Description of *Gluconacetobacter sacchari* sp. nov., a new species of acetic acid bacterium isolated from the leaf sheath of sugar cane and from the pink sugar-cane mealy bug

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A new species of the genus *Gluconacetobacter*, for which the name *Gluconacetobacter sacchari* sp. nov. is proposed, was isolated from the leaf sheath of sugar cane and from the pink sugar-cane mealy bug, *Saccharicoccus sacchari*, found on sugar cane growing in Queensland and northern New South Wales, Australia. The nearest phylogenetic relatives in the α -subclass of the *Proteobacteria* are *Gluconacetobacter liquefaciens* and *Gluconacetobacter diazotrophicus*, which have 98.8–99.3% and 97.9–98.5% 16S rDNA sequence similarity, respectively, to members of *Gluconacetobacter sacchari*. On the basis of the phylogenetic positioning of the strains, DNA reassociation studies, phenotypic tests and the presence of the Q10 ubiquinone, this new species was assigned to the genus *Gluconacetobacter*. No single phenotypic characteristic is unique to the species, but the species can be differentiated phenotypically from closely related members of the acetic acid bacteria by growth in the presence of 0.01% malachite green, growth on 30% glucose, an inability to fix nitrogen and an inability to grow with the L-amino acids asparagine, glycine, glutamine, threonine and tryptophan when D-mannitol was supplied as the sole carbon and energy source. The type strain of this species is strain SRI 1794^T (= DSM 12717^T).

Keywords: *Gluconacetobacter sacchari*, taxonomy, phylogeny, acetic acid bacteria, pink sugar-cane mealy bug

INTRODUCTION

The acetic acid bacteria are Gram-negative, obligately aerobic, rod-shaped bacteria, the habitats and biology of which are relatively well-known, due to the considerable economic impact the bacteria have on industry (Swings, 1992). An extensive literature about these organisms has accumulated since the first description of acetic acid bacteria by Persoon (1822). Relationships among the acetic acid bacteria were first

studied genotypically by DNA–rRNA hybridization (Gillis & De Ley, 1980) and then by DNA–DNA hybridization (Yamada & Kondo, 1984; Entani *et al.*, 1985; Micales *et al.*, 1985; Urakami *et al.*, 1989; Sievers *et al.*, 1992). The first sequencing of rRNA genes was conducted on the 5S rRNA gene (Bulygina *et al.*, 1992) and, subsequently, a paper on the phylogenetic positioning of species of the genera *Acetobacter*, *Gluconobacter* and *Acidomonas* among acidophilic bacteria in the α -subclass of the *Proteobacteria*, based on comparative 16S rRNA gene sequence analysis, was published (Sievers *et al.*, 1994a). There have been several publications on the taxonomy of the acetic acid bacteria (Yamada & Kondo, 1984; Sievers *et al.*, 1994b; Yamada *et al.*, 1997). In 1984, Yamada & Kondo divided the members of *Acetobacter* at the subgeneric level on the basis of differences in their ubiquinone systems and introduced the new

The GenBank accession numbers for the 16S rRNA gene sequences of the strains reported in this study are: SRI 244, AF127391; SRI 1990, AF127392; SRI 1957, AF127393; SRI 1939, AF127394; SRI 1994, AF127395; SRI 1995, AF127396; SRI 1212, AF127397; SRI 1214, AF127398; SRI 1321, AF127399; SRI 1941, AF127400; SRI 1206, AF127401; SRI 1205, AF127402; IFR 101, AF127403; SRI 1951, AF127404; SRI 1230, AF127405; SRI 1216, AF127406; SRI 1794^T, AF127407; SRI 1853, AF127408; SRI 1255, AF127409; SRI 1953, AF127410; IF 9701, AF127411; IF 2-6, AF127412; and IF 9645, AF127413.

subgenus *Gluconoacetobacter* (ex Asai) Yamada and Kondo, with the type species *Gluconoacetobacter liquefaciens*, for organisms equipped with the Q10 ubiquinone. The Q9-equipped species were, meanwhile, accommodated in the subgenus *Acetobacter* of the genus *Acetobacter*. Recently, Yamada *et al.* (1997) proposed the elevation of the subgenus *Gluconoacetobacter* to the generic level, on the basis of partial base sequences of the 16S rRNA gene. The species containing the Q9 ubiquinone remain in the genus *Acetobacter*. The name *Gluconoacetobacter* was orthographically incorrect and has recently been corrected to *Gluconacetobacter* (Yamada *et al.*, 1998), in accordance with Rule 61 of the International Code of Nomenclature of Bacteria. The corrected name will be used throughout this paper.

This paper describes the finding of a new species of *Gluconacetobacter*, isolated from the leaf sheath of sugar cane and from the pink sugar-cane mealy bug, found in the leaf sheath of various sugar cane varieties growing in Queensland, Australia. The pink sugar-cane mealy bug, *Saccharicoccus sacchari* (Cockerell) (Homiptera: Pseudococcidae), is common to all sugar-cane-growing countries and is almost the exclusive species of mealy bug on commercially grown cane (Inkerman *et al.*, 1986). Our research was conducted to extend the work of Ashbolt & Inkerman (1990), who found that the pink sugar-cane mealy bug supports the growth of populations of bacteria appearing to resemble members of the *Acetobacteraceae*. Several strains were studied by Gillis *et al.* (1989) and identified as *Acetobacter diazotrophicus*. However, as we have been unable to detect nitrogen fixation in some mealy-bug isolates, we have undertaken studies to clarify the taxonomic position of these bacteria.

On the basis of 16S rRNA gene sequence information, phenotypic characteristics, fatty acid composition, DNA-DNA hybridization and past research, we conclude that the organisms isolated from the leaf sheath of sugar cane and from the pink sugar-cane mealy bug constitute a new species in the genus *Gluconacetobacter*, for which we propose the name *Gluconacetobacter sacchari* sp. nov.

METHODS

Bacterial strains and cultivation. The strains included in this study and their sources are listed in Table 1. These strains were grown and maintained on either glucose/yeast extract/calcium carbonate (GYC) agar (De Ley *et al.*, 1984), which consisted of 50 g glucose, 10 g yeast extract, 30 g calcium carbonate and 25 g agar l⁻¹ distilled water, or on WL nutrient agar (Oxoid), which contained 4 g yeast extract, 4 g tryptone, 50 g glucose, 0.55 g K₂HPO₄, 0.425 g KCl, 0.125 g CaCl₂, 0.125 g MgSO₄, 0.0025 g FeCl₃, 0.0025 g MnSO₄, 0.022 g bromocresol green and 15 g agar l⁻¹ distilled water. Cultivation was at 28 °C for 3–4 d.

