This article was published in International Journal of Systemic and Evolutionary Microbiology, 64, 2407-2415, 2014
http://dx.doi.org/10.1099/ijs.0.058354-0

Acetobacter sicerae sp. nov., isolated from cider and kefir, and identification of species of the genus *Acetobacter* by *dnaK*, *groEL* and *rpoB* sequence analysis

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Five acetic acid bacteria isolates, awK9_3, awK9_4 (5LMG 27543), awK9_5 (5LMG 28092), awK9_6 and awK9_9, obtained during a study of micro-organisms present in traditionally produced kefir, were grouped on the basis of their MALDI-TOF MS profile with LMG 1530 and LMG 1531^T, two strains currently classified as members of the genus *Acetobacter*. Phylogenetic analysis based on nearly complete 16S rRNA gene sequences as well as on concatenated partial sequences of the housekeeping genes *dnaK*, *groEL* and *rpoB* indicated that these isolates were representatives of a single novel species together with LMG 1530 and LMG 1531^T in the genus *Acetobacter*, with *Acetobacter aceti, Acetobacter nitrogenifigens*, *Acetobacter oeni* and *Acetobacter estunensis* as nearest phylogenetic neighbours. Pairwise similarity of 16S rRNA gene sequences between LMG 1531^T and the type strains of the above-mentioned species were 99.7 %, 99.1 %, 98.4 % and 98.2 %, respectively. DNA–DNA hybridizations confirmed that status, while amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) data indicated that LMG 1531^T, LMG 1530, LMG 27543 and LMG 28092 represent at least two different strains of the novel species. The major fatty acid of LMG 1531^T and LMG 27543

was $C_{18:1}\omega 7c$. The major ubiquinone present was Q-9 and the DNA G+C contents of LMG 1531^T and LMG 27543 were 58.3 and 56.7 mol%, respectively. The strains were able to grow on D-fructose and D-sorbitol as a single carbon source. They were also able to grow on yeast extract with 30 % D-glucose and on standard medium with pH 3.6 or containing 1 % NaCl. They had a weak ability to produce acid from D-arabinose. These features enabled their differentiation from their nearest phylogenetic neighbours. The name *Acetobacter sicerae* sp. nov. is proposed with LMG 1531^T (=NCIMB 8941^T) as the type strain.

Acetic acid bacteria (AAB) are Gram-negative, coccoid or rod-shaped, obligately aerobic bacteria that are ubiquitous in the environment. They occur in sugary and alcoholic, slightly acidic niches including several traditional fermented foods and beverages (Kersters *et al.*, 2006; Lisdiyanti *et al.*, 2003). From the latter sources, strains of members of the genus *Acetobacter* in particular are isolated (Lisdiyanti *et al.*, 2003).

Strain LMG 1531^T, a non-cellulose-producing mutant of strain LMG 1530, which was isolated from cider (Shimwell & Carr, 1958), is phenotypically similar and phylogenetically related to *Acetobacter aceti* (Cleenwerck *et al.*, 2002; Gossele´ *et al.*, 1983; Shimwell & Carr, 1958), but was excluded from that species based on amplified fragment length polymorphism (AFLP) and $(GTG)_5$-PCR fingerprint data and its low DNA–DNA relatedness value ($\leq$60 %) with true *A. aceti* strains (Cleenwerck *et al.*, 2009; De Vuyst *et al.*, 2008; Papalexandratou *et al.*, 2009).

During a study of micro-organisms present in a concentrated, industrially produced kefir made with syrup as an additional carbon source and ready for bottling and consumption, acetic acid bacteria were isolated as follows. The kefir sample was serially diluted to 10^{-6} in physiological water (0.85 %, w/v, NaCl) and plated onto acetic acid medium (AAM) agar [1 %, w/v, D-glucose; 1.5 %, w/v, bacteriological peptone (Oxoid); 0.8 %, w/v, yeast extract (Oxoid); 0.3 %, v/v, acetic acid; 0.5 %, v/v, ethanol; 0.32 %, v/v, hydrochloric acid and 1.5 %, w/v, agar (Lisdiyanti *et al.*, 2001)], containing 200 p.p.m. cycloheximide and 5 p.p.m. amphotericin B. Acetic acid, ethanol, hydrochloric acid, cycloheximide and amphotericin B were added to the isolation medium after sterilization. Inoculated media were incubated aerobically at 30 ºC for 5 days. Isolates were dereplicated by matrix-assisted laser desorption/ ionization-time-of-flight (MALDI-TOF) MS, a fast and accurate technique for identification of many bacteria species including AAB (Andrés-Barrao *et al.*, 2013; Anhalt & Fenselau, 1975; Claydon *et al.*, 1996; Krishnamurthy & Ross, 1996), using the method described previously (Wieme *et al.*, 2012). Five isolates showed identical mass spectra with a high level of similarity towards those of LMG 1531^T and LMG 1530, which was indicative of relatedness at the species level (Fig. 1 and Fig. S1 available in the online Supplementary Material). Two of these isolates, awK9_4 and awK9_5, were selected as representatives for further investigation and deposited in the Belgian Co-ordinated Collection of Micro-organisms/Laboratorium voor Micro- biologie Ghent (BCCM/LMG) Bacteria Collection as

LMG 27543 and LMG 28092, respectively.

