to those of *G. liquefaciens* NBRC 12388^T (99.0 % similarity), *G. sacchari* SRI 1794^T (98.4 %) and *G. diazotrophicus* ATCC 49037^T (98.7 %). In contrast, strain K8617-1-1b^T was related most closely to *G. liquefaciens* NBRC 12388^T (98.8 %), *G. sacchari* SRI 1794^T (98.8 %) and *G. diazotrophicus* ATCC 49037^T (98.6 %). The level of 16S rRNA gene sequence similarity between strain K5929-2-1b^T and strains K5929-1-1b, K8617-3-4b, K8617-7-3b and K5929-3-1b was 100 %, whereas that between strains K8617-1-1b^T and K5929-2-1b^T was 99.5 %. Levels of 16S rRNA gene sequence similarity between the novel isolates and representatives of other genera in the family *Acetobacteraceae* were lower than 97 %. The neighbour-joining phylogenetic trees depicted in Fig. 1 and Fig. S1, and strains K5929-2-1b^T (and K5929-1-1b, K8617-3-4b, K8617-7-3b and K5929-3-1b) and K8617-1-1b^T were included in a major cluster containing *G. liquefaciens*, the type species of the genus, with 92 % bootstrap support (Fig. S1), but formed an independent subgrouping on their own that was tightly clustered away from the type species. The six novel isolates could be divided phylogenetically into two groups, represented by strains K5929-2-1b^T and K8617-1-1b^T, respectively. Recently, the novel genus *Ameyamaea* (Yukphan *et al.*, 2009) was proposed in the family *Acetobacteraceae*. According to these authors, this genus is related phylogenetically to the genera *Gluconacetobacter* and *Tanticharoenia* (Yukphan *et al.*, 2008). However, the Kitora Tumulus isolates could clearly be differentiated from these two genera based on the results of phylogenetic analysis and levels of 16S rRNA gene sequence similarity.

Cell shape was observed after growth on mannitol agar medium, containing (w/v): 2.5 % mannitol, 0.5 % yeast extract, 0.3 % peptone and 2.0 % agar, adjusted to pH 6.0 (Asai *et al.*, 1964), at 30 °C for 24 h. Gram staining was carried out by using a Favour G Gram-stain kit (Nissui) according to the manufacturer's instructions. Motility and flagellation were observed with cells cultured on agar slants for 18–24 h at 20 °C. The arrangement of flagella was observed with a JEM-2000EX transmission electron microscope (JEOL) and flagella were negatively stained with uranyl acetate at 100 kV. Physiological and biochemical characterization was performed according to the methods described by Asai *et al.* (1964), Yamada *et al.* (1976) and Swings *et al.* (1992).

Cells of strains K5929-2-1b^T and K8617-1-1b^T were Gram-negative, rod-shaped, aerobic, asporogenous, and motile by means of peritrichous flagella (Fig. 2). They were catalase-positive and oxidase-negative. Strain K5929-2-1b^T produced a water-soluble brown pigment on GYC agar,

Fig. 1. Neighbour-joining phylogenetic tree based on the nearly complete 16S rRNA gene sequences of the six novel isolates and the type strains of recognized species of the genus *Gluconacetobacter*. Bootstrap values (percentages of 1000 replications) of >50% are shown at the respective branch points. *Acetobacter acetii* NCIB 8621^T was used as an outgroup. Bar, 5 changes per 1000 nt positions. An expanded version of this tree is shown in Fig. S1.

Fig. 2. Transmission electron micrographs of cells of strains K5929-2-1b^T (a) and K8617-1-1b^T (b), both revealing peritrichous flagella. The two isolates were cultivated on mannitol agar at 20 °C for 18 h. Bars, 1.0 μm.

whereas strain K8617-1-1b^T did not. Growth occurred at 15–30 °C but not at 37 °C (optimum 20–30 °C). The pH range for growth was 3.5–6.8. The isolates were capable of acid formation from both glucose and ethanol. Both sodium acetate and sodium lactate were oxidized. Growth was observed on glutamate agar and mannitol agar, but not in 10% ethanol. Strains K5929-2-1b^T and K8617-1-1b^T grew on LGI medium (Dutta & Gachhui, 2007) at 30 °C, which contained no combined nitrogen.

