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Description of *Acetobacter oboediens* sp. nov. and *Acetobacter pomorum* sp. nov., two new species isolated from industrial vinegar fermentations

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Two strains of *Acetobacter* sp., LTH 2460^T and LTH 2458^T, have been isolated from running red wine and cider vinegar fermentations, respectively. Taxonomic characteristics of the isolates were investigated. Comparative analysis of the 16S rRNA sequences revealed > 99% similarity between strain LTH 2460^T and the type strains of the related species *Acetobacter europaeus* and *Acetobacter xylinus* and between strain LTH 2458^T and *Acetobacter pasteurianus*. On the other hand, low levels of DNA relatedness (< 34%) were determined in DNA–DNA similarity studies. This relatedness below the species level was consistent with specific physiological characteristics permitting clear identification of these strains within established species of acetic acid bacteria. Based on these results, the names *Acetobacter oboediens* sp. nov. and *Acetobacter pomorum* sp. nov. are proposed for strains LTH 2460^T and LTH 2458^T, respectively. The phylogenetic positions of the new species are reflected by a 16S rRNA-based tree. Furthermore, a 16S rRNA-targeted oligonucleotide probe specific for *A. oboediens* was constructed.

Keywords: *Acetobacter oboediens* sp. nov., *Acetobacter pomorum* sp. nov., vinegar fermentation, acetic acid bacteria

INTRODUCTION

In man-made substrates, acetic acid bacteria are involved in food spoilage and indigenous fermentations to produce vinegar from alcohol-containing liquids such as wine and beer. Modern fermentations are performed in submerged processes which allow final acetic acid concentrations of up to 20% to be achieved. The usual substrates for vinegar fermentations in Germany are cider, wine and diluted distilled ethanol. Strains isolated from vinegar fermentations have been allotted to species of the genus *Acetobacter*, e.g. *Acetobacter aceti*, *Acetobacter hansenii*, *Acetobacter pasteurianus* and *Acetobacter xylinus* (Kittelmann *et al.*, 1989; Swings, 1992). In addition, Sievers *et al.* (1992) described *Acetobacter europaeus* as a new species which represents the main flora component in industrial vinegar fermenters in Central Europe. It is remarkable that the majority of strains isolated from vinegar fermentations are not suitable for preservation (Sievers & Teuber, 1995). Recently, a method for starter preparation was described (Sokollek & Hammes, 1997) and the applicability of this approach to cultivate and preserve various isolates from industrial vinegar fermentations was reported (Sokollek *et al.*, 1998). Two of these isolates, strains LTH 2460^T and LTH 2458^T, could not be allotted to any species of the genera *Acetobacter* and *Gluconobacter*. In this communication, characterization of the taxonomic position of these isolates, based on physiological properties in combination with 16S rRNA sequence analysis and DNA similarity studies, is described.

METHODS

Bacterial strains and growth conditions. The following strains were used. *Acetobacter* sp. LTH 2460^T (Sokollek & Hammes, 1997) was isolated from a red wine vinegar fermentation at a total concentration [i.e. percentage (w/v) acetic acid plus percentage (v/v) ethanol] of 11·4%. *Acetobacter* sp. LTH 2458^T (Sokollek *et al.*, 1998) was isolated

The EMBL accession numbers for the 16S rDNA sequences reported in this paper are AJ001631 (*Acetobacter oboediens* strain LTH 2460^T) and AJ001632 (*Acetobacter pomorum* strain LTH 2458^T).

