J. Microbiol. Biotechnol. (2004), **14**(2), 276–283

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Gluconacetobacter persimmonis sp. nov., Isolated from Korean Traditional Persimmon Vinegar

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Received: February 17, 2003
Accepted: June 9, 2003

Abstract Screening was performed to isolate cellulose-producing microorganisms from the Korean traditional fermented persimmon vinegar. The resulting strain, KJ 145^T, was then taxonomically investigated by phenotypic characterization, particularly chemotaxonomic, and by phylogenetic inference based on a 16S rDNA sequence analysis including other related taxa. Strain KJ 145^T was found to grow rapidly and form pale white colonies with smooth to rough surfaces on a GYC agar. Strain KJ 145^T also produced acetate from ethanol, and was tolerable to 10% ethanol in SM medium. In a static culture, a thick cellulose pellicle was produced, and in GYC broth, the strain grew at temperatures ranging from 28 to 40°C with an optimum pH of 4.0. The genomic DNA G+C content of strain KJ 145^T was 61.9 mol%, and the predominant ubiquinone was Q10 as the major quinone and Q9 as the minor quinone. The major cellular fatty acids were $C_{16:0}$ and the sum in feature 7 ($C_{18:1}$ w9c, w12t and/or w7c). A 16S rRNA-targeted oligonucleotide probe specific for strain KJ 145^T was constructed, and the phylogenetic position of the new species was derived from a 16S rDNA-based tree. When comparing the 16S rDNA nucleotide sequences, strain KJ 145^T was found to be most closely related to *G. hansenii* LMG 1527^T (99.2%), although KJ 145^T was still distinct from *G. hansenii* LMG 1527^T and *G. xylinus* LMG 1515^T in certain phenotypic characteristics. Therefore, on the basis of 16S rDNA sequences and taxonomic characteristics, it is proposed that strain KJ 145^T should be placed in the genus *Gluconacetobacter* as a new species, *Gluconacetobacter persimmonis* sp. nov., under the type-strain KJ 145^T (=KCTC =10175BP^T=KCCM=10354^T).

Key words: *Gluconacetobacter persimmonis* sp. nov., taxonomy, persimmon vinegar fermentation, 16S rDNA sequence analysis, phylogeny

