Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora*) and *Erwinia stewartii* to the Genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov. and *Pantoea stewartii* (Smith 1898) comb. nov., Respectively, and Description of *Pantoea stewartii* subsp. *indologenes* subsp. nov.

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Eight *Erwinia stewartii* strains, 11 *Erwinia ananas* strains, and 7 *Erwinia uredovora* strains, as well as 9 phenotypically similar *Erwinia herbicola* strains and *Enterobacter agglomerans* LMG 5342, were compared by examining electropherograms prepared from their soluble proteins and were grouped into nine protein electrophoretic groups. The levels of DNA relatedness among these electrophoretic groups were determined spectrophotometrically from the renaturation rates at 74°C of the DNAs of 13 selected strains. The representatives from five protein electrophoretic groups, including *E. ananas* LMG 2665^T (T = type strain) and *E. uredovora* LMG 2667^T, exhibited 76 to 100% DNA binding to each other and constituted DNA hybridization group 2665. All *E. stewartii* strains (including LMG 2715^T) were electrophoretically very similar; representatives of this species exhibited 93 to 99% DNA binding to each other and constituted DNA hybridization subgroup 2715. The strains belonging to the remaining three protein electrophoretic groups exhibited 94 to 96% DNA binding to each other and formed DNA hybridization subgroup 2632. The latter two subgroups were 60 to 83% (average, 73%) interrelated and exhibited 30 to 39% DNA binding to group 2665. It is proposed that *E. ananas* and *E. uredovora* should be united in a single species, which should be classified in the genus *Pantoea* as *Pantoea ananas* (Serrano 1928) comb. nov.; its type strain is strain LMG 2665 (= NCPPB 1846). This species also includes DNA hybridization group VI of Brenner et al. (D. J. Brenner, G. R. Fanning, J. K. Leete Knutson, A. G. Steigerwalt, and M. I. Krichevsky, Int. J. Syst. Bacteriol. 34:45–55, 1984). The transfer of *E. stewartii* to the genus *Pantoea* and the creation of two separate subspecies within *Pantoea stewartii* (Smith 1898) comb. nov. are also proposed. *Pantoea stewartii* subsp. *stewartii* (Smith 1898) comb. nov. (synonym, *Erwinia stewartii*) contains the strains belonging to subgroup 2715, and its type strain is strain LMG 2715 (= NCPPB 2295); and *Pantoea stewartii* subsp. *indologenes* subsp. nov. contains the strains belonging to subgroup 2632, and its type strain is strain LMG 2632 (= NCPPB 2280). As determined by principal-component analysis of the cellular fatty acid compositions, *P. ananas*, *P. stewartii* subsp. *stewartii*, and *P. stewartii* subsp. *indologenes* are separated from each other mainly by differences in the relative contents of *cis*-9-hexadecenoic acid (C_{16:1 cis 9}), cyclo-heptadecanoic acid (C_{17:0 cyclo}), and straight-chain octadecenoic acids (C_{18:1}). *P. stewartii* subsp. *stewartii* can also be differentiated from *P. ananas* and *P. stewartii* subsp. *indologenes* by its inability to produce indole, to utilize citrate, to grow on *cis*-aconitate, and to form acid from seven carbohydrates. *P. stewartii* subsp. *indologenes* can also be separated from *P. ananas* by its inability to form acid from sorbitol and α -methyl-D-mannoside. Descriptions of *P. ananas* and *P. stewartii* and its two subspecies are given, and the description of the genus *Pantoea* is emended.

In 1972 Ewing and Fife (16) proposed the name *Enterobacter agglomerans* to accommodate the “herbicola group” (13) of the genus *Erwinia*. This taxon included the species *Erwinia herbicola*, *Erwinia milletiae*, *Erwinia ananas*, *Erwinia uredovora*, and (according to Brenner [6] and Richard [39]) *Erwinia stewartii*, as well as *Escherichia adecarboxylata* and other phenotypically similar strains of clinical origin. Many authors demonstrated the extreme heterogeneity of the “*Erwinia herbicola*-*Enterobacter agglomerans*” complex on phenotypic (18, 32–34, 45) as well as genotypic levels (5, 7, 19, 26, 29). Some of the members of this complex were placed in new genera. *Escherichia adecarboxylata* was renamed *Leclercia adecarboxylata* (44), despite its affinities with *Enterobacter cloacae* and *Enterobacter amnigenus* (26). Subjective synonymy of the species names *Enterobacter agglomerans*, *Erwinia herbicola*, and *Erwinia milletiae* was suspected on phenotypic grounds (18, 34, 45) and was

confirmed by the high levels (>90%) of DNA binding observed (5, 29) and the similarity of the electrophoretic protein patterns of the type strains of these species (33). It was proposed (19) that a new genus *Pantoea* should be established, with type species *Pantoea agglomerans*, to include the type strains of *Enterobacter agglomerans*, *Erwinia herbicola*, and *Erwinia milletiae*, as well as the other strains belonging to electrophoretic protein profile groups I and III to VI of Beji et al. (5) and DNA hybridization group XIII of Brenner et al. (7). Gavini et al. (19) proposed the name *Pantoea dispersa* for the strains of their DNA hybridization group 14589, in which they included DNA hybridization group III of Brenner et al. (7). These authors suggested that other members of the “*Erwinia herbicola*-*Enterobacter agglomerans*” complex might also be included in the genus *Pantoea*, but lacked comparative data for a sufficient number of strains to do so. More recently, three new species isolated from fruits and soils were described and classified in the genus *Pantoea* on the basis of phenotypic and chemo-

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systematic characteristics (*Pantoea punctata*, *Pantoea citrea*, and *Pantoea terrea*) (27).

Synonymy of the names *Erwinia ananas* and *Erwinia uredovora* has been suspected (14, 34, 45) on phenotypic grounds, but has not yet been confirmed by DNA hybridization data. In the numerical analysis of Verdonck et al. (45), *Erwinia uredovora* and *Erwinia ananas* strains were mixed up with several (obviously misnamed) *Erwinia herbicola* strains and *Enterobacter agglomerans* CDC 6076-69 (= LMG 5342), a reference strain of DNA hybridization group VI of Brenner et al. (7). In this paper, we describe the inter- and intraspecific relationships of *Erwinia ananas*, *Erwinia uredovora*, and *Erwinia stewartii* strains and other phenotypically similar strains. On the basis of our data and data from previous papers (5, 7, 19, 29), we propose the new combinations *Pantoea ananas* (synonyms, *Erwinia ananas* and *Erwinia uredovora*) and *Pantoea stewartii* and describe a new subspecies, *Pantoea stewartii* subsp. *indologenes*.

MATERIALS AND METHODS

Strains used. The 36 strains used in this study are listed in Table 1. For comparison, *Pantoea agglomerans* LMG 1286^T (= NCTC 9381^T = ATCC 27155^T) (T = type strain) and LMG 2565 (= NCPPB 2971), *Pantoea dispersa* LMG 2603^T (= ATCC 14589^T) and LMG 2749 (= Lille 214-6) (5, 19), and *Erwinia amylovora* LMG 2024^T (= NCPPB 683^T) were included in some of the experiments.

Polyacrylamide gel electrophoresis of soluble proteins. Protein electropherograms were prepared as described previously (33).

DNA base composition. Mean guanine-plus-cytosine (G+C) contents were determined by the thermal denaturation method (10, 31).

DNA-DNA hybridizations. To prepare DNA, cells were grown at 28°C for 48 h in Roux flasks on a medium containing 1% tryptone (Oxoid), 1% proteose peptone (Oxoid), 0.15% K₂HPO₄, 0.3% MgSO₄ · 7H₂O, 1.0% glycerol, and 2.5% agar in distilled water. DNA was prepared by the method of Marmur (30). Percentages of DNA binding were determined spectrophotometrically from renaturation rates by the method of De Ley et al. (11). The optimal renaturation temperature, calculated from the G+C content with the equation of De Ley et al. (11), was approximately 74°C in double-strength standard saline citrate buffer.

