INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Apr. 1992, p. 203–210 Vol. 42, No. 2
0020-7713/92/020203-08$02.00/0

Pantoea punctata sp. nov., *Pantoea citrea* sp. nov., and *Pantoea terrea* sp. nov. Isolated from Fruit and Soil Samples

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A total of 37 bacterial strains with the general characteristics of the family *Enterobacteriaceae* were isolated from fruit and soil samples in Japan as producers of 2,5-diketo-D-gluconic acid from D-glucose. These organisms were phenotypically most closely related to the genus *Pantoea* (F. Gavini, J. Mergaert, A. Beji, C. Mielearek, D. Izard, K. Kersters, and J. De Ley, Int. J. Syst. Bacteriol. 39:337–345, 1989) and were divided into three phenotypic groups. We selected nine representative strains from the three groups for an examination of DNA relatedness, as determined by the S1 nuclease method at 60°C. Strain SHS 2003^T (T = type strain) exhibited 30 to 41 and 28 to 33% DNA relatedness to the strains belonging to the strain SHS 2006^T group (strains SHS 2004, SHS 2005, SHS 2006^T, and SHS 2007) and to the strains belonging to the strain SHS 2008^T group (strains SHS 2008^T, SHS 2009, SHS 2010, and SHS 2011), respectively. Strain SHS 2006^T exhibited 38 to 46% DNA relatedness to the strains belonging to the strain SHS 2008^T group. The levels of DNA relatedness within the strain SHS 2006^T group and within the strain SHS 2008^T group were more than 85 and 71%, respectively. Strain SHS 2003^T, SHS 2006^T, and SHS 2008^T DNAs exhibited less than 18% binding to *Pantoea dispersa* ATCC 14589^T and *Pantoea agglomerans* ATCC 27155^T DNAs. On the basis of phenotypic characteristics, DNA base compositions, and the results of DNA relatedness studies, the nine strains which we studied were considered to be new species of the genus *Pantoea*, and the names *Pantoea citrea*, *Pantoea punctata*, and *Pantoea terrea* are proposed for strains belonging to the strain SHS 2003^T, SHS 2006^T, and SHS 2008^T groups, respectively. *P. citrea* SHS 2003^T, *P. punctata* SHS 2006^T, and *P. terrea* SHS 2008^T have been deposited in the American Type Culture Collection as strains ATCC 31623^T ("*Erwinia citreus*"), ATCC 31626^T ("*Erwinia punctata*"), and ATCC 31628^T ("*Erwinia terreus*"), respectively.

As reported previously (24), we isolated from various fruit and soil samples 42 bacterial strains that effectively produce within 24 h 2,5-diketo-D-gluconic acid (DKGA) from 30% D-glucose solutions via D-gluconic acid and 2-keto-D-gluconic acid. These organisms are gram-negative, oxidase-negative, facultatively anaerobic, fermentative bacterial strains with the general characteristics of the family *Enterobacteriaceae* (3). On the basis of the characteristics of the *Enterobacteriaceae* described by Brenner (3), our 42 strains were initially thought to be related to species belonging to the genus *Erwinia* or to *Enterobacter agglomerans*. They were divided into three phenotypic groups and were tentatively named "*Erwinia citreus*," "*Erwinia punctata*," and "*Erwinia terreus*" (24).

After these tentative new species names were published, we conducted further detailed investigations of other characteristics by using 37 of the 42 isolates (24) (referred to as the DKGA-producing strains). As a result, we concluded that these 37 strains are members of the *Erwinia herbicola-Enterobacter agglomerans* complex, because they are phenotypically related to the anaerogenic group of *Enterobacter agglomerans* described by Ewing and Fife (9) and to DNA hybridization groups II, III, and IV of Brenner et al. (4).

Recently, Gavini et al. (11) analyzed the *Erwinia herbicola-Enterobacter agglomerans* complex on the basis of phenotypic characteristics and DNA-DNA hybridization data and proposed the new genus *Pantoea* for some of the strains that are members of this complex, which includes the type strains of *Erwinia herbicola* (strain ATCC 14589) and *Enterobacter agglomerans* (strain ATCC 27155).

To clarify the taxonomic position of the DKGA-producing strains, we examined their phenotypic characteristics and carried out DNA-DNA hybridization experiments with nine representative DKGA-producing strains and strains belonging to the *Erwinia herbicola-Enterobacter agglomerans* complex, including *Pantoea dispersa* ATCC 14589^T (T = type strain) and *Pantoea agglomerans* ATCC 27155^T. In this paper we describe the DKGA-producing strains as new species of the genus *Pantoea*. On the basis of phenotypic and chemosystematic characteristics, we propose the names *Pantoea citrea* sp. nov., *Pantoea punctata* sp. nov., and *Pantoea terrea* sp. nov. for our DKGA-producing strains.