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Bacteria in the family *Acetobacteraceae* (alpha subdivision of Proteobacteria) are phenotypically characterized by their ability to grow at low pH and by their ability to oxidize ethanol to acetic acid. Traditionally, the *Acetobacteraceae* family has been divided into the genera *Acetobacter* and *Gluconobacter* [4, 19]. However, Yamada *et al.* [28] recently proposed dividing acetic acid bacteria into four genera such as *Acetobacter*, *Acidomonas*, *Gluconobacter*, and *Gluconacetobacter* (formerly *Acetobacter*) on the basis of their 16S rRNA gene sequences. Yet, the classification of the acetic acid bacteria group is still a subject of controversy. For example, Sokollek *et al.* [16] described *Acetobacter oboediens* LTH 2460^T, while Boescht *et al.* [2] described *Acetobacter intermedius* TF2^T, whereas the type strains for both species are actually included within the cluster of the genus *Gluconacetobacter* in a phylogenetic tree constructed on the basis of the 16S rRNA gene sequences identified by Sokollek *et al.* [16] and Boescht *et al.* [2]. Furthermore, the type strains of seven other species are also located in the cluster of the genus *Gluconacetobacter*, including *G. intermedius* TF2^T [2, 30], *G. diazotrophicus* LMG 7603^T [6, 28], *G. europaeus* DSM 6160^T [16, 28], *G. hansenii* LMG 1527^T [7, 28], *G. liquefaciens* LMG 1382^T [7, 28], *G. xylinus* LMG 1515^T [11, 24, 26, 27, 28, 29], and *G. oboediens* LTH 2460^T [16, 30]. For the industrial production of cellulose and microbial cellulose, the current study showed a bacterium possessing a high ability to produce cellulose and uniform structure of cellulose pellicle from the Korean traditionally fermented persimmon vinegar. The resulting strain, KJ 145^T, and *G. hansenii* LMG1527^T and *G. xylinus* LMG1515^T are then investigated as to their chemotaxonomic characteristics and phylogenetically analyzed based on the nucleotide sequences of their 16S rRNA gene. As a result, strain KJ 145^T is designated as a new species, *Gluconacetobacter persimmonis* sp. nov. and deposited in the KCTC (10175BP^T) and KCCM (10354^T), Korea.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Persimmon vinegar samples obtained from the fermentation tanks in several areas across Korea were collected and plated on GYC agar (5 g glucose, 1 g yeast extract, 3 g $CaCO_3$, and 2 g agar per liter of distilled water). A bacterial strain that formed a clear zone of calcium carbonate around the colonies was isolated from GYC agar at 30°C that had been inoculated into a Hestrin & Schramm (HS) medium (20 g glucose, 5 g yeast extract, 5 g peptone, 2.7 g Na_2HPO_4 and 1.15 g sodium citrate per liter of distilled water, adjusted to pH 6.0) [17]. The most potent cellulose-producing bacterial strain was selected and designated KJ 145^T. To investigate its morphological and physiological characteristics, strain KJ 145^T was cultivated in GYC medium. A cell mass for analyzing the ubiquinone and fatty acid methyl ester (FAME) was obtained from the GYC agar at 30°C, while a biomass of strain KJ 145^T for DNA extraction was obtained from growth in liquid GYC medium. The strain was cultivated at 30°C on a horizontal shaker at 150 rpm, and the broth culture microscopically checked for purity before harvesting by centrifugation. The reference strains for the 16S rDNA sequence analysis included *Acetobacter aceti* DSM 3508^T (T=Type strain), *Acetobacter pasteurianus* LMG 1262^T, *Acetobacter pomorum* LTH 2458^T, *Gluconacetobacter intermedius* DSM 11804^T, *Gluconacetobacter diazotrophicus* LMG 7603^T, *Gluconacetobacter europaeus* DSM 6160^T, *Gluconacetobacter hansenii* LMG 1527^T, *Gluconacetobacter liquefaciens* LMG 1382^T, *Gluconacetobacter xylinus* LMG 1515^T, *Gluconacetobacter oboediens* LTH 2460^T, *Acidomonas methanolica* LMG 1668^T, *Gluconobacter asali* IFO 3276^T, *Gluconobacter cerinus* IFO 3267^T, *Gluconobacter frateurii* IFO 3264^T, *Gluconobacter oxydans* DSM 3503^T, *Acidocella facilis* ATCC 35904^T, *Acidocella aminolytica* JCM 8796^T, *Acidiphilum cryptum* ATCC 33463^T, *Rhodopila globiformis* DSM 161^T, *Rhodospirillum rubrum* ATCC 11170^T, *Magnetospirillum magnetotacticum* DSM 3856^T, and *Escherichia coli* [3].

Morphological and Physiological Characteristics

The morphology of cells was examined by light microscopy and scanning electron microscopy (SEM). Gram staining was performed using a Merck kit (Germany). Negative staining of the cells was achieved with 1% phosphotungstic acid. To observe the motility, hanging-drop technique was used. The ability to oxidize ethanol into acetic acid and overoxidize acetate and lactate into CO_2 and H_2O was observed using the method of Frateur as described by Swings [19]. Ketogenesis forming glycerol, growth on a glutamate agar, and the assimilation of ammoniacal nitrogen were all investigated based on the methods described by Asai *et al.* [1]. The utilization of various substrates as the sole carbon and energy source was tested as described by Shirling and Gottlieb [14]. Most of the substrates were tested at a concentration of 1% (w/v) with the exception of glycerol (0.1%, w/v). The acid production from D-glucose, *myo*-inositol, D-sorbitol, D-mannose, L-rhamnose, sucrose, melibiose, D-xylose, and D-amygdalin was determined as described by Takeuchi and Hatano [21]. Most of the substrates were tested at a concentration of 1% (w/v). An API 20E kit (bio-Mérieux Co., Ltd., France) was used to examine the catalase, oxidase, citrate, urea, lactose, arginine, H_2S, indole, acetyl-methyl carbinol, nitrate and gelatin liquefaction. The effects of temperature and pH on the growth of strain KJ 145^T were tested in GYC broth and recoded after 7 days at 30°C.