A nearly complete 16S rRNA gene sequence was determined for strains LMG 27543, LMG 28092 and LMG 1530 as described previously (Snauwaert *et al.*, 2013). The sequences were compared with 16S rRNA gene sequences of LMG 1531^T (AJ419840) and the type strains of the species of the genus *Acetobacter* with validly published names retrieved from the EMBL database or determined as part of the present study (i.e. *Acetobacter nitrogenifigens* LMG 23498^T, HG424425) using the BioNumerics 5.1 software (Applied Maths). Strains LMG 1531^T and LMG 1530 were identified as the closest relatives of LMG 27543 and LMG 28092, both with ≥ 99.9 % pairwise sequence similarity, while *A. aceti* and *A. nitrogenifigens* were found to be the most closely related species with validly published names, exhibiting 99.7 and 99.1 % pairwise sequence similarity with LMG 27543, respectively. Similarities to other species of the genus *Acetobacter* were below 98.7 %. The 16S rRNA gene sequences of LMG 27543, LMG 28092, LMG 1530 and LMG 1531^T and of all type strains of species of the genus *Acetobacter* were aligned against the SILVA bacteria database using the Mothur pipeline (Quast *et al.*, 2013; Schloss *et al.*, 2009). Subsequently, phylogenetic trees based on 1312 – 1320 nt were reconstructed with MEGA6 using the maximum-likelihood (ML) and neighbour- joining (Felsenstein, 1981; Saitou & Nei, 1987) methods.

The statistical reliability of the topology of the trees was evaluated by bootstrap analysis (Felsenstein, 1985). Both trees showed generally the same topology, and therefore only the ML tree is shown (Fig. 2).

For species of the genus *Gluconacetobacter* and related taxa, sequences of the housekeeping genes *dnaK*, *groEL* and *rpoB* show a higher resolution than the 16S rRNA gene (Cleenwerck *et al.*, 2010). In the present study, partial sequences of these housekeeping genes were therefore determined for representative strains of all the species of the genus *Acetobacter* with validly published names and for strains LMG 27543 and LMG 1531^T (Table S1), using the approach described previously (Cleenwerck *et al.*, 2010). The obtained sequences were translated into amino acid sequences in MEGA6 and were aligned using MUSCLE under default parameters (Edgar, 2004). Subsequently, the alignments were retro-transcribed to their respective nucleotide sequences. The sequences of the three genes were concatenated (1614 bp) and a phylogenetic tree was reconstructed with MEGA6 using the maximum-likelihood model (Fig. 3). The DNA substitution GTR+G+I was selected under the Bayesian Information Criterion (Nei & Kumar, 2000; Tamura *et al.*, 2013). A concatenated tree based on amino acid sequences (538 aa) of the above-mentioned sequences was also reconstructed, with substitution model LG+G (Fig. S2a). Bootstrap values lower than 70 % were removed (Tindall *et al.*, 2010). *Acetobacter cibinongensis*, *Acetobacter orientalis*, *Acetobacter papayae* and *Acetobacter peroxydans* were not included in this tree as sequences of *dnaK* of the latter three species and *rpoB* of the former two species could not be obtained. Phylogenetic trees based on *groEL* sequences (528 bp) and corresponding amino acid sequences (176 aa), which include all species of the genus *Acetobacter*

with validly published names, are shown in Fig. S2b and c. Both nucleotide-sequence-based trees showed topologies similar to the 16S rRNA gene-based tree, but with a higher discriminatory power. The housekeeping gene sequences enabled differentiation of nearly all species of the genus *Acetobacter*. Only strains of the closely related species *Acetobacter cerevisiae* and *Acetobacter malorum* as well as *Acetobacter tropicalis* and *Acetobacter senegalensis* were intermixed. The concatenated tree based on amino acid sequences showed a topology similar to those of the nucleotide-sequence-based trees, with only a few differences, i.e. *Acetobacter senegalensis* and *Acetobacter tropicalis* were differentiated, while *Acetobacter lovaniensis* as well as *Acetobacter pomorum* were not differentiated from *Acetobacter fabarum* and *Acetobacter pasteurianus*, respect- ively. Trees based on amino acid sequences of *dnaK*, *groEL* and *rpoB* separately showed a lower taxonomical resolution (shown for *groEL* in Fig. S2b and c). These trees are less informative and thus less useful for the differentiation of AAB. In the concatenated-amino-acid-sequences-based tree, strains LMG 27543 and LMG 1531^T were clearly differentiated from *A. aceti*. Differentiation was noticed at amino acid positions 25 and 131 of *dnaK* and position 23 of *rpoB*. Overall, strains LMG 27543 and LMG 1531^T grouped together on a branch separate from all established species but close to *A. aceti*, indicating that they represented a single novel species within the genus *Acetobacter*. Additionally, their nucleotide sequences were not identical, indicating that they were different strains.