Phenotypic tests. Tests in API 20E and API 50CHE systems (bioMérieux, La Balme les Grottes, Montalieu Vercieu, France) were performed at 30°C by the procedures described previously (34). API 20E results were coded and interpreted with the APILAB V3.1.1 computer program. The following characteristics were also tested: motility; pigment production on nutrient agar medium (Oxoid); gas production from glucose (in a 1% peptone-water solution supplemented with 1% D-glucose); phenylalanine deaminase activity; acid production on Jordan's tartrate medium; utilization of malonate and mucate (15); growth at different temperatures (in a medium containing 0.5% yeast extract, 0.5% neutralized peptone, and 1% D-glucose); tetrathionate reductase activity (28); DNase activity (on Oxoid medium supplemented with 0.01% toluidine blue); hydrolysis of Tween 20, Tween 40, Tween 60, and Tween 80 (41); pectate degradation (37); and growth on medium containing acetate, *cis*-aconitate, DL-4-aminobutyrate, DL-3-hydroxybutyrate, *meso*-tartrate, or D-malate (tested on the medium of Ayers et al. [3], which contained 0.25% MgSO₄ · 7H₂O, 0.25% KCl,

0.125% NH₄H₂PO₄, 0.5% carbon source substrate, and 2% agar).

Fatty acid analyses. A quantitative analysis of cellular fatty acid compositions was performed by using the gas-liquid chromatographic procedure described by Alcorn et al. (2), with the modifications described below. Fatty acid methyl esters were extracted with a mixture of hexane and methyl-*iso*-butylether. Fatty acid methyl esters were analyzed with a Hewlett-Packard model 5890A gas chromatograph and were identified by using the Microbial Identification System (MIS) software (version 3.5) (Microbial ID, Inc., Newark, Del.). A principal-component analysis was carried out by using the same software package.

RESULTS

Comparison of protein electropherograms. Protein electropherograms were prepared for the 36 strains listed in Table 1 and for *Pantoea agglomerans* LMG 1286^T and *Pantoea dispersa* LMG 2603^T and were compared by visually inspecting the normalized photographs. Among the strains listed in Table 1, nine protein profile groups, each of which had a characteristic protein profile, could be recognized (Fig. 1 and 2). These groups contained strains with similar protein patterns. In some groups variations in the intensities of the protein bands in the upper part of the gel (e.g., protein profile group II) or the lower part of the gel (protein profile group VIII) were obvious, while in other groups the protein patterns were almost identical (protein profile group I).

Protein profile groups I and II each contained four strains of *Erwinia ananas*. Protein profile group II included type strain LMG 2665, as well as *Erwinia herbicola* LMG 2101. Protein profile groups III and IV each contained three *Erwinia uredovora* strains; protein profile group IV included type strain LMG 2676. *Enterobacter agglomerans* LMG 5342 (= CDC 6070-69; a reference strain belonging to DNA hybridization group VI of Brenner et al. [7]), *Erwinia herbicola* LMG 2628, and *Erwinia uredovora* LMG 2679 constituted protein profile group V (Fig. 1).

Protein profile groups VI and VIII contained three and four *Erwinia herbicola* strains, respectively. Protein profile group VII consisted of three *Erwinia ananas* strains. Finally, all *Erwinia stewartii* strains, including type strain LMG 2715, were electrophoretically similar and constituted protein profile group IX (Fig. 2).

The electrophoretic protein profiles of groups I to IX were significantly different from those of *Pantoea agglomerans* LMG 1268^T and *Pantoea dispersa* LMG 2603^T (Fig. 1 and 2).

DNA base compositions. The DNA base compositions of four strains were determined in duplicate. The differences between the duplicate test results were less than 0.5 mol% in each case. The G+C contents of the DNAs of *Erwinia ananas* LMG 2665^T and LMG 2673 and *Erwinia herbicola* LMG 2630 and LMG 2632 were 55.0, 54.5, 54.7, and 56.4 mol%, respectively.

DNA-DNA hybridizations. In order to determine the genotypic relationships among the different electrophoretic protein profile groups, 13 representative strains were selected for DNA-DNA hybridization experiments. Representative strains of *Pantoea agglomerans* and *Pantoea dispersa* were included for comparison. The results are shown as a sorted matrix of percentages of DNA binding in Fig. 3; the percentages are averages of two to four determinations. Among the strains belonging to protein profile groups I to VII two major DNA hybridization groups could be distinguished.

DNA hybridization group 2665 contained all seven strains

TABLE 1. Strains used, their sources, and their API 20E profile numbers

Group	Species name as received	Strain ^a	Strain designation as received ^a	Isolated in:	Isolated from:	API 20E profile ^b
<i>Pantoea ananas</i> (Serrano 1928) comb. nov.						
Protein profile group I	<i>Erwinia ananas</i>	LMG 2666	Graham BG1	Hawaii	<i>Ananas comosus</i>	1245773
	<i>Erwinia ananas</i>	LMG 2667	Graham BG2	Hawaii	<i>Ananas comosus</i>	1245773
	<i>Erwinia ananas</i>	LMG 2668	Graham MF28	Hawaii	<i>Ananas comosus</i>	1245563
Protein profile group II	<i>Erwinia ananas</i>	LMG 2669	Graham Y1	Hawaii	<i>Ananas comosus</i>	1245563
	<i>Erwinia herbicola</i>	LMG 2101	Graham G144	India	<i>Oryza sativa</i>	1245163
	<i>Erwinia ananas</i>	LMG 2664	NCPPB 544	Hawaii	<i>Ananas comosus</i>	1245773
	<i>Erwinia ananas</i>	LMG 2665 ^{Tc,d}	NCPPB 1846 ^{Tc,d}	Brazil	<i>Ananas comosus</i>	1245573
	<i>Erwinia ananas</i>	LMG 2670	NCPPB 545	Hawaii	<i>Ananas comosus</i>	1245373
	<i>Erwinia ananas</i>	LMG 2672	HIM 597-3		<i>Ananas comosus</i>	1245573
Protein profile group III	<i>Erwinia uredovora</i>	LMG 2675	NCPPB 391	Hungary	<i>Puccinia graminis</i> f. sp. <i>tritici</i>	1245773
	<i>Erwinia uredovora</i>	LMG 2677	NCPPB 802	Hungary	<i>Puccinia graminis</i> f. sp. <i>tritici</i>	1247773
	<i>Erwinia uredovora</i>	LMG 2681	NCPPB 1440	Hungary	<i>Puccinia graminis</i> f. sp. <i>tritici</i>	1247773
Protein profile group IV	<i>Erwinia uredovora</i>	LMG 2676 ^{Td}	NCPPB 800 ^{Td}	United States	<i>Puccinia graminis</i> f. sp. <i>tritici</i>	1247573
	<i>Erwinia uredovora</i>	LMG 2678	NCPPB 1044	Zimbabwe	<i>Puccinia graminis</i> f. sp. <i>tritici</i>	1244773
	<i>Erwinia uredovora</i>	LMG 2680	NCPPB 1419	Hungary	<i>Puccinia graminis</i> f. sp. <i>tritici</i>	1244773
Protein profile group V	<i>Erwinia herbicola</i>	LMG 2628	HIM 577-8		<i>Musa</i> sp.	1244773
	<i>Erwinia uredovora</i>	LMG 2679	NCPPB 1416	Hungary	<i>Puccinia graminis</i> f. sp. <i>tritici</i>	1245773
	<i>Enterobacter agglomerans</i>	LMG 5342	CDC 6070-69	United States	Human clinical isolate	1245773
<i>Pantoea stewartii</i> subsp. <i>indologenes</i> subsp. nov.						
Protein profile group VI	<i>Erwinia herbicola</i>	LMG 2630	NCPPB 1877		<i>Cyamopsis tetragonolobus</i>	1045163
	<i>Erwinia herbicola</i>	LMG 2631	NCPPB 2275	India	<i>Pennisetum americanum</i>	1245163
	<i>Erwinia herbicola</i>	LMG 2635	NCPPB 2283	India	<i>Pennisetum americanum</i>	1245163
Protein profile group VII	<i>Erwinia ananas</i>	LMG 2671	NCPPB 1845	Hawaii	<i>Ananas comosus</i>	1245163
	<i>Erwinia ananas</i>	LMG 2673	PDDCC 1517	Hawaii	<i>Ananas comosus</i>	1245163
	<i>Erwinia ananas</i>	LMG 2674	PDDCC 1518	Hawaii	<i>Ananas comosus</i>	1245163
Protein profile group VIII	<i>Erwinia herbicola</i>	LMG 2629	NCPPB 1562	India	<i>Pennisetum americanum</i>	1244163
	<i>Erwinia herbicola</i>	LMG 2632 ^{Tc}	NCPPB 2280 ^{Tc}	India	<i>Setaria italica</i>	1245163
	<i>Erwinia herbicola</i>	LMG 2633	NCPPB 2281	India	<i>Setaria italica</i>	1244163
	<i>Erwinia herbicola</i>	LMG 2634	NCPPB 2282	India	<i>Pennisetum americanum</i>	1245163
<i>Pantoea stewartii</i> subsp. <i>stewartii</i> (Smith 1898) comb. nov.						
Protein profile group IX	<i>Erwinia stewartii</i>	LMG 2712	NCPPB 449	United States	<i>Zea mays</i> var. <i>rugosa</i>	1004162
	<i>Erwinia stewartii</i>	LMG 2713	NCPPB 1553	United States	<i>Zea mays</i>	1004162
	<i>Erwinia stewartii</i>	LMG 2714	NCPPB 2294	United States	<i>Zea mays</i>	1005162
	<i>Erwinia stewartii</i>	LMG 2715 ^{Tc,d}	NCPPB 2295 ^{Tc,d}	United States	<i>Zea mays</i> var. <i>rugosa</i>	1004162
	<i>Erwinia stewartii</i>	LMB 2716	PDDCC 270	United States	<i>Zea mays</i> var. <i>rugosa</i>	1004162
	<i>Erwinia stewartii</i>	LMG 2717	PDDCC 722	United States	Beetle	1005162
	<i>Erwinia stewartii</i>	LMG 2718	PDDCC 724	United States		1004162
	<i>Erwinia stewartii</i>	LMG 2719	PDDCC 5929	United States	Corn flea beetle	1004162