Analyses of Cellular Fatty Acids and Quinines

The ubiquinones were extracted and isolated according to the method of Yamada and Kondo [31]. The purified ubiquinones were then identified according to their retention times in HPLC. The analytical systems were as described by Tamaoka *et al.* [22] with slight modifications: instrument, EuroChrom 2000 LC Controller; pump, KNAUER K-1001; column, COSMOSIL-Pack $5C_{18}$-AR (4.6Ø×150 mm, Nacalai tesque Co., Ltd., Japan); UV, 275 nm; elutent, methanol/isopropanol (2:1); flow rate, 1 ml/min. The whole-cell fatty acids and main types of isoprenoid quinone were determined using biomass grown on GYC agar at 30°C for 7 days. The fatty acids were extracted and analyzed according to the instructions of Microbial Identification System (MIDI; Microbial ID).

Isolation of Chromosomal DNA

The chromosomal DNA was extracted and purified according to the method of Saito and Miura [12].

Determination of the G+C Content

The DNA G+C content was determined using the method of Tamaoka and Komagata [23]. The DNA was hydrolyzed and the resultant nucleotides analyzed by reversed-phase HPLC.

PCR Amplification and Sequencing of 16S rDNA

Two primers described by Stackebrandt and Liesack [18], 9F [5'-GAGTTTGATCCTGGCTCAG-3'; positions 9-27 (*Escherichia coli* 16S rRNA numbering)] and 1542R (5'-AGAAAGGAGGTGATCCAGCC-3'; 1542-1525), were used for amplification of the 16S rRNA gene. The 5' ends of the two primers were phosphorylated using T4 polynucleotide kinase (New England Biolabs) according to the instructions of a Strandase ssDNA preparation kit (Novagen). The 16S rRNA gene was amplified as described by Yoon *et al.* [32] using phosphorylated 9F plus non-phosphorylated 1542R and phosphorylated 1542R plus non-phosphorylated 9F, respectively. The ssDNA

templates produced were used directly for the sequencing reaction. The sequencing was performed as described by Kim *et al.* [10] using α-^{35}S-labelled dATP and a DNA sequencing kit (US Biochemical) according to the manufacturer's instructions. The sequencing primers were derived from conserved regions of the 16S rRNA gene sequences of eubacteria. The forward primers used were 373F [5'-AATGGGCGCAAGCCTGAT-3'; positions 373–390 (*E. coli* 16S rRNA numbering)], 792F (5'-ATACCCT-GGTAGTCCAC-3'; 792–808), and 1392F (5'-GTACAC-ACCGCCCGT-3'; 1392–1406). The reverse primers used were 330R (5'-GTGTCTCAGTCCCAGTGT-3'; 330–313), 536R (5'-GWATTACCGCGGCKGCTG-3'; 536–519), 926R (5'-CCGTCAATTCMTTTRAG-3'; 926–910), 1406R (5'-ACGGGCGGTGTGTRC-3'; 1406–1392), and 1512R (5'-ACGGHTACCTTGTTACGACTT-3'; 1512–1492).