from a cider vinegar fermentation at a total concentration of 6·1 %. An additional eight strains were isolated from various vinegar fermentations (Sokollek *et al.*, 1998). The type strains of species of the genera *Acetobacter* and *Gluconobacter* were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). All but one of the type strains (*A. europaeus*) were grown on YPM medium containing (l^{-1}): yeast extract, 5 g; peptone, 3 g; and mannitol, 25 g. Solid YPM medium was obtained by adding 1 % agar. Acetic acid–ethanol (AE) medium and reinforced AE (RAE) medium (Sokollek & Hammes, 1997) were used as growth media for *A. europaeus* and strains isolated from vinegar fermentations, respectively. Ethanol and acetic acid were added to the autoclaved basal medium at the concentrations indicated in parentheses, e.g. AE medium (4a/3e) contains 4 ml glacial acetic acid and 3 ml absolute ethanol added to 100 ml basal medium. Cultures were inoculated with a loopful of culture from agar plates. Shaking cultures were incubated on a rotary shaker at 200 r.p.m. in 1 l double-baffled Erlenmeyers containing 200 ml medium. Surface cultures on agar plates were incubated at 30 °C in an atmosphere of > 90 % relative humidity.

Pre-cultures for physiological tests were obtained by growing *A. europaeus* on AE agar (4a/3e), *A. xylinus* on YPM agar, and *A. pasteurianus* as well as the isolates on RAE agar (1a/2e). For characterization of growth in the presence of up to 8 % (w/v) acetic acid, the isolates were pre-grown on AE agar (4a/3e).

Physiological characterization. Growth in the presence of acetic acid was tested in AE broth containing 3 % (v/v) ethanol and 0, 1, 2, 3, 4, 5, 6, 7 and 8 % (w/v) acetic acid, respectively. The cultures were incubated at 30 °C for 10 d by aeration. The formation of 2- and 5-ketogluconic acid was investigated in RAE broth (1a/2e) after 7 d incubation at 30 °C; 2- and 5-ketogluconic acid were detected by TLC (Gosselé *et al.*, 1980). Growth on acetate at pH 2·5 was tested in basis bouillon which contained (l^{-1}): peptone, 10 g; and yeast extract, 10 g. Acetic acid (15 g l^{-1}) was added to the medium and the pH was adjusted with HCl. Utilization of lactate was examined in basis bouillon supplemented with glacial acetic acid (10 ml l^{-1}) and sodium lactate (20 g l^{-1}). Growth in the presence of 30 % (w/v) glucose was determined in the basis bouillon supplemented with glacial acetic acid (10 ml l^{-1}) and glucose (300 g l^{-1}). Glucose, lactate and gluconic acid were assayed enzymically using Boehringer Mannheim test kits. Formation of cellulose was tested in non-agitated cultures at 25 °C in RAE broth (0a/0e) and (1a/2e). For the study of utilization of various carbon sources, the following media were employed: (i) salt agar, containing (l^{-1}) 0·2 g $(NH_4)_2HPO_4$, 0·1 g $MgSO_4 . 7H_2O$, 0·1 g $Ca_5(PO_4)_3(OH)$, 2 ml glacial acetic acid and 10 g agar; and (ii) yeast extract/peptone agar, containing (l^{-1}) 2 g yeast extract, 1 g peptone, 5 ml glacial acetic acid and 10 g agar. Carbon sources were added at a concentration of 20 g l^{-1} or 20 ml l^{-1}, respectively. The cultures were incubated at 30 °C and > 90 % relative humidity for 14 d. Cultures grown on the glycerol-containing media were investigated for the formation of dihydroxyacetone (Entani *et al.*, 1985). Colonies from fructose- or glucose-containing agar plates were subjected to the ferric chloride reaction as described by Entani *et al.* (1985). All investigations were performed twice for reproducibility testing.

Quinone system. Cells grown in 200 ml RAE broth (1a/2e) were harvested, 5 ml 90 % 2-propanol was added to the precipitate, and the mixture was incubated at 30 °C for 2 h. The extract was evaporated to dryness under a stream of nitrogen and redissolved in 1 ml methanol. Quinones were separated by reverse phase HPLC using the Spherisorb ODS2 column (RP-18, 250 × 4·6 mm, 3 μm; GROM) at 25 °C and monitored at 275 nm. Methanol/2-propanol (75:25, v/v) was used as eluent at a flow rate of 1 ml min^{-1}. Authentic standards for ubiquinone Q-9 and Q-10 were obtained from Fluka. For ubiquinone Q-8 identification, an extract of *Frateuria aurantia* DSM 6220^T grown in RAE broth (0a/0e) was used.