^a Abbreviations: CDC, Centers for Disease Control, Atlanta, Ga.; Graham, D. C. Graham, Department of Agriculture and Fisheries for Scotland, Agricultural Scientific Services, Edinburgh, Scotland; HIM, Hygiene-Institut und Medizinisch-Untersuchungsamt, University of Marburg, Marburg/Lahn, Germany; LMG, Culture Collection, Laboratorium voor Microbiologie, Ghent, Belgium; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, England; PDDCC, Plant Diseases Division Culture Collection, Auckland, New Zealand.

^b After 24 h of incubation at 30°C.

^c Type strain of proposed *Pantoea* species or subspecies.

^d Type strain of *Erwinia* species (42).

FIG. 1. Normalized protein electropherograms for 18 strains of *Pantoea ananas* and the type strains of *Pantoea agglomerans* and *Pantoea dispersa*. Lane a, *Pantoea agglomerans* LMG 1286^T; lanes b through s, *Pantoea ananas* LMG 2666, LMG 2667, LMG 2668, LMG 2669, LMG 2101, LMG 2664, LMG 2670, LMG 2672, LMG 2665^T, LMG 2675, LMG 2681, LMG 2677, LMG 2680, LMG 2678, LMG 2676, LMG 2679, LMG 2628, and LMG 5342, respectively; lane t, *Pantoea dispersa* LMG 2603^T.

belonging to protein profile groups I to V tested. It included type strains *Erwinia uredovora* LMG 2676 and *Erwinia ananas* LMG 2665. Strains belonging to this DNA hybridization group exhibited 76 to 100% DNA binding to each other. The levels of interspecies DNA binding of the *Erwinia uredovora* and *Erwinia ananas* strains in this group were as high as 99%, and the level of DNA binding was 79% when the DNAs of the type strains were hybridized.

The second DNA hybridization group contained the reference strains of protein profile groups VI to IX. Within this DNA hybridization group two genomic subgroups could be recognized, each consisting of three strains which exhibited more than 93% DNA binding to each other. The levels of DNA binding calculated between members of the two subgroups ranged from 60 to 83%. Subgroup 2632 contained the

strains belonging to protein profile groups VI to VIII, and subgroup 2715 contained the strains of *Erwinia stewartii* tested (protein profile group IX), including type strain LMG 2715.

Representatives of DNA hybridization group 2665 exhibited 30 to 32% DNA binding to *Erwinia stewartii* LMG 2715^T, a member of DNA hybridization subgroup 2715, and 32 to 39% DNA binding to strains belonging to DNA hybridization subgroup 2632.

Strains of *Pantoea agglomerans* and *Pantoea dispersa* exhibited 18 to 34% DNA binding to members of DNA hybridization group 2665 and DNA hybridization subgroups 2715 and 2632.

Phenotypic features. The results of the phenotypic tests are shown in Table 2.

FIG. 2. Normalized protein electropherograms for 18 strains of *Pantoea stewartii* and the type strains of *Pantoea agglomerans* and *Pantoea dispersa*. Lane a, *Pantoea agglomerans* LMG 1286^T; lanes b through k, *Pantoea stewartii* subsp. *indologenes* LMG 2631, LMG 2635, LMG 2630, LMG 2673, LMG 2674, LMG 2671, LMG 2629, LMG 2634, LMG 2632^T, and LMG 2633, respectively; lanes l through s, *Pantoea stewartii* subsp. *stewartii* LMG 2715^T, LMG 2717, LMG 2714, LMG 2712, LMG 2719, LMG 2713, LMG 2716, and LMG 2718, respectively; lane t, *Pantoea dispersa* LMG 2603^T.

Protein profile group	Species name as received	Strain no. LMG																													
I	<i>Erwinia ananas</i>	2668	100																												
II	<i>Erwinia ananas</i>	2665 ^T	90	100																											
II	<i>Erwinia ananas</i>	2670	-	89	100	<i>Pantoea ananas</i>																									
V	<i>Erwinia uredovora</i>	2679	-	79	89	100	(DNA hybridization group 2665)																								
III	<i>Erwinia uredovora</i>	2681	-	94	97	100	100																								
IV	<i>Erwinia uredovora</i>	2680	-	83	99	100	91	100																							
IV	<i>Erwinia uredovora</i>	2676 ^T	76	79	-	85	100	96	100																						
VI	<i>Erwinia herbicola</i>	2630	-	-	38	-	-	-	-	100	<i>Pantoea stewartii</i> subsp. <i>indologenes</i>																				
VII	<i>Erwinia ananas</i>	2673	-	32	39	-	-	-	-	94	100	(DNA hybridization subgroup 2632)																			
VIII	<i>Erwinia herbicola</i>	2632	-	-	-	-	-	-	-	100	96	100																			
IX	<i>Erwinia stewartii</i>	2713	-	-	-	-	-	-	-	-	71	83	100	<i>Pantoea stewartii</i> subsp. <i>stewartii</i>																	
IX	<i>Erwinia stewartii</i>	2715 ^T	-	30	31	-	32	-	-	60	76	79	93	100	(DNA hybridization subgroup 2715)																
IX	<i>Erwinia stewartii</i>	2718	-	-	-	-	-	-	-	-	73	74	97	99	100																
	<i>Pantoea agglomerans</i>	1286 ^T	-	-	-	-	-	-	-	-	-	-	-	22	-	100	<i>Pantoea</i>														
	<i>Pantoea agglomerans</i>	2565	25	34	-	34	-	-	-	-	28	-	-	24	22	88	100	<i>agglomerans</i>													
	<i>Pantoea dispersa</i>	2603 ^T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	<i>Pantoea</i>												
	<i>Pantoea dispersa</i>	2749	18	-	-	-	-	-	-	-	18	-	-	-	-	-	26	87	100	<i>dispersa</i>											

FIG. 3. Sorted matrix of average percentages of DNA binding for 17 *Pantoea* strains. The percentages obtained from homologous reactions were, by definition, set at 100%. Strains LMG 2665, LMG 2632, and LMG 2715 are type strains of the proposed *Pantoea* species and subspecies.