Characteristics that differentiate the two proposed novel species from each other and from their closest phylogenetic neighbours are detailed in Table 1. Strain K5929-2-1b^T could be distinguished from strain K8617-1-1b^T by the production of a water-soluble brown pigment on GYC agar, the ability to grow on 0.1% sorbitol and *myo*-inositol as sole carbon sources, ketogenesis from glycerol and the ability to produce 2,5-diketogluconic acid from D-glucose. Strain K5929-2-1b^T could be distinguished from *G. liquefaciens* based on the ability to produce 5-ketogluconic acid from D-glucose. Strain K8617-1-1b^T could be distinguished from *G. liquefaciens* by the ability to produce a water-soluble brown pigment on GYC agar, and the ability to produce 5-ketogluconic acid and 2,5-diketogluconic acid from D-glucose (Table 1).

Fatty acid profiles were determined from cells of the six novel isolates and five reference strains grown for 48 h at 30 °C under aerobic conditions on DSM medium 105, *Gluconobacter oxydans* medium (per litre: 100 g glucose, 10 g yeast extract, 20 g CaCO₃ and 15 g agar; adjusted to

Table 1. Differential characteristics of strains K5929-2-1b^T and K8617-1-1b^T and their phylogenetically nearest relatives in the genus *Gluconacetobacter*

Taxa: 1, strain K5929-2-1b^T; 2, strain K8617-1-1b^T; 3, *G. liquefaciens*; 4, *G. diazotrophicus*; 5, *G. sacchari*; 6, *G. johannae*; 7, *G. azotocaptans*. All taxa shown were: motile with peritrichous flagella (only data for the novel isolates are from the present study); negative for growth on 10% ethanol; catalase-positive and oxidase-negative; oxidized acetate and lactate to CO₂ and water; positive for acid production from glucose and ethanol; negative for acid production from D-mannitol and sorbitol; positive for growth on glutamate agar and mannitol agar; positive for growth on ethanol as sole carbon source; negative for growth on methanol as sole carbon source; and negative for formation of H₂S. The major fatty acid and ubiquinone in all taxa were C_{18:1}ω7c and Q-10, respectively. Data for all strains were obtained in this study under the same conditions except where indicated. ND, No data.

Characteristic	1	2	3	4	5	6	7
Production of:							
Water-soluble brown pigment on GYC plates	+	–	+*	+†	+‡	+§	+§
γ-Pyrones from D-fructose	+	+	+	+	+	–	–
Ketogenesis from glycerol	+	–	+	–	–	–	–
5-Ketogluconic acid from D-glucose	–	–	+	–	+	–	–
2-Ketogluconic acid from D-glucose	+	+	+	+	+	–	–
2,5-Diketogluconic acid from D-glucose	+	–	+	+	+	+	+
Growth on N-free media	+	+	–	+	ND	+	+
Acid production from 1-propanol	+	+	+	+	+	–	–
Growth on sole carbon sources							
Sodium acetate	+	+	–	+	+	+	–
D-Sorbitol	+	–	–	+	–	+	+
myo-Inositol	+	–	–	–	–	–	–
DNA G + C content (mol%)	64.7	65.4	64.6	66	66	58§	64§

*Data from Yamada *et al.* (1997).

†Data from Gillis *et al.* (1989).

‡Data from Franke *et al.* (1999).

§Data from Fuentes-Ramírez *et al.* (2001).

pH 6.8, see http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium105.pdf). Cellular fatty acid methyl esters were prepared and analysed by GC (HP6890 series GC system; Hewlett Packard) according to the instructions given for the MIDI Microbial Identification system version 4.5, and profiles were compared with the database in the Sherlock System 4.5 (Microbial ID; MIDI).

For analyses of respiratory quinones, cells were grown in medium containing (w/v): 2.5 % mannitol, 0.5 % yeast extract and 0.3 % peptone. Cultures were shaken for 72 h at 28 °C. Quinone extraction and analysis were carried out as described previously (Nishijima *et al.*, 1997).