AFLP DNA fingerprinting was performed on strains LMG 27543, LMG 1530 and LMG 28092 as previously described (Castro *et al.*, 2013). The obtained DNA fingerprints were compared with AFLP profiles of AAB present in a BCCM/ LMG in-house database (Cleenwerck *et al.*, 2009). The strains formed a cluster with LMG 1531^T separate from the related species (Fig. 4a), confirming the MLSA results. In addition, the cluster showed two distinct DNA fingerprint types (with LMG 1530 and LMG 1531^T forming the first type and LMG 27543 and LMG 28092 forming the second type), indicating that LMG 1531^T, LMG 1530, LMG 27543 and LMG 28092 represent at least two different strains.

Random amplified polymorphic DNA (RAPD) analysis was performed on strains LMG 1531^T, LMG 1530, LMG 27543 and LMG 28092 as previously described (Williams *et al.*, 1990). Using primer RAPD-270, three different band patterns were obtained. Those of LMG 1530 and its mutant LMG 1531^T showed a few differences, while no clear differences were found for LMG 27543 and LMG 28092 (Fig. 4b). The latter isolates were obtained from the same sample at the same time and are most probably reisolates of the same strain.

DNA – DNA hybridizations were performed between strains LMG 1531^T and LMG 27543 and with their nearest neigh- bours, *A. aceti* and *A. nitrogenifigens*, to confirm the single novel species status of the two strains. Genomic DNA was extracted using the large-scale method described previously (Cleenwerck *et al.*, 2002). DNA – DNA hybridizations were performed at 46 ºC using a modification (Goris *et al.*, 1998) of the microwell plate method (Ezaki *et al.*, 1989). Reciprocal reactions (A→B and B→A) were performed for each DNA pair. A high DNA – DNA relatedness

was found between strains LMG 27543 and LMG 1531^T (88 %) and a low relatedness (< 70 %) to the type strains of *A. aceti* LMG 1504^T (< 53 %) and *A. nitrogenifigens* LMG 23498^T (< 15 %) (Table S2). The DNA – DNA hybridization data therefore confirmed that strains LMG 1531^T and LMG 27543 were representative of a single novel species. The DNA G+C content of strains LMG 1531^T and LMG 27543 was 58.3 and 56.7 mol%, respectively, which is consistent with DNA G+C contents of members of the genus *Acetobacter* (Cleenwerck *et al.*, 2008; Iino *et al.*, 2012).

The whole-cell fatty acid methyl ester composition was determined for strains LMG 1531^T and LMG 27543 and

A. aceti LMG 1504^T using an Agilent Technologies 6890N gas chromatograph. Cultivation of the strains, fatty acid extraction and analysis of the fatty acid methyl esters were performed according to the recommendations of the Microbial Identification System, Sherlock version 3.10 (MIDI). Fatty acids were extracted from cultures grown in AAM for 48 h at 28 ºC under aerobic conditions. The peaks of the profiles were identified using the TSBA50 identification library version 5.0 (MIDI, Hewlett Packard). The predominant fatty acid was $C_{18:1}\upsilon 7c$ (54.2 – 58.3 %), while the following fatty acids were present in lower percentages (above 1 %): $C_{16:0}$ (11.2 – 11.65 %), $C_{14:0}$ 2-OH (10.56 – 12.85%), $C_{16:0}$ 2-OH (4.33 – 5.77%), $C_{18:0}$ (3.71 – 4.14%), $C_{16:0}$ 3-OH (3.07 – 3.44%), $C_{18:0}$ 3-OH (3.07 – 3.23 %) and $C_{14:0}$ (1.93 – 2.72 %) (Table 1). The fatty acid methyl ester data were consistent with those reported for the species of the genus *Acetobacter* with validly published names by Spitaels *et al.* (2014), generated using the same method from cultures also grown on AAM at 28 ºC under aerobic conditions, for 24 to 72 h, depending on the strain. The analysis of respiratory quinones of LMG 1531^T was performed as described previously (Vaz-Moreira *et al.*, 2007) using the method of Tindall (1989). The major ubiquinone present was Q-9, which was consistent with previous studies showing that Q-9 ubiquinone enables the members of the genus *Acetobacter* to be differentiated from the members of other genera (Yamada & Yukphan, 2008).