API 20E seven-digit codes. The strains belonging to protein profile groups I to IX were tested in the API 20E system, and the seven-digit codes obtained are shown in Table 1. These codes did not correlate with the electrophoretic groups.

The eight different codes obtained for protein profile groups I to V (DNA hybridization group 2665) were correctly identified as *Erwinia ananas* or *Erwinia uredovora* codes by the APILAB profile recognition program. Both codes obtained for the *Erwinia stewartii* strains were identified as *Erwinia mallotivora* codes. The three codes obtained for the strains belonging to protein profile groups VI to VIII (DNA hybridization subgroup 2632) were identified as *Erwinia ananas* or *Erwinia uredovora* codes. In most cases reactions in additional tests (yellow pigment; gas production from D-glucose; esculin hydrolysis; acid production from α -methyl-D-glucoside, lactose, D-xylose, and trehalose) had to be used to differentiate the strains used in this study from *Enterobacter agglomerans*, *Klebsiella oxytoca*, *Serratia plymuthica*, and other *Erwinia* species.

Fatty acid analyses. The relative cellular fatty acid compositions of all of the strains were determined and are shown in Table 3. The groups defined by DNA hybridization characteristics and the type strains of *Pantoea agglomerans*, *Pantoea dispersa*, and *Erwinia amylovora* were separated in the principal-component analysis by using principal components 1 and 2 (Fig. 4). The separation of these groups and type strains was based mainly on differences in relative contents of *cis*-9-hexadecenoic acid ($C_{16:1\text{ cis }9}$), cyclo-heptadecanoic acid ($C_{17:0\text{ cyclo}}$), and straight-chain octadecenoic acids ($C_{18:1}$) (Table 3, summed feature 7). DNA hybridization group 2665 and DNA hybridization subgroup 2632 exhibited greater similarity in their fatty acid methyl ester compositions to each other than to DNA hybridization subgroup 2715. *Erwinia ananas* strains belonging to protein

profile groups I and II intermingled with the *Erwinia uredovora* strains in the analysis. The standard MIS library (version 3.5) identified the strains belonging to protein profile groups I to V (DNA hybridization group 2665) as *Erwinia uredovora*, with the genus *Cedecea* as the second choice, or vice versa. All of the *Erwinia stewartii* strains were identified as such. For the strains belonging to protein profile groups VI to VIII (DNA hybridization subgroup 2632) the MIS program gave low identification scores (<0.4) and could not differentiate among the genus *Cedecea*, *Enterobacter cloacae*, *Citrobacter diversus*, and *Erwinia uredovora*.

DISCUSSION

Seven strains received as *Erwinia ananas* and *Erwinia uredovora* (including their type strains [strains LMG 2665 and LMG 2676, respectively]) exhibited 76 to 100% DNA binding to each other and constituted DNA hybridization group 2665 (Fig. 3). The existence of five different electrophoretic groups within this DNA hybridization group (Fig. 1) indicates that the taxonomic resolution of the electrophoretic technique which we used is at an intraspecific level. The high levels of DNA binding observed between strains which produced similar or identical protein patterns (89, 96, and 93 to 99% within protein profile groups II, IV, and IX, respectively [Fig. 3]) are in accordance with previous observations (5, 19, 26). Consequently, we assign all of the strains belonging to protein profile groups I to V to DNA hybridization group 2665. Five strains belonging to these protein profile groups (strains LMG 2668 [= ICPB EA181], LMG 2628 [= ATCC 23822], LMG 2676 [= ICPB XU102], LMG 2677 [= ICPB XU104], and LMG 5342) were classified in DNA hybridization group VI by Brenner et al. (7). Therefore, we concluded that DNA hybridization group VI of

TABLE 2. Phenotypic characteristics of *Pantoea ananas* (DNA hybridization group 2665), *Pantoea stewartii* subsp. *stewartii* (DNA hybridization subgroup 2715), and *Pantoea stewartii* subsp. *indologenes* (DNA hybridization subgroup 2632)

Characteristic ^a	<i>Pantoea ananas</i>		<i>Pantoea stewartii</i> subsp. <i>stewartii</i>		<i>Pantoea stewartii</i> subsp. <i>indologenes</i>	
	No. of strains positive/no. of strains tested	Reaction of type strain LMG 2665	No. of strains positive/no. of strains tested	Reaction of type strain LMG 2715	No. of strains positive/no. of strains tested	Reaction of type strain LMG 2632
Production of yellow pigment	17/18	+	8/8	+	9/10	+
Growth at:						
4°C	16/18	+	2/8	—	8/10	+
37°C	9/17	—	2/7	—	7/8	+
41°C	4/17	—	0/7	—	3/8	—
44°C	1/17	—	0/7	—	2/8	—
Motility	17/18	+	0/8	—	8/10	+
Gelatin liquefaction	4/18	—	0/8	—	0/10	—
Indole production	18/18	+	0/8	—	10/10	+
Acetoin production	15/18	+	2/8	—	8/10	+
Nitrate reduced to nitrite	7/18	—	0/8	—	0/10	—
Hydrolysis of esculin	9/18	+	0/8	—	10/10	+
DNase	9/18	—	2/8	+	2/10	—
Citrate utilization	18/18	+	0/8	—	9/10	+
Malonate utilization	1/18	—	0/8	—	0/10	—
Mucate utilization	7/18	—	0/8	—	2/10	—
Hydrolysis of:						
Tween 20	18/18	+	8/8	+	7/10	+
Tween 60	18/18	+	8/8	+	9/10	+
Tween 80	7/18	+	0/8	—	0/10	—
Growth on:						
<i>cis</i> -Aconitate	18/18	+	0/8	—	10/10	+
DL-4-Aminobutyrate	7/18	+	0/8	—	8/10	+
DL-3-Hydroxybutyrate	1/18	+	0/8	—	0/10	—
<i>meso</i> -Tartrate	17/18	+	7/8	—	10/10	+
D-Malate	17/18	+	8/8	+	10/10	+
Acid produced from:						
D-Mannose	17/18	+	8/8	+	10/10	+
L-Rhamnose	14/18	+	0/8	—	4/10	+
Cellobiose	18/18	+	0/8	—	10/10	+
Maltose	17/18	+	0/8	—	10/10	+
Lactose	17/18	+	0/8	—	10/10	+
Melibiose	16/18	+	8/8	+	10/10	+
Gentiobiose	10/18	+	0/8	—	5/10	+
Raffinose	15/18	+	8/8	+	10/10	+
α -Methyl-D-mannoside	16/18	+	0/8	—	0/10	—
α -Methyl-D-glucoside	0/18	—	0/8	—	1/10	—
Arbutin	17/18	+	0/8	—	10/10	+
Salicin	16/18	+	0/8	—	10/10	+
Amygdalin	6/18	+	0/8	—	3/10	—
Glycerol	17/18	+	0/8	—	8/10	+
D-Arabitol	18/18	+	0/8	—	10/10	+
Dulcitol	0/18	—	0/8	—	1/10	—
<i>meso</i> -Inositol	13/18	+	0/8	—	3/10	+
Sorbitol	15/18	+	0/8	—	0/10	—
D-Gluconate	9/18	+	0/8	—	3/10	—
2-Keto-D-gluconate	3/18	—	0/8	—	2/10	—
5-Keto-D-gluconate	5/18	+	0/8	—	4/10	+