Phylogenetic Analysis

The 16S rDNA sequence of strain KJ 145^T was aligned with 16S rRNA/16S rDNA sequences of *Gluconacetobacter* species and various other related reference strain taxa using CLUSTAL W software [25]. The other reference sequences were obtained from the GenBank database, where the nucleotide sequences are available under the following accession numbers: *A. aceti* DSM 3508^T (X74066), *A. pasteurianus* LMG 1262^T (AJ007834), *A. pomorum* LTH 2458^T (AJ001632), *G. intermedius* DSM 11804^T (Y14694), *G. diazotrophicus* LMG 7603^T (X75618), *G. europaeus* DSM 6160^T (Z21936), *G. hansenii* LMG 1527^T (X75620), *G. liquefaciens* LMG 1382^T (X75617), *G. xylinus* LMG 1515^T (X75619), *G. oboediens* LTH 2460^T (AJ001631), *A. methanolica* LMG 1668^T (X77468), *G. asali* IFO 3276^T (X80165), *G. cerinus* IFO 3267^T (X80775), *G. frateurii* IFO 3264^T (X82290), *G. oxydans* DSM 3503^T (X73820), *A. facilis* ATCC 35904^T (AH001559), *A. aminolytica* JCM 8796^T (D30771), *A. cryptum* ATCC 33463^T (D30773), *R. globiformis* DSM 161^T (D86513), *R. rubrum* ATCC 11170^T (D30778), *M. magnetotacticum* DSM 3856^T (Y10110), and *E. coli* (V00348). The 16S rDNA similarity values were calculated from the alignment. Gaps at the 5' and 3' ends of the alignment were omitted from further analyses. Evolutionary distance matrices were calculated using the algorithm of Jukes and Cantor [8] with the DNADIST program within the PHYLIP package [5]. A phylogenetic tree was constructed using the neighbour-joining method [13] from a distance matrix calculated by CLUSTAL W [25]. The stability of the relationships was assessed by a bootstrap analysis of 1000 data sets using the programs SEQBOOT, DNADIST, NEIGHBOR, and DONSENSE from the PHYLIP package.

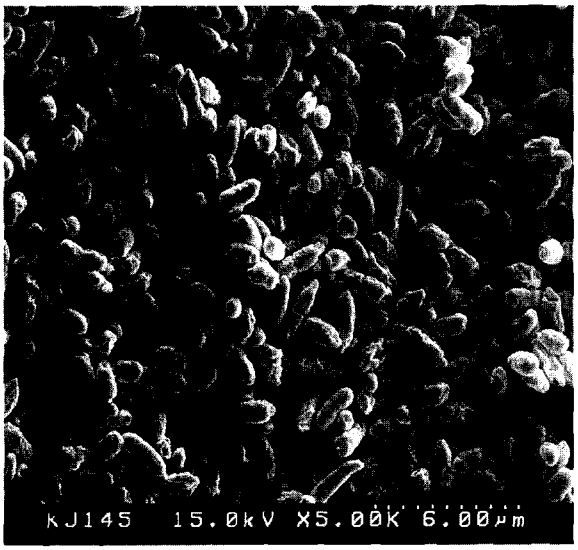
Nucleotide Sequence Accession Numbers

The GenBank/EMBL accession numbers for the reference 16S rDNA sequences used in the phylogenetic analysis are shown in Fig. 2. The 16S rDNA nucleotide sequences for strain KJ 145^T have been submitted to be DDBJ database under the following accession number: AB095100.

Results and Discussion

Morphological Characteristics

Strain KJ 145^T producing bacterial cellulose was isolated from the Korean traditional fermented persimmon vinegar. Strain KJ 145^T was found to be gram-negative, non-spore-forming, and non-motile. The cells were long rods



(A)

(B)

Fig. 1. Scanning electron micrograph (SEM) of strain KJ 145^T grown on GYC agar for 7 days at 30°C. Bars, 6 µm (A) and 1 µm (B).

Table 1. Morphological characteristics of strain KJ 145^T and type-strains from *Gluconacetobacter* species.

Portions observed	KJ 145T	*G. xylinus* (LMG 1515^T)	*G. hansenii* (LMG 1527^T)
Gram staining	-	-	-
Cell shape	long rod	rod	long rod
Cell size (μm)	0.6-0.7×1.4-1.8	0.3-0.4×0.7-0.8	0.6-0.8×1.3-1.6
Motility	-	-	-
Colony characteristics (GYC agar)			
Shape	entire, circular convex to flat	entire, circular convex to flat	entire, circular convex to flat
Color	pale white	pale white	pale white
Surface	smooth to rough	smooth to rough	smooth to rough
Transparency	opacity	opacity	opacity

The color codes correspond to those in National Bureau of Standards (Kelly and Judd, 1976).

measuring 0.6-0.7×1.4-1.8 μm (Fig. 1) in GYC medium at 30°C and occurred singly, in pairs or occasionally in short chains. After incubation on GYC agar for 7 days, the colonies were pale white, smooth to rough, convex, opaque, and approximately 3 to 4 mm in diameter (Table 1). The morphological properties of the isolated strain KJ 145^T were determined according to *Bergey's Manual of Systematic Bacteriology* [4].

