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Transfer of *Pectobacterium chrysanthemi* (Burkholder *et al.* 1953) Brenner *et al.* 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov.

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A collection of 75 strains of *Pectobacterium chrysanthemi* (including all biovars and pathovars) and the type strains of *Brenneria paradisiaca* (CFBP 4178^T) and *Pectobacterium cypripedii* (CFBP 3613^T) were studied by DNA–DNA hybridization, numerical taxonomy of 121 phenotypic characteristics, serology and 16S rRNA gene-based phylogenetic analyses. From analysis of 16S rRNA gene sequences, it was deduced that *P. chrysanthemi* strains and *B. paradisiaca* CFBP 4178^T formed a clade distinct from the genera *Pectobacterium* and *Brenneria*; therefore, it is proposed to transfer all the strains to a novel genus, *Dickeya* gen. nov. By DNA–DNA hybridization, the strains of *P. chrysanthemi* were distributed among six genomic species: genomospecies 1 harbouring 16 strains of biovar 3 and four strains of biovar 8, genomospecies 2 harbouring 16 strains of biovar 3, genomospecies 3 harbouring two strains of biovar 6 and five strains of biovar 5, genomospecies 4 harbouring five strains of biovar 2, genomospecies 5 harbouring six strains of biovar 1, four strains of biovar 7 and five strains of biovar 9 and genomospecies 6 harbouring five strains of biovar 4 and *B. paradisiaca* CFBP 4178^T. Two strains of biovar 3 remained unclustered. Biochemical criteria, deduced from a numerical taxonomic study of phenotypic characteristics, and serological reactions allowed discrimination of the strains belonging to the six genomic species. Thus, it is proposed that the strains clustered in these six genomic species be assigned to the species *Dickeya zeae* sp. nov. (type strain CFBP 2052^T = NCPPB 2538^T), *Dickeya dadantii* sp. nov. (type strain CFBP 1269^T = NCPPB 898^T), *Dickeya chrysanthemi* comb. nov. (subdivided into two biovars, bv. *chrysanthemi* and bv. *parthenii*), *Dickeya dieffenbachiae* sp. nov. (type strain CFBP 2051^T = NCPPB 2976^T), *Dickeya dianthicola* sp. nov. (type strain CFBP 1200^T = NCPPB 453^T) and *Dickeya paradisiaca* comb. nov., respectively.

INTRODUCTION

Pectobacterium chrysanthemi (Burkholder *et al.* 1953) Brenner *et al.* 1973 emend. Hauben *et al.* 1998 was reinstated in the genus *Pectobacterium* Waldee 1945 after a long period of time during which *Erwinia chrysanthemi* Burkholder *et al.* 1953, its homotypic synonym (same type

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the type strains of *D. paradisiaca*, *D. dadantii*, *D. dianthicola*, *D. dieffenbachiae* and *D. zeae* are respectively AF520710, AF520707, AF520708, AF520712 and AF520711.

strain), was being used according to successive descriptions in *Bergey's Manual of Determinative Bacteriology* (Burkholder, 1957) and *Bergey's Manual of Systematic Bacteriology* (Lelliott & Dickey, 1984).

The species *E. chrysanthemi* was created for the *Chrysanthemum morifolium* hollow stalk agent (Burkholder *et al.*, 1953). Similar bacteria were subsequently isolated from soft rots and wilts of numerous diseased plant species (CABI, 2001). After extensive biochemical studies (Martinec & Kocur, 1963; Graham, 1964, 1972; Dye, 1969) and for the elaboration of the Approved Lists (Skerman *et al.*, 1980), all isolates were finally gathered into a single species, *E. chrysanthemi*. For convenience, phytobacteriologists divided *E. chrysanthemi* into six pathovars, pv. *chrysanthemi*, pv. *dianthicola*, pv. *dieffenbachiae*, pv. *parthenii*, pv. *zeae* and pv. *paradisiaca* (Young *et al.*, 1978; Lelliott & Dickey, 1984). *E. chrysanthemi* pv. *paradisiaca* was renamed *Brenneria paradisiaca* (Hauben *et al.*, 1998) on the basis of 16S rRNA gene sequences, whereas *E. chrysanthemi* remained clustered with members of the genus *Pectobacterium*. In order to designate in this study all strains formerly included in *E. chrysanthemi sensu stricto* (Lelliott & Dickey, 1984), i.e. belonging either to *P. chrysanthemi* or to *B. paradisiaca*, the expression '*E. chrysanthemi* complex' will be used.