^a Acid production data (API 50CHE tests) were recorded after 48 h, API 20E test data (gelatin liquefaction; indole, acetoin, and hydrogen sulfide production; nitrate reduction; arginine dihydrolase; lysine and ornithine decarboxylases; tryptophan deaminase; β -D-galactosidase; urease; and citrate utilization) were recorded after 24 h, and data for the other tests were recorded after 3 days. Incubation was at 30°C, unless stated otherwise. All *Pantoea ananas* and *Pantoea stewartii* strains were positive in the following tests: β -D-galactosidase; hydrolysis of Tween 40; growth on L-aspartate; and acid production from L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, sucrose, trehalose, N-acetyl-D-glucosamine, and D-mannitol. They were all negative in the following tests: production of hydrogen sulfide; gas production from D-glucose; arginine dihydrolase; lysine and ornithine decarboxylases; phenylalanine and tryptophan deaminases; tetrathionate reductase; urease; oxidase; Jordan's tartrate; pectate degradation; growth on acetate; and acid production from D-arabinose, L-xylose, D-lyxose, L-sorbose, D-tagatose, D-fucose, L-fucose, D-turanose, melezitose, inulin, starch, glycogen, β -methyl-D-xyloside, *meso*-erythritol, adonitol, *meso*-xylitol, and L-arabitol.

TABLE 3. Cellular fatty acid compositions of *Pantoea ananas* (DNA hybridization group 2665), *Pantoea stewartii* subsp. *stewartii* (DNA hybridization subgroup 2715), *Pantoea stewartii* subsp. *indologenes* (DNA hybridization subgroup 2632), *Pantoea agglomerans* LMG 1286^T, *Pantoea dispersa* LMG 2603^T, and *Erwinia amylovora* LMG 2024^T, expressed as percentages of the total fatty acids

Fatty acid	% in:					
	<i>Pantoea ananas</i> (18 strains)	<i>Pantoea stewartii</i> subsp. <i>stewartii</i> (8 strains)	<i>Pantoea stewartii</i> subsp. <i>indologenes</i> (10 strains)	<i>Pantoea agglomerans</i> LMG 1286 ^T	<i>Pantoea dispersa</i> LMG 2603 ^T	<i>Erwinia amylovora</i> LMG 2024 ^T
10:0 3 OH			<1 (2) ^a			
12:0	3.3–4.6	3.4–4.8	3.9–5.0	3.8	4.2	5.1
12:0 3 OH	<1 (13)		<1 (1)			
13:0	<1 (6)					
14:0	1.2–5.8	0–5.1 (6)	1.3–2.8	6.0	3.0	4.9
14:0 2 OH	0–3.3 (17)		2.2–3.0		2.1	
15:0	0–5.1 (16)		<1 (8)	1.1	<1	<1
15:0 3 OH	<1 (1)					
16:0	26.9–34.1	24.3–29.6	29.3–32.1	27.1	30.8	34.0
16:1 <i>cis</i> 9	17.2–26.7	11.9–15.4	18.7–22.9	24.2	10.6	24.5
17:0	0–4.6 (17)	<1 (1)	0.5–1.1	<1	<1	1.5
17:0 cyclo ^b	2.2–11.2		1.4–3.5	13.2	11.3	9.6
17:1 ^b	<1 (5)					
18:0	<1 (17)	1.5–2.4	<1		<1	
18:1 iso ^b	0–1.7 (12)	0–1.0 (3)	0.5–3.3			
19:0 cyclo C11-12	<1 (9)				1.7	
20:1 <i>trans</i> 11		<1 (6)				
Summed feature 2 ^c	<1 (9)					
Summed feature 3 ^d	7.5–10.6	8.6–14.6	7.8–11.2	11.2	9.0	9.1
Summed feature 7 ^e	14.2–19.9	35.7–41.4	21.8–25.4	11.6	24.4	9.8
Unknown 13.961 ^f	<1 (14)		<1		<1	
Unknown 14.503 ^f	0–1.4 (10)	<1 (6)	<1	1.0	<1	<1
Unknown 15.549 ^f	<1 (3)		<1 (2)			

^a If a fatty acid was not detected in all strains of a taxon, the number of strains in which it was detected is given in parentheses.

^b The position of the double bond or cyclopropane ring is unknown.

^c Fatty acids 15:1 iso (position of double bond unknown) and 13:0 3 OH cannot be separated by using gas chromatography and the MIS system and are designated summed feature 2.

^d Fatty acids 16:1 iso (position of double bond unknown) and 14:0 3 OH cannot be separated by using gas chromatography and the MIS system and are designated summed feature 3.

^e Fatty acids 18:1 *trans* 6, 18:1 *trans* 9, and 18:1 *cis* 11 cannot be separated by using gas chromatography and the MIS system and are designated summed feature 7.

^f Designations are the equivalent chain lengths relative to the lengths of known, straight-chain fatty acids.

Brenner et al. and our DNA hybridization group 2665 are the same.

From the high level of genotypic relatedness (79% DNA binding) between the type strains of *Erwinia ananas* and *Erwinia uredovora*, it follows that these species names are subjective synonyms. *Erwinia ananas* Serrano 1928 is a senior synonym of *Erwinia uredovora* (Pon et al. 1954) Dye 1963 and therefore should be given nomenclatural priority. The contention that these names are synonyms is supported by the results of numerical analyses (14, 34, 45), in which the type strains, as well as other strains, consistently were placed in the same phena, and by the similarity of the fatty acid compositions (Fig. 4) (2). *Erwinia ananas* has been identified as a cause of rot of pineapple, but no supplementary evidence that *Erwinia ananas* is the causative agent of the disease has been presented since the original report of Serrano (40). *Erwinia uredovora* was described as a *Puccinia graminis* pathogen (38). Although the two nomenspecies belonged to different protein electrophoretic profile groups, they each intermingled with strains that were from other ecological origins, including clinical origins, and were received as *Erwinia herbicola* or *Enterobacter agglomerans*. There seem to be no phenotypic features by which these organisms can be differentiated from each other (34, 45), and no subgrouping can be recognized from the DNA-DNA hybridization or fatty acid data. Thus, there is hardly any taxonomic or phytopathological support for the proposal of

FIG. 4. Principal-component analysis of fatty acid compositions of 38 *Pantoea* strains and *Erwinia amylovora* LMG 2024^T, performed by using principal component 1 and principal component 2. Symbols: ●, *Pantoea ananas*; ○, *Pantoea stewartii* subsp. *indologenes*; ▲, *Pantoea stewartii* subsp. *stewartii*; □, other strains. The positions of the type strains are indicated.

TABLE 4. Differential characteristics of *Pantoea* species

Characteristic	<i>Pantoea ananas</i>	<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	<i>Pantoea stewartii</i> subsp. <i>indologenes</i>	<i>Pantoea agglomerans</i>	<i>Pantoea dispersa</i>	<i>Pantoea citrea</i>	<i>Pantoea punctata</i>	<i>Pantoea terrea</i>
API 20E tests								
Nitrate reduced to nitrite	d ^a	—	—	+	—	+	+	+
Indole production	+	—	+	—	—	—	—	—
Citrate utilization	+	—	+	d	+	+	+	+
β-Galactosidase	+	+	+	+	+	+	—	—
API 50CHE tests								
Acid produced from glycerol	+	—	[+]	[—]	d	+	+	+
Acid produced from D-arabitol	+	—	+	d	+	[+]	—	—
Acid produced from sorbitol	[+]	—	—	—	—	[—]	—	[—]
Acid produced from cellobiose	+	—	+	d	+	—	—	—
Acid produced from maltose	+	—	+	+	+	+	[—]	[—]
Acid produced from lactose	+	—	+	[—]	[—]	+	—	—
Acid produced from α-methyl-D-mannoside	[+]	—	—	—	—	—	—	—
Acid produced from arbutin	+	—	+	+	—	[—]	—	+
Acid produced from salicin	+	—	+	+	[—]	[—]	—	+
Acid produced from raffinose	[+]	+	+	—	—	—	d	d
Acid produced from D-turanose	—	—	—	—	+	—	—	—
Acid produced from D-fucose	—	—	—	—	—	+	+	+
Hydrolysis of esculin	d	—	+	+	+	—	[—]	+
Other tests								
Motility	+	—	[+]	+	+	—	—	+
Malonate utilization	—	—	—	+	—	—	—	—
Phenylalanine deaminase	—	—	—	+	—	—	—	—
Growth on <i>cis</i> -aconitate	+	—	+	+	+	NT	NT	NT

^a +, at least 90% of the strains are positive; —, at least 90% of the strains are negative; [+], 76 to 89% of the strains are positive; [—], 11 to 25% of the strains are positive; d, 26 to 75% of the strains are positive; NT, not tested. Data were obtained after 24 h (API 20E tests, motility), 48 h (API 50CHE tests), or 3 days (other tests) of incubation at 30°C. Data for *Pantoea citrea*, *Pantoea punctata*, and *Pantoea terrea* are from reference 27.