Isolation of strains from mealy bugs. New bacterial strains to supplement an existing collection at the Sugar Research Institute, Mackay, Queensland, Australia (Table 1), were

isolated from the pink sugar-cane mealy bug (*Saccharicoccus sacchari*) found in the leaf sheath pocket of sugar cane from different sites throughout Queensland, Australia, over the period 1995–1997. Mealy bugs were placed in 1.5 ml 0.05% peptone water (pH 3) and homogenized using a sterile swab stick and vortexing. Serial dilutions of the homogenized suspension were made and samples were spread on WL nutrient agar plates adjusted to pH 3, which contained 100 mg cycloheximide l⁻¹ to inhibit fungal contamination. Colonies resembling *Gluconacetobacter* were selected from the isolation plates and isolated in pure culture.

Phenotypic characterization. GYC and WL nutrient agar plates were used for the pre-culture of bacterial strains, and cultures were incubated for 3–4 d at 28 °C prior to test inoculations. The formation of water-soluble brown pigments on GYC agar plates and the formation of hydrogen sulfide from L-cysteine were determined by the method of Swings *et al.* (1992). Bacterial motility was tested by growth through a Craigie tube in semi-solid medium (0.3% WL nutrient agar) and confirmed microscopically by motility in a hanging-drop preparation. Flagellar morphology was determined by transmission electron microscopy after negative staining for 30 s with 1% ammonium molybdate containing 0.003% bacitracin. The Gram stain reaction was conducted using the method of Skerman (1967), modified by the addition of 5% polyvinylpyrrolidone to the iodine reagent (bioMérieux). The oxidase test was performed using 1% (w/v) N,N,N',N'-tetramethyl 1,4-phenylenediamine hydrochloride (Kovacs, 1956) and confirmed using oxidase identification sticks (Oxoid). Catalase activity was detected by the production of O₂ from 3% H₂O₂. Glycerol ketogenesis was observed using the method and medium of Carr (1968). Nitrate reduction was tested from nitrate peptone water (10 g peptone, 2 g KNO₃ l⁻¹ distilled water; pH 7), using the method of Skerman (1967). The production of acid from sorbitol, D-mannitol and propanol was tested according to the method of Asai *et al.* (1964). Oxidation of ethanol was tested using the medium of Frateur (1950) and acetate and lactate oxidation were tested using the medium of Asai *et al.* (1964). Growth in the presence of 0.01% malachite green was tested using the medium of Gosselé *et al.* (1983). The medium of Entani *et al.* (1985) was used to test for the ability of strains to grow in 6% ethanol. Growth in 30% glucose was tested according to a modified method of De Ley *et al.* (1984), in which the medium contained 0.3% peptone, 0.5% yeast extract and 30% glucose. The use of the L-amino acids asparagine, glycine, glutamine, threonine and tryptophan as sole nitrogen sources for growth was tested using the medium of Swings *et al.* (1992). Growth with carbon sources was tested in Hoyer's medium lacking mannitol (De Ley *et al.*, 1984), with filter-sterilized carbon sources included at final concentrations of 0.1%. The production of 2-ketogluconic, 5-ketogluconic and 2,5-diketogluconic acids was tested by detection using HPLC, following growth in a medium containing 0.3% yeast extract and 3% glucose at 28 °C for 1–7 d (Blake *et al.*, 1984).

The acetylene reduction assay was tested on bacteria grown on the semi-solid, nitrogen-poor medium LGI-P (Cavalcante & Döbereiner, 1988), using 10% (v/v) acetylene, according to the method of Li & Macrae (1991).

The numerical analysis program NTSYS-pc version 1.6 (Applied Biostatistics) was used to analyse the phenotypic characteristics (Dice coefficient) and phenograms were constructed using the unweighted pair-group method with averages (UPGMA).

Table 1. Bacterial strains used in this study

Abbreviations: ACM, Australian Collection of Microorganisms, Department of Microbiology, The University of Queensland, St Lucia, Australia; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IF, Ingrid Franke; LMG, Culture Collection Laboratorium voor Microbiologie, University of Ghent, Belgium; SRI, Sugar Research Institute, Mackay, Australia.

Species	Strain	Source
<i>Acetobacter aceti</i>	ACM 17	ACM
	SRI 1321	Mealy bug, Queensland, SRI
<i>Acetobacter pasteurianus</i>	ACM 2866	ACM
<i>Gluconacetobacter diazotrophicus</i>	LMG 7603 ^T	LMG
	SRI 1205	Mealy bug, Queensland, SRI
	SRI 1206	Mealy bug, Queensland, SRI
	SRI 1212	Mealy bug, Queensland, SRI
	SRI 1990	Mealy bug, Hawaii, SRI
<i>Gluconacetobacter liquefaciens</i>	LMG 1381 ^T	LMG
	SRI 244	Sugar cane, Queensland, SRI
	SRI 1957	Non- <i>Saccharicoccus sacchari</i> mealy bug, marble-diseased pineapple, The Philippines, SRI
	SRI 1994	Mealy bug, Hawaii, SRI
<i>Gluconacetobacter hansenii</i>	DSM 5602 ^T	DSMZ
<i>Gluconacetobacter xylinus</i>	ACM 19	ACM
Novel mealy-bug taxon	SRI 1216	Sugar cane, Queensland, SRI
	SRI 1230	Sugar cane, Queensland, SRI
	SRI 1255	Sugar cane, Queensland, SRI
	SRI 1794 ^T	Sugar cane, Queensland, SRI
	SRI 1801	Sugar cane, Queensland, SRI
	SRI 1853	Mealy bug, Queensland, SRI
	SRI 1908	Mealy bug, Queensland, SRI
	SRI 1951	Mealy bug, Queensland, SRI
	SRI 1953	Mealy bug, New South Wales, SRI
	IF 2-6	Mealy bug, Queensland, this study
	IF 9645	Mealy bug, Queensland, this study
	IF 9700	Mealy bug, Queensland, this study
	IF 9701	Mealy bug, Queensland, this study
Unidentified bacteria	SRI 1214	Mealy bug, Queensland, SRI
	SRI 1313	Mealy bug, Queensland, SRI
	SRI 1793	Sugar cane, Queensland, SRI
	SRI 1939	Mealy bug, Queensland, SRI
	SRI 1941	Mealy bug, Queensland, SRI
	SRI 1995	Mealy bug, Hawaii, T. Taniguchi
	[<i>Acetobacter</i>] IFR 101	Vinegar acidifier, IFR, Norwich, UK

Determination of ubiquinones. Ubiquinones were analysed according to the procedure of Hippe *et al.* (1997), with modifications. Freeze-dried bacterial cells were extracted twice with chloroform/methanol (2:1 v/v). Combined extracts were dried using a Turbo Vap Evaporator (Zymark), operated at 40 °C with nitrogen gas sparging. The extracts were resuspended in a chloroform/water/methanol solution (2:2:1) and centrifuged to separate polysaccharides from the quinones. The chloroform phase was transferred to a clean tube, the chloroform was evaporated and the resulting quinone preparation was dissolved in acetone.