MATERIALS AND METHODS

Bacterial strains. We used 37 of the 42 previously isolated (24) DKGA-producing strains (5 strains of "*Erwinia citreus*," 7 strains of "*Erwinia punctata*," and 25 strains of "*Erwinia terreus*") for phenotypic characterization. Nine representative strains were used to examine DNA-DNA relatedness and DNA base composition. The nine DKGA-producing strains and 30 reference strains used in this study are listed in Table 1. All of the strains were grown on nutrient agar slants at 30°C for 24 h.

Characterization. The morphological and biochemical characteristics of the DKGA-producing strains were investigated by using previously described methods (24) and by using API 20E and API 50CHE systems (Analytab Products, Montalieu-Vercieu, France), as described by Mergaert et al. (21). Growth factor requirements were determined by using

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TABLE 1. Strains used in this study

Group	Species name as deposited in the ATCC[a]	Strain
Strains assigned by us to:		
P. citrea	"*Erwinia citreus*"	SHS 2003^T (= ATCC 31623^T)
P. punctata	"*Erwinia punctata*"	SHS 2004 (= ATCC 31624)
	"*Erwinia punctata*"	SHS 2005 (= ATCC 31625)
	"*Erwinia punctata*"	SHS 2006^T (= ATCC 31626^T)
	"*Erwinia punctata*"	SHS 2007 (= ATCC 31627)
P. terrea	"*Erwinia terreus*"	SHS 2008^T (= ATCC 31628^T)
	"*Erwinia terreus*"	SHS 2009 (= ATCC 31629)
	"*Erwinia terreus*"	SHS 2010 (= ATCC 31630)
	"*Erwinia terreus*"	SHS 2011 (= ATCC 31631)
Strains belonging to the genus *Pantoea*		
P. agglomerans	*Enterobacter agglomerans*	ATCC 27155^T
P. dispersa	*Erwinia herbicola*	ATCC 14589^T
Strains belonging to the genus *Enterobacter*[b]		
Phenotypic biogroup 3 (DNA hybridization group IV)	*Enterobacter agglomerans*	ATCC 27998
Phenotypic biogroup 4	*Enterobacter agglomerans*	ATCC 27995
Phenotypic biogroup 5	*Enterobacter agglomerans*	ATCC 27994
Phenotypic biogroup 5 (DNA hybridization group VII)	*Enterobacter agglomerans*	ATCC 29904
Phenotypic biogroup 5	*Enterobacter agglomerans*	ATCC 27992
Phenotypic biogroup 6 (DNA hybridization group VIII)	*Enterobacter agglomerans*	ATCC 29919
Phenotypic biogroup 7	*Enterobacter agglomerans*	ATCC 27990
Phenotypic biogroup G1 (DNA hybridization group IX)	*Enterobacter agglomerans*	ATCC 29918
	Enterobacter intermedium	ATCC 33110^T
	Enterobacter sakazakii	ATCC 29544^T
	Enterobacter cloacae	ATCC 13047^T
	Enterobacter gergoviae	ATCC 33028^T
Strains belonging to the genus *Erwinia*	*Erwinia stewartii*	ATCC 8199^T
	Erwinia ananas	ATCC 33244^T
	Erwinia uredovora	ATCC 19321^T
	Erwinia carotovora	ATCC 15390
	Erwinia quercina	ATCC 29282
	Erwinia cypripedii	ATCC 29267
	Erwinia rhapontici	ATCC 29284
	Erwinia tracheiphila	ATCC 33245^T
	Erwinia rubrifaciens	ATCC 29293
	Erwinia chrysanthemi	ATCC 11662
Other species of the family *Enterobacteriaceae*	*Hafnia alvei*	ATCC 29926
	Hafnia alvei	ATCC 29927
	Cedecea davisae	ATCC 33431^T
	Cedecea lapagei	ATCC 33432^T
	Cedecea neteri	ATCC 33855^T
	Citrobacter diversus	ATCC 27156
	Citrobacter freundii	ATCC 8090
	Escherichia coli K-12	ATCC 10798
	Serratia marcescens	IFO 3046[c]

[a] ATCC, American Type Culture Collection, Rockville, Md.
[b] Phenotypic biogroups of Ewing and Fife (9) and DNA hybridization groups of Brenner et al. (4).
[c] IFO, Institute for Fermentation, Osaka, Japan.

the method of Clowes and Hayes (6). Flagellation was observed by using the method of Hiller et al. (12) and a transmission electron microscope. The activities of D-gluconate and 2-keto-D-gluconate dehydrogenases were estimated by using the methods of Ameyama (1) and Bouvet et al. (2) and *Serratia marcescens* IFO 3046 and *Escherichia coli* ATCC 10798 as positive and negative control strains, respectively.

Numerical analysis. A total of 52 phenotypic characteristics (API 20E and API 50CHE system tests and two oxidation tests, the test for gluconate dehydrogenase activity and the test for 2-keto-D-gluconate dehydrogenase activity described by Bouvet et al. [2]) were used in our numerical analysis of 37 DKGA-producing strains. The following characteristics common to all of the strains tested were excluded from the numerical analysis: acid production from D-glucose, D-mannose, L-arabinose, D-ribose, D-xylose, D-galactose, D-fructose, and *N*-acetylglucosamine (positive for all strains); acid production from D-arabinose, L-xylose, and L-fucose (negative for all strains); and lysine decarboxylase activity, ornithine decarboxylase activity, urease activity, oxidase activity, and H_2S production (negative for all strains). The data were coded in binary notation, and computations were performed. The similarity between each pair of strains was calculated by using the simple matching coefficient, and clustering was achieved as described by Colwell and Austin (8).