Young et al. (47) that these species should be classified as separate pathovars of the same species.

Six strains constituted a second DNA hybridization group, as they exhibited 60 to 100% DNA binding to each other. Within this DNA hybridization group two subgroups were recognized (Fig. 3). DNA hybridization subgroup 2715 (93 to 99% DNA binding) contained three *Erwinia stewartii* strains (including type strain LMG 2715), which were members of protein profile group IX (Fig. 2). The genotypic, electrophoretic, and fatty acid homogeneity (Table 3 and Fig. 4) of *Erwinia stewartii* confirms the phenotypic data (Table 2) (14, 34, 45). The other DNA hybridization subgroup, DNA hybridization subgroup 2632 (94 to 100% DNA binding), included strains which were received as *Erwinia herbicola* or *Erwinia ananas* and were members of protein profile groups VI to VIII (Fig. 2 and 3). Strain LMG 2630 of DNA hybridization subgroup 2632 exhibited 60% DNA binding to subgroup 2715 (*Erwinia stewartii*) strains, and the other two subgroup 2632 strains exhibited 71 to 83% DNA binding to subgroup 2715 (*Erwinia stewartii*) strains. In the delineation of the bacterial species, 70% DNA binding was recommended as the minimum level of genotypic relatedness within a species, with a difference between the melting point of the homologous hybrid and the melting point of the heterologous hybrid of 5°C or less (46). Taking into account that our hybridization experiments were carried out at a restrictive temperature (74°C), at which only fragments with high levels of sequential homology are allowed to reassociate (22), it follows that DNA hybridization subgroup 2715 (*Erwinia stewartii*) and DNA hybridization subgroup 2632 should be united in a single species. On the other hand, subgroup 2632 is phenotypically similar to DNA hybridization group 2665 (Tables 2 and 3) (45). It has been recom-

mended that phenotypic characteristics should be allowed to override the phylogenetic concept of species only in a few exceptional cases and that a distinct genospecies that cannot be differentiated from another genospecies on the basis of any known phenotypic property should not be named (46). However, subgroup 2632 can be differentiated from group 2665 by its electrophoretic protein patterns (Fig. 1 and 2), by its overall fatty acid profile (Fig. 4), by a higher straight-chain octadecenoic acid (C_{18:1}) (Table 3, summed feature 7) content in its total cellular acid profile, and by its inability to produce acid from sorbitol and α-methyl-D-mannoside. In the numerical analysis of Verdonck et al. (45), the two groups were separated into phena 12 and 13, which had a small overlap (two strains).

The considerable differences between subgroups 2632 and 2715 in biochemical characteristics (Tables 2 and 4), fatty acid composition (Table 3 and Fig. 4), and possibly also phytopathological features warrant the classification of these taxa as two subspecies within the same species. There are other examples, although they are not as extreme, of biochemically active and inactive sets of strains that are genotypically highly related and classified in the same species (e.g., the subspecies of *Klebsiella pneumoniae* [36]). In numerical analyses of the phenotypic features of *Erwinia* strains (14, 34, 45), *Erwinia stewartii* consistently grouped near other biochemically less active *Erwinia* species, such as *Erwinia amylovora*, *Erwinia mallotivora*, and *Erwinia rubrifaciens*. It is not impossible that during the course of adaptation and specialization of *Erwinia stewartii* to its parasitic way of life on *Zea mays* and in its vector, the corn flea beetle, the ability to carry out some biochemical activities which had become superfluous for its survival was lost.

The generic position of *Erwinia ananas* (synonym, *Er-*

winia uredovora) and *Erwinia stewartii* was questioned by Beji et al. (5) and Gavini et al. (19). These authors found that *Pantoea agglomerans* and *Pantoea dispersa* exhibited levels of DNA binding to the type strains of *Erwinia ananas* (56 and 39%), *Erwinia uredovora* (51 and 47%), and *Erwinia stewartii* (46 and 51%) that were as high as the levels of DNA binding to each other and exhibited less than 28% DNA binding to *Erwinia amylovora* and *Erwinia carotovora*. Lind and Ursing (29) observed 53, 48, and 41% DNA binding between a representative strain of *Pantoea agglomerans* (see reference 19) and the type strains of *Erwinia ananas*, *Erwinia uredovora*, and *Erwinia stewartii*, respectively. Brenner et al. (7) found that their DNA hybridization group VI (corresponding to our DNA hybridization group 2665) exhibited 43 to 48% DNA binding to DNA hybridization groups XIII (*Pantoea agglomerans* [19]) and III (*Pantoea dispersa* [19]) and less than 23% DNA binding to *Erwinia amylovora* and *Erwinia carotovora*. Kageyama et al. (27) used the S1 endonuclease method at 60°C, which gives binding ratios that are at least 10% lower than those obtained by the hydroxyapatite method (at 60°C) between strains that are related at less than species level (23). In their hybridization experiments with labeled DNAs from the type strains of *Pantoea agglomerans* and *Pantoea dispersa*, the type strains of *Erwinia ananas*, *Erwinia uredovora*, and *Erwinia stewartii* exhibited higher levels of DNA binding (30 to 40%) than strains of *Pantoea citrea*, *Pantoea punctata*, and *Pantoea terrea* did (16 to 25%) (27). In our experiments, in which a more restrictive incubation temperature (74°C) was used, DNA hybridization group 2665 exhibited 30 to 32% DNA binding to *Erwinia stewartii* LMG 2175^T and 32 to 39% DNA binding to DNA hybridization subgroup 2632 strains (Fig. 3). Strains belonging to these groups and subgroups exhibited 18 to 34% DNA binding to *Pantoea agglomerans* and *Pantoea dispersa* strains (Fig. 3). In the phenotypic analyses of Dye (14), Mergaert et al. (34), and Verdonck et al. (45) DNA hybridization group 2665 and subgroup 2632 strains, as well as strains now classified as *Pantoea agglomerans* and *Pantoea dispersa*, exhibited more phenotypic similarities to each other and to *Erwinia cypripedii* than to other *Erwinia* species. *Erwinia stewartii* grouped together with some species of the "amylovora group" sensu Dye (12). The apparent phenotypic homogeneity of the latter group, which included the species *Erwinia amylovora*, *Erwinia mallotivora*, *Erwinia salicis*, *Erwinia rubrifaciens*, *Erwinia nigrifluens*, *Erwinia tracheiphila*, and *Erwinia quercina*, is not reflected genotypically (4, 8, 35) and is probably due to the occurrence of many negative, but not redundant, features in the numerical analyses.

We propose that DNA hybridization group 2665 should be classified in the genus *Pantoea* as *Pantoea ananas* (Serrano 1928) comb. nov. [synonyms, *Erwinia ananas* Serrano 1928, *Erwinia uredovora* (Pon et al. 1954) Dye 1963], which includes DNA hybridization group VI of Brenner et al. (7), and that DNA hybridization subgroup 2715 and subgroup 2632 should be united in one species, *Pantoea stewartii* (Smith 1898) comb. nov., with two subspecies, *Pantoea stewartii* subsp. *stewartii* (Smith 1898) comb. nov. (synonym, *Erwinia stewartii*) (for DNA hybridization subgroup 2715) and *Pantoea stewartii* subsp. *indologenes* subsp. nov. (for DNA hybridization subgroup 2632).

