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Reclassification of non-pigmented *Erwinia herbicola* strains from trees as *Erwinia billingiae* sp. nov.

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Twenty-two *Erwinia*-like strains, isolated from trees since the late fifties and belonging to a distinct phenotypic group with resemblance to *Pantoea agglomerans*, were further characterized by conventional biochemical tests, the BIOLOG metabolic fingerprinting system and fatty acid analysis. Their phylogenetic positions were determined by comparing the 16S rRNA gene sequence of a representative strain to available sequences of *Erwinia*, *Pantoea*, *Pectobacterium* and *Brenneria* species. The strains were shown to belong to the genus *Erwinia*, with *Erwinia rhapontici* and *Erwinia persicina* as the closest phylogenetic relatives. The name *Erwinia billingiae* sp. nov. is proposed (type strain LMG 2613^T) and a description of the species is given.

Keywords: *Erwinia billingiae* sp. nov., taxonomy, phylogenetic analysis

INTRODUCTION

More than thirty years ago, Billing & Baker (1963) described the characteristics of 20 non-pigmented *Erwinia*-like isolates (which they referred to as DC isolates) isolated from stem cankers, diseased blossoms or immature fruits of pear, apple, cherry, hawthorn and elm, during a routine diagnosis of fire blight. They concluded that these strains belonged to an ill-defined '*lathyri–herbicola*' group within the genus *Erwinia*, a former vernacular name of *Erwinia herbicola* (now reclassified as *Pantoea agglomerans* by Gavini *et al.*, 1989).

In an extensive numerical analysis of the phenotypic features of the genus *Erwinia*, using the API 20E and API 50CHE biochemical characterization systems, Verdonck *et al.* (1987) classified 421 strains assigned to 21 species in 27 phena. Eighteen DC isolates of Billing & Baker (1963) constituted a separate well-delineated phenon 11, together with three non-pigmented strains isolated from hawthorn in France. Subsequently, a fourth French isolate from apple was shown to possess the same phenotype (Mergaert, 1989). Other non-pigmented DC strains, isolated by Billing & Baker (1963), grouped with *Rahnella aquatilis* and *Erwinia rhapontici*. Phenon 11 of Verdonck *et al.* (1987) was clearly separated from the other phena, including the phena that have now been reclassified in *Pantoea* as *Pantoea agglomerans* (synonym *Erwinia herbicola*, *Erwinia milletiae*, phenon 8), *Pantoea dispersa* (phenon 10) (Gavini *et al.*, 1989), *Pantoea ananatis* (phenon 12), *Pantoea stewartii* subsp. *stewartii* (phenon 29) and *Pantoea stewartii* subsp. *indologenes* (phenon 13) (Mergaert *et al.*, 1993).

The phenotypic homogeneity of the DC strains of phenon 11 of Verdonck *et al.* (1987) was confirmed by PAGE analysis of soluble proteins (protein group 10 of Mergaert, 1989), while the four French strains, under pairwise analysis, constituted two slightly different protein patterns (protein groups 8 and 9). DNA–DNA hybridizations revealed 82–93% binding between DNAs prepared from three strains that were representative of each of the protein electrophoretic groups, less than 29% with strains representative for *Pantoea agglomerans*, *Pantoea dispersa*, *Pantoea ananatis* and *Pantoea stewartii* (Mergaert, 1989), and 10% with *Erwinia amylovora* LMG 2024^T (J. Mergaert, unpublished results). The exact taxonomic position of phenon 11 has remained unknown until now.

Modern taxonomy is performed ideally within a polyphasic framework, with 16S rDNA sequence-based phylogeny as a backbone (Vandamme *et al.*, 1993). The phylogenetic position of the genus *Erwinia* and other plant-associated *Enterobacteriaceae* has recently been investigated by 16S rDNA sequence analysis (Kwon *et al.*, 1997; Hauben *et al.*, 1998). Both groups of authors concluded that the former *Erwinia*

The EMBL accession number for the 16S rDNA sequence of *Erwinia billingiae* LMG 2613^T is Y13249.