Strains LMG 1531^T and LMG 27543 were subjected to phenotypic tests to identify characteristics enabling their differentiation from the established species of the genus *Acetobacter*, using methods described previously (Cleenwerck *et al.*, 2002; 2007). The production of 2-keto-D-gluconic acid and 5-keto-D-gluconic acid from D-glucose was determined as reported by Spitaels *et al.* (2014). The type strains of *A. aceti*, *A. nitrogenifigens* and *Acetobacter oeni* were investigated when appropriate, concurrently with strain LMG 1531^T and LMG 27543. Strains LMG 1531^T and LMG 27543 could be differentiated from their nearest phylogenetic neighbour species based on their ability to grow on D-fructose and D-sorbitol as the sole carbon source; their ability to grow on yeast extract with 30 % D-glucose and on standard medium [5 %, w/v, D-glucose; 0.5 %, w/v, yeast extract (Oxoid)] with pH 3.6 or containing 1 % NaCl; and their weak acid production from D- arabinose (Table 2, Table S3). The production of cellulose was examined

by boiling cell pellicle in 5 % NaOH for 2 h (Navarro *et al.*, 1999). Only strain LMG 1530 produced a cellulose pellicle.

In conclusion, the results presented above demonstrate that strains LMG 1531^T, LMG 1530, LMG 27543 and LMG 28092

represent a single novel species that can be differentiated genotypically and phenotypically from the currently established species of the genus *Acetobacter*. Therefore, we propose to classify them as the novel species *Acetobacter sicerae* sp. nov., with strain LMG 1531^T as the type strain.

Description of *Acetobacter sicerae* sp. nov.

Acetobacter sicerae (si.ce9rae. L. gen. n. *sicerae* of a fermented liquor, intended to mean of cider).

Cells are Gram-stain-negative, motile, coccoid rods, approximately 1 μm wide and 1.5 – 2.5 μm long. Cells occur singly or in pairs. Catalase and oxidase activity is present. On LMG medium 404 agar 5 %, w/v, D-glucose; 1 %, w/v, yeast extract (Oxoid) and 1.5 %, w/v agar, colonies are round, smooth, beige and slightly raised, with a diameter of approximately 1 mm after 2 days of incubation. Able to produce 2-keto-D-gluconic and 5-keto-D-gluconic acid from D-glucose. Able to grow on D-fructose, D-sorbitol and glycerol as single carbon sources, but not on maltose or methanol. Able to grow on ammonium as sole nitrogen source with ethanol as carbon source. Able to grow on yeast extract containing 30 % D-glucose and on standard medium with pH 3.6 or containing 1 % NaCl. Able to produce acid from D-arabinose weakly. The predominant fatty acid is $C_{18:1}\omega 7c$; other fatty acids present in significant amounts are $C_{14:0}$ 2-OH, $C_{16:0}$ 2-OH and $C_{18:0}$.

The type strain, LMG 1531^T (5NCIMB 8941^T), is a non- cellulose-producing mutant from the peritrichous flagel- lated strain LMG 1530, which was isolated by J. Carr from cider (Shimwell & Carr, 1958). The DNA G+C content of the type strain is 58.3 %.

Acknowledgements

Leilei Li has a PhD grant from the Chinese Scholarship Council and Ghent University Co-Funding. The MLSA work was supported by funds from the European Community's Seventh Framework Programme (FP7, 2007 – 2013), Research Infrastructures Action, under the grant agreement no. FP7-228310 (EMbaRC project). The BCCM/LMG Bacteria Collection is supported by the Federal Public Planning Service – Science Policy, Belgium. Katrien Engelbeen and Marjan De Wachter are acknowledged for help with the DNA – DNA hybridizations and the AFLP data, respectively.

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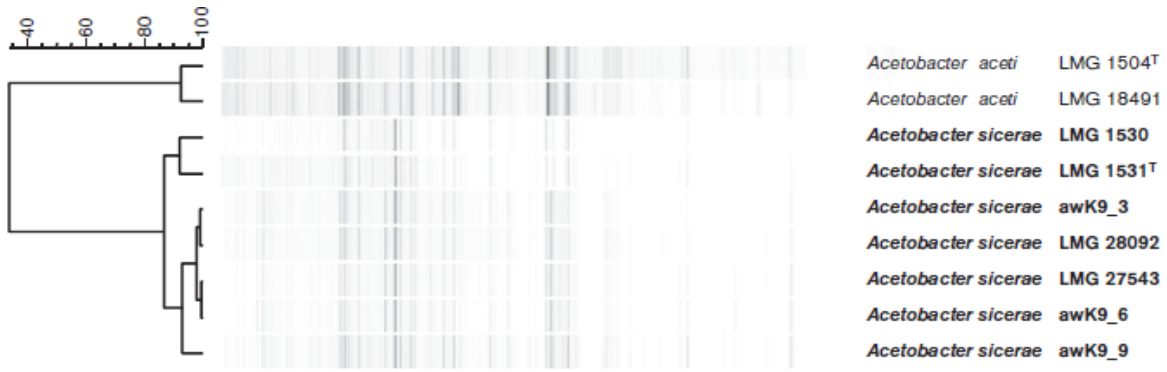

Fig. 1. MALDI-TOF MS profiles of *Acetobacter sicerae* sp. nov. and its closest phylogenetic relative, *Acetobacter aceti*. The dendrogram was derived from UPGMA of the fingerprints with levels of linkage expressed as Pearson correlation coefficients.