Phenotypic diversity of strains of the *E. chrysanthemi* complex was demonstrated using classical tests, auxanograms and API galleries (Hildebrand *et al.*, 1978; Dickey, 1979; Dickey & Victoria, 1980; Thomson *et al.*, 1981; Verdonck *et al.*, 1987). However, the practice of using pathogenicity tests to define the affiliation of a strain to a given pathovar proved difficult to implement (Dickey, 1981; Janse & Ruissen, 1988). As an alternative, nine biovars were proposed to characterize all strains of the complex by unambiguous differential biochemical tests (Samson & Nassan-Agha, 1978; Samson *et al.*, 1987, 1990; Ngwira & Samson, 1990). Complementary methods, such as serology and DNA typing, revealed either uniformity or diversity within the *E. chrysanthemi* complex. A major O-serogroup (1) recognized many isolates from many plant species (Samson, 1973; Yakrus & Schaad, 1979; Dickey *et al.*, 1984). Other distinct O-serogroups were described that each recognized a few strains (Samson, 1973; Samson & Nassan-Agha, 1978; Dickey *et al.*, 1984, 1987; Samson *et al.*, 1987, 1990). Ribotyping and PCR-RFLP studies showed large genomic heterogeneity of isolates of the *E. chrysanthemi* complex and allocated the strains to different groups that could be related to the biovar classification (Boccara *et al.*, 1991; Nassar *et al.*, 1994, 1996). The overall diversity of isolates of the *E. chrysanthemi* complex was pointed out again by amplified fragment length polymorphism (AFLP) studies (Avrova *et al.*, 2002), but none of these works allowed clarification of its taxonomy.

Initial DNA relatedness studies (Brenner *et al.*, 1977), although undertaken on a small number of strains, revealed that *P. chrysanthemi* could be divided into four DNA relatedness groups: one with *Dieffenbachia* isolates, one with *Chrysanthemum morifolium* and *Parthenium* sp. isolates, one with maize isolates and one with a sugarcane isolate. The purpose of the present study was to scrutinize the whole diversity of a large collection of strains belonging to the *E. chrysanthemi* complex by using phenotypic characteristics, DNA–DNA hybridization, serology and 16S rRNA gene sequence analysis. The outcomes of this study were (i) the assignment of strains previously characterized as *P. chrysanthemi* and *B. paradisiaca* to a novel genus, *Dickeya*, and (ii) the delineation of six species within the novel genus.

METHODS

Bacterial strains. A total of 75 representative strains of *P. chrysanthemi* and the type strains of *B. paradisiaca* (CFBP 4178^T) and *Pectobacterium cypripedii* (CFBP 3613^T) were used in this study (Table 1). The strains were isolated from various host plants and locations all over the world. The identity of the strains as belonging to the *E. chrysanthemi* complex was confirmed by pectinolytic activity, production of indole, utilization of (+)-L-arabinose, malonate and (+)-D-malate and non-utilization of trehalose (Gardan *et al.*, 2003).