Table 1. Strains investigated and assigned to *Erwinia billingiae*

Strains were received as *Erwinia herbicola*. Abbreviations and sources: CUETM, Collection de l'Unité d'Ecotoxicologie Microbienne, Villeneuve d'Ascq, France; LMG, Culture Collection Laboratorium voor Microbiologie, Gent, Belgium; NCPPB, National Collection of Plant-pathogenic Bacteria, Harpenden, UK.

LMG strain no.	Strain designation as received, original strain designation	Isolator, year of isolation, country of isolation, plant source
2606	NCPPB 652, Billing E28	E. Billing, 1959, UK, *Pyrus communis*
2607	NCPPB 653, Billing E36	E. Billing, 1958, UK, *Pyrus communis*
2608	NCPPB 654, Billing E39	E. Billing, 1958, UK, *Pyrus communis*
2609	NCPPB 655, Billing E42	E. Billing, 1958, UK, *Pyrus communis*
2610	NCPPB 657, Billing E48	E. Billing, 1958, UK, *Crataegus laevigata*
2611	NCPPB 659, Billing E55	E. Billing, 1958, UK, *Crataegus laevigata*
2612	NCPPB 660, Billing E56	E. Billing, 1958, UK, *Crataegus laevigata*
2613^T	NCPPB 661^T, Billing E63^T	E. Billing, 1959, UK, *Pyrus communis*
2614	NCPPB 662, Billing E71	E. Billing, 1959, UK, *Pyrus communis*
2615	NCPPB 663, Billing E72	E. Billing, 1959, UK, *Pyrus communis*
2616	NCPPB 664, Billing E73	E. Billing, 1959, UK, *Pyrus communis*
2617	NCPPB 665, Billing E78	E. Billing, 1959, UK, *Pyrus communis*
2618	NCPPB 666, Billing E79	E. Billing, 1959, UK, *Pyrus communis*
2619	NCPPB 1261, Baker 109	L. A. E. Baker, 1959, UK, *Malus sylvestris*
2620	NCPPB 1263, Baker 111	L. A. E. Baker, 1959, UK, *Crataegus laevigata*
2621	NCPPB 1264, Baker 112	L. A. E. Baker, 1959, UK, *Pyrus communis*
2622	NCPPB 1265, Baker 113	L. A. E. Baker, 1959, UK, *Prunus avium*
2623	NCPPB 1266, Baker 114	L. A. E. Baker, 1960, UK, *Ulmus* sp.
2624	CUETM 79-74, Paulin 168.17	J. P. Paulin, before 1979, France, *Crataegus* sp., lesion, associated with *Erwinia amylovora*
2625	CUETM 79-255, Paulin 217-8	J. P. Paulin, before 1979, France, *Crataegus* sp., leaf surface
2626	CUETM 79-90, Paulin 238-3	J. P. Paulin, before 1979, France, *Crataegus* sp., associated with *Erwinia amylovora*
2641	CUETM 79-95, Paulin B.908	J. P. Paulin, before 1979, France, *Malus sylvestris*, leaf surface

species belong to four different phylogenetic lineages, which were each given genus status by Hauben *et al.* (1998). The first branch contained the type species *E. amylovora* together with *E. rhapontici*, *Erwinia persicina*, *Erwinia psidii*, *Erwinia mallotivora* and *Erwinia tracheiphila*. The five *Erwinia carotovora* subspecies formed the core of a second lineage together with *Erwinia chrysanthemi* and *Erwinia cypripedii* and were reallocated in the genus *Pectobacterium*. A third branch contained the species *Erwinia alni*, *Erwinia nigrifluens*, *Erwinia paradisiaca*, *Erwinia quercina*, *Erwinia rubrifaciens* and *Erwinia salicis*; a new genus *Brenneria* was proposed to incorporate these species. Finally, the classification of *Pantoea agglomerans*, *Pantoea ananatis* and the two subspecies of *Pantoea stewartii* in a separate genus was confirmed by phylogenetic analysis. Hauben *et al.* (1998) showed that each of the genera *Erwinia*, *Pectobacterium* and *Brenneria* are characterized by genus-specific signature nucleotides in their 16S rRNA gene sequences. These phylogenetic data were used as a framework to determine the phylogenetic position of the strains of phenon 11 of Verdonck *et al.* (1987) and to recognize them as a new species within the genus *Erwinia sensu stricto* Hauben *et al.* (1998), for which the name *Erwinia billingiae* is proposed.