Table 2. Comparison of physiological characteristics for strain KJ 145^T and type-strains from *Gluconacetobacter* species.

Characteristics	KJ 145^T	*G. xylinus* (LMG 1515^T)	*G. hansenii* (LMG 1527^T)
Catalase	+	+	+
Urease	-	-	-
Cytochrome oxidase	-	-	-
Nitrate to nitrite	-	-	-
Methyl red test	+	-	+
ONPG (β-galactosidase)	-	-	+
VP test	-	-	+
Gelatin liquefaction	-	-	-
Arginine dehydrolase	-	-	+
Lysine decarboxylase	-	-	+
Tryptophane deaminase	-	-	-
Sodium citrate utilization	-	-	+
Indole pyruvic acid production	-	-	-
H_2S production	-	-	-
Indole production	-	-	-
Acid produced from			
amygdalin	+	+	-
arabinose	+	-	+
glucose	+	+	+
inositol	±	±	-
mannose	+	+	+
melibiose	+	-	+
rhamnose	+	-	-
sorbitol	+	-	-
sucrose	+	-	+
xylose	+	+	+
Overoxidation of ethanol	+	+	+
Oxidation of acetate	+	+	+
Oxidation of lactate	+	+	+
Ketogenesis form glycerol	+	+	+
Cellulose formation	+	+	+
H_2S formation	-	-	-
Brown pigmentation on GYC agar	-	-	-
γ-Pyrone from 3% glucose	-	-	-
γ-Pyrone from 3% fructose	+	-	-

All results are from current study. Symbols: +, positive reaction; -, negative reaction; ±, weekly positive reaction.

Cultural and Physiological Characteristics

Strain KJ 145^T was biochemically characterized using an API 20E kit (bio Mérieux) and API CORYNE and API ZYM systems according to the manufacturer's instructions. The biochemical properties of isolate KJ 145^T and the type strains of the *Gluconacetobacter* species, *G. hansenii* LMG1527^T and *G. xylinus* LMG1515^T, are summarized in Table 2. The isolate KJ 145^T overoxidized acetate and lactate into CO_2 and H_2O. Yet, the oxidase test was negative, and nitrate was not reduced to nitrite. Strain KJ 145^T was able to grow on glutamate and mannitol agar, yet not with ethanol and glycerol as the sole carbon source. However, strain KJ 145^T did grow on SM medium (5 g yeast extract and 50 g glucose per liter of distilled water) with an additional 10% ethanol. As regards the use of various compounds as sole carbon and energy source, strain KJ 145^T utilized ethanol, D-galactose, D-glucose, D-mannose, and sucrose as shown in Table 2. The strain also tested positive for catalase, methyl red test, and the formation of cellulose. Acid was produced from all the tested substrates (arbinose, D-amygdalin, D-glucose, D-mannose, melibiose, L-rhamnose, D-sorbitol, sucrose, and D-xylose). A negative reaction was recorded for urease, indole, ONPG, VP test, gelatin liquefaction, H_2S, sodium

Table 3. Cellular fatty acid composition (%) of isolates KJ 145^T and *G. xylinus* LMG 1515^T.

Fatty acid	KJ 145^T	*G. xylinus* LMG 1515^T
	Fatty acid composition (%)	
$C_{14:0}$	5.21	5.94
$C_{16:0}$	10.35	17.16
$C_{18:0}$	2.93	2.13
$C_{14:0}$ 2OH	4.75	3.15
$C_{16:0}$ 2OH	7.65	4.93
$C_{16:0}$ 3OH	2.71	2.16
Summed feature 3	0.66	0.91
Summed feature 7	65.74	55.81

For fatty acid methyl ester (FAME) analysis, strain KJ 145^T was grown for 5 days on GYC agar (Difco).