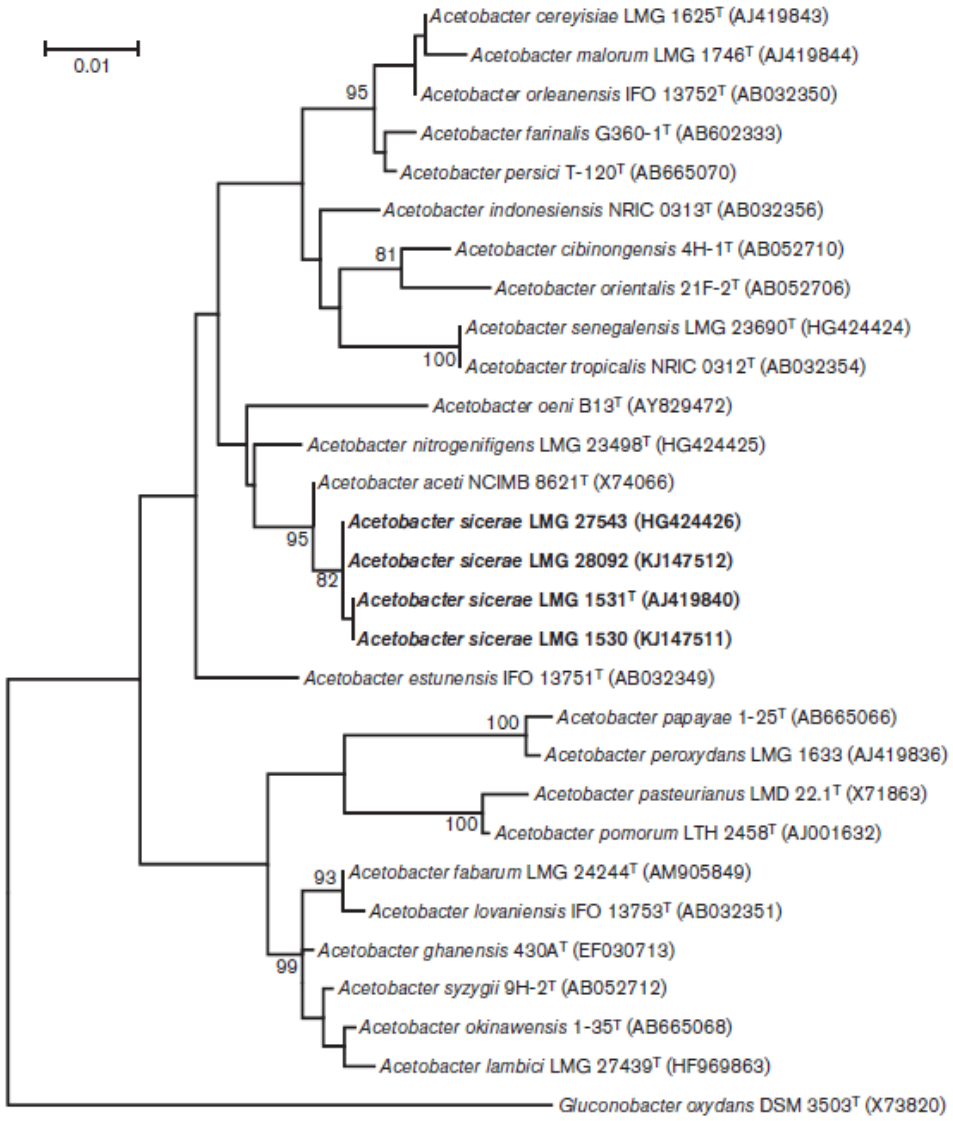

Fig. 2. Maximum-likelihood tree based on nearly complete 16S rRNA gene sequences (1312–1320 nt) showing the phylogenetic position of *Acetobacter sicerae* sp. nov. within the genus *Acetobacter*. The robustness of the branching is indicated by bootstrap values calculated for 1000 subsets. Bar, 0.01 % sequence divergence.