METHODS

Strains investigated. The 22 strains investigated and hereafter classified as *E. billingiae* are listed in Table 1.

Phenotypic tests. Tests in the API 20E and API 50CHE systems (bioMérieux) were performed by previously described procedures (Mergaert *et al.*, 1984). Acid production from carbohydrates and aesculin hydrolysis (API 50CHE tests) were recorded after 48 h. API 20E tests (gelatin liquefaction, indole, acetoin and hydrogen sulphide production, nitrate reduction, arginine dihydrolase, lysine and ornithine decarboxylase, tryptophan deaminase, β-D-galactosidase, urease and citrate utilization) were recorded after 24 h. API 20E and API 50CHE results were also interpreted with the APILAB identification program (version V3.1.1).

Table 2. Biochemical and physiological characteristics of *Erwinia billingiae*

+, Positive reaction; −, negative reaction. All *E. billingiae* strains were positive in the following tests: ortho-nitrophenyl-β-galactosidase, nitrate reduction to nitrite, acid production from L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, *N*-acetyl-glucosamine, trehalose, gentiobiose, D-gluconate, growth at 4 °C, growth on aspartate and D-malate. They were all negative in the following tests: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, hydrogen sulphide production, urease, tryptophan deaminase, indole production, oxidase, gelatin liquefaction, acid from glycerol, *meso*-erythritol, D-arabinose, L-xylose, adonitol, methyl β-D-xyloside, sorbose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, *meso*-xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, gas from glucose, growth at 41 °C, malonate utilization, tetrathionate reduction, Jordan's tartrate, Tween 80 hydrolysis, pectate degradation, yellow pigment on nutrient agar, growth on acetate and DL-aminobutyrate.

Feature	Most common result	LMG strains differing from common result
Voges–Proskauer	+	2609
Acid produced from:		
Ribose	+	2614
meso-Inositol	−	2606–2608, 2610, 2611, 2619, 2623, 2625, 2641
D-Sorbitol	+	2607, 2620, 2622
Arbutin, salicin	+	2619, 2620
Cellobiose	−	2624
Maltose	+	2611, 2615, 2621
Sucrose	−	2623–2626
D-Arabitol	+	2615
2-Keto-D-gluconate	−	2609, 2625, 2626
5-Keto-D-gluconate	+	2620
Aesculin hydrolysis	+	2619, 2620
Mucate utilization	−	2607, 2609, 2619, 2625
Growth at 37 °C	−	2608
Phenylalanine deaminase	−	2617–2623, 2641
Hydrolysis of:		
Tween 20	+	2614, 2615, 2619–2625, 2641
Tween 40	+	2624, 2625
Tween 60	+	2614, 2624, 2625
Deoxyribonuclease	−	2608
Growth on:		
cis-Aconitate	+	2609–2613^T, 2615, 2618, 2621, 2622
DL-3-Hydroxybutyrate	−	2612, 2613^T
meso-Tartrate	+	2616, 2624

Other phenotypic tests, listed in Table 2, were carried out as previously described and read after 3 d (Mergaert *et al.*, 1993). Incubation was at 30 °C, unless stated otherwise.

BIOLOG metabolic fingerprinting. Strains were grown on Trypticase Soy Broth (BBL), supplemented with 1·5% Bacteriological Agar No. 1 (Oxoid). Suspensions were prepared and GN microplates (BIOLOG) were inoculated as recommended by the manufacturer. After 24 h incubation at 28 °C, the absorbances obtained in the wells were read with a microplate reader (Molecular Devices). The absorbance results were transformed to positive, borderline and negative scores and interpreted by the BIOLOG MicroStation System software program (release 3.50).