DNA isolation. For the isolation of chromosomal DNA, cells (1 g wet weight) were suspended in 10 ml saline/EDTA buffer (0·15 M NaCl, 50 mM EDTA, pH 8·0) containing 30 mg lysozyme and 0·2 mg RNase. After incubation at 37 °C for 2 h, the lysis of cells was completed by adding 25 % (w/v) SDS to obtain a final concentration of 2 % (w/v). Subsequently, the concentration of NaCl was adjusted to 1 M with a 5 M stock solution. For protein digestion, 30 mg proteinase K was added and the mixture was incubated at 65 °C overnight and finally treated once with phenol/chloroform and twice with chloroform (Sambrook *et al.*, 1989). The DNA was precipitated with ethanol and dissolved in 0·1 × SSC (Sambrook *et al.*, 1989) to obtain a final concentration of 0·5–1·0 mg ml^{-1}. For degradation of RNA, 40 μg RNase ml^{-1} was added to the solution and incubated at 37 °C for 1 h. Subsequently, the DNA solution was adjusted to 1 × SSC and 1 % (w/v) SDS with 10 × SSC and 10 % (w/v) SDS, respectively. Proteinase K (80 μg ml^{-1}) treatment was performed at 65 °C for 2 h. The DNA was purified by repeated phenol/chloroform extraction, precipitated with ethanol and dissolved in 0·1 × SSC to obtain a concentration of 0·5–1·0 mg ml^{-1}.

DNA hybridization. For determination of DNA similarities, 5 μg chromosomal DNA was denatured (0·05 × SSC and 0·4 M NaOH, 25 °C, 20 min) and transferred to a nylon membrane (Qiabrane; Qiagen) using a dot-blot apparatus (Schleicher & Schüll). The DNA was immobilized at 80 °C for 1 h. The accessibility of DNA was determined by hybridization with the radiolabelled 16S rDNA bacterial probe EUB 338 (Amann *et al.*, 1990) as described previously (Hertel *et al.*, 1992). The probe-specific hybridization temperature and washing temperature were 45 and 55 °C, respectively. Radioactive labelling of the probe was carried out with the Ready-To-Go T4 Polynucleotide Kinase kit (Pharmacia) and [γ-^{32}P]ATP. Genomic DNA was labelled with the Random Primers (hexamers) DNA Labelling System (Gibco-BRL) and [α-^{32}P]dCTP. Hybridization was performed at 65 °C for 48 h in the hybridization solution described by Hertel *et al.* (1992). The membranes were washed three times for 20 min in 0·1 × SSC, 0·1 % SDS at 70 °C. Autoradiograms were digitized with a video documentation system (E.A.S.Y.; Herolab). The DNA similarities were calculated using WinCam software (Cybertech) and the accessibility of immobilized DNA for hybridization was taken into consideration.

The radioactive dot-blot hybridization with the organism-specific oligonucleotide probe was performed as described by Hertel *et al.* (1992) with the following modifications: chromosomal DNA and Qiabrane nylon membranes were used and labelling of the oligonucleotide probe was carried out with the Ready-To-Go T4 Polynucleotide Kinase kit and [γ-^{32}P]ATP.

Sequencing of 16S rDNA. 16S rDNA was amplified *in vitro* with *Taq* polymerase in a GeneAmp 2400 PCR system

(Perkin-Elmer). The PCR product was purified using the QIAquick PCR Purification kit (Qiagen). The T7 Sequencing kit (Pharmacia) was used for direct sequencing.

Sequence data analysis. The rRNA sequences were added to an alignment of about 1000 primary structures of 16S rRNA. Phylogenetic analysis was performed by applying maximum-parsimony and maximum-likelihood approaches. The respective tools of the ARB program package (Springer *et al*., 1992) were used for alignment, calculation of similarities and tree reconstruction, evaluation and drawing.

DNA base composition. The DNA G+C contents were determined according to the thermal denaturation method (Marmur & Doty, 1962). The modified equation of De Ley (1970) was applied for calculation of the values.