Cellular fatty acid analysis of strains K5929-2-1b^T and K8617-1-1b^T showed that C_{18:1}ω7c was the most abundant component (58.7–63.1 % of the total) (Table 2). Analysis of the respiratory quinones revealed that both strains K5929-2-1b^T and K8617-1-1b^T contained ubiquinones, with Q-10 as the major quinone (accounting for >90 %) and Q-9 as a minor component (<10 %). These are common characteristics of strains belonging to the genus *Gluconacetobacter* (Yamada *et al.*, 1969; Yamada & Yukphan, 2008). The reference type strains of *Gluconacetobacter* species used in this study had C_{18:1}ω7c and Q-10 as major components.

For determination of the G + C content of the genomic DNA and for DNA–DNA hybridization experiments, the genomic DNA of the six novel isolates and of the type strains of the three most closely related species, i.e. *G. liquefaciens* NBRC 12388^T, *G. diazotrophicus* DSM 5601^T and *G. sacchari* DSM 12717^T, was extracted and purified according to the combined methods of Marmur (1961) and Saito & Miura (1963). The genomic DNA was digested to nucleotides by nuclease P1, by using a DNA-GC kit (Seikagaku Kogyo) according to the procedures described by Katayama-Fujimura *et al.* (1984). The G + C content of the DNA was determined by HPLC (LC-10; Shimadzu) with an RP Aqueous column (4.6 × 250 mm; Nomura Chemical) and a UV-VIS spectrophotometric detector (SPD-10AV; Shimadzu) at 270 nm. Levels of DNA–DNA relatedness were determined by the fluorometric hybridization method on a microdilution plate (Ezaki *et al.*, 1989) at 52 °C in the presence of 50 % formamide.

The results of the DNA G + C content determination and DNA–DNA hybridization experiments are shown in Table S2. The DNA G + C contents of the six novel isolates were in the range 64.7–65.4 mol%. These values are consistent with the G + C contents of the type strains of *G. liquefaciens*, *G. diazotrophicus* and *G. sacchari* determined herein

Table 2. Cellular fatty acid compositions of the novel strains and the type strains of closely related *Gluconacetobacter* species

Strains: 1, K5929-2-1b^T; 2, K5929-1-1b; 3, K5929-3-1b; 4, K8617-3-4b; 5, K8617-7-3b; 6, K8617-1-1b^T; 7, *G. liquefaciens* NBRC 12388^T; 8, *G. diazotrophicus* DSM 5601^T; 9, *G. sacchari* DSM 12717^T; 10, *G. azotocaptans* DSM 13594^T; 11, *G. johannae* DSM 13595^T. The content of each fatty acid is expressed as a percentage of the total. Fatty acids present at lower than 0.5% in all strains are not shown; —, not detected. Data were obtained in this study by using the same cultivation conditions (DSM medium 105 containing 10% glucose at 30 °C for 2 days) and the same analytical method (MIDI system).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
Saturated											
C _{12:0}	—	—	—	—	—	0.6	2.1	1.2	1.1	1.3	0.9
C _{14:0}	3.0	4.9	3.7	2.9	2.6	4.1	4.1	4.5	2.2	3.1	3.3
C _{16:0}	11.2	15.8	11.2	11.5	9.8	9.3	8.4	7.4	9.2	14.0	10.5
C _{17:0}	—	—	0.5	—	—	0.6	—	—	—	—	0.3
C _{18:0}	3.4	0.8	3.2	3.7	5.2	3.4	2.3	1.4	1.4	1.2	1.4
Unsaturated											
C _{18:1ω7c}	62.8	58.7	61.3	62.4	62.0	63.1	62.6	64.3	63.8	60.7	63.7
Hydroxylated											
C _{14:0} 2-OH	5.5	6.8	5.3	5.2	4.8	4.5	4.3	5.5	5.8	5.9	6.3
C _{16:0} 2-OH	7.1	4.7	7.7	7.0	7.2	6.3	7.7	8.4	8.1	5.3	5.5
C _{18:1} 2-OH	0.9	—	1.2	0.7	—	2.2	—	0.9	1.8	—	0.8
C _{16:0} 3-OH	2.8	2.9	3.1	2.9	3.0	2.7	3.3	3.0	3.4	3.4	3.4
C _{18:0} 3-OH	0.9	0.6	1.0	1.1	1.3	0.9	1.3	0.9	1.1	1.2	1.2
C _{19:0ω8c} cyclo	1.4	1.9	0.8	1.5	2.9	0.6	2.1	1.4	0.9	2.2	1.1
Summed features*											
2	1.0	1.2	1.1	1.1	1.2	1.2	1.8	1.1	1.3	1.1	1.4
3	—	1.9	—	—	—	—	—	—	—	0.8	0.4