Fatty acid analysis. Quantitative analysis of cellular fatty acid compositions was performed by a GLC procedure as previously described (Mergaert *et al.*, 1993). The resulting profiles were identified with the Microbial Identification Software (MIS; MIDI) using the TSBA database (version 3.90). For comparison, the following reference strains were included: *E. amylovora* LMG 2024^T, *E. tracheiphila* LMG 2707^T, *E. mallotivora* LMG 2708^T, *E. rhapontici* LMG 2688^T, *E. psidii* LMG 7039, *Pantoea agglomerans* LMG 1286^T, *Pantoea dispersa* LMG 2603^T, *Pantoea ananatis* LMG 2665^T, *Pantoea stewartii* subsp. *stewartii* LMG 2715^T, *Pantoea stewartii* subsp. *indologenes* LMG 2632^T, *Brenneria salicis* LMG 2698^T, *Brenneria nigrifluens* LMG 2694^T, *Brenneria quercina* LMG 2724^T and *Brenneria rubrifaciens* LMG 2709^T.

Phylogenetic analysis. DNA preparation, PCR amplification, sequencing of 16S rRNA genes, 16S rRNA gene sequence comparisons and phylogenetic analysis were car-

ried out as described previously (Hauben *et al.*, 1998). Calculation of global alignment, global similarity values and cluster analysis were done using the GENECOMPAR software (Applied Maths) taking into account the homologous nucleotide positions after discarding all unknown bases and with a gap cost of 0%. Bootstrap values were calculated after 1000 resamplings.

DNA–DNA hybridizations. DNA was prepared from *E. billingiae* LMG 2613^T, *E. rhapontici* LMG 2688^T and *E. persicina* LMG 11254^T, as described previously (Mergaert *et al.*, 1993). Percentages of DNA binding were determined spectrophotometrically from renaturation rates by the method of De Ley *et al.* (1970). The optimal renaturation temperature, calculated from the G+C content, was approximately 74·5 °C in double-strength standard saline–citrate buffer and a concentration of 48·5 μg DNA ml^{-1} was used.

RESULTS

Biochemical and physiological characteristics

Biochemical and physiological features of the 22 *E. billingiae* strains are given in Table 2. Most of the strains were identified as *Pantoea agglomerans* by the APILAB recognition program.

BIOLOG metabolic fingerprinting

Reproducibilities (correlation coefficient, *r*) of the raw absorbances, recorded with the microplate reader, and of the scores obtained after transformation of triplicate BIOLOG GN plates of strain LMG 2613^T were above 0·95 and 0·94, respectively. The triplicate microplates of strain LMG 2613^T were also interpreted visually. Gower similarity coefficients calculated between the scores obtained by visual reading and those obtained with the reader, after transformation to 1 (positive), 0.5 (borderline) and 0 (negative), were 86–95%. All *E. billingiae* strains showed respiration in the presence of the following 39 carbon sources: dextrin, glycogen, *N*-acetyl-D-glucosamine, L-arabinose, D-arabitol, D-fructose, D-galactoside, α-D-glucose, *meso*-inositol, maltose, D-mannitol, methyl β-D-glucoside, D-psicose, L-rhamnose, D-trehalose, turanose, methyl pyruvate, mono-methyl succinate, acetic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, D-saccharic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-asparagine, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, L-serine, urocanic acid, inosine, thymidine, glycerol, glucose 1-phosphate and glucose 6-phosphate. None of the strains showed

Table 3. Cellular fatty acid composition of *Erwinia billingiae* and type and reference strains from *Erwinia*, *Pantoea* and *Brenneria* species

Abbreviations: ND, not detected; SD, standard deviation; TR, trace amount (<1·0%). Fatty acid compositions of individual strains are means of 2–11 replicates. Only traces of the following fatty acids were detected in *E. billingiae* strains (the maximum amount obtained in other strains is given in parentheses, if >1%): 10:0 3-OH (3·7%), 12:0 2-OH (5·6%), 12:0 3-OH (4·8%), 13:0, 14:0 2-OH (2·7%), 17:0 10-methyl, 17:1 ω8*cis*, 18:0 (2·0%), 19:0 cyclo ω8*cis* (1·7%), summed feature 2, summed feature 9, unknown equivalent chain-length (ECL) 13·961 and unknown ECL 14·503. Fatty acids that could not be identified by the MIS system were designated by their ECL relative to the length of known, straight-chain fatty acids. Fatty acids that could not be separated by GC and the MIS system were designated as summed features. Summed feature 2 contained one or more of the following fatty acids: 15:1 iso (position of the double bond unknown) and 13:0 3-OH. Summed feature 3 contained one or more of the following fatty acids: 16:1 iso (position of the double bond unknown), 14:0 3-OH, 12:0 aldehyde and an unknown fatty acid ECL 10·928. Summed feature 4 contained one or more of the following fatty acids: 16:1 ω7*cis* and 15:0 iso 2-OH. Summed feature 7 contained one or more of the following fatty acids: 18:1 ω7*cis*, 18:1 ω9*trans* and 18:1 ω12*trans*. Summed feature 9 contained one or more of the following fatty acids: 19:0 cyclo ω10*cis* and unknown fatty acids ECL 18·846 and ECL 18·858.