DNA extraction and DNA–DNA hybridization. Extraction and purification of DNA were performed as described by Brenner *et al.* (1982). Native DNA of five strains of *P. chrysanthemi* (CFBP 2048^T, CFBP 2052^T, CFBP 1269^T, CFBP 2051^T, CFBP 2015) and of *B. paradisiaca* CFBP 3477 was labelled *in vitro* by nick translation with tritium-labelled nucleotides (Amersham Biosciences). The S1 nuclease/trichloroacetic method was used as described by Crosa *et al.* (1973) with the modifications of Grimont *et al.* (1980). The ΔT_m (thermal stability of reassociated DNA) was determined by using the method of Crosa *et al.* (1973).

Phenotypic tests and numerical taxonomy. Twenty-two conventional tests and assimilation of carbon sources using Biotype 100 strips (bioMérieux) were determined as described by Sutra *et al.* (2001). A total of 121 tests were included in a numerical taxonomy analysis for 68 strains of *P. chrysanthemi*, *B. paradisiaca* and *P. cypripedii*. The Jaccard coefficient was used for calculating the distance matrix and UPGMA was used to perform cluster analysis (Sneath & Sokal, 1973). From the numerical analysis performed with TAXONUM software, calculation of a diagnostic ability coefficient allowed the selection of discriminatory phenotypic tests (Descamp & Véron, 1981).

Serology. Polyclonal antibodies were produced in rabbits as described previously (Samson *et al.*, 1987). Care was taken to ensure that the immunizing bacteria, as well as the tested bacteria, were motile, in order to ascertain the serotypes of the flagella (H antigens) (Guillorit-Rondeau *et al.*, 1996). Twelve O-serogroups and five H-types were delineated on the basis of indirect immunofluorescent staining of the bacterial cells, by preparing antiserum 174 with CFBP 2048^T, antiserum 35 with CFBP 1236, antiserum 71 with CFBP 1451, antiserum 74 with CFBP 1502, antiserum 85 with CFBP 1496, antiserum 231 with CFBP 2052^T, antiserum 228 with CFBP 1278, antiserum 263 with CFBP 1531, antiserum 268 with CFBP 1277, antiserum 273 with CFBP 1528, antiserum 277 with CFBP 3805 and antiserum 306 with CFBP 3804. The first typing step of the bacteria was performed after mixing the antisera (used in a 200-fold dilution) which contained the same H antibodies in order to establish the H-type. Antisera of each mixture of O-antibodies that gave a positive reaction for the cell walls (lipopolysaccharide recognition) were then tested separately to obtain the

Table 1. Strains of the *E. chrysanthemi* complex (*Pectobacterium chrysanthemi* and *Brenneria paradisiaca*) used in this study, listed according to their biovar type

Serotyping results were determined in this study.