Ubiquinones were separated by TLC using silica gel 60 F₂₅₄-coated plastic plates (Merck). The running phase employed was petroleumbenzene/diethyl ether (85:15) and the dried plate was examined under UV light for quinones, which were cut from the plate and eluted with diethyl ether. The solvent was evaporated and quinones were resuspended in 2-propanol and then filtered through a 0.2 µm Dyna Gard hollow-fibre syringe. Purified quinones were examined by HPLC and separated on a reverse-phase column (Ecocart 125-3 silica gel column with RP-18, end-capped) maintained at 40 °C. The eluent was acetonitrile/2-propanol (65:35 v/v).

Fig. 1. Dendrogram showing the relationship of isolates determined by a numerical analysis of their phenotypic characteristics. *Glac.*, *Gluconacetobacter*.

at a flow rate of 0.5 ml min⁻¹ and quinones were identified by their retention times using quinone extracts of known composition as standards.

Fatty acid composition. Cellular fatty acids from bacterial strains were identified using the Microbial Identification system MIDI (Hewlett Packard). Bacteria were grown on GYC plates containing 10% glucose for 48 h and the instructions of the manufacturer were followed exactly for fatty acid extraction and analysis.

16S rRNA gene sequencing. DNA was extracted from bacterial strains suspended in a 0.15 M NaCl/0.1 M EDTA buffer and cell lysis was induced by the addition of 5 µl lysozyme (0.3 µg µl⁻¹) and incubation at 37 °C for 20 min. Proteinase K (15 µl of a 1% solution in distilled water) and SDS (10 µl of a 10% solution) were added and the preparation was incubated at 60 °C for 30 min. Nucleic acids were purified by extraction with an equal volume of phenol (water-saturated) and subsequently by extraction with an equal volume of chloroform. An aliquot of the aqueous phase was used directly in PCR. The 16S rRNA gene was amplified in a 100 µl reaction mixture containing 1 × buffer [67 mM Tris/HCl (pH 8.5), 16.6 mM (NH₄)₂SO₄, 0.45% (v/v) Triton X-100, 200 µg gelatin ml⁻¹], 1.5 mM MgCl₂, 200 µM dNTPs, 0.25 µM of each of the primers 27f and 1525r (Lane, 1991) and 1 U of *Tth* plus DNA polymerase (Biotech Australia). Reactions were overlaid with mineral

oil to prevent evaporation. A Perkin-Elmer Cetus model 480 thermal cycler was programmed for PCR amplifications and the program was started with a 3 min 96 °C denaturation step, followed by the addition of *Tth* plus polymerase at 72 °C. The thermal profile consisted of 28 cycles of 94 °C for 1 min, 72 °C for 2 min and 48 °C for 1 min, followed by an extension phase of 48 °C for 1 min and 72 °C for 5 min. Amplification products were electrophoresed at 5 V cm⁻¹ for 30 min on a 1% agarose gel and visualized by ethidium-bromide staining under UV light.

Purified PCR products were sequenced directly using the *Taq* Dye Terminator cycle sequencing kit (Applied Biosystems) according to the instructions of the manufacturer. The sequencing primers 27f, 319r, 357f, 519r, 530f, 907r, 926f, 1100r, 1114f, 1525r, 1492r (Lane, 1991) and 803f (Stackebrandt & Charfreitag, 1990) were used. Extension products were purified using a phenol/chloroform extraction method (ABI) and sequences were determined with an Applied Biosystems model 373A sequencer.

The 16S rRNA gene sequences determined were manually aligned with each other and with other reference 16S rRNA gene sequences using the ae2 sequence editor (Maidak *et al.*, 1997). All ambiguous sites were removed from the alignment prior to phylogeny inference. Evolutionary distances between pairs of DNA sequences were determined using the algorithm of Jukes & Cantor (1969) in the DNADIST program

Table 2. Salient characteristics that differentiate the novel mealy-bug isolates from closely related species of *Gluconacetobacter* and *Acetobacter*

Characteristic	Novel mealy-bug isolates	<i>G. diazotrophicus</i> LMG 7603 ^T	<i>G. liquefaciens</i> LMG 1381 ^T	<i>G. europaeus</i> *	<i>G. hansenii</i> *	<i>G. xylinus</i> ACM 19	<i>A. aceti</i> ACM 17	<i>A. pasteurianus</i> ACM 2866
Formation of water-soluble brown pigment on GYC plate	+	+	+	NA	—	—	—	—
Acetylene reduction	—	+	—	NA	—	—	—	—
Glycerol ketogenesis	—	—	+	NA	+	—	+	—
Formation of acid from glucose	+	+	+	NA	+	+	+	+
Formation from D-glucose of:								
2-Ketogluconic acid	+	+	+	d	d	+	+	+
5-Ketogluconic acid	+	+	+	d	d	+	+	+
2,5-Diketogluconic acid	+	+	+	NA	—	—	—	—
Oxidation of ethanol	+	+	+	+	+	+	+	+
Acid production from:								
D-Mannitol	—	—	—	NA	—	—	—	—
Sorbitol	—	—	—	—	d	—	—	—
1-Propanol	+	+	+	NA	+	+	+	+
Growth on the following carbon sources:								
Ethanol	+	+	+	+	—	—	+	+
Methanol	—	—	—	—	—	—	—	—
Dulcitol	—	—	—	NA	d	—	—	—
Sodium acetate	+	+	+	NA	—	—	+	+
Nitrate reduction	—	—	—	NA	d	—	+	—
Growth in 6% ethanol	—	—	—	+	—	—	+	+
Growth on 30% glucose	+	+	—	—	—	—	—	—
Motility	+	+	+	NA	—	+	+	+
Gram stain	—	—	—	NA	—	—	—	—
Oxidase reaction	—	—	—	NA	—	—	—	—
Catalase reaction	+	+	+	NA	+	—	+	+
Sodium acetate oxidation	+	+	+	NA	+	+	+	+
Sodium lactate oxidation	+	+	+	NA	+	+	+	+
Formation of H ₂ S	—	—	—	NA	—	—	—	—
Growth on the following in the presence of D-mannitol as a carbon source:								
L-Asparagine	—	+	+	NA	+	—	—	—
L-Glycine	—	+	+	NA	d	—	—	—
L-Glutamine	—	+	+	NA	+	—	+	—
L-Threonine	—	—	+	NA	—	—	—	—
L-Tryptophan	—	+	+	NA	—	—	—	—
Growth on glutamate agar	+	+	+	NA	NA	—	+	—
Growth on mannitol agar	+	+	+	NA	d	+	+	+
Growth on 0.01% malachite-green agar	+	—	+	NA	NA	—	—	—
G+C content of DNA (mol%)	62–67	61–63	62–65	56–58	58–63	55–63	56–60	53–63
Ubiquinone type	Q10	Q10	Q10	Q10	Q10	Q10	Q9	Q9