Fatty acid methyl ester	Total fatty acids (%) in:					
	Erwinia billingiae			Other *Erwinia* sp.	*Pantoea* sp.	*Brenneria* sp.
	22 strains		LMG 2613^T	Range	Range	Range
	Mean ± SD	Range				
12:0	4·1 ± 0·5	3·2–5·2	4·7	3·2–5·9	3·3–4·4	TR–3·6
14:0	5·6 ± 0·4	4·8–6·3	5·8	TR–5·8	TR–5·8	5·8–11·6
15:0	1·3 ± 0·6	TR–2·3	1·1	ND–TR	ND–1·6	ND–TR
16:0	26·3 ± 1·6	21·7–28·8	28·3	26·9–33·4	27·3–31·3	30·1–32·1
17:0	2·0 ± 0·8	TR–3·6	1·5	ND–1·2	ND–2·7	ND
17:0 cyclo*	10·2 ± 4·8	1·1–16·3	11·8	ND–13·6	ND–13·2	3·2–10·8
Summed feature 3	12·3 ± 2·0	9·7–19·4	13·5	ND–12·2	8·5–10·5	8·3–10·0
Summed feature 4	23·9 ± 5·2	17·0–34·8	21·5	22·7–33·7	10·8–24·4	23·3–30·8
Summed feature 7	11·1 ± 2·7	7·6–18·0	8·8	6·3–16·9	12·6–36·9	9·0–15·5

* The position of the cyclopropane ring is unknown.

respiration on the following 15 carbon sources: *i*-erythritol, xylitol, β-hydroxybutyric acid, α-keto-valeric acid, malonic acid, sebacic acid, hydroxy-L-proline, L-phenylalanine, L-pyroglutamic acid, DL-carnitine, γ-aminobutyric acid, phenylethylamine, putrescine, 2-aminoethanol or 2,3-butanediol. Positive or borderline reactions were obtained for formic acid, L-alanyl-glycine, L-glutamic acid, L-proline or uridine. Negative results or borderline reactions were obtained for lactulose, *p*-hydroxyphenylacetic acid, α-keto-butyric acid, itaconic acid, α-cyclodextrin, adonitol, cellobiose, γ-hydroxybutyric acid, *N*-acetyl-D-galactosamine, D-raffinose and L-leucine.

Different reactions (positive, borderline and negative) were obtained for the remaining carbon sources: D-mannose, D-melibiose, α-D-lactose, sucrose, L-fucose, D-sorbitol, gentiobiose, L-aspartic acid, L-alanine, D-alanine, D-serine, L-threonine, D-glucosaminic acid, L-ornithine, DL-lactic acid, *cis*-aconitic acid, citric acid, α-ketoglutaric acid, propionic acid, quinic acid, α-hydroxybutyric acid, alaninamide, Tween 40, Tween 80 and DL-α-glycerolphosphate. The strains were identified as *Pantoea agglomerans* by the pattern recognition program.

Fatty acid analysis

The fatty acid compositions of *E. billingiae* strains and type and reference strains of the genera *Erwinia*, *Pantoea* and *Brenneria* are given in Table 3. Reproducibilities (correlation coefficient, *r*) of the fatty acid profiles obtained in fivefold from *E. billingiae* LMG 2613^T were 0·95–0·99. The fatty acid profiles of the *E. billingiae* strains resemble those of reference strains of other *Erwinia* species and *Pantoea* and *Brenneria* strains. Their main fatty acids included hexadecanoic acid (16:0), octadecenoic acid (18:1), a varying amount of cyclopropane-hexadecanoic acid (17:0 cyclo), and large amounts of fatty acids that could not be identified unambiguously by the methods used (summed features 3 and 4, see Table 3 legend). They generally contained less octadecanoic acid and higher amounts of fatty acids summed in feature 3, as compared to the reference strains of other *Erwinia* species and strains of *Pantoea* and *Brenneria*. By principal analysis using combinations of principal components 1, 2, 3 and 4, the fatty acid profiles obtained with the *E. billingiae* strains could be separated from the profiles obtained with the reference strains, except for *Pantoea agglomerans* LMG 1286^T (data not shown). The *E. billingiae* strains were recognized as *Salmonella typhimurium*, *Pantoea agglomerans* or *R. aquatilis* by the MIS program.