Quinones. Quinones were examined by using the methods described by Yamada et al. (27) and Collins et al. (7).

G+C contents of DNAs. To isolate DNAs for guanine-plus-cytosine (G+C) content determinations, all of the strains tested were grown in GYP medium (0.5% glycerol, 0.5% yeast extract [Difco], 0.3% Bacto Peptone [Difco], 0.1% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$; pH 7.0) at 30°C. DNA was

TABLE 2. Phenotypic characteristics of *P. citrea*, *P. punctata*, and *P. terrea*

Characteristic	*P. citrea*		*P. punctata*		*P. terrea*	
	% of positive strains (n = 5)	Strain SHS 2003^T	% of positive strains (n = 7)	Strain SHS 2006^T	% of positive strains (n = 25)	Strain SHS 2008^T
Motility	0	−[a]	0	−	100	+
Growth at 36°C	100	+	100	+	100	+
Growth at 41°C	0	−	14	−	0	−
Growth in KCN	0	−	0	−	0	−
Gas produced from glucose	0	−	0	−	0	−
Reducing compounds produced from sucrose	0	−	100	+	100	+
Methyl red test	100	+	100	+	100	+
Voges-Proskauer test	100	W	100	W	100	+
Citrate (Simmons)	100	+	100	+	100	+
Citrate (Christensen)	100	+	100	+	100	+
ONPG (β-galactosidase)[b]	100	+	0	−	0	−
Catalase activity	100	+	100	+	100	+
Pectate degradation	0	−	0	−	0	−
Oxidase activity	0	−	0	−	0	−
Indole	0	−	0	−	0	−
Nitrate reduction	100	+	100	+	92	+
Gelatin liquefaction	0	−	0	−	0	−
Phenylalanine deaminase activity	0	−	0	−	0	−
Tryptophan deaminase activity	0	−	0	−	0	−
H_2S produced on triple sugar iron	0	−	0	−	0	−
H_2S produced from cysteine	100	+	100	+	100	+
L-Glutamate decarboxylase activity	0	−	100	+	0	−
Arginine dihydrolase activity	100	+	100	+	0	−
Lysine decarboxylase activity	0	−	0	−	0	−
Ornithine decarboxylase activity	0	−	0	−	0	−
Urease activity (Christensen)	0	−	0	−	0	−
Lipase activity	0	−	0	−	0	−
DNase activity	0	−	0	−	0	−
Oxidation of gluconate	100	+	100	+	100	+
Oxidation of 2-keto-D-gluconate	100	+	100	+	100	+
Utilization of:						
Acetate	100	+	0	−	100	+
Fumarate	100	+	100	+	100	+
Malate	100	+	100	+	100	+
Succinate	100	+	100	+	100	+
Benzoate	0	−	0	−	0	−
Oxalate	0	−	0	−	0	−
Propionate	0	−	0	−	0	−
Formate	100	+	86	−	100	+
D-Galacturonate	0	−	0	−	0	−
Lactate	100	+	0	−	100	+
Malonate	0	−	0	−	0	−
Tartrate	0	−	0	−	0	−

[a] +, positive reaction; −, negative reaction; W, weak reaction.
[b] ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

extracted and purified by using the method described by Marmur (19). DNA base compositions were determined by high-performance liquid chromatography (22), using a commercial kit (DNA-GC kit; Yamasa Shoyu Co., Ltd., Chiba, Japan).

DNA-DNA hybridization. Labeled DNA was prepared with [1′,2′,5-^{3}H]dCTP by nick translation, using a commercial kit (Takara Shuzou Co., Ltd., Kyoto, Japan). DNA relatedness values were determined by the S1 nuclease procedure as described by Johnson (14).

RESULTS

Phenotypic characterization. Morphological and biochemical characteristics of 37 DKGA-producing strains are shown in Tables 2 and 3.

All of the DKGA-producing strains were gram-negative, facultatively anaerobic, nonsporeforming, straight, short rods. The five "*Erwinia citreus*" strains and the seven "*Erwinia punctata*" strains were nonmotile, and their cells were 0.8 to 1.2 by 1.0 to 2.9 and 1.1 to 1.3 by 1.3 to 2.3 μm, respectively, when they were grown on nutrient agar for 16 h at 20°C. All 25 "*Erwinia terreus*" strains were motile; 4 of these strains (strains SHS 2008^T, SHS 2009, SHS 2010, and SHS 2011) had one or two lateral flagella, and their cells were 0.8 to 0.9 by 1.2 to 2.0 μm. Figure 1 shows an electron micrograph of a cell of representative strain SHS 2008^T of "*Erwinia terreus*." The cells of strain SHS 2008^T were estimated to be 0.8 by 1.9 μm and had only one flagellum.