*Summed feature represents group of two or three fatty acids that could not be separated by GLC using MIDI system. Summed feature 3: *iso*-$C_{16:1}$ and/or $C_{14:0}$-3OH. Summed feature 7: $C_{18:1}$ w9c, w12t and/or w7c.

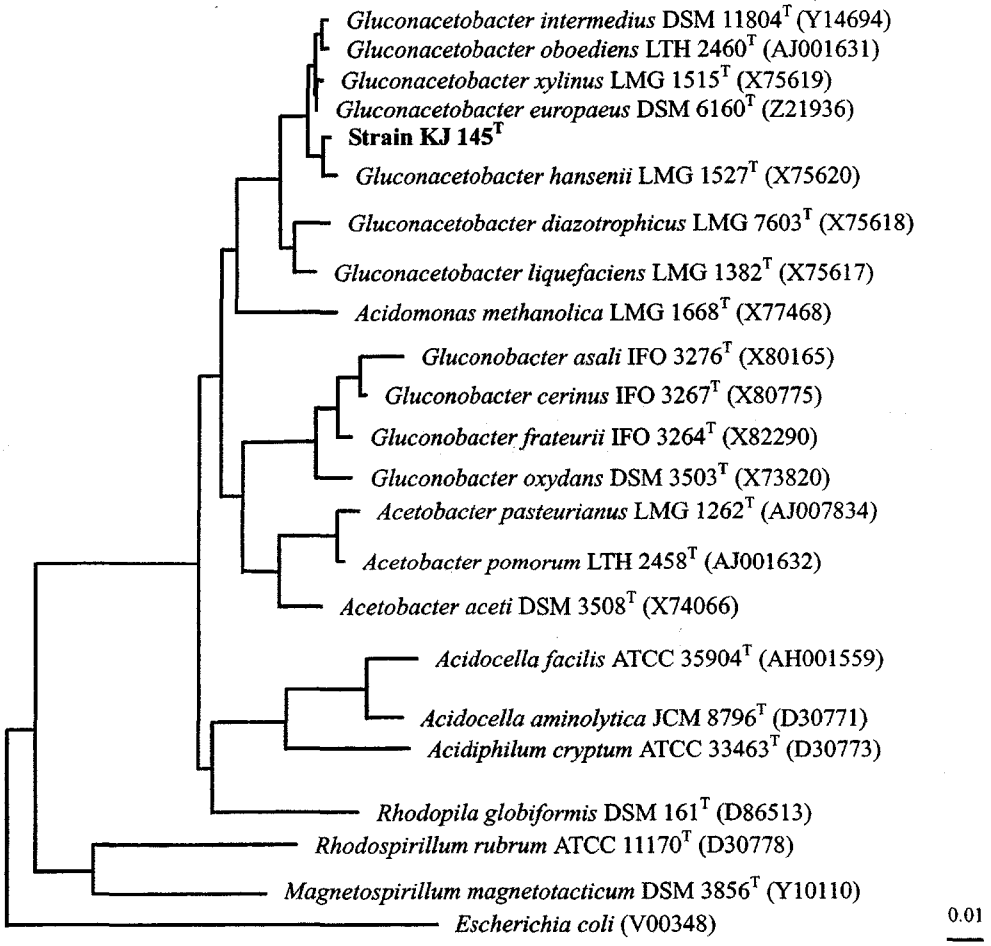


Fig. 2. Phylogenetic tree showing relationship between strain KJ 145^T and related species.

Table 4. Percentage 16S rDNA/16S rRNA similarity between strain KJ 145^T and reference strains used in phylogenetic analysis.