Phylogenetic analysis

An estimated 97·1% of the total 16S rDNA primary sequence was determined for *E. billingiae* LMG 2613^T, comprising a continuous stretch of 1497 bases, ranging from positions 28–1524 (*Escherichia coli* numbering) of the 16S rRNA gene sequence (Brosius *et al.*, 1981).

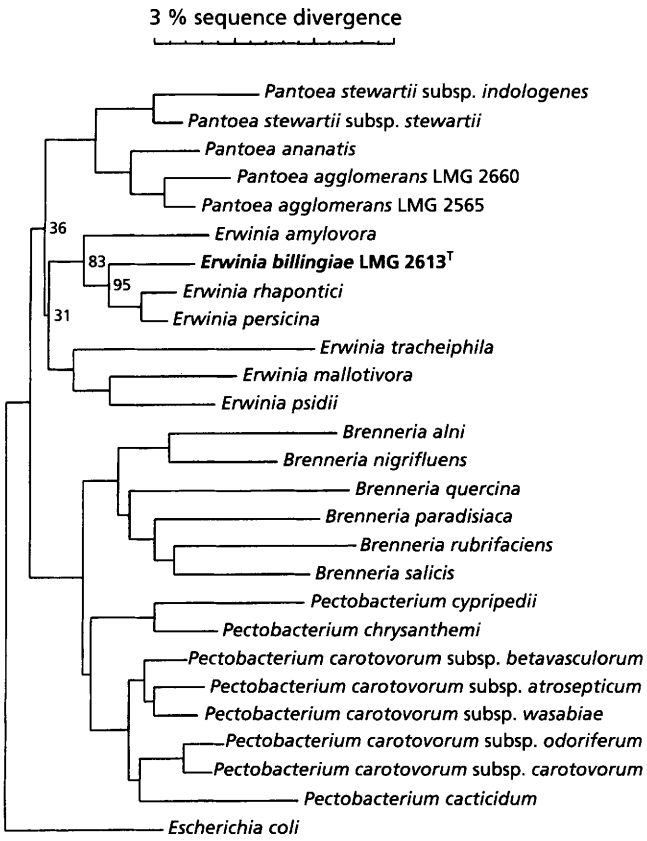

Fig. 1. Neighbour-joining dendrogram depicting the estimated phylogenetic relationships among species of the genera *Pantoea*, *Erwinia*, *Pectobacterium* and *Brenneria*, based on global comparisons of nearly complete 16S rRNA gene sequences. The position of *E. billingiae* LMG 2613^T is given in bold and the bootstrap values of the involved branches are shown. Strain designations for other species and sequence accession numbers are as given by Hauben *et al.* (1998). The distance between the species is obtained by summing the lengths of the connecting horizontal branches using the sequence divergence scale at the top of the figure.

The overall 16S rDNA gene sequence similarities of *E. billingiae* LMG 2613^T to representatives of the genera *Erwinia*, *Pantoea*, *Brenneria* and *Pectobacterium* were used to construct the phylogenetic tree shown in Fig. 1. *E. billingiae* showed highest overall 16S rRNA gene sequence similarity to *E. persicina* (98·2%) and *E. rhapontici* (98·0%), and similarities of 96·9, 96·3 and 96·2% to *E. amylovora*, *E. mallitovora* and *E. psidii*, respectively, classified in *Erwinia* by Hauben *et al.* (1998), and means of 95·6% to *Pantoea* species, 95·7% to species classified in the genus *Pectobacterium*, and 93·8% to species classified in the genus *Brenneria*. The 16S rRNA gene of strain LMG 2613^T possessed the signature nucleotides identical to those described for the genus *Erwinia* (Hauben *et al.*, 1998), i.e. A, A, C, G, G, C, G, G, G, C, G, C, C, C and G at positions 408, 594, 598, 639, 646, 839, 847, 987, 988, 989, 1216, 1217, 1218, 1308 and 1329, respectively, according to the *Escherichia coli* 16S rRNA gene sequence numbering (Brosius *et al.*, 1981).