The colonies were pale beige to pale reddish yellow and did not produce the yellow pigment that has been observed in *P. agglomerans* ATCC 27155^T and *P. dispersa* ATCC 14589^T.

The DKGA-producing strains grew on nutrient agar at 8 to 39°C, but not at temperatures below 5°C and above 41°C (except for strain SHS 2007). Abundant growth was ob-

TABLE 3. Acid production from various carbohydrates by *Pantoea* species, as determined by using the API 50CHE system

Acid production from:	*P. citrea*		*P. punctata*		*P. terrea*		*P. agglomerans* ATCC 27155^T	*P. dispersa* ATCC 14589^T
	% of positive strains (n = 5)	Strain SHS 2003^T	% of positive strains (n = 7)	Strain SHS 2006^T	% of positive strains (n = 25)	Strain SHS 2008^T		
Glycerol	100	+[a]	100	+	100	+	+	+
meso-Erythritol	20	−	0	−	0	−	−	−
D-Arabinose	0	−	0	−	0	−	−	−
L-Arabinose	100	+	100	+	100	+	+	+
D-Ribose	100	+	100	+	100	+	+	+
D-Xylose	100	W	100	+	100	+	+	+
L-Xylose	0	−	0	−	0	−	−	−
Adonitol	0	−	0	−	8	−	−	−
β-Methyl-D-xyloside	0	−	0	−	8	−	−	−
D-Galactose	100	+	100	+	100	+	+	+
D-Glucose	100	+	100	+	100	+	+	+
D-Fructose	100	+	100	+	100	+	+	+
D-Mannose	100	+	100	+	100	+	+	+
L-Sorbose	0	−	0	−	8	−	−	−
L-Rhamnose	0	−	0	−	0	−	+	+
Dulcitol	0	−	0	−	4	−	−	−
meso-Inositol	0	−	0	−	0	−	W	+
D-Mannitol	100	+	0	−	0	−	+	+
D-Sorbitol	20	−	0	−	12	−	−	−
α-Methyl-D-mannoside	0	−	0	−	4	−	−	−
α-Methyl-D-glucoside	20	−	0	−	8	−	−	−
N-Acetyl-D-glucosamine	100	+	100	+	100	+	+	+
Amygdalin	0	−	0	−	0	−	−	+
Arbutin	20	−	0	−	100	+	+	−
Esculin	0	−	14	−	100	+	+	−
Salicin	20	−	0	−	100	+	+	−
Cellobiose	0	−	0	−	4	−	+	+
Maltose	100	+	14	−	24	−	+	+
Lactose	100	+	0	−	4	−	−	W
Melibiose	100	+	100	+	100	+	W	+
Sucrose	0	−	100	+	100	+	+	+
Trehalose	100	+	100	+	100	+	+	+
Inulin	0	−	0	−	4	−	−	−
Melezitose	0	−	14	−	4	−	−	−
Raffinose	0	−	29	+	32	−	−	−
Starch	0	−	0	−	12	−	−	−
Glycogen	0	−	0	−	8	−	−	−
D-Xylitol	20	−	14	−	8	−	−	−
β-Gentiobiose	100	+	100	+	100	+	W	+
D-Turanose	0	−	0	−	4	−	−	−
D-Lyxose	20	−	14	−	0	−	−	−
D-Tagatose	100	+	0	−	0	−	−	−
D-Fucose	100	+	100	+	100	+	+	+
L-Fucose	0	−	0	−	0	−	−	−
D-Arabitol	80	+	0	−	0	−	+	+
L-Arabitol	0	−	0	−	4	−	−	−
D-Gluconate	100	W	100	W	100	W	+	W
2-Keto-D-gluconate	0	−	0	−	0	−	+	W
5-Keto-D-gluconate	100	+	100	+	100	+	−	+

[a] +, positive reaction; −, negative reaction; W, weak reaction.

served at temperatures between 20 and 34°C and at pH values between 6.0 and 7.5. Either nicotinic acid or nicotinamide was required for growth.

The DKGA-producing strains were fermentative and negative for oxidase and pectinase activities. They were positive for citrate utilization as determined by the Simmons and Christensen procedures, but exhibited very weak activity or were negative for this characteristic as determined by the API 20E test. H_2S was produced from L-cysteine, but not on triple sugar iron slants.

All of the DKGA-producing strains were clearly positive for both D-gluconate dehydrogenase activity and 2-keto-D-gluconate dehydrogenase activity and produced DKGA from D-gluconate via 2-keto-D-gluconate. D-Gluconate dehydrogenase activity was present in *Erwinia ananas* ATCC 33244^T, *Erwinia cypripedii* ATCC 29267, *Erwinia rhapontici* ATCC 29284, *Erwinia uredovora* ATCC 19321^T, *P. dispersa* ATCC 14589^T, *P. agglomerans* ATCC 27155^T and four strains of *Enterobacter agglomerans* (strains ATCC 27995, ATCC 27992, ATCC 29919, and ATCC 27998). 2-Keto-D-gluconate dehydrogenase activity was observed only in *Erwinia cypripedii* ATCC 29267 among the members of the genera *Erwinia* and *Enterobacter* which we examined. These results agree with those of Bouvet et al. (2).