RESULTS

Ten strains of acetic acid bacteria were isolated from running industrial vinegar fermentations in southern Germany (Sokollek *et al*., 1998). Two of the unidentified isolates, LTH 2460^T and LTH 2458^T, were especially suitable for preservation. The plasmid profiles obtained from these strains differed from each other. The profiles were compared with those obtained from the acetator cultures used as the source for isolation. Identical plasmid profiles were found indicating that the isolates represented the predominant strains of the acetators. Moreover, strain LTH 2458^T could be isolated from two independently running cider vinegar fermenters. Based on these results, strains LTH 2460^T and LTH 2458^T were subjected to further taxonomic investigations.

16S rDNA sequence analysis

The 16S rDNA of strains LTH 2460^T and 2458^T was directly sequenced using amplified DNA. The 16S rRNA sequences were compared with those of the type strains of *Acetobacteraceae* (Sievers *et al*., 1994a, b, 1995). Similarities of $>99\%$ were found for the sequence of strain LTH 2460^T compared to those of *A. europaeus* and *A. xylinus*, corresponding to seven and eleven base changes, respectively. With regard to *A. hansenii*, the similarity was only 98·2 %. The 16S rRNA sequence of strain LTH 2458^T showed the highest similarity to that of *A. pasteurianus*. A deletion of one base pair as well as five base changes were detected. On the other hand, a similarity of only 97·3 % was determined between the 16S rRNA of LTH 2458^T and *A. aceti*. Based on these sequence data, the phylogenetic tree depicted in Fig. 1 was constructed, reflecting the position of the strains within the genera *Acetobacter* and *Gluconobacter*.

Design and application of oligonucleotide probes

Results from comparison of the 16S rRNA sequences were used to construct the oligonucleotide probe 16S/2460 (5′ GGCTCGGTGTCACCACCT 3′) which is specific for strain LTH 2460^T. The specificity of the probe was evaluated by dot-blot hybridization to membrane-bound genomic DNA of the type strains of

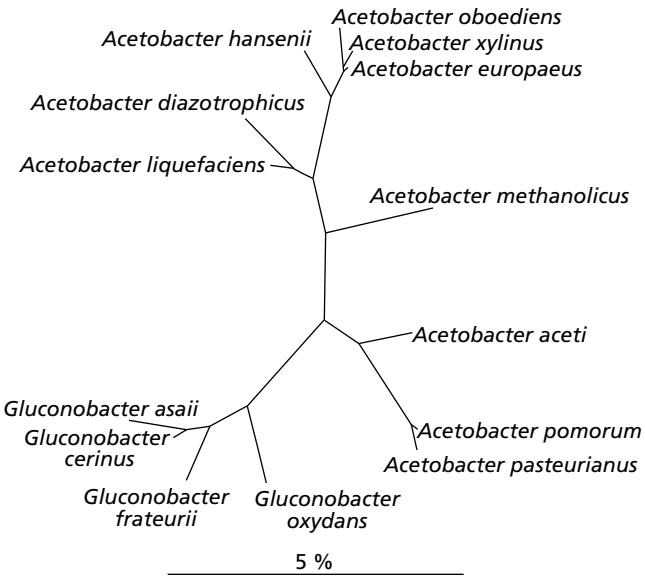

Fig. 1. 16S rRNA-based tree reflecting the phylogenetic position of *A. oboediens* (LTH 2460^T) and *A. pomorum* (LTH 2458^T) within the acetic acid bacteria. A tree was constructed which is based on a maximum-likelihood tree and a data set containing all available almost complete 16S rRNA sequences from acetic acid bacteria and selected reference organisms from other phylogenetic groups. All positions were used for the calculation. The tree topology was corrected according to the results of distance matrix and maximum-parsimony analysis. The bar indicates 5 % estimated sequence divergence.

Acetobacteraceae (Sievers *et al*., 1994a) and the 10 strains isolated from industrial vinegar fermentations (Sokollek *et al*., 1998). Specific temperatures of 45 and 58 °C were defined for hybridization and stringent washing, respectively. Probe 16S/2460 hybridized exclusively to the DNA of strain LTH 2460^T.