*Summed features represent two or more components that cannot be separated by GLC with the MIDI system. Summed feature 2 comprises iso-C_{16:1} I and/or C_{14:0} 3-OH; summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1 ω 7c}.

and with published data (Yamada *et al.*, 1997; Gillis *et al.*, 1989; Franke *et al.*, 1999). Reciprocal hybridization experiments were performed with the six novel isolates and the type strains of *G. liquefaciens*, *G. diazotrophicus* and *G. sacchari*. In each assay, each combination was tested three times and mean percentages are indicated in Table S2. Levels of DNA–DNA relatedness obtained were greater than 80% between each combination of the novel isolates except strain K8617-1-1b^T. The level of DNA–DNA relatedness between strains K5929-2-1b^T and K8617-1-1b^T was below 51%. Levels of DNA–DNA relatedness of $\leq 28\%$ were found between strain K5929-2-1b^T and each of the type strains of *G. liquefaciens*, *G. diazotrophicus* and *G. sacchari*, and also below 21% between strain K8617-1-1b^T and each of the type strains of *G. liquefaciens*, *G. diazotrophicus* and *G. sacchari*. These values clearly indicated that strains K5929-2-1b^T and K8617-1-1b^T represented two novel genospecies assignable to the genus *Gluconacetobacter* based on the 70% recommended guideline for species delineation (Wayne *et al.*, 1987).

Polychrome mural paintings of both Takamatsuzuka and Kitora Tumuli were drawn directly onto thin calcium carbonate plaster in the stone chambers (e.g. Kigawa *et al.*, 2009). Many members of the *G. liquefaciens* group have been isolated from plant material such as fruits, flowers, sugar cane and coffee plants, whereas strains of the *G. xylinus* group have largely been isolated from fermented foods such as vinegar, nata de coco (fermented coconut water) and fermented teas (Yamada & Yukphan, 2008). There is no record of isolation of acetic acid bacteria from ancient buildings, particularly mural paintings or plaster walls. The isolation of *Gluconacetobacter* bacteria in this study is thus of note. In addition, our previous experiments (Kigawa *et al.*, 2009) revealed that strain K5929-2-1b^T dissolved calcium carbonate and produced a water-soluble brownish pigment in GYC medium. Other experiments (Sano *et al.*, 2010) have also shown that production of acetic acid by these six *Gluconacetobacter* isolates (170–2200 mg l^{−1}) was higher than that exhibited by *G. diazotrophicus* DSM 5601^T (90 mg l^{−1}). As a result, these isolates caused a large decrease in pH (i.e. by up to 3.85 pH units). These data suggest that *Gluconacetobacter* species are involved in the biodeterioration of mural paintings and plaster. Further discussions will be made elsewhere by R. Kigawa and others.

In conclusion, the results of our polyphasic systematic approach support the recognition of two novel species of the genus *Gluconacetobacter* (*Acetobacteraceae*, *Rhodospirillales*, *Alphaproteobacteria*), for which we propose the names *Gluconacetobacter tumulicola* sp. nov. and *Gluconacetobacter asukensis* sp. nov.

Description of *Gluconacetobacter tumulicola* sp. nov.

Gluconacetobacter tumulicola [tu.mu.li'co.la. L. n. *tumulus* tumulus or tomb; L. suff. *-cola* (from L. n. *incola*) dweller; N.L. n. *tumulicola* tumulus dweller].