Description of *Pantoea ananas* (Serrano 1928) comb. nov. *Pantoea ananas* (*Erwinia ananas* Serrano 1928) (a'na.nas. M. L. n. *Ananas*, generic name of the pineapple). The description below is based on the data obtained for the 18 strains listed in Table 1. Most strains grow at 4°C, some

strains grow at 37°C, a few strains grow at 41°C, but no strain grows at 44°C. Indole is produced, and citrate is utilized. β -Galactosidase positive; ornithine decarboxylase, arginine dihydrolase, and phenylalanine deaminase negative. Most strains grow on L-aspartate, *cis*-aconitate, *meso*-tartrate, and D-malate, but not on acetate and DL-3-hydroxybutyrate. Acid is produced from L-arabinose, sucrose, cellobiose, D-arabitol, and D-mannitol and generally also from D-mannose, lactose, arbutin, and glycerol, but not from D-lyxose, L-sorbose, D-tagatose, D-fucose, melezitose, inulin, starch, glycogen, β -methyl-D-xyloside, *meso*-erythritol, or L-arabitol or on Jordan's tartrate medium. The biochemical and nutritional characteristics at 30°C are shown in Tables 2 and 4 through 6, and the cellular fatty acid composition is shown in Table 3. The main fatty acids are (in order of decreasing relative content) hexadecanoic acid (C_{16:0}), *cis*-9-hexadecenoic acid (C_{16:1 cis 9}), and straight-chain octadecenoic acids (C_{18:1}). The G+C contents of the DNAs of 10 strains range from 53.1 to 55.2 mol% (9, 17, 24, 43; this study). Some strains (synonym, *Erwinia ananas*) are thought (40) to cause rot of *Ananas comosus* fruitlets, although this is doubted (13, 21). Other strains (synonym, *Erwinia uredovora*) attack the uredia of the wheat pathogen *Puccinia graminis* (38). Some strains have been isolated from seeds of *Oryza sativa* and *Musa* sp. or are of clinical origin. The type strain is strain LMG 2665 (= NCPPB 1846 = ATCC 33244 = PDDCC 1850 = ICPB EA175); its characteristics are shown in Table 2, and the G+C content of its DNA is 55.0 mol%.

Description of *Pantoea stewartii* (Smith 1898) comb. nov. *Pantoea stewartii* [*Erwinia stewartii* (Smith 1898) Dye 1963] (stew.art'i.i. M. L. gen. n. *stewartii*, of Stewart; named after F. C. Stewart). The description below is based on the data obtained for the 18 strains listed in Table 1. The species is divided into two subspecies; the common characteristics are shown in Tables 2 and 4 through 6. β -Galactosidase positive; arginine dihydrolase, ornithine decarboxylase, phenylalanine deaminase, urease, and tetrathionate reductase negative. Tween 40 is hydrolyzed, but Tween 80 is not hydrolyzed. Growth occurs on L-aspartate and D-malate, but not on acetate or DL-3-hydroxybutyrate. Gelatin is not liquefied. Nitrate is not reduced to nitrite. Malonate is not utilized. Acid is produced from L-arabinose, sucrose, and D-mannitol, but not from L-lyxose, L-sorbose, D-tagatose, D-fucose, melezitose, α -methyl-D-mannoside, sorbitol, inulin, starch, glycogen, β -methyl-D-xyloside, *meso*-erythritol, adonitol, *meso*-xylytol, or L-arabitol or on Jordan's tartrate medium. Isolated from *Z. mays*, several other grasses, *A. comosus*, and beetles. The G+C contents of the DNAs of five strains range from 53.6 to 56.4 mol% (43; this study). The type strain is strain LMG 2715 (= NCPPB 2295 = ATCC 8199 = ICMP 257 = DSM 30176 = ICPB SS11 = IMET 11187); its characteristics are shown in Table 2, and the G+C content of its DNA is 55.1 mol% (43).

Description of *Pantoea stewartii* subsp. *stewartii* (Smith 1898) comb. nov. The subspecies has all of the characteristics of the species. The description below is based on the data obtained for the eight strains listed in Table 1. A few strains grow at 4°C, some strains grow at 37°C, and no strain grows at 41°C. The biochemical and nutritional characteristics at 30°C and characteristics that distinguish this subspecies from *Pantoea stewartii* subsp. *indologenes* are shown in Tables 2 and 4. Nonmotile. No indole is produced. Esculin is not hydrolyzed. Citrate and mucate are not utilized. No growth occurs on *cis*-aconitate and DL-4-aminobutyrate. No acid is produced from L-rhamnose, cellobiose, maltose, lactose, gentiobiose, α -methyl-D-glucoside, arbutin, salicin, amygdal-

lin, glycerol, D-arabitol, dulcitol, meso-inositol, D-gluconate, 2-keto-D-gluconate, or 5-keto-D-gluconate. The cellular fatty acid composition is shown in Table 3. The main fatty acids are (in order of decreasing relative content) straight-chain octadecenoic acids ($C_{18:1}$), hexadecanoic acid ($C_{16:0}$), and *cis*-9-hexadecenoic acid ($C_{16:1 \text{ cis } 9}$). The G+C contents of the DNAs of two strains are 54.6 and 55.1 mol% (43). Strains of *Pantoea stewartii* subsp. *stewartii* (synonym, *Erwinia stewartii*) are the causative agents of Stewart's bacterial wilt of corn, a vascular disease of *Z. mays*, and exist in the insect vector, the corn flea beetle *Chaetocnema pulicaria* (1). The type strain is the same as the type strain of the species.

Description of *Pantoea stewartii* subsp. *indologenes* subsp. nov. *Pantoea stewartii* subsp. *indologenes* (in.do.lo'gen.es. M. L. n. *indolum*, indole; Gr. n. *genmao*, to produce; M. L. adj. *indologenes*, indole producing). The subspecies has all of the characteristics of the species. The description below is based on the data obtained for the 10 strains listed in Table 1. Many strains grow at 4 and 37°C and some strains grow at 41°C, but few strains grow at 44°C. The biochemical and nutritional characteristics at 30°C and characteristics that distinguish this subspecies from *Pantoea stewartii* subsp. *stewartii* are shown in Tables 2 and 4. Indole is produced. Esculin is hydrolyzed. Growth occurs on *cis*-aconitate and *meso*-tartrate. Acid is produced from cellobiose, maltose, lactose, arbutin, salicin, and D-arabitol. The cellular fatty acid composition is shown in Table 3. The main fatty acids are (in order of decreasing relative content) hexadecanoic acid ($C_{16:0}$), straight-chain octadecenoic acids ($C_{18:1}$), and *cis*-9-hexadecenoic acid ($C_{16:1 \text{ cis } 9}$). The G+C contents of the DNAs of three strains are 53.6 mol% (43), 54.5 mol%, and 56.4 mol% (this study). Strains of *Pantoea stewartii* subsp. *indologenes* are thought to cause leaf spot on foxtail millet (*Setaria italica*) and pearl millet (*Pennisetum americanum*) and rot of *A. comosus*; one strain was isolated from cluster bean (*Cyamopsis tetragonolobus*). The type strain is strain LMG 2632 (= NCPPB 2280 = ICMP 77); its characteristics are shown in Table 2, and the G+C content of its DNA is 56.4 mol%.