DNA similarity

Quantitative DNA–DNA reassociation studies were performed with the membrane-bound DNA of strains LTH 2460^T, LTH 2458^T, *A. europaeus* DSM 6160, *A. xylinus* DSM 6513 and *A. pasteurianus* DSM 3509. The species used for comparison were chosen on the basis of their relatedness revealed in Fig. 1. The labelled DNA of the type strains were employed. As shown in Table 1, the isolates exhibited levels of DNA relatedness with the type strains of below 34 %.

Physiological and biochemical characterizations

The taxonomically relevant physiological properties of strains LTH 2460^T and LTH 2458^T and of *Acetobacter* species (Sievers *et al*., 1992) are compiled in Table 1. Remarkably, strain LTH 2460^T grew in the presence of 30 % (w/v) glucose and accumulated high amounts [13 % (w/v)] of gluconic acid. In addition, unlike *A. europaeus*, the strain did not require acetic acid for growth in AE broth. The ability to grow in the presence

Table 1. Characteristics of strains LTH 2460^T and LTH 2458^T permitting their differentiation from other *Acetobacter* species

+, Positive; d, some strains positive; (+), weak positive reaction; −, negative; ND, not determined. 1, *A. europaeus*; 2, *A. xylinus*; 3, LTH 2460^T; 4, *A. pasteurianus*; 5, LTH 2458^T; 6, *A. aceti*; 7, *A. hansenii*; 8, *Acetobacter liquefaciens*; 9, *A. diazotrophicus*; and 10, *A. methanolicus*.

Characteristic	1	2	3*	4	5*	6	7	8	9	10
Growth on 3% (v/v) ethanol in the presence of 4–8% (w/v) acetic acid	+	−	+	−	−	−	−	−	ND	−
Growth without acetic acid	−	+	+	+	+	+	+	+	ND	+
Growth on acetate at pH 2·5	+	−	+	−	−	−	−	−	ND	−
DNA–DNA homology with:*										
A. europaeus	100	16	34	< 10	< 10	ND	ND	ND	ND	ND
A. xylinus	37	100	25	< 10	< 10	ND	ND	ND	ND	ND
A. pasteurianus	17	< 10	< 10	100	17	ND	ND	ND	ND	ND
Formation of ketogluconic acids from D-glucose:										
2-Ketogluconic acid	d	+	+	d	−	+	d	d	+	−
5-Ketogluconic acid	d	+	−	−	−	+	d	d	−	−
Growth in the presence of:										
Ethanol	+	−	+	d	+	+	−	+	+	(+)
Lactate	d	ND	+	d	+	+	d	+	(+)	−
30% (w/v) D-Glucose†	−	−	+	−	+	−	−	−	+	−
DNA G+C content (mol%)	56–58	55–63	59·9	53–63	50·5	56–60	58–63	62–65	61–63	62

* Data obtained in this work; all other data were taken from Sievers *et al.* (1992).

† Strain LTH 2458^T formed ⩽ 1% (w/v) gluconic acid in contrast to 13% (w/v) produced by strain LTH 2460^T.

Table 2. Utilization of carbon sources

−, No growth; +, reduced growth; ++, good growth; +++, excellent growth.

Carbon source	Salt agar		Yeast extract/peptone agar		
	A. pasteurianus DSM 3509^T	LTH 2458^T	*A. xylinus* DSM 6513^T	*A. europaeus* DSM 6160^T	LTH 2460^T
Methanol	−	+	−	−	−
Ethanol	−	++	−	−	−
n-Propanol	−	+++	−	−	−
D-Glucose	+	+++	+++	++	++
D-Fructose	+	+++	+	+++	++
Maltose	++	+++	+	++	+
D-Ribose	+	+++	−	++	++
D-Xylose	++	+++	+	+	−
Sucrose	−	−	+	−	+++
Sorbitol	++	+++	++	−	−
D-Mannitol	++	+++	+++	−	−
Glycerol	+++	+++	++	−	++
L-Lactate	−	−	−	−	++
D-Gluconate	−	−	+	−	+++

of 30% (w/v) glucose was also found for strain LTH 2458^T but this culture accumulated less than 1% (w/v) gluconic acid. Analyses of the ubiquinone systems revealed Q-10 and small amounts of Q-9 for strain LTH 2460^T, whereas for strain LTH 2458^T, the ubiquinones Q-9 and Q-8 were the major and minor components, respectively. The DNA G+C contents of strains LTH 2460^T and LTH 2458^T were 59·9 and 50·5 mol%, respectively.