+, Positive; —, negative; d, some strains positive; NA, result not available.

* Data from De Ley *et al.* (1984), Uhlig *et al.* (1986), Gillis *et al.* (1989), Sievers & Teuber (1995), Yamada *et al.* (1997) and Sokollek *et al.* (1998).

of the PHYLIP phylogenetic analysis software package (Felsenstein, 1993). Phylogenetic trees were constructed from evolutionary distances using the neighbour-joining method of Saitou & Nei (1987). Trees based on maximum parsimony were constructed using the DNAPARS program of PHYLIP (Felsenstein, 1993). Bootstrapping analysis using the program SEQBOOT (in PHYLIP) was undertaken to test the statistical reliability of the topology of the distance- and parsimony-generated trees using 100 bootstrap resamplings of the data.

The following 16S rRNA gene sequences were obtained from GenBank for use in the phylogenetic analyses: *Acetobacter aceti* JCM 7641, D30768; [*Acetobacter*] *intermedius* DSM 11804^T, Y14694; [*Acetobacter*] *oboediens* DSM 11826^T, AJ001631; *Acetobacter pasteurianus* LMG 1262^T, X71863; *Acetobacter pomorum* DSM 11825^T, AJ001632; *Acidomonas methanolica* LMG 1668^T, X77468; *Gluconacetobacter diazotrophicus* DSM 7603^T, X75618; *Gluconacetobacter europaeus* DSM 6160^T, Z21936; *Gluconacetobacter hansenii* LMG

1527^T, X75620; *Gluconacetobacter liquefaciens* LMG 1382^T, X75617; *Gluconacetobacter xylinus* LMG 1515^T, X75619; *Gluconobacter asaii* LMG 1390^T, X80165; *Gluconobacter cerinus* LMG 1368^T, X80775; *Gluconobacter frateurii* LMG 1365^T, X82290; *Gluconobacter oxydans* DSM 3503^T, X73820; and *Rhodopila globiformis* DSM 161^T, D86513.

DNA isolation and characterization. Genomic DNA for DNA–DNA hybridization studies and for G+C content determination was isolated by chromatography on hydroxyapatite, using the method of Cashion *et al.* (1977). DNA was dialysed against 2 × SSC and DNA quality and quantity was determined spectroscopically using a microprocessor-controlled Gilford 2600 spectrophotometer. HPLC was used to determine the G+C content, according to the method of Mesbah *et al.* (1989).

DNA–DNA hybridizations were conducted using the method of De Ley *et al.* (1970), as modified by Huss *et al.* (1983) and Escara & Hutton (1980). A thermal-denaturation

profile was determined using the Gilford 2600 spectrophotometer, equipped with a thermoelement, including four cuvettes with integrated temperature control, a thermo-programmer (2527-R) and an automatic cuvette changer. The midpoint of the thermal denaturation curve (T_m) was determined graphically by the computer and the optimal temperature of renaturation (T_{or}) was determined using the equation: $T_{or} = 0.5T_m + 65.2 - 25$ (Escara & Hutton, 1980; Huss *et al.*, 1983). Renaturation was monitored by following A_{260} and involved the denaturation of DNA ($50 \mu\text{g ml}^{-1}$) at 99°C before renaturation at the T_{or} , 64°C . Levels of DNA-DNA relatedness were determined by recording absorbances every 15 s for 30 min and using the formula of De Ley *et al.* (1970), with the computer program TRANSFER.BAS of Jahnke (1992), where the percentage of DNA homology is determined as $100 \times (4V_M - V_A + V_B) / [2 \times \sqrt{(V_A \times V_B)}]$. In this formula, V is the renaturation rate, A is DNA from strain A, B is DNA from strain B and M is a mixture of equal amounts of DNA from strains A and B.

RESULTS

Isolation of bacteria

All mealy bugs examined were found to support a population of acidophilic bacteria and yeasts, and filamentous fungal growth was rarely seen due to the incorporation of cycloheximide in the isolation medium. Bacterial colonies that resembled the growth of previous mealy-bug isolates, represented by strain SRI 1794^T, *Gluconacetobacter liquefaciens* or *Gluconacetobacter diazotrophicus*, on WL nutrient-agar plates (khaki-brown colonies producing large amounts of acid to change the colour of agar plates from aqua to yellow) were selected and isolated as pure cultures. New isolates included in this study are shown in Table 1 (IF numbers).

Phenotypic relationships

Twenty-four isolates were phenotypically characterized and the data were analysed by numerical analysis (Fig. 1). Strains SRI 244 and SRI 1957 clustered with the type strain of *Gluconacetobacter liquefaciens* (92 and 86% similarity, respectively) and strains SRI 1206, SRI 1212 and SRI 1990 clustered with the type strain of *Gluconacetobacter diazotrophicus* (92, 92 and 89% similarity, respectively). Twelve strains, including three new isolates IF 2-6, IF 9700 and IF 9645, formed a highly related cluster independent of existing type strains included in the analysis. Strain SRI 1994 clustered with this group of isolates, but on a separate branch. All other unidentified isolates (except SRI 1321, which grouped with *Gluconacetobacter xylinus*) belonged to separate taxa and were not associated with the known species included.