DNA–DNA hybridizations

DNA–DNA binding ratios between strain LMG 2613^T, *E. rhapontici* LMG 2688^T and *E. persicina* LMG 11254^T, were less than 25%.

DISCUSSION

The strains listed in Table 1 constitute a homogeneous taxon on the basis of phenotypic (Verdonck *et al.*, 1987; Mergaert, 1989), chemotaxonomic (Mergaert, 1989; Table 4) and genotypic analysis (Mergaert, 1989). Although they phenotypically and chemotaxonomically resemble *Pantoea agglomerans*, and were often identified as such by commercial identification systems, DNA hybridization experiments have shown that they do not belong to this species (Mergaert, 1989). These data, together with the 16S rDNA sequence comparisons and additional DNA hybridization experiments with strain LMG 2613^T, indicate that the strains listed in Table 1 should be classified in a new species, situated within the genus *Erwinia sensu stricto* Hauben *et al.* (1998), for which the name *Erwinia billingiae* is proposed. Useful characteristics to differentiate *E. billingiae* from the phylogenetically related species *E. persicina* and *E. rhapontici*, and to other phenotypically neighbouring species of *Erwinia*, *Pantoea* and *Pectobacterium* (Verdonck *et al.*, 1987), are given in Table 4. *E. billingiae* can easily be differentiated from the other *Erwinia* species (*sensu stricto* Hauben *et al.*, 1998) by the limited number of carbon sources from which acid is produced by the latter species (Verdonck *et al.*, 1987).

Table 4. Differential characteristics of *Erwinia billingiae* and some biochemically similar species

+, Positive reactions for at least 90% of the strains; −, negative reactions for at least 90% of the strains; d, 11–89% positive. Data for *E. persicina* are from Hao *et al.* (1990), for *E. rhapontici* and *Pectobacterium cypripedii* from Verdonck *et al.* (1987), and for *Pantoea agglomerans* and *Pantoea dispersa* from Mergaert (1989).1, *Erwinia billingiae*; 2, *Erwinia rhapontici*; 3, *Erwinia persicina*; 4, *Pantoea agglomerans*; 5, *Pantoea dispersa*; 6, *Pectobacterium cypripedii*.

Characteristic	1	2	3	4	5	6
API 20E tests						
Acetoin production	+	+	+	+	+	−
Citrate utilization	−	d	+	d	+	+
Nitrate reduced to nitrite	+	d	+	+	−	−
API 50CHE tests						
Acid produced from:						
D-Xylose	+	−	−	+	+	+
Cellobiose	−	+	+	d	+	−
Lactose	−	+	+	d	d	−
Melibiose	−	+	+	d	d	d
D-Turanose	−	+	−	−	−	−
Gentiobiose	+	+	−	d	+	−
Raffinose	−	+	+	−	−	−
D-Arabitol	+	−	−	d	+	−
Amygdalin	−	+	−	−	d	−
Arbutin	+	+	+	+	−	+
D-Gluconate	+	d	−	d	d	−
5-Keto-D-gluconate	+	d	−	−	+	d
N-Acetylglucosamine	+	+	−	+	+	+
Aesculin hydrolysis	+	+	+	+	−	+
Other tests:						
Yellow pigment	−	−	−	+	−	−
Pink pigment	−	+	+	−	−	−

Description of *Erwinia billingiae* sp. nov.

Erwinia billingiae (bil.ling'i.ae. N.L. fem. gen. n. *billingiae* of Billing, named after Eve Billing who first isolated these organisms).