Species (Taxon)	(%) Sequence similarity																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1. Strain KJ 145^T																						
2. *Acetobacter aceti* DSM 3508^T	95.2																					
3. *Acetobacter pasteurianus* LMG 1262^T	94.6	96.8																				
4. *Acetobacter pomorum* LTH 2458^T	94.7	97.2	99.4																			
5. *Gluconacetobacter intermedius* DSM 11804^T	98.5	95.7	95.3	95.4																		
6. *Gluconacetobacter diazotrophicus* LMG 7603^T	97.1	95.8	95.3	95.4	96.6																	
7. *Gluconacetobacter europaeus* DSM 6160^T	98.8	95.5	94.9	95.1	99.5	97.0																
8. *Gluconacetobacter hansenii* LMG 1527^T	99.2	95.0	94.3	94.5	98.2	97.0	98.6															
9. *Gluconacetobacter liquefaciens* LMG 1382^T	97.1	95.9	95.5	95.7	97.2	98.5	97.4	97.0														
10. *Gluconacetobacter xylinus* LMG 1515^T	98.7	95.3	94.6	94.7	99.2	96.7	99.6	98.3	97.2													
11. *Gluconacetobacter oboediens* LTH 2460^T	98.5	95.6	95.1	95.3	99.8	96.5	99.5	98.2	97.0	99.1												
12. *Acidomonas methanolica* LMG 1668^T	95.5	95.2	94.7	95.0	95.8	96.0	95.8	95.3	96.6	95.5	95.6											
13. *Gluconobacter asali* IFO 3276^T	93.2	94.7	93.6	94.0	94.0	94.0	93.7	93.2	93.9	93.6	93.8	93.8										
14. *Gluconobacter cerinus* IFO 3267^T	94.1	95.4	94.3	94.8	94.9	94.7	94.7	94.1	94.8	94.4	94.7	94.7	98.6									
15. *Gluconobacter frateurii* IFO 3264^T	94.5	95.5	94.8	95.3	95.4	95.0	95.0	94.4	94.9	94.9	95.3	95.3	98.0	98.8								
16. *Gluconobacter oxydans* DSM 3503^T	94.6	95.9	94.9	95.3	95.4	94.6	94.9	94.4	94.4	94.7	95.3	95.1	97.0	97.9	98.0							
17. *Acidocella facilis* ATCC 35904^T	91.5	92.2	91.4	91.8	92.0	92.1	92.0	91.3	92.5	91.7	91.8	91.5	90.9	91.7	91.7	92.4						
18. *Acidocella aminolytica* JCM 8796^T	92.2	92.9	92.1	92.5	92.5	92.5	92.2	91.9	93.1	92.0	92.3	91.7	91.1	91.9	91.9	92.5	98.0					
19. *Acidiphilum cryptum* ATCC 33463^T	92.3	91.9	90.9	91.3	92.8	92.2	92.5	92.0	92.4	92.4	92.6	92.1	90.0	90.8	91.1	91.5	94.0	94.2				
20. *Rhodopila globiformis* DSM 161^T	94.2	92.7	92.2	92.3	93.8	94.1	93.6	93.6	94.0	93.5	93.6	93.3	91.3	92.2	92.2	92.4	92.2	92.6	92.8			
21. *Rhodospirillum rubrum* ATCC 11170^T	85.7	85.9	85.4	85.6	85.3	85.6	85.7	85.5	85.8	85.6	85.2	85.4	85.0	85.9	85.9	85.7	84.2	84.4	84.9	85.4		
22. *Magnetospirillum magnetotacticum* DSM 3856^T	86.9	86.6	86.2	86.6	87.0	86.6	87.0	86.6	86.5	86.8	86.8	85.9	85.9	86.8	86.9	87.6	86.5	86.4	86.0	86.4	86.4	
23. *Escherichia coli*	81.4	81.8	81.1	81.1	82.0	81.4	82.2	81.4	81.8	81.9	82.0	80.9	80.3	81.3	80.9	81.1	80.3	80.7	79.8	81.1	82.2	81.7

citrate, arginine dehydrolase, lysine decarboxylase, tryptophane deaminase, and indole pyruvic acid. The strain produced dihydroxyacetone from glycerol and γ-pyrones from fructose, yet did not produce γ-pyrones from glucose. In GYC broth, the strain grew at temperatures ranging from 28 to 40°C with an optimum at pH 4.0.