Members of the major cluster of novel mealy-bug isolates were shown to be 100% similar on the basis of the tests conducted (Table 2). They are Gram-negative, ellipsoidal-to-rod-shaped cells, motile by peritrichous

Fig. 2. Electron micrograph of strain SRI 1794^T, showing cellular morphology and peritrichous flagella.

flagella (Fig. 2) and produce a water-soluble brown pigment on GYC agar plates. Like all other reference strains tested, these novel isolates were capable of acid formation from glucose and the oxidation of ethanol. Members of the taxon were oxidase-negative, catalase-positive, unable to reduce nitrates, incapable of nitrogen fixation as determined by acetylene reduction and did not form hydrogen sulfide. Both sodium acetate and sodium lactate were oxidized. There was no growth in 6% ethanol, although bacteria did grow in 30% glucose. Growth occurred on glutamate agar, on mannitol agar and in the presence of 0.01% malachite green. There was no acid production from D-mannitol or sorbitol, but acid was formed from L-propanol. Organisms were capable of growth on 0.1% ethanol and sodium acetate as sole carbon sources, but not on 0.1% methanol or dulcitol. No strains were able to utilize the amino acids L-asparagine, L-glycine, L-glutamine, L-threonine or L-tryptophan as sole nitrogen sources with D-mannitol as a carbon source. Bacteria of this taxon produced 2-keto, 5-keto and 2,5-diketogluconic acids after 1–2 d growth.

Fatty acid composition

The predominant fatty acid found in all the acetic acid bacteria tested, both reference strains and mealy-bug isolates, was the straight-chain unsaturated $C_{18:1\omega7c}$

Table 3. Cellular fatty acid composition of members of the novel taxon of mealy-bug isolates, type strains of *Gluconacetobacter* and *Acetobacter* and other bacteria isolated from mealy bugs

Percentages of total fatty acids are shown. Additional fatty acids found in smaller amounts in various isolates included: 0.69% 3-OH C_{10:0} in SRI 1313; 0.83% C_{12:0} in SRI 1990; 0.44% C_{17:1ω6c} in SRI 1794^T; 1.05% C_{17:0} in *G. liquefaciens* LMG 1381^T; 0.53% C_{17:0} in SRI 1794^T; 0.28% 2-OH C_{16:1} in SRI 1939; 1.25% C_{18:2ω6,9c} in SRI 1957; and 0.79% C_{19:1ω12t} in SRI 1939. –, Not detected.

Strain	C _{14:0}	2-OH C _{14:0}	3-OH C _{14:0}	C _{16:1ω7c}	C _{16:0}	2-OH C _{16:0}	3-OH C _{16:0}	C _{18:1ω7c}	C _{18:0}	C _{19:0cycloω8c}	2-OH C _{18:1}	C _{20:3ω6,9,12}
<i>A. aceti</i>												
ACM 17	2.25	6.18	1.14	0.70	11.55	2.04	1.61	70.61	1.68	0.75	–	1.48
SRI 1321	1.18	2.89	2.05	–	10.52	8.80	2.47	63.30	1.35	–	6.32	1.11
<i>G. diazotrophicus</i>												
LMG 7603 ^T	6.08	4.79	0.98	–	7.18	6.84	2.44	66.22	1.45	2.45	1.58	–
SRI 1206	7.43	2.24	0.78	–	8.32	6.64	2.00	67.76	2.04	1.24	1.54	–
SRI 1212	6.27	3.29	0.90	0.45	7.22	6.69	1.92	68.05	1.55	1.48	1.45	0.72
SRI 1990	6.14	3.41	–	–	8.33	7.64	2.13	65.73	1.88	–	–	–
<i>G. liquefaciens</i>												
LMG 1381 ^T	4.43	2.97	1.80	–	8.07	7.52	3.35	61.49	2.81	3.76	1.43	1.33
SRI 244	5.80	1.41	1.23	–	9.35	5.64	2.35	66.14	2.62	1.52	2.90	1.03
SRI 1957	5.55	2.25	1.11	1.03	8.27	6.15	2.28	67.62	1.57	–	2.92	–
SRI 1994	5.57	1.80	1.23	–	8.50	6.41	2.24	67.38	1.96	1.16	2.94	0.81
Novel mealy-bug taxon												
SRI 1216	3.12	4.53	1.39	–	9.32	7.31	3.15	62.14	2.26	1.10	4.62	1.07
SRI 1230	2.26	4.83	1.42	–	9.40	7.94	2.96	63.61	1.78	1.08	3.74	0.99
SRI 1255	2.19	4.29	1.46	–	10.37	7.74	2.74	63.40	1.87	1.59	3.16	1.20
SRI 1794 ^T	2.38	5.16	1.46	0.68	11.37	8.31	2.74	60.67	1.30	1.11	2.77	1.08
SRI 1801	3.35	3.53	1.27	–	9.62	6.69	2.78	64.13	2.12	1.16	4.45	0.90
SRI 1853	3.71	5.19	1.48	0.66	15.87	9.43	3.56	49.09	5.11	0.89	–	–
SRI 1908	2.87	4.41	1.38	–	10.48	7.27	2.46	64.29	1.95	0.93	2.79	1.17
SRI 1939	1.19	4.86	1.57	0.66	10.59	5.74	2.22	63.65	2.63	0.61	4.06	1.16
SRI 1951	3.81	3.92	0.98	–	10.48	7.03	2.93	63.86	2.03	–	3.82	1.14
SRI 1953	3.12	4.22	1.50	0.86	9.21	7.96	3.19	61.43	1.96	1.12	4.18	1.26
IF 2-6	2.80	4.73	1.20	0.81	10.32	7.17	2.40	64.25	1.79	1.55	2.21	0.78
IF 9645	3.03	4.84	1.30	0.90	9.44	7.76	2.94	63.39	1.26	1.18	3.06	0.91
IF 9700	2.82	5.28	1.51	–	9.02	8.88	2.87	63.36	1.13	0.88	3.37	0.88
Unidentified bacteria												
SRI 1313	1.06	4.37	2.58	–	6.97	7.22	3.09	59.29	1.12	–	12.15	1.46
SRI 1793	0.87	4.24	2.44	–	7.89	7.04	2.71	61.36	1.33	–	10.77	1.34
SRI 1941	–	1.68	1.48	–	8.83	7.81	3.48	59.03	5.06	–	12.63	–
SRI 1995	–	–	1.36	–	9.07	7.13	3.58	69.8	0.97	1.34	5.84	0.89

acid, which accounted for 49–71% of the fatty acid content (Table 3). Other fatty acids common to all members of the new taxon of bacteria were C_{14:0} (2.2–3.8%), C_{16:0} (9.0–15.9%), C_{18:0} (1.1–5.1%), 2-OH C_{14:0} (3.5–5.3%), 2-OH C_{16:0} (6.7–9.4%), 3-OH C_{14:0} (1.0–1.5%) and 3-OH C_{16:0} (2.4–3.6%). The fatty acid compositions of *Acetobacter aceti* and *Gluconacetobacter liquefaciens* are in agreement with earlier studies of Yamada *et al.* (1981) and Urakami *et al.* (1989). Principal-component analysis of fatty acid profiles grouped all members of the novel taxon except for SRI 1853 together and separate from *Gluconacetobacter liquefaciens* and *Gluconacetobacter diazotrophicus*.