The description is based on the data obtained for the 22 strains listed in Table 1. The species shows the general characteristics of the *Enterobacteriaceae* and of the genus *Erwinia*, as described by Hauben *et al.* (1998). Cells are Gram-negative, oxidase-negative, catalase-positive motile rods, and ferment glucose without formation of gas. Strains grow at 4 °C, rarely at 37 °C, but not at 41 °C, and have an optimal growth temperature of 28–30 °C. Colonies grown on nutrient agar are circular, low convex with entire margins, translucent and not pigmented. Nitrate is reduced to nitrite and strains exhibit β-galactosidase activity, but not arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease or tryptophan deaminase activities, and rarely deoxyribonuclease activity. Citrate and malonate are not utilized and indole and hydrogen sulphide are not produced. Gelatin is not liquefied, pectate is not degraded, and Tween 80 is not hydrolysed. Tetrathionate is not reduced. Acetoin is usually produced. Acid is produced from L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, mannitol, *N*-acetyl-glucosamine, trehalose, gentiobiose and D-gluconate, but not from glycerol, *meso*-erythritol, D-arabinose, L-xylose, adonitol, methyl β-D-xyloside, sorbose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, *meso*-xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose or L-arabitol, or on Jordan's tartrate medium. Strains grow on aspartate and D-malate as sole carbon sources, but not on acetate or DL-aminobutyrate. Other biochemical and physiological characteristics are given in Table 2 and in the Results. The fatty acid composition is given in Table 3. *Erwinia billingiae* strains were isolated from stem cankers, diseased blossoms and immature fruits mainly of rosaceous trees, often in association with plant pathogens, and are considered as secondary invaders rather than primary pathogens (Billing & Baker, 1963). The DNA G+C contents of three strains are 54·1–55·1 mol% (Starr & Mandel, 1969; J. Mergaert, unpublished results). The type strain is LMG 2613^T (= NCPPB 661^T = Billing E63^T).

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REFERENCES

Billing, E. & Baker, L. A. E. (1963). Characteristics of *Erwinia*-like organisms found in plant material. *J Appl Bacteriol* **26**, 59–65.

Brosius, J., Dull, T. J., Sleeter, D. D. & Noller, H. F. (1981). Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* **148**, 107–127.

De Ley, J., Cattoir, H. & Reynaert, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Gavini, F., Mergaert, J., Beji, A., Mielcarek, C., Izard, D., Kersters, K. & De Ley, J. (1989). Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. *Int J Syst Bacteriol* **39**, 337–345.

Hao, M. V., Brenner, D. J., Steigerwalt, A. G., Kosako, Y. & Komagata, K. (1990). *Erwinia persicinus*, a new species isolated from plants. *Int J Syst Bacteriol* **40**, 379–383.

Hauben, L., Moore, E. R. B., Vauterin, L., Steenackers, M., Mergaert, J., Verdonck, L. & Swings, J. (1998). Phylogenetic position of phytopathogens within the *Enterobacteriaceae*. *Syst Appl Microbiol* **21**, 384–397.

Kwon, S.-W., Go, S.-J., Kang, H.-W., Ryu, J.-C. & Jo, J.-K. (1997). Phylogenetic analysis of *Erwinia* species based on 16S rRNA gene sequences. *Int J Syst Bacteriol* **47**, 1061–1067.

Mergaert, J. (1989). A taxonomic study of the '*Erwinia herbicola–Enterobacter agglomerans* complex'. *Academiae Analecta Mede K Acad Wet Lett Schone Kunsten Belg KL Wet* **51**, 1–23.

Mergaert, J., Verdonck, L., Kersters, K., Swings, J., Boeufgras, J. M. & De Ley, J. (1984). Numerical taxonomy of *Erwinia* species using API systems. *J Gen Microbiol* **130**, 1893–1910.

Mergaert, J., Verdonck, L. & Kersters, K. (1993). 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. *Int J Syst Bacteriol* **43**, 162–173.

Starr, M. P. & Mandel, M. (1969). DNA base composition and taxonomy of phytopathogenic and other enterobacteria. *J Gen Microbiol* **56**, 113–123.

Vandamme, P., Pot, B., Gillis, M., De Vos, P., Kersters, K. & Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev* **60**, 407–438.

Verdonck, L., Mergaert, J., Rijckaert, C., Swings, J., Kersters, K. & De Ley, J. (1987). The genus *Erwinia*: a numerical analysis of phenotypic features. *Int J Syst Bacteriol* **37**, 4–18.