Strain		Biovar	Pathovar	Host plant	Geographical origin, year of isolation	Results of serotyping
CFBP no.	Other designation(s)					
722		1		*Lycopersicon esculentum*	France, 1965	O:1, H:a
795		1	*dianthicola*	*Dianthus* sp.	France, 1965	O:1, H:a
1200Ta*	NCPPB 453^{T}	1	*dianthicola*	*Dianthus caryophyllus*	UK, 1956	O:1, H:a
1888		1		*Solanum tuberosum*	France, 1978	O:1, H:a
1982		1		*Dahlia* sp.	France, 1972	O:1, H:a
3265		1		*Cichorium intybus*	France, 1983	O:1, H:a
3702		1		*Cynara scolymus*	France, 1984	O:1, H:a
1247		2	*dieffenbachiae*	*Dieffenbachia picta*	USA, 1957	O:1, H:a
1360		2	*dieffenbachiae*	*Dieffenbachia* sp.	France, 1972	O:1, H:?
2051^{Tb}	NCPPB 2976^{T}	2	*dieffenbachiae*	*Dieffenbachia* sp.	USA, 1957	O:1, H:a
3694		2		*Lycopersicon esculentum*	Cuba, 1987	O:1, H:?
3698		2		*Musa* sp.	Cuba, 1987	O:1, H:a
1269^{T}	NCPPB 898^{T}, Hayward B374^{T}	3		*Pelargonium capitatum*	Comoros, 1960	O:1, H:a
1277	NCPPB 1863	3	*zeae*	*Zea mays*	USA, 1966	O:9, H:e
1278	NCPPB 1121	3		*Ananas comosus*	Malaysia, 1961	O:7, H:a
1496		3	*zeae*	*Zea mays*	France, 1973	O:5, H:e
1502		3	*zeae*	*Zea mays*	France, 1973	O:4, H:d
1533		3	*zeae*	*Zea mays*	Italy, 1970	O:?, H:?
1537		3		*Saccharum officinarum*	Australia	O:7, H:?
1613		3		*Euphorbia pulcherrima*	France, 1974	O:1, H:a
1871		3		*Musa* sp.	Ivory Coast, 1976	O:?, H:?
1884		3		*Brachiaria ruziziensis*	Guyana (Fr.), 1979	O:11, H:a
2018		3		*Saintpaulia ionantha*	France, 1977	O:1, H:a
2052^{Tc}	NCPPB 2538^{T}	3	*zeae*	*Zea mays*	USA, 1970	O:6, H:a
2268		3		*Solanum tuberosum*	Australia, 1978	O:6, H:a
3695		3	*zeae*	*Zea mays*	Cuba, 1987	O:1, H:?
3697		3		*Ipomoea batatas*	Cuba, 1987	O:1, H:a
3707		3		water	Israel, 1986	O:1, H:?
3780		3		*Dianthus* sp.	Italy	O:1, H:a
3781		3		*Dianthus* sp.	Italy	O:1, H:a
3782		3		*Dianthus* sp.	Italy	O:1, H:a
3783		3		*Dianthus* sp.	Italy	O:1, H:a
3804		3		*Nicotiana tabacum*	Cuba	O:12, H:d
3805		3	*zeae*	*Zea mays*	Senegal, 1986	O:11, H:a
3855	Lemattre 3937	3		*Saintpaulia ionantha*	France, 1977	O:1, H:a
4148	ICMP 7077	3		*Oryza sativa*	Japan, 1978	O:?, H:?
4149	ICMP 7078	3		*Oryza sativa*	Japan, 1978	O:?, H:?
4150	ICMP 7079	3		*Oryza sativa*	Japan, 1978	O:?, H:a
4151	ICMP 1566	3		*Philodendron scandens*	USA, 1959	O:6, H:d
4152	ICMP 9156	3		*Philodendron* sp.	Greece, 1985	O:6, H:d
4153	NCPPB 454	3		*Philodendron* sp.	USA, 1985	O:1, H:d
4176	NCPPB 2339	3		*Chrysanthemum morifolium*	USA, 1970	O:6, H:a
4177		3		*Musa paradisiaca*	Jamaica, 1970	O:1, H:a
4180		3		*Musa paradisiaca*	Panama, 1972	O:1, H:d
6466		3		*Ananas comosus*	Martinique (Fr.), 1991	O:10, H:?
6467		3		*Musa* sp.	Martinique (Fr.), 1994	O:1, H:a
1445		4	*paradisiaca*	*Musa paradisiaca*	Colombia, 1972	O:3, H:?
1446		4	*paradisiaca*	*Musa paradisiaca*	Colombia, 1972	O:3, H:?
1451		4	*paradisiaca*	*Musa paradisiaca*	Colombia, 1972	O:3, H:?
3477^{d}	ICMP 2349, LMG 2545	4	*paradisiaca*	*Musa paradisiaca*	Colombia, 1968	O:3, H:?

Table 1. cont.