16S rRNA gene sequence analysis

Near-complete 16S rRNA gene sequences (1341–1420 nucleotides) were determined for 23 strains of acetic acid bacteria, including 10 strains of the novel taxon. Fig. 3 shows a dendrogram constructed from evolutionary distances using the neighbour-joining method, based on 1241 nucleotide positions (ambiguous nucleotides omitted). Bootstrap values supported the topology of the tree. The parsimony tree (not shown) reflected the topology of the neighbour-joining tree. Phylogenetically, the acetic acid bacteria form two major divisions, one containing all species of the genus *Gluconacetobacter* and the second containing

Fig. 3. Neighbour-joining tree depicting the phylogenetic relationships of mealy-bug isolates among the Acetobacteraceae determined from 16S rRNA gene sequence similarities. The scale bar represents 1 nucleotide substitution per 100 nucleotides.

separate lineages for the genera *Gluconobacter*, *Acetobacter* and *Acidomonas*. Two subdivisions were evident within the genus *Gluconacetobacter*, the first including members of the novel mealy-bug taxon, *Gluconacetobacter diazotrophicus* and *Gluconacetobacter liquefaciens* and the other containing *Gluconacetobacter europaeus*, *Gluconacetobacter hansenii*, *Gluconacetobacter xylinus* and some newly sequenced and misidentified *Acetobacter* species, [*Acetobacter*] *oboediens* and [*Acetobacter*] *intermedius*, which should be reassigned to the genus *Gluconacetobacter*. Strains of the new mealy-bug-isolate taxon clustered together and separately from other species of the genus *Gluconacetobacter*. All sequenced members of the taxon were found to be almost identical, with strains sharing 99.5–100% sequence similarity. The nearest relative of this group is *Gluconacetobacter liquefaciens*, which, although seemingly a distinct phylotype, shares 98.8–99.3% sequence similarity with members of the mealy-bug taxon. There is 97.9–98.5% sequence similarity between members of the mealy-bug taxon and *Gluconacetobacter diazotrophicus*, and progressively lower similarity to the other species of *Gluconacetobacter*.

bacter; *Gluconacetobacter xylinus* (97%), *Gluconacetobacter europaeus* (97.2%) and *Gluconacetobacter hansenii* (96.9%). The closest relative of the *Acetobacteraceae* family was found to be *Rhodopila globiformis*. Unique nucleotide positions in the 16S rRNA gene were found in isolates of the novel mealy-bug taxon and in no other members of the genus *Gluconacetobacter*. The G at position 648 (*Escherichia coli* numbering) was characteristic for members of the novel taxon, and the bases U, U and A at positions 264, 265 and 647 were characteristic for members of the novel taxon and found in no other members of the genus *Gluconacetobacter*, with the exception of strains SRI 1941 (U at position 265, A at position 647) and SRI 1939 (U at position 264). These strains appear to be the first representatives of undescribed species of *Gluconacetobacter* (SRI 1941) and *Acetobacter* (SRI 1939).

DNA characterization

The base composition ranges of the novel taxon of mealy-bug isolates and their nearest phylogenetic relatives are shown in Table 2. All members of the novel taxon tested were found to have G+C contents ranging from 62.1 to 67.3 mol%. DNA base compositions of the type strains determined in this study were found to resemble published values closely (*Gluconacetobacter diazotrophicus*, 62 ± 0.4 mol%, *Gluconacetobacter liquefaciens*, 62.1 ± 0.3 mol%, and *Acetobacter aceti*, 60.7 ± 0.1 mol%) (De Ley *et al.*, 1984; Swings *et al.*, 1992). Values for isolates related to the type strains were similar to the G+C contents obtained for the reference strains of *Gluconacetobacter diazotrophicus* (SRI 1205, 66 ± 0.2 mol%, SRI 1212, 66 ± 0.1 mol%, and SRI 1990, 66.3 ± 0.4 mol%) and *Gluconacetobacter liquefaciens* (SRI 244, 66.8 ± 0.4 mol%, SRI 1957, 65 ± 0.2 mol%, and SRI 1994, 65.1 ± 0.1 mol%).

The levels of DNA–DNA reassociation among members of the acetic acid bacteria studied (Table 4) indicate that the novel mealy-bug isolates belong to a new species. Hybridization values of 78–100% were obtained for all reassociation experiments conducted between various strains belonging to the taxon. In contrast, there was only 46% relatedness between the DNA of the novel taxon reference strain, SRI 1794^T, and *Gluconacetobacter liquefaciens* and 39% relatedness with *Gluconacetobacter diazotrophicus*, the two nearest phylogenetic relatives determined by 16S rDNA sequence similarity. There was 52% relatedness between another member of the novel taxon, IF 9645, and *Gluconacetobacter liquefaciens*. Strains SRI 244, SRI 1957 and SRI 1994 showed 69–72% relatedness to the type strain of *Gluconacetobacter liquefaciens* and 85–100% relatedness among each other, and strains SRI 1205, SRI 1212 and SRI 1990 showed 85–89% relatedness to the type strain of *Gluconacetobacter diazotrophicus*.

Table 4. DNA–DNA relatedness among members of the novel taxon of mealy-bug isolates, *Gluconacetobacter* and *Acetobacter* strains and other bacteria isolated from mealy bugs

Percentage DNA binding is shown.