For further physiological characterization of the isolates, the utilization of various carbon sources was

studied on supplemented salt agar and yeast extract/peptone agar. Both media contained low concentrations of acetic acid. It was observed that acetic acid *per se* did not permit any growth. As shown in Table 2, strain LTH 2458^T can be distinguished from the type strain of *A. pasteurianus* by its ability to grow on ethanol and n-propanol with ammonium as the sole nitrogen source. Remarkably, strain LTH 2458^T grew on methanol as carbon source. Upon streaking on agar only a few (about 70) colonies were obtained, but the methanol-adapted cells of these colonies formed dense streaks and grew well on methanol within 7–10 d. In contrast to *A. pasteurianus* DSM 3509, strain LTH 2458^T formed dihydroxyacetone upon growth on glycerol. This property was also found for strain LTH 2460^T and for *A. xylinus* DSM 6513, whereas *A. europaeus* DSM 6160 did not grow on glycerol. Moreover, strain LTH 2460^T grew well on sucrose and gluconate; this was not the case for *A. europaeus*. The characteristics of strain LTH 2460^T differed from those of the type strain of *A. xylinus* in that strain LTH 2460^T was able to grow on sorbitol and mannitol. Finally, no growth on n-butanol and no ferric chloride reaction on glucose- and fructose-containing agar were observed for any of the cultures.

DISCUSSION

Using improved culture techniques for acetic acid bacteria (Entani *et al.*, 1985), it was possible to study in more detail the fermentation flora involved in vinegar fermentations. With these techniques, Entani *et al.* (1985) characterized an organism involved in submerged vinegar fermentations and later on Sievers *et al.* (1992) described a species, *A. europaeus*, present in vinegar fermenters in Central Europe. These techniques were used in this investigation and revealed a diversity in the fermentation flora in fermenters run with various substrates (Sokollek & Hammes, 1997; Sokollek *et al.*, 1998). Two isolates were characterized on the genomic and physiological level and it was observed that these could not be allotted to any one of the species of the family *Acetobacteraceae*.

The study of the 16S rRNA revealed a close overall sequence similarity of $>99\%$ between strain LTH 2460^T and *A. europaeus* as well as *A. xylinus*. A similar degree of relatedness was also observed between strain LTH 2458^T and *A. pasteurianus*. The detected sequence differences allowed construction of a specific probe to clearly identify strain LTH 2460^T. On the other hand, as the base exchanges were more evenly distributed, it was not possible, in spite of a relatively high overall sequence difference, to obtain a probe specific for LTH 2458^T. The comparative rRNA sequence analysis at a high level of phylogenetic relatedness can only provide differentiating information and is not sufficient to be used for species definition. It is accepted (Stackebrandt & Goebel, 1994) that the superior method for phylogenetic investigations at and below the species level is the quantitative hybridization analysis of genomic DNA. The DNA–DNA hybridization data clearly indicated a relatedness of the isolates to other *Acetobacteraceae* below the species level. The relatedness was $<34\%$ between strain LTH 2460^T and *A. europaeus* as well as *A. xylinus*, and $<17\%$ between strain LTH 2458^T and *A. pasteurianus*.