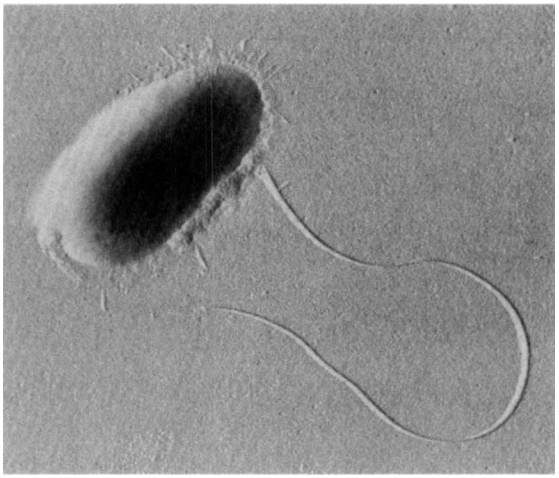

FIG. 1. Electron micrograph of *P. terrea* SHS 2008^T (= ATCC 31628^T). The cell was 0.8 by 1.9 μm.

Numerical analysis. Figure 2 shows our results in the form of a simplified dendrogram. The 37 DKGA-producing strains were separated into the following three phenotypic groups: the "*Erwinia citreus*" group (5 strains, with representative strain SHS 2003^T); the "*Erwinia punctata*" group (7 strains, including strains SHS 2006^T, SHS 2004, SHS 2005, and SHS 2007, with representative strain SHS 2006^T); and the "*Erwinia terreus*" group (25 strains, including strains SHS 2008^T, SHS 2009, SHS 2010, and SHS 2011, with representative strain SHS 2008^T). The simple matching coefficients for the 5 strains belonging to the "*Erwinia citreus*" group, for the 7 strains belonging to the "*Erwinia punctata*" group, and for the 25 strains belonging to the "*Erwinia terreus*" group were 93.6, 96.2, and 94.0%, respectively. The simple matching coefficient when the "*Erwinia punctata*" and "*Erwinia terreus*" groups were examined was 85.8%. The "*Erwinia citreus*" group exhibited 75.2% similarity to the "*Erwinia punctata*" and "*Erwinia terreus*" groups. The level of similarity of the DKGA-producing strains to *P. agglomerans* ATCC 27155^T and *P. dispersa* ATCC 14589^T was 68.4%, a value that was higher than the levels of similarity to the *Erwinia* strains (63.1%) and the *Enterobacter agglomerans* strains (63.9%). *P. agglomerans* ATCC 27155^T was most closely related to the "*Erwinia terreus*" strains (73.8%), and *P. dispersa* ATCC 14589^T exhibited a higher level of similarity to the "*Erwinia citreus*" group (70.8%) than to the "*Erwinia punctata*" (66.6%) and "*Erwinia terreus*" (64.2%) groups.

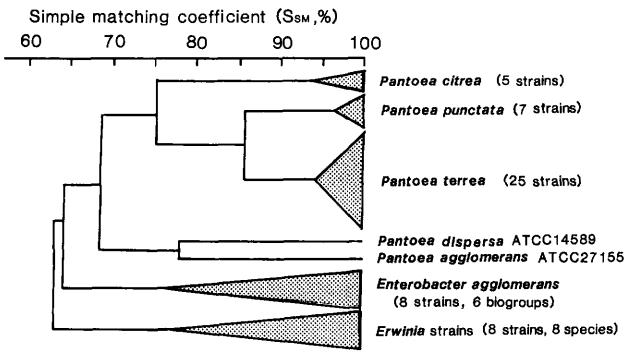

FIG. 2. Simplified dendrogram obtained by using the unweighted pair group average method of Colwell and Austin (8).

Quinones. The major isoprenoid side chain of the DKGA-producing strains was ubiquinone Q8.

G+C contents of DNAs. The DNA base compositions of nine representative DKGA-producing strains are shown in Table 4.

DNA hybridization. "*Erwinia citreus*" SHS 2003^T, "*Erwinia punctata*" SHS 2006^T, and "*Erwinia terreus*" SHS 2008^T were selected for preparation of labeled DNAs for DNA hybridization experiments. Relative DNA binding data are shown in Table 4.

P. dispersa ATCC 14589^T and *P. agglomerans* ATCC 27155^T exhibited levels of relatedness of less than 18% and less than 13% to the three representative DKGA-producing strains (strains SHS 2003^T, SHS 2006^T, and SHS 2008^T), respectively. Nine DKGA-producing strains exhibited less than 24% and less than 25% DNA binding to *P. dispersa* ATCC 14589^T and *P. agglomerans* ATCC 27155^T, respectively, when labeled DNAs from the *Pantoea* strains were used. Eight other strains belonging to the *Erwinia herbicola-Enterobacter agglomerans* complex exhibited levels of relatedness of less than 18% to the three representative DKGA-producing strains. The DNA-binding levels for strains belonging to other *Erwinia* and *Enterobacter* species were less than 20% and less than 14%, respectively. DNA-binding levels of less than 13% were observed for strains belonging to other species of the family *Enterobacteriaceae*.