Strain	1	3	6	7	8	9	10	12	13	14
1. SRI 1794 ^T	100									
2. SRI 1216	78		82							
3. SRI 1230	100									
4. SRI 1853	83	83								
5. SRI 1951	86			90						
6. IF 2-6	88									
7. IF 9645	97				52			43		
8. <i>G. liquefaciens</i> LMG 1381 ^T	46									
9. SRI 244	38				69			38		
10. SRI 1957					71	85				
11. SRI 1994					72	92	100			
12. <i>G. diazotrophicus</i> LMG 7603 ^T	39				38					
13. SRI 1205								87		
14. SRI 1212								89	90	
15. SRI 1990								85	88	96
16. <i>A. aceti</i> ACM 17	23				18			30		
17. SRI 1939	29				28			30		
18. SRI 1941	34				23			37		

DISCUSSION

A polyphasic study of acetic acid bacteria isolated from the sugar cane leaf sheath and from the pink sugar-cane mealy bug has revealed a new species of the genus *Gluconacetobacter*, for which we propose the name *Gluconacetobacter sacchari* sp. nov., and has also shown the presence of its nearest phylogenetic relatives, *Gluconacetobacter liquefaciens* and *Gluconacetobacter diazotrophicus*, in this environment. The 16S rDNA sequences of all strains of the new taxon were very similar (>99.5% sequence similarity), despite having been isolated over a 17-year period from various geographical regions.

The phylogenetic tree generated in this study is mainly in agreement with the 5S rRNA gene sequence study by Bulygina *et al.* (1992) of several *Acetobacter* and *Gluconobacter* strains and is in accordance with the 16S rRNA gene study of Sievers *et al.* (1994a), showing essentially two main lineages. A recent study of partial 16S rRNA gene sequences and ubiquinone content led to the elevation of the *Acetobacter* subgenus *Gluconoacetobacter* (Yamada & Kondo, 1984) to the generic level (Yamada *et al.*, 1997). The number of base differences between *Acetobacter aceti* and *Gluconacetobacter liquefaciens* was found to be greater than the number between *Acetobacter aceti* and *Gluconobacter oxydans*. This indicated that the phylogenetic distance between members of the subgenera *Acetobacter* and *Gluconacetobacter* of the genus *Acetobacter* is greater than that between members of the subgenus *Acetobacter* of the genus *Acetobacter* and the

genus *Gluconobacter*. Our alignment of the almost complete 16S rDNA sequences from the same strains of acetic acid bacteria indicated a nearly identical phylogeny and clearly confirms the findings of Yamada *et al.* (1997). Ubiquinone content in these organisms was also found to differ. While *Acidomonas* and members of *Gluconacetobacter* and *Gluconobacter* were equipped with the Q10 ubiquinone, members of the genus *Acetobacter* were equipped with the Q9 ubiquinone. The presence of Q10 as ubiquinone in the mealy-bug isolates strongly supports the inclusion of the new species in the genus *Gluconacetobacter* (Yamada *et al.*, 1997) with the organisms it shares the greatest similarity to on the basis of 16S rRNA gene sequence information. There was only one position in the 16S rRNA gene (G at 648, *E. coli* numbering) that was unique within the genus *Gluconacetobacter* to members of the novel taxon of mealy-bug isolates, and three positions (U, U and A, at positions 264, 265 and 647, *E. coli* numbering) that were unique within the genus *Gluconacetobacter* to members of the novel taxon of mealy-bug isolates with the exception of the unidentified strains SRI 1941 (U at position 265, A at position 647) and SRI 1939 (U at position 264).

DNA–DNA hybridization is acknowledged to be the superior method for the elucidation of relationships between closely related taxa at the species level and the newer technique of gene sequence analysis has been found most often to confirm DNA reassociation results (Fox *et al.*, 1992; Stackebrandt & Goebel, 1994). Bacteria demonstrating >70% DNA hom-

ology are regarded as belonging to the same species and would be expected to exhibit 97.5% or greater 16S rRNA gene sequence similarity (Stackebrandt & Goebel, 1994). All mealy-bug isolates belonging to the novel taxon were found to have a high level of DNA homology with each other (>78%) and low levels of homology with the most closely related species of the genus *Gluconacetobacter* (determined by rRNA phylogeny). This is evidence for the designation of a new species of *Gluconacetobacter*.

The G + C content determinations for the strains of the new species were found to be slightly higher than the 51–65 mol% range originally described for the genus (De Ley *et al.*, 1984; Yamada & Kondo, 1984). Most of the strains tested were found to have a G + C content between 65.1 and 67.3 mol%, although one strain, SRI 1853, had a G + C content of 62.1 mol%. This strain also had a different fatty acid profile from the other strains of the species, but did not differ phenotypically, nor in the 16S rRNA gene sequence. Its inclusion in the species is further supported by high DNA reassociation levels with other members of the new species. A number of different methods have been used for the determination of G + C content and results have been found to differ depending on the method used (Tamaoka & Komagata, 1984; Fahmy *et al.*, 1985). For this reason, the fact that the G + C contents obtained for isolates of the species were slightly higher than those recorded previously for the *Acetobacteraceae* is not considered to be a barrier to their inclusion in the family.

All strains of the new species were identical by phenotypic characterization and shared similar chemotaxonomic features. No exclusive phenotypic character differentiated the new species from all other related bacteria; however, tests such as growth in the presence of 0.01% malachite green, the inability to fix nitrogen, growth on 30% glucose (a characteristic previously specific for *Gluconacetobacter diazotrophicus*, another bacterium isolated from sugar cane) and the inability to grow on various L-amino acids in the presence of D-mannitol as carbon source allowed the differentiation of the species from other species of the genera *Gluconacetobacter* and *Acetobacter*. Numerical analysis of the phenotypic characteristics placed the mealy-bug isolates in a separate group. The results obtained for the various reference strains included in this study mostly supported the results of previous researchers (De Ley *et al.*, 1984; Cavalcante & Döbereiner, 1988), although there were a few discrepancies. We found that *Gluconacetobacter diazotrophicus* was able to grow with L-glycine and L-glutamine in the presence of D-mannitol as carbon source. We also found that *Gluconacetobacter xylinus* ACM 19, a non-type strain, was incapable of glycerol ketogenesis and that *Acetobacter aceti* ACM 17, also a non-type strain, was capable of nitrate reduction. A high level of sensitivity was obtained when detecting ketogluconic acids formed from glucose, and low levels of 5-ketogluconic acid were detected in all bacteria