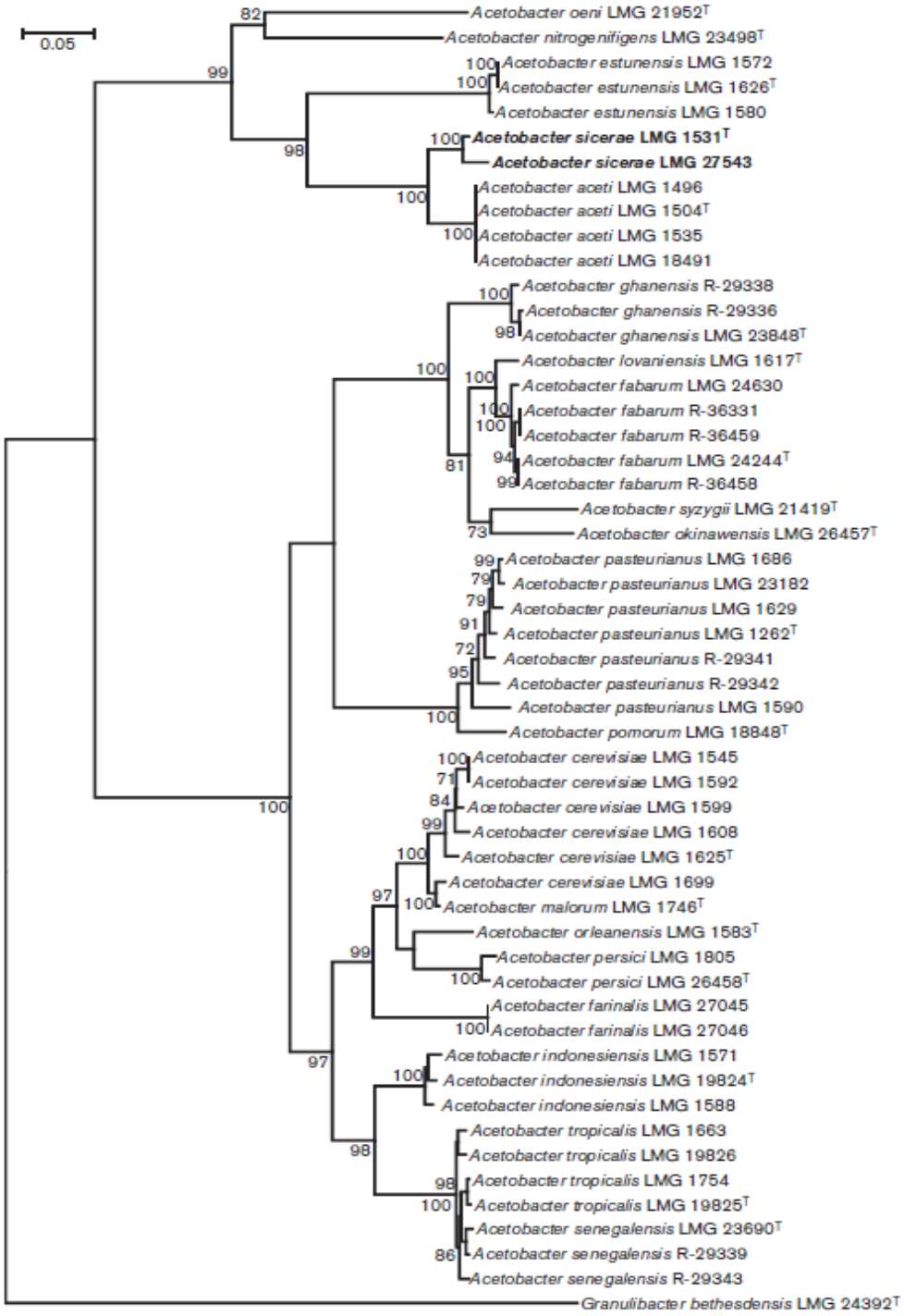

Fig. 3. Maximum-likelihood tree based on concatenated sequences (1614 bp) of three housekeeping gene fragments [*dnaK* (522 bp), *groEL*(528 bp) and *rpoB*(564 bp)] showing the phylogenetic position of *Acetobacter sicerae* sp. nov. within the genus *Acetobacter*. The type strain of *Granulibacter bethesdensis* was used as outgroup. Numbers at branching points are percentage bootstrap values based on 1000 replications. The sequence accession numbers for *dnaK*, *groEL* and *rpoB* gene sequences are provided in Table S1. Bar, 0.05 % sequence divergence.