Cells are Gram-negative rods measuring 0.7–0.8 × 1.2–2.0 µm and are motile by peritrichous flagella. Strictly aerobic, catalase-positive and oxidase-negative. A water-soluble brown pigment is produced on GYC agar. Lactate and acetate are oxidized to CO₂ and H₂O. Acetic acid is not required for growth. Grows at pH 3.0–6.8 and at 15–30 °C (optimum 20–30 °C). Acid is produced from glucose, 1-propanol and ethanol, but not from D-mannitol or sorbitol. Ketogenesis occurs in the presence of glycerol. 2-Ketogluconic acid and 2,5-diketogluconic acid are produced from D-glucose, but 5-ketogluconic acid is not. γ -Pyrone is produced from D-fructose. Grows on glutamate agar and mannitol agar, but not on 10% ethanol. Growth occurs on 0.1% ethanol, sodium acetate, 1-propanol, meso-ribose, D-mannitol, sorbitol, myo-inositol, D-ribose, D-fructose, D-glucose, calcium DL-gluconate, sucrose and sodium DL-lactate as sole carbon sources, but not on methanol, L-arabitol, meso-xylitol, dulcitol, D-xylose, D-lyxose, D-fucose, L-arabinose, D-galactose, D-mannose, L-sorbose, cellobiose, maltose,

raffinose, dextrin, sodium malonate, sodium L-malate or sodium citrate. Growth occurs on nitrogen-free LGI medium at 30 °C. The ubiquinone system consists of Q-10 as the major component and Q-9 as a minor component. C_{18:1}ω7c is the major fatty acid.

The type strain, K5929-2-1b^T (=JCM 17774^T=NCIMB 14760^T), was isolated from a black viscous substance in a plaster hole at the centre of the ceiling in the stone chamber of the Kitora Tumulus in Asuka village, Nara Prefecture, Japan. The DNA G+C content of the type strain is 64.7 mol%. K5929-1-1b (=JCM 17773), K8617-3-4b (=JCM 17776), K8617-7-3b (=JCM 17777) and K5929-3-1b (=JCM 17775) are additional strains of the species.

Description of *Gluconacetobacter asukensis* sp. nov.

Gluconacetobacter asukensis (a.suk.en'sis. N.L. masc. adj. *asukensis* of or belonging to Asuka village, referring to the isolation of the type strain from Asuka village).

Cells are Gram-negative rods measuring 0.8–0.9 × 1.2–2.0 µm and are motile by peritrichous flagella. Strictly aerobic, catalase-positive and oxidase-negative. A water-soluble brown pigment is not produced on GYC agar. Lactate and acetate are oxidized to CO₂ and H₂O. Acetic acid is not required for growth. Grows at pH 3.0–6.8 and at 15–30 °C (optimum 20–30 °C). Acid is produced from glucose, 1-propanol and ethanol, but not from D-mannitol or sorbitol. Ketogenesis occurs in the presence of glycerol. 2-Ketogluconic acid is produced from D-glucose, but 5-ketogluconic acid and 2,5-diketogluconic are not. γ-Pyrone is produced from D-fructose. Grows on glutamate agar and mannitol agar, but not on 10 % ethanol. Growth occurs on 0.1 % ethanol, sodium acetate, 1-propanol, *meso*-ribitol, D-mannitol, D-ribose, D-fructose, D-glucose, calcium DL-gluconate, sucrose and sodium DL-lactate as sole carbon sources, but not on methanol, L-arabitol, *meso*-xylitol, sorbitol, *myo*-inositol, dulcitol, D-xylose, D-lyxose, D-fucose, L-arabinose, D-galactose, D-mannose, L-sorbose, cellobiose, maltose, raffinose, dextrin, sodium malonate, sodium L-malate or sodium citrate. Growth occurs on nitrogen-free LGI medium at 30 °C. The ubiquinone system consists of Q-10 as the major component and Q-9 as a minor component. C_{18:1}ω7c is the major fatty acid.

The type strain, K8617-1-1b^T (=JCM 17772^T=NCIMB 14759^T), was isolated from a brown viscous gel on the north-east area of the ceiling in the stone chamber of the Kitora Tumulus in Asuka village, Nara Prefecture, Japan. The DNA G+C content of the type strain is 65.4 mol%.

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