Chemotaxonomic Characteristics

Strain KJ 145^T contained a dehydrogenated ubiquinone, with Q10 as the major quinone and Q9 as the minor quinone. The ubiquinone profile of strain KJ 145^T was similar to that of *G. liquefaciens* LMG 1382^T [28] where isoprenoid composition was concerned. The cellular fatty acid profile of strain KJ 145^T was characterized by the predominance of the sum in feature 7 (65.74%, $C_{18:1}$ w9c, w12t and/or w7c) followed by $C_{16:0}$ (10.35%, saturated fatty acid), whereas *G. xylinus* LMG 1515^T was characterized by the predominance of the sum in feature 7 (55.81%) and the occurrence of a significant amount of $C_{16:0}$ (17.16%) (Table 3). The summed features represent groups of two or three fatty acids that could not be separated by GLC using a MIDI system. The cellular fatty acid pattern of strain KJ 145^T was similar to that of *G. xylinus* LMG 1515^T. However, no branched saturated or 10-methyl fatty acids were detected (Table 3). Strain KJ 145^T had a 61.9 mol% G+C content (as determined by HPLC). The genomic DNA G+C content in other strains from the genus *Gluconacetobacter* ranges from 56 to 64 mol%.

Phylogenetic Analysis

An almost complete 16S rDNA sequence of 1,444 nucleotides (approx. 94% of the *Escherichia coli* sequence) was determined for strain KJ 145^T. Since the morphology, ubiquinone system, cellular fatty acid profile, and genomic DNA G+C content all suggested that strain KJ 145^T is a member of the genus *Gluconacetobacter* as described above, the reference sequences were selected from species belonging to this phylogenetic group. Figure 2 shows the phylogenetic tree derived from the 16S rDNA sequences of 7 type-strains of validly described *Gluconacetobacter* species, and other members of the family *Acetobacteraceae*. The phylogenetic analysis, as shown in Fig. 2, revealed that strain KJ 145^T was a member of the genus *Gluconacetobacter*, and most closely related to *G. hansenii* LMG 1527^T. However, it would seem that *G. hansenii* LMG 1527^T should be transferred to *A. orleanensis*, as it oxidizes acetate and lactate, has Q9 as its major ubiquinone, and exhibits a high level of 16S rDNA similarity with the type-strain of *A. orleanensis*. Furthermore, it was clear that strain KJ 145^T was distinct from *R. rubrum* ATCC 11170^T, *A. facilis* ATCC 35904^T, *G. asali* IFO 3276^T, and *A. aceti* DSM 3508^T. Among the *Gluconacetobacter* species, *G. hansenii* LMG 1527^T exhibited the highest similarity (99.2%) to strain KJ 145^T (Table 4), although they differed in terms of biochemical and chemotaxonomic characteristics. Therefore, strain KJ 145^T would appear to be a new *Gluconacetobacter* species. Accordingly, in the current study, strain KJ 145^T was isolated from traditional Korean fermented persimmon vinegar and could not be identified as any known species of the acetic acid bacteria. As such, based on a phylogenetic trees constructed using near-complete 16S rRNA gene sequences, strain KJ 145^T was included within the cluster of the genus *Gluconacetobacter* as a new species under the name *Gluconacetobacter persimmonis* (per. sim. mo'. nis. N. L. gen n. persimmonis of persimmon, a tropical tree) sp. nov. Moreover, since the bacterial cellulose production of this strain was found to be very stable, it is expected that strain KJ 145^T will be used in a variety of industrial applications related to cellulose production. Consequently, further studies on a production system for strain KJ 145^T are currently underway including the medium conditions, fermentation apparatus, breeding the microorganism, and other factors.

Acknowledgments

This work was supported by the Korean Ministry of Science & Technology (MOST) and Korea Science and Engineering Foundation (KOSEF, R12-2001-044-00002-0) through the Center for Traditional Microorganism Resources (TMR) at Keimyung University.

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