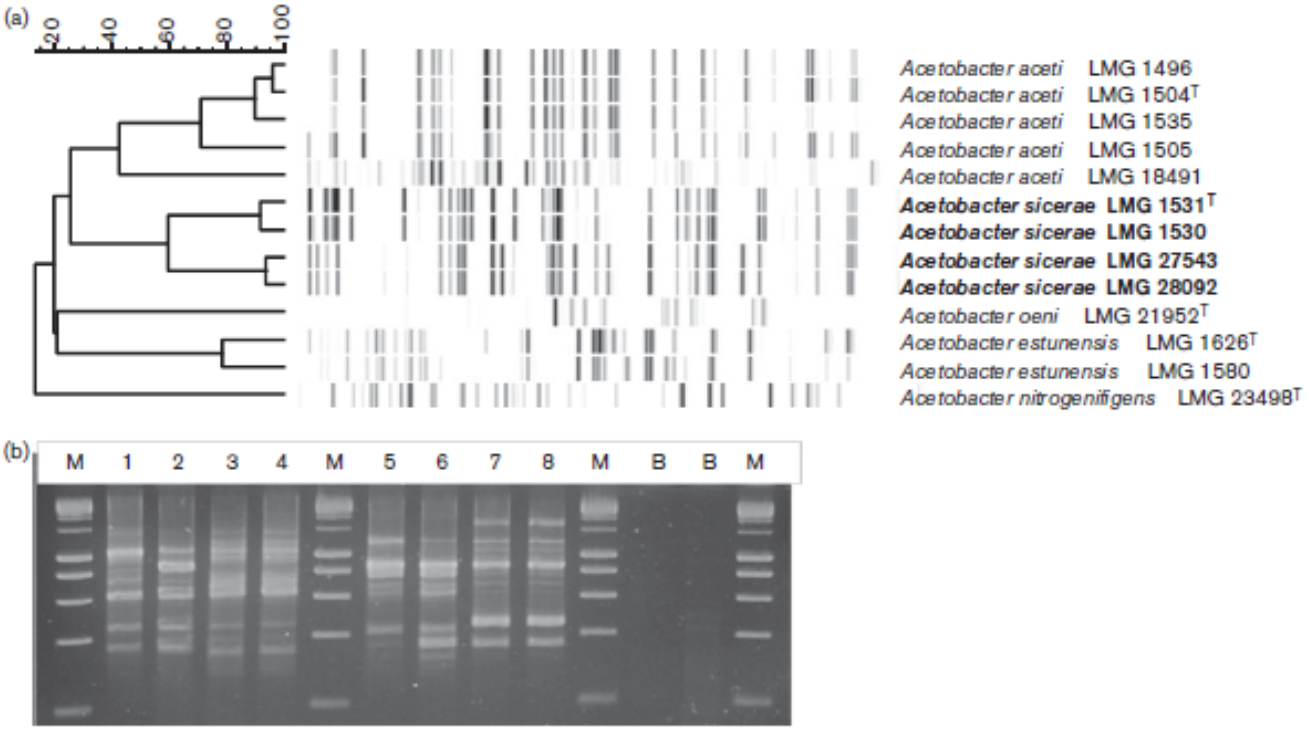

Fig. 4. (a) AFLP fingerprints of *Acetobacter sicerae* sp. nov. strains and their closest phylogenetic relatives. The dendrogram was derived from UPGMA of the fingerprints with levels of linkage expressed as Dice similarity coefficients; (b) RAPD fingerprints of *Acetobacter sicerae* sp. nov. strains LMG 1530, LMG 1531^T, LMG 27543 and LMG 28092. Lanes 1–4, RAPD patterns obtained using primer RAPD-270 (5′-TGCGCGCGGG-3′) for LMG 1530, LMG 1531^T, LMG 27543 and LMG 28092, respectively. Lanes 5–8, RAPD patterns obtained using primer RAPD-272 (5′-AGCGGGCCAA-3′) for LMG 1530, LMG 1531^T, LMG 27543 and LMG 28092, respectively. Lane M, reference marker; lane B, blank. LMG 1530 and LMG 1531^T show much similarity (lanes 1 and 2 and lanes 4 and 5) but also a few differences, while LMG 27543 and LMG 28092 show no clear differences.

Table 1. Cellular fatty acid contents (percentages) of *Acetobacter sicerae* sp. nov. (data in bold type) and all type strains of species of the genus *Acetobacter*

Strains: 1, *A. sicerae* sp. nov. LMG 1531^T; 2, *A. sicerae* sp. nov. LMG 27543; 3, *A. aceti* LMG 1504^T; 4, *A. nitrogenifigens* LMG 23498^T; 5, *A. oeni* LMG 21952^T; 6, *A. estunensis* LMG 1626^T; 7, *A. pomorum* LMG 18848^T; 8, *A. pasteurianus* LMG 1262^T; 9, *A. senegalensis* LMG 23690^T; 10, *A. tropicalis* LMG 19825^T; 11, *A. indonesiensis* LMG 19824^T; 12, *A. papayae* LMG 26456^T; 13, *A. fabarum* LMG 24244^T; 14, *A. ghanensis* LMG 23848^T; 15, *A. syzygii* LMG 21419^T; 16, *A. okinawensis* LMG 26457^T; 17, *A. lovaniensis* LMG 1617^T; 18, *A. peroxydans* LMG 1635^T; 19, *A. cerevisiae* LMG 1625^T; 20, *A. cibinongensis* LMG 21418^T; 21, *A. orleanensis* LMG 1583^T; 22, *A. persici* LMG 26458^T; 23, *A. malorum* LMG 1746^T; 24, *A. orientalis* LMG 21417^T; 25, *A. farinalis* LMG 26772^T; 26, *A. lambici* LMG 27439^T. –, Not detectable or trace amount (<1%). Data for *A. sicerae* LMG 1531^T, LMG 27543 and *A. aceti* LMG 1504^T were generated as part of this study. Other data were taken from Spitaels *et al.* (2014). Cultivation conditions prior to fatty acid extraction were identical for all strains, except for the duration of cultivation, which varied from 24 h to 72 h depending on the strain.