Emended description of the genus *Pantoea* Gavini, Mer-gaert, Beji, Mielcarek, Izard, Kersters, and De Ley 1989. *Pantoea* (Pan.toe'a. Gr. adj. *pantoios*, of all sorts and sources; M.L. fem. n. *Pantoea*, [bacteria] from diverse [geographical and ecological] sources). The description below is based on the data of Beji et al. (5), Gavini et al. (19), and Kageyama et al. (27) and the data in this paper. Gram-negative, noncapsulated, nonsporeforming, straight rods that are 0.5 to 1.3 by 1.0 to 3.0 µm. Cells are motile and peritrichous or are nonmotile. Cultures grow well on nutrient agar at 30°C. Colonies on nutrient agar are smooth, translucent, and more or less convex with entire margins. Colonies are yellow, pale beige to pale reddish yellow, or nonpigmented. Facultatively anaerobic and oxidase negative. No gas is produced from D-glucose. No lysine decarboxylase, tryptophan deaminase, or urease activity. Pectate is not degraded, and hydrogen sulfide is not produced from thio-sulfate. Acid is produced from D-xylose, D-ribose, D-galactose, D-fructose, trehalose, and *N*-acetyl-D-glucosamine, but not from D-arabinose, L-xylose, L-fucose, or D-turanose. Isolated from plants, seeds, fruits, soils, and water, as well as from humans (wounds, blood, urine, internal organs) and animals, in several parts of the world. Some strains are (or have been thought to be) phytopathogenic. The G+C contents of the DNAs range from 49.7 to 60.6 mol% (9, 17, 19, 25, 27, 43; this study). The type species is *Pantoea agglom-*

TABLE 5. Differential characteristics of *Pantoea ananas*, *Pantoea stewartii*, and *Erwinia* species

Species	Yellow pigment	API 20E tests				API 50C/HE tests			
		β-D-Galactosidase	Indole production	Nitrate reduced to nitrite	Acid produced from sorbitol	Acid produced from α-methyl-D-mannoside	Acid produced from melibiose	Acid produced from raffinose	Acid produced from D-turanose
<i>Pantoea ananas</i>	+	+	+	d	[+]	[+]	[+]	[+]	-
<i>Pantoea stewartii</i>	+	+	D	-	-	-	+	D	- ^b
<i>Erwinia carotovora</i>	-	D	-	+	-	-	D	[+]	-
<i>Erwinia chrysanthemi</i>	-	+	+	+	-	-	[+]	[+]	-
<i>Erwinia paradisiaca</i>	-	+	+	+	-	-	+	+	-
<i>Erwinia cacticola</i>	-	+	-	+	-	-	-	-	-
<i>Erwinia cryptopeti</i>	-	+	-	[+]	d	-	d	+	+
<i>Erwinia thapsigalis</i>	-	+	-	[+]	[+]	-	+	+	+
<i>Erwinia nigraflava</i>	-	-	-	-	-	-	-	-	-
<i>Erwinia amylovora</i>	-	-	-	-	d	-	-	-	-
<i>Erwinia malitiosa</i>	-	+	-	+	+	-	+	+	-
<i>Erwinia persici</i>	-	+	-	+	-	-	+	+	-
<i>Erwinia quercina</i>	-	-	-	-	-	-	-	-	-
<i>Erwinia salicis</i> and <i>Erwinia rubrifaciens</i>	-	-	-	-	-	-	-	-	+

^a +, at least 90% of the strains are positive; -, at least 90% of the strains are negative; [d], 76 to 89% of the strains are positive; [-], 11 to 25% of the strains are positive; D, differential characteristic for the subspecies. Data were obtained after 24 h (API 20E tests), 48 h (API 50C/HE tests), or 3 days (yellow pigment production) of incubation at 30°C. Data for *Erwinia carotovora* subsp. *wasabiae* are from reference 20, data for *Erwinia cacticola* are from reference 2, data for *Erwinia persici* are from reference 24, and data for the other *Erwinia* species are from reference 45.

^b No data were available for *Erwinia carotovora* subsp. *wasabiae*.

TABLE 6. Differential characteristics of *Pantoea ananas*, *Pantoea stewartii*, and other members of the family *Enterobacteriaceae*^a

Species	Yellow pigment	Indole production	Nitrate reduced to nitrite	Esculin hydrolysis	Gas produced from D-glucose	Ornithine decarboxylase	Malonate utilization	Mucate utilization	Acid produced from sorbitol	Acid produced from D-arabitol
<i>Pantoea ananas</i>	^b +	+	d	d	—	—	—	—	[+]	+
<i>Pantoea stewartii</i>	+	D	—	—	—	—	—	—	—	D
<i>Escherichia adecarboxylata</i>	+	+	+	+	+	—	d	+	—	+
<i>Escherichia coli</i>	—	+	+	d	+	d	—	+	+	—
<i>Enterobacter cloacae</i>	—	—	+	d	+	+	[+]	[+]	+	[—]
<i>Enterobacter sakazakii</i>	+	[—]	+	+	+	+	[—]	—	—	—
<i>Citrobacter diversus</i>	—	+	+	—	+	+	+	+	+	+
<i>Serratia plymuthica</i>	—	—	+	+	—	—	—	—	d	—
<i>Klebsiella oxytoca</i>	—	+	+	+	+	—	+	+	+	+
<i>Rahnella aquatilis</i>	—	—	+	+	[+]	—	+	d	+	—
<i>Ewingella americana</i>	—	—	+	+	—	—	—	—	—	+
<i>Cedecea davisae</i>	—	—	+	d	[+]	+	[+]	—	—	+

^a Data from reference 6.^b +, at least 90% of the strains are positive; —, at least 90% of the strains are negative; [+], 76 to 89% of the strains are positive; [—], 11 to 25% of the strains are positive; d, 26 to 75% of the strains are positive; D, differential characteristic for the subspecies. Data were obtained after 24 to 72 h of incubation at 30°C (*Pantoea* species) or 36°C (other species).

erans (Beijerinck 1888) Gavini, Mergaert, Beji, Mielcarek, Izard, Kersters, and De Ley 1989.

The differential characteristics of *Pantoea ananas*, *Pantoea stewartii* subsp. *stewartii*, and *Pantoea stewartii* subsp. *indologenes* are shown in Tables 2 and 4. *Pantoea stewartii* subsp. *stewartii* and *Pantoea stewartii* subsp. *indologenes* can be further differentiated from *Pantoea ananas* by the higher percentages of straight-chain octadecenoic acids (C_{18:1}) (Table 3, summed in feature 7) in their fatty acid compositions. Unlike *Pantoea stewartii* subsp. *indologenes*, *Pantoea stewartii* subsp. *stewartii* lacks 2-hydroxytetradecanoic acid (C_{14:0} 2 OH) and cyclo-heptadecanoic acid (C_{17:0} cyclo). Characteristics useful for differentiation from other *Pantoea* species, *Erwinia* species, and other members of the *Enterobacteriaceae* are shown in Tables 4 through 6, respectively.

Data presented above or published previously (5, 19, 34) demonstrate that the API 20E commercial kits are not sufficient for the identification of most *Pantoea* and *Erwinia* species. An updating of the APILAB identification program is needed, and several other tests, some of which are contained in the API 50CHE system, have to be used to differentiate *Pantoea ananas*, *Pantoea stewartii* subsp. *stewartii*, and *Pantoea stewartii* subsp. *indologenes* from each other and from other taxa (Tables 4 through 6). The use of the MIS data base for the identification of the fatty acid profiles seems promising; *Pantoea ananas* and *Pantoea stewartii* subsp. *stewartii* were correctly identified, while the newly proposed taxon *Pantoea stewartii* subsp. *indologenes* produced an unidentified, unique fatty acid profile.

Of the 13 DNA hybridization groups of Brenner et al. (7), 5 have been named; group III has been described as *Pantoea dispersa* (19), group VI has been described as *Pantoea ananas* (this paper), group XI has been described as *Escherichia adecarboxylata* (26), group XIII has been described as *Pantoea agglomerans* (5, 19), and group V has been provisionally classified as *Pantoea* sp. (5, 19). The taxonomic positions of DNA hybridization groups I, II, IV, VII to X, and XII of Brenner et al. (7) remain to be elucidated.

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