From the 16S rRNA-based tree of relatedness of acetic acid bacteria, it is evident that isolates LTH 2460^T and LTH 2458^T belong to different clusters. Strain LTH 2460^T is related to *A. europaeus*, *A. xylinus* and *A. hansenii*, whereas strain LTH 2458^T falls into the cluster comprising *A. pasteurianus* and *A. aceti*. The high degree of similarity of some species within the clusters is reflected in the short branches, e.g. for *A. europaeus*. Remarkably, the ubiquinone system of strains LTH 2460^T and LTH 2458^T is consistent with the systems of species within the corresponding clusters (Swings, 1992).

Investigation of the physiological properties revealed that the isolates are characterized by remarkable and prominent properties. For strain LTH 2460^T these included the following. (i) Growth in the presence of 30% (w/v) glucose. This property is shared with strain LTH 2458^T but is only described otherwise as a characteristic of the species *Acetobacter diazotrophicus* (Swings, 1992). (ii) Growth on ethanol in AE broth in the presence of 0–8% (w/v) acetic acid. The ability to grow in the absence of acetic acid differentiates strain LTH 2460^T from *A. europaeus*. *A. europaeus* is also present in high acetic acid vinegar fermentations and was shown to be dependent on the presence of acetic acid in the growth medium (Sievers *et al.*, 1992). The related species *A. xylinus*, on the other hand, can be differentiated from strain LTH 2460^T by its inability to grow on acetic acid at pH 2·5.

The following prominent properties of strain LTH 2458^T were identified. (i) Growth in the presence of 30% (w/v) glucose and (ii) growth on methanol. The latter property is characteristic of *A. methanolicus* (Swings, 1992); some strains of *A. pasteurianus* were also reported to share that property (De Ley *et al.*, 1984).

The data obtained revealed that the strains represent two new species. The names *Acetobacter oboediens* and *Acetobacter pomorum* are proposed for strains LTH 2460^T and LTH 2458^T, respectively. The descriptions refer to the type strains of the new species and do not repeat phenotypic features of the genus *Acetobacter* (De Ley *et al.*, 1984).

Description of *Acetobacter oboediens* sp. nov.

Acetobacter oboediens (ob.oe′diens. L. adj. *oboediens* obedient).

Cells are 0·8–1·2 μm by 1·3–1·6 μm, non-motile, occurring mainly in pairs. Colonies are round, regular, umbonate, soft, glossy and beige with a diameter of 1–2 mm on RAE agar (1a/2e). Grows on 3% (v/v) ethanol in the presence of up to 8% (w/v) acetic acid

in AE broth. Grows in the presence of 30% (w/v) glucose with a strong accumulation of gluconic acid ($\geqslant 130$ g l^{-1}). Forms 2-ketogluconic acid from glucose. Grows well on glucose, fructose, ribose, glycerol, sodium lactate, potassium gluconate and sucrose on yeast extract/peptone agar. Forms dihydroxyacetone from glycerol. Ferric chloride reaction on glucose and fructose negative. Cellulose is not formed. The ubiquinone system consists of Q-10 as major and Q-9 as minor components. The DNA G+C content is 59·9 mol%. Strain LTH 2460^T (= DSM 11826^T) is the type strain and was isolated from a submerged red wine vinegar fermentation at a factory in the southern part of Germany.

Description of *Acetobacter pomorum* sp. nov.

Acetobacter pomorum (po.mo′rum. L. neut. n. *pomum* fruit; L. gen. pl. neut. n. *pomorum* of the fruits).

Cells are 0·8–1·2 μm by 1·3–1·6 μm, non-motile, occurring mainly in pairs. Colonies are round, regular, convex, soft to liquid, glossy and beige with a diameter of 0·8–1·5 mm on RAE agar (1a/2e). Grows on 3% (v/v) ethanol in the presence of up to 4% (w/v) acetic acid in AE broth. Grows in the presence of 30% (w/v) glucose with a weak gluconic acid formation (< 10 g l^{-1}). 2- or 5-Ketogluconic acid are not formed from glucose. Grows well with ammonium as the sole nitrogen source on ethanol, n-propanol, glucose, fructose, maltose, ribose, xylose, sorbitol, mannitol and glycerol and has reduced growth on methanol on salt agar. Forms dihydroxyacetone from glycerol. Ferric chloride reaction on glucose and fructose negative. Cellulose is not formed. The ubiquinone system has Q-9 as the major and Q-8 as the minor components. The DNA G+C content is 50·5 mol%. Strain LTH 2458^T (= DSM 11825^T) is the type strain and was isolated from a submerged cider vinegar fermentation at a factory in the southern part of Germany.