Strain	$C_{14:0}$	$C_{14:0}$ 2-OH	$C_{16:0}$	$C_{16:0}$ 2-OH	$C_{16:0}$ 3-OH	$C_{18:0}$	$C_{18:0}$ 3-OH	$C_{18:1}\omega7c$	$C_{19:0}$ cyclo $\omega8c$
1	**1.9**	**10.6**	**11.7**	**4.3**	**3.1**	**4.1**	**3.1**	**58.3**	–
2	**2.7**	**12.9**	**11.2**	**5.8**	**3.4**	**3.7**	**3.2**	**54.2**	–
3	4.3	21.8	11.7	14.5	3.7	–	–	40.4	–
4	–	16.2	10.3	23.1	5.5	–	2.3	33.4	1.8
5	1.2	8.7	9.8	10.6	4.4	4.4	8.0	48.1	–
6	3.6	5.4	11.6	4.0	2.6	4.2	3.3	61.7	1.9
7	6.3	15.4	8.2	12.5	7.6	–	4.8	41.4	–
8	3.9	16.5	8.2	13.0	6.9	1.4	3.0	42.8	–
9	2.8	14.1	8.0	13.2	7.4	1.9	6.1	33.8	–
10	1.5	9.2	10.0	8.3	4.5	2.5	3.7	52.8	–
11	1.9	6.0	11.0	10.2	4.6	4.1	4.0	53.8	1.0
12	5.5	9.4	11.4	13.3	3.7	3.5	1.6	46.3	2.6
13	6.0	2.1	8.5	10.0	3.1	2.6	1.0	61.5	3.1
14	4.5	3.8	9.3	9.6	2.6	3.5	1.5	61.4	2.0
15	6.1	2.5	10.7	7.9	2.4	3.3	1.2	60.9	2.2
16	4.6	2.6	9.5	10.3	2.9	4.4	1.5	59.3	2.2
17	6.0	1.1	9.0	9.1	2.2	2.0	0.9	65.4	1.8
18	2.0	9.2	9.7	10.2	2.2	2.2	0.5	60.0	2.6
19	0.9	3.9	11.1	5.4	2.2	5.9	3.6	63.0	1.0
20	1.0	2.3	11.0	4.5	3.8	5.1	4.1	62.1	1.9
21	1.2	5.1	11.2	7.4	2.6	4.0	1.8	64.6	–
22	1.1	4.4	11.4	6.6	2.3	3.9	1.9	64.8	–
23	–	5.2	10.7	6.4	2.6	4.7	3.1	61.5	–
24	2.2	6.5	10.3	6.3	4.7	3.1	1.6	61.9	1.6
25	–	3.2	13.0	8.2	2.4	6.1	3.0	58.1	3.1
26	1.1	1.2	10.5	8.8	1.9	8.3	1.2	59.3	2.1

Table 2. Differential characteristics for *Acetobacter sicerae* sp. nov. from the phylogenetically closest species of the genus *Acetobacter*

Taxa: 1, *Acetobacter sicerae* sp. nov. (LMG 1531^T and LMG 27543) 2, *A. aceti* (four strains, including LMG 1504^T); 3, *A. nitrogenifigens* LMG 23498^T; 4, *A. oeni* LMG 21952^T; 5, *A. estunensis* LMG 1626^T. Data were obtained in this study, unless indicated otherwise. +, Positive; −, negative; W, weakly positive; V, variable (the result for the type strain is given in parentheses); SM, standard medium.

Characteristic	1	2	3	4	5
Formation from D-glucose					
5-Keto-D-gluconic acid	+	+*	+†	+*	−*
2-Keto-D-gluconic acid	+	+*	−†	−*	+*
Growth in ammonium with ethanol	+	+	+	−	+
Growth in 10 % ethanol	−	−	+	+	−
Growth on yeast extract + 30 % D-glucose	+	−	+	−	−
Growth on carbon sources					
D-Fructose	+	−	+	+	+
D-Sorbitol	+	V (−)	−	−	+
Acid production from D-arabinose	W‡	V (+)	+	+	+
Growth on SM with 1 % NaCl	+	−	−	+	−
Growth on SM at pH 3.6	+	V (W)	−	+	W

*Data taken from Cleenwerck *et al.* (2008).
†Data taken from Spitaels *et al.* (2014).
‡Colour change was observed, with a pH range between pH 5.98 and 6.05, while + was described as a colour change and a pH measurement lower than pH 5.9 (Gosselé, 1982).