DISCUSSION

The DKGA-producing strains which we investigated are gram negative, facultatively anaerobic, fermentative, and oxidase negative and belong to the family *Enterobacteriaceae* as described by Brenner (3). They were initially classified in the genus *Erwinia* or as *Enterobacter agglomerans* on the basis of the descriptions of Lelliot and Dickey (18) and Richard (23) and the criteria for biological identification described by Brenner (3).

As shown in Tables 2 and 3, the DKGA-producing strains had characteristics similar to those of the *Erwinia herbicola-Enterobacter agglomerans* complex described by Ewing and Fife (9) and by Brenner et al. (4). In addition, on the basis of the results of a numerical analysis in which we used the APILAB program and the API 20E and API 50CHE systems, we identified the DKGA-producing strains as uncertain representatives of *Erwinia* spp. or *Enterobacter agglomerans*. These facts indicated that the DKGA-producing strains could be included in the *Erwinia herbicola-Enterobacter agglomerans* complex.

Many investigators have reported that the *Erwinia herbicola-Enterobacter agglomerans* complex is heterogeneous and thus contains various kinds of bacterial strains that have different phenotypes and genotypes (4, 5, 10, 17, 20, 21, 25). On the basis of the results of a numerical phenotypic analysis, Gavini et al. (10) divided 169 strains belonging to the *Erwinia herbicola-Enterobacter agglomerans* complex into five major groups and 15 subgroups, and Verdonck et al. (25) divided 529 strains belonging to or related to the *Erwinia herbicola-Enterobacter agglomerans* complex into 33 phenotypic groups. Brenner et al. (4) reclassified 90 strains of the *Erwinia herbicola-Enterobacter agglomerans* complex into 13 DNA hybridization groups on the basis of DNA-DNA hybridization results.

Recently, Gavini et al. (11) proposed the genus *Pantoea* for 69 strains, including *Erwinia herbicola* ATCC 14589^T (*P.*

TABLE 4. Levels of DNA relatedness among *P. citrea*, *P. punctata*, *P. terrea*, and related strains

Strain	G+C content (mol%)	% of relative binding at 60°C with labeled DNA from:				
		P. citrea SHS 2003^T	*P. punctata* SHS 2006^T	*P. terrea* SHS 2008^T	*P. agglomerans* ATCC 27155^T	*P. dispersa* ATCC 14589^T
P. citrea SHS 2003^T	49.7	100	43	41	21	21
P. punctata SHS 2004	50.0	41	115	43	17	22
P. punctata SHS 2005	50.1	41	105	45	22	21
P. punctata SHS 2006^T	50.3	30	100	39	15	19
P. punctata SHS 2007	50.1	34	85	40	16	17
P. terrea SHS 2008^T	51.9	28	38	100	19	22
P. terrea SHS 2009	51.1	33	44	99	25	20
P. terrea SHS 2010	51.0	30	40	71	18	24
P. terrea SHS 2011	51.4	29	46	88	25	23
Species belonging to the genus *Pantoea*						
P. agglomerans ATCC 27155^T	55.6	12	13	12	100	43
P. dispersa ATCC 14589^T	57.8	18	15	9	39	100
Other strains belonging to the *Erwinia herbicola-Enterobacter agglomerans* complex						
Enterobacter agglomerans ATCC 29919		10	18	15	37	31
Enterobacter agglomerans ATCC 29918		18	15	9	21	24
Enterobacter agglomerans ATCC 27998		18	14	13	38	42
Enterobacter agglomerans ATCC 29904		8	8	11	21	33
Enterobacter agglomerans ATCC 27994		10	3	4		
Enterobacter agglomerans ATCC 27992		10	3	8		
Enterobacter agglomerans ATCC 27990		9	3	6		
Enterobacter agglomerans ATCC 27995		5	3	2		
Other *Erwinia* species						
Erwinia stewartii ATCC 8199^T		20	18	11	34	30
Erwinia ananas ATCC 33244^T		16	15	14	40	36
Erwinia uredovora ATCC 19321^T		11	12	10	39	33
Erwinia carotovora ATCC 15390		13	13	19		
Erwinia quercina ATCC 29282		7	10	7		
Erwinia cypripedii ATCC 29267		7	3	1		
Erwinia rhapontici ATCC 29284		15	2	4		
Erwinia rubrifaciens ATCC 29293		0	0	2		
Erwinia chrythantemi ATCC 11662		0	0	0		
Other *Enterobacter* species						
Enterobacter intermedium ATCC 33110^T		14	10	14		
Enterobacter sakazakii ATCC 29544^T		3	10	9		
Enterobacter cloacae ATCC 13047^T		10	4	5		
Enterobacter gergoviae ATCC 33028^T		0	6	4		
Other members of the *Enterobacteriaceae*						
Hafnia alvei ATCC 29926		10	11	6		
Hafnia alvei ATCC 29927		7	6	3		
Cedecea davisae ATCC 33431^T		8	7	13		
Cedecea lapagei ATCC 33432^T		1	5	4		
Cedecea neteri ATCC 33855^T		3	3	5		
Citrobacter diversus ATCC 27156		4	5	0		
Citrobacter freundii ATCC 8090		2	3	6		