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REFERENCES

Amann, R. I., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R. & Stahl, D. A. (1990). Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl Environ Microbiol* **56**, 1919–1925.

De Ley, J. (1970). Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J Bacteriol* **101**, 738–754.

De Ley, J., Gillis, M. & Swings, J. (1984). Family VI. *Acetobacteraceae*. In *Bergey's Manual of Systematic Bacteriology*, pp. 267–278. Edited by N. R. Krieg & J. G. Holt Baltimore: Williams & Wilkins.

Entani, E., Ohmori, S., Masai, H. & Suzuki, K.-I. (1985). *Acetobacter polyoxogenes* sp. nov., a new species of an acetic acid bacterium useful for producing vinegar with high acidity. *J Gen Appl Microbiol* **31**, 475–490.

Gosselé, F., Swings, J. & De Ley, J. (1980). A rapid, simple and simultaneous detection of 2-keto-, 5-keto- and 2,5-diketogluconic acids by thin-layer chromatography in culture media of acetic acid bacteria. *Zentbl Bakteriol Hyg 1 Abt Orig C*, 178–181.

Hertel, C., Ludwig, W. & Schleifer, K. H. (1992). Introduction of silent mutations in a proteinase gene of *Lactococcus lactis* as a useful marker for monitoring studies. *Syst Appl Microbiol* **15**, 447–452.

Kittelmann, M., Stamm, W. W., Follmann, H. & Trüper, H. G. (1989). Isolation and classification of acetic acid bacteria from high percentage vinegar fermentations. *Appl Microbiol Biotechnol* **30**, 47–52.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sievers, M. & Teuber, M. (1995). The microbiology and taxonomy of *Acetobacter europaeus* in commercial vinegar production. *J Appl Bacteriol Symposium* (Suppl.) **79**, 84S–95S.

Sievers, M., Sellmer, S. & Teuber, M. (1992). *Acetobacter europaeus* sp. nov., a main component of industrial vinegar fermenters in Central Europe. *Syst Appl Microbiol* **15**, 386–392.

Sievers, M., Ludwig, W. & Teuber, M. (1994a). Phylogenetic positioning of *Acetobacter*, *Gluconobacter*, *Rhodopila* and *Acidiphilium* species as a branch of acidophilic bacteria in the α-subclass of *Proteobacteria* based on 16S ribosomal DNA sequences. *Syst Appl Microbiol* **17**, 189–196.

Sievers, M., Ludwig, W. & Teuber, M. (1994b). Revival of the species *Acetobacter methanolicus* (ex Uhlig *et al*. 1986) nom. rev. *Syst Appl Microbiol* **17**, 352–354.

Sievers, M., Gaberthül, C., Boesch, C., Ludwig, W. & Teuber, M. (1995). Phylogenetic position of *Gluconobacter* species as a coherent cluster separated from all *Acetobacter* species on the basis of 16S ribosomal RNA sequences. *FEMS Microbiol Lett* **126**, 123–126.

Sokollek, S. J. & Hammes, W. P. (1997). Description of a starter culture preparation for vinegar fermentation. *Syst Appl Microbiol* **20**, 481–491.

Sokollek, S. J., Hertel, C. & Hammes, W. P. (1998). Cultivation and preservation of vinegar bacteria. *J Biotechnol* (in press).

Springer, N., Ludwig, W., Drozanski, V., Amann, R. & Schleifer, K. H. (1992). The phylogenetic status of *Sarcobium lyticum*, an obligate intracellular parasite of small amoebae. *FEMS Microbiol Lett* **96**, 199–202.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Swings, J. (1992). The Genera *Acetobacter* and *Gluconobacter*. In *The Prokaryotes*, 2nd edn, pp. 2268–2286. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.