tested. This was not expected for *Acetobacter pasteurianus* or *Gluconacetobacter diazotrophicus*. Ketogluconic acids were formed by *Gluconacetobacter diazotrophicus*, *Gluconacetobacter liquefaciens* and members of the new species after 24 h growth, but only after 7 d growth for *Gluconacetobacter xylinus*, *Acetobacter pasteurianus* and *Acetobacter aceti*. Yamada *et al.* (1997) reported that growth on mannitol agar can be used to differentiate between the genera *Acetobacter*, *Gluconobacter*, *Gluconoacetobacter* and *Acidomonas*. In our study, all *Acetobacter* and *Gluconacetobacter* species grew on mannitol. None of the species tested were capable of acid formation from mannitol. Members of the novel taxon exhibit a combination of properties that have not been described previously for any member species of the *Acetobacteraceae*. However, it is clear that the phenotypic relationships revealed in the phenogram generated by numerical analysis do not reflect the phylogenetic relationships determined by 16S rRNA sequence analysis. The novel taxon falls in the same lineage as *Acetobacter aceti*, *Gluconacetobacter xylinus* and *Acetobacter pasteurianus* and, in the 16S rRNA gene phylogenetic tree, the novel mealy-bug isolates clustered closer to *Gluconacetobacter diazotrophicus* and *Gluconacetobacter liquefaciens* and more distantly to the *Gluconacetobacter xylinus* group and the *Acetobacter* and *Gluconobacter* groups. Strain SRI 1794^T, representative of the species, has higher phenotypic similarity to *Acetobacter pasteurianus* (81%) than to *Gluconacetobacter diazotrophicus* (79%) or *Gluconacetobacter liquefaciens* (76%). These conflicting relationships indicate the inability of phenotypic characters to determine phylogenetic relationships amongst members of the *Acetobacteraceae*, which resulted in the need for its recent taxonomic revision (Yamada *et al.*, 1997).

Gillis *et al.* (1989) published a description of a new nitrogen-fixing acetic acid bacterium, *Acetobacter diazotrophicus*. This paper included a paragraph mentioning that sugar cane isolates from Australia belong to the species *Acetobacter diazotrophicus* on the basis of their growth characteristics, protein gel electropherograms and nitrogen-fixing ability. The strains included in their study were the SRI strains 244, 1205, 1206, 1212, 1214 and 1216 (M. Gillis, personal communication). Only three of these strains (SRI 1205, SRI 1206 and SRI 1212) were found in our study to group with *Gluconacetobacter diazotrophicus* on the basis of 16S rRNA gene sequence analysis, phenotypic tests (including acetylene reduction as a test for nitrogen fixation) and DNA–DNA hybridization. Of the other strains, we found strain SRI 1216 to belong to the new species according to 16S rRNA gene sequence similarity and the absence of acetylene reduction activity, strain SRI 244 to belong to *Gluconacetobacter liquefaciens* and strain SRI 1214 to group most closely with *Acidomonas methanolica*, a result also found by M. Gillis (personal communication).

Results of fatty acid analyses have been found most

often to support 16S rRNA gene phylogeny (Klatte *et al.*, 1994; Kämpfer *et al.*, 1997). In our study, the results appear also to be consistent with phylogenetic data, although not all *Acetobacter* and *Gluconacetobacter* reference species were included in the analysis. The fatty acid compositions of bacteria tested were in agreement with the results obtained by Yamada *et al.* (1981) for *Acetobacter* and *Gluconacetobacter*, where $C_{18:1\omega7c}$ was the major fatty acid constituent and $C_{16:0}$ and 2-OH $C_{16:0}$ were also present in significant amounts. The fatty acid $C_{18:1\omega7c}$ comprised 60–64% of the total fatty acid content of the mealy-bug isolates, except for SRI 1853, in which $C_{18:1\omega7c}$ represented only 49% of the total fatty acid composition. The fatty acid 2-OH $C_{18:1}$ was missing from the profile of SRI 1853, a fatty acid present in all other members in small amounts (2.2–4.6%). In contrast, SRI 1853 had raised levels of $C_{16:0}$ and $C_{18:0}$. These results and the lower G+C content obtained for SRI 1853 indicate the unique phenotypic properties of this strain. Although there was no single fatty acid found in significant levels in the new species and absent in all other organisms, bacteria of the new species (except SRI 1853) did cluster together and separately from other strains by principal-component analysis of fatty acid data. Microbial fatty acid composition of lipids is highly dependent on environmental conditions and standardization in the entire culturing and extraction procedure is critical for reproducibility (Mukwaya & Welch, 1989). The small amount of variation in fatty acid profiles between the different mealy-bug isolates is therefore not significant.

Description of *Gluconacetobacter sacchari* sp. nov.

Gluconacetobacter sacchari (sacchar'i. M.L. gen. n. *sacchari*, pertaining to the genus *Saccharum*, sugar cane, from which the organism was isolated).

Cells are ellipsoidal to rod-shaped, approximately $1.3\text{--}2.2 \times 0.7\text{--}0.9\text{ }\mu\text{m}$ in size and occur singly, in pairs or in short chains. Cells are motile by peritrichous flagella. Resting stages are not formed. Strictly aerobic. Gram-negative, oxidase-negative and catalase-positive. Produce brown water-soluble pigments on GYC medium and khaki-brown colonies on WL nutrient agar plates. High levels of acid are produced on both these media. Optimum temperature for growth is around 28–30 °C. Nitrates are not reduced to nitrite, hydrogen sulfide is not formed and microaerophilic nitrogen fixation as determined by acetylene reduction is negative. Acid is formed from glucose and ethanol is oxidized. Both sodium acetate and sodium lactate are oxidized. Isolates do not grow in 6% ethanol but do grow in 30% glucose. Isolates grow on glutamate agar, on mannitol agar and in the presence of 0.01% malachite green. Acid is produced from propanol, but not from D-mannitol or sorbitol. Isolates grow using 0.1% ethanol and sodium acetate as sole carbon sources, but not 0.1% methanol or dulcitol. Members of the species do not grow on the amino acids L-

asparagine, L-glycine, L-glutamine, L-threonine or L-tryptophan with D-mannitol as the carbon source. Produce 2-keto, 5-keto and 2,5-diketogluconic acids. $C_{18:1\omega7c}$ is the major cellular fatty acid, with 2-OH $C_{16:0}$ and $C_{16:0}$ present in smaller but significant amounts. The ubiquinone type is Q10. The mean G+C content (as determined by HPLC) ranges from 62 to 67 mol%. The bacteria were isolated from the leaf sheath of sugar cane and from the pink sugar-cane mealy bug, found living in the leaf sheath of sugar cane in Queensland, Australia. The type strain is SRI 1794^T (= DSM 12717^T), which has a G+C content of 65 mol% and has all the characteristics given above for the species.

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