dispersa ATCC 14589^T) and *Enterobacter agglomerans* ATCC 27155^T (*P. agglomerans* ATCC 27155^T). Our DKGA-producing strains are phenotypically related to the genus *Pantoea*, as shown in Tables 2 and 3 and Fig. 2. However, they differ from the species *P. agglomerans* and *P. dispersa* proposed by Gavini et al. (11) as follows: *P. agglomerans* ATCC 27155^T and *P. dispersa* ATCC 14589^T exhibit less than 18% DNA binding to the DKGA-producing strains (Table 4). Phenotypically, the DKGA-producing strains can be distinguished from *P. agglomerans* and *P. dispersa* by the characteristics shown in Table 5. The G+C contents of the DNAs of the DKGA-producing strains are 49.7 to 51.9 mol% and differ from those of *P. agglomerans* ATCC 27155^T (55.6 mol%) and *P. dispersa* ATCC 14589^T (57.8 mol%).

The nine representative DKGA-producing strains were divided into three DNA-DNA hybridization groups, which were formerly referred to as the "*Erwinia citreus*," "*Erwinia punctata*," and "*Erwinia terreus*" groups (24) (Table 4). "*Erwinia citreus*" SHS 2003^T exhibited 43 and 41% DNA binding to "*Erwinia punctata*" SHS 2006^T and "*Erwinia terreus*" SHS 2008^T, respectively. "*Erwinia terreus*" strains were 38 to 46% related to "*Erwinia punctata*" SHS 2006^T. The levels of DNA relatedness within the species were more than 71%. The criteria for DNA relatedness as described by Wayne et al. (26) and Johnson (15) indicate that "*Erwinia citreus*," "*Erwinia punctata*," and "*Erwinia terreus*" are separate species. These three species can also be differentiated phenotypically, as shown in Table 5. The G+C contents of the DNAs of "*Erwinia citreus*" SHS 2003^T, four strains of "*Erwinia punctata*," and four strains of "*Erwinia terreus*" are 49.7, 50.0 to 50.3, and 51.0 to 51.9 mol%, respectively, compared with values of 57.8 mol% for *P. dispersa* ATCC 14589^T and 55.6 mol% for *P. agglomerans* ATCC 27155^T.

Recently, Bouvet et al. (2) studied the taxonomic diversity

TABLE 5. Differential characteristics of *Pantoea* species

Characteristic	*P. citrea*	*P. punctata*	*P. terrea*	*P. agglomerans*[a]	*P. dispersa*[a]
Yellow pigment	–[b]	–	–	+	d
Motility	–	–	+	+	+
Growth at 41°C	–	–	–	–	+
Growth in KCN	–	–	–	(+)	+
Gelatin liquefaction	–	–	–	+	+
β-Galactosidase activity	+	–	–	+	+
L-Arginine dihydrolase activity	+	+	–	–	–
2-Keto-D-gluconate dehydrogenase activity	+	+	+	–	–
Utilization of:					
Acetate	+	–	+	–	
Lactate	+	–	+		+
Malonate	–	–	–	+	–
Acid production from:					
L-Rhamnose	–	–	–	+	+
Mannitol	+	–	–	+	+
Lactose	+	–	–	d	–
Maltose	+	(–)	(–)	+	+
Melibiose	+	+	+	–	d
Raffinose	–	d	d	d	–
Sucrose	–	+	+	+	+
Salicin	(–)	–	+	+	–
Arbutin	(–)	–	+		
Esculin	–	(–)	+		

[a] Data from reference 11.

[b] +, positive for 90 to 100% of the strains; (+), positive for 75 to 89% of the strains; d, positive for 26 to 74% of the strains; (–), positive for 11 to 25% of the strains; –, positive for 0 to 10% of the strains.

of the D-glucose oxidation pathways in the *Enterobacteriaceae* and found that five enterobacterial species, *Erwinia cypripedii*, *Ewingella americana*, *Rahnella aquatilis*, *Serratia marcescens*, and *Tatumella ptyseos*, produce DKGA from D-glucose. P. Grimont (11a) has suggested that "*Erwinia punctata*" and "*Erwinia terreus*" might be similar to *T. ptyseos* in nutritional patterns. However, "*Erwinia punctata*" and "*Erwinia terreus*" differ from *T. ptyseos* as described by Hollis et al. (13) in the following characteristics: acid production from D-xylose and L-arabinose, arginine dihydrolase activity, phenylalanine deaminase activity, methyl red reaction, Voges-Proskauer reaction, esculin hydrolase activity, citrate utilization, resistance to penicillin (10 U), and viability and colony size on Trypticase soy agar. In addition, the G+C content of *T. ptyseos* is 53 to 54 mol% (13), a value that is higher than the values for our three species (49.7 to 51.9 mol%).

The strains of "*Erwinia terreus*" have one or two lateral flagella, while the members of the family *Enterobacteriaceae* are defined as being motile and peritrichous (except for members of the genus *Tatumella*) (3). However, an *Erwinia herbicola* strain with a few lateral flagella has been described by Komagata et al. (16). The flagella of "*Erwinia terreus*" originate on the lateral side of the cell, not on the polar side. The flagella of *T. ptyseos* have been reported to be lateral, subpolar, or polar (13).

For the reasons mentioned above, we conclude that the nine representative DKGA-producing strains belong to the genus *Pantoea* and propose the following three new species of the genus: *Pantoea citrea*, *Pantoea punctata*, and *Pantoea terrea* for strain SHS 2003^T of "*Erwinia citreus*," for strains SHS 2006^T, SHS 2004, SHS 2005, and SHS 2007 of "*Erwinia punctata*," and for strains SHS 2008^T, SHS 2009, SHS 2010, and SHS 2011 of "*Erwinia terreus*," respectively. Table 5 shows the characteristics that differentiate *P. citrea*, *P. punctata*, *P. terrea*, and the two *Pantoea* species proposed by Gavini et al. (11).

Description of *Pantoea citrea* sp. nov. *Pantoea citrea* (ci'tre.a. M.L. adj. *citrea*, of citrus). Gram-negative, noncapsulated, nonsporeforming, nonmotile rods (0.8 to 1.2 by 1.0 to 2.9 μm) that occur singly and in pairs. Colonies grown on nutrient agar at 30°C for 2 days are pale beige to pale reddish yellow. The organism is facultatively anaerobic, but anaerobic growth is weak. Fermentative. Abundant growth occurs at 20 to 34°C and at pH 6.0 to 7.5. No growth occurs at pH values below 5.0 and above 9.0. Either nicotinic acid or nicotinamide is required for growth. Other biological and nutritional characteristics at 30°C are shown in Tables 2 and 3. The characteristics that differentiate this species from other species of the genus are shown in Table 5. The G+C content of the DNA of strain SHS 2003^T is 49.7 mol%, as determined by a chemical method (high-performance liquid chromatography). The type strain is strain SHS 2003 (= ATCC 31623), which was isolated from a mandarin orange in Japan.

Description of *Pantoea punctata* sp. nov. *Pantoea punctata* (punc. ta' ta. L. n. *punctum*, a point; M.L. adj. *punctata*, full of points). Gram-negative, noncapsulated, nonsporeforming, straight rods (1.1 to 1.2 by 1.3 to 2.3 μm) that occur singly and in pairs. The cells are nonmotile. Colonies grown on nutrient agar at 30°C for 2 days are pale beige to pale reddish yellow. This organism is facultatively anaerobic, but anaerobic growth is weak. Fermentative. Abundant growth occurs at 20 to 34°C and at pH 6.0 to 7.5. No growth occurs at pH values below 5.0 and above 9.0. Either nicotinic acid or nicotinamide is required for growth. Yellow pigment is not produced on nutrient agar. Other biological and nutritional characteristics at 30°C are shown in Tables 2 and 3. All strains are differentiated from other species of the genus by the characteristics shown in Table 5.

The G+C contents of the DNAs of four strains range from 50.0 to 50.3 mol% (the G+C content of strain SHS 2006^T is 50.3 mol%), as determined by a chemical method (high-performance liquid chromatography). The type strain is

strain SHS 2006 (= ATCC 31626), which was isolated from a mandarin orange in Japan.

Description of *Pantoea terrea* sp. nov. *Pantoea terrea* (ter're.a. L. n. *terra*, soil; L. adj. *terrea*, of soil). Gram-negative, noncapsulated, nonsporeforming, straight rods (0.8 to 0.9 by 1.2 to 2.0 μm) that occur singly and in pairs. The cells are motile by means of one or two lateral flagella. Colonies grown on nutrient agar at 30°C for 2 days are pale beige to pale reddish yellow. This organism is facultatively anaerobic, but anaerobic growth is weak. Fermentative. Abundant growth occurs at 20 to 34°C and at pH 6.0 to 7.5. No growth occurs at pH values below 5.0 and above 9.0. Either nicotinic acid or nicotinamide is required for growth. Other biological and nutritional characteristics at 30°C are shown in Tables 2 and 3. All strains are differentiated from other species of the genus by the characteristics shown in Table 5. The G+C contents of the DNAs of four strains range from 51.0 to 51.9 mol% (the G+C content of strain SHS 2008^T is 51.9 mol%), as determined by a chemical method (high-performance liquid chromatography). The type strain is strain SHS 2008 (= ATCC 31628), which was isolated from soil in Japan.

ACKNOWLEDGMENT

We thank Kazuo Komagata, Tokyo University of Agriculture, for many helpful discussions.

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