Strain		Biovar	Pathovar	Host plant	Geographical origin, year of isolation	Results of serotyping
CFBP no.	Other designation(s)					
3696		4		*Musa* sp.	Cuba, 1987	O:3, H:?
3699		4	*zeae*	*Zea mays*	Cuba, 1987	O:3, H:?
4178^T	NCPPB 2511^T	4	*paradisiaca*	*Musa paradisiaca*	Colombia, 1970	O:3, H:?
1346		5	*chrysanthemi*	*Chrysanthemum maximum*	Italy, 1969	O:2, H:?
2048^T	NCPPB 402^T	5	*chrysanthemi*	*Chrysanthemum morifolium*	USA, 1958	O:1, H:b
3262		5		*Cichorium intybus*	France, 1981	O:1, H:b
3700		5		water	France, 1974	O:1, H:?
3701		5		*Lycopersicon esculentum*	France, 1981	O:1, H:?
3703		5		*Helianthus annuus*	France, 1986	O:1, H:b
1236	NCPPB 1861	6	*parthenii*	*Parthenium argentatum*	USA, 1945	O:2, H:c
1245		6		*Philodendron oxycardium*	USA, 1959	O:?, H:d
1270[e]	NCPPB 516	6	*parthenii*	*Parthenium argentatum*	Denmark, 1957	O:2, H:c
3704		6		*Cynara scolymus*	Réunion (Fr.), 1986	O:1, H:a
1276	NCPPB 1385	7		*Dahlia* sp.	Romania, 1962	O:1, H:?
2015[f]		7		*Solanum tuberosum*	France, 1975	O:1, H:a
3705		7		*Solanum tuberosum*	Switzerland, 1986	O:1, H:a
3706		7		*Cichorium intybus*	Switzerland, 1986	O:1, H:a
1447[g]	NCPPB 2546	8	*zeae*	*Zea mays*	India, 1969	O:?, H:?
1528	NCPPB 2541	8	*zeae*	*Zea mays*	USA, 1966	O:10, H:a
1531		8	*zeae*	*Zea mays*	USA, 1966	O:8, H:a
3708		8	*zeae*	*Zea mays*	USA, 1986	O:?, H:a
1805[h]		9		*Kalanchoe blossfeldiana*	Denmark, 1977	O:1, H:?
2598		9		*Kalanchoe blossfeldiana*	Switzerland, 1982	O:1, H:a
2982		9		*Kalanchoe blossfeldiana*	France, 1987	O:1, H:a
4155		9		*Kalanchoe blossfeldiana*	The Netherlands, 1985	O:1, H:a
4156		9		*Kalanchoe blossfeldiana*	The Netherlands, 1985	O:1, H:a

*Reference strains are indicated as follows: *a*, pv. *dianthicola* and biovar 1; *b*, pv. *dieffenbachiae* and biovar 2; *c*, pv. *zeae* and biovar 3; *d*, pv. *paradisiaca* (*B. paradisiaca*) and biovar 4; *e*, pv. *parthenii* and biovar 6; *f*, biovar 7; *g*, biovar 8; *h*, biovar 9.

precise O-serogroup. The reactions were of a plus/minus type without cross-reactions between the O-serogroups, as explained by Janse & Ruissen (1988).

Phylogenetic analyses. The 16S rRNA gene sequences of *P. chrysanthemi* strains CFBP 1200^T, CFBP 1269^T, CFBP 1270, CFBP 2051^T and CFBP 2052^T and *B. paradisiaca* CFBP 4178^T were determined and the sequences were aligned by comparison within our database of 66 000 already aligned bacterial 16S rRNA gene sequences. Selection of related sequences was according to previous phylogenetic analyses of the entire database and BLAST searches with CFBP 1269^T, CFBP 4178^T, CFBP 2052^T and *Brenneria rubrifaciens* LMG 2709^T against the latest release of EBI (http://www.ebi.ac.uk/). Alignments were refined manually within this subset of related sequences. Successive phylogenetic trees (from 250 to 19 sequences) were then constructed to determine to which subset of the global tree the new sequences could be related. When several sequences were available for a type strain, all sequences were included in the preliminary analysis (they often differed by a few nucleotides) and, if they formed a clade, a single one was chosen for the tree presented; this procedure led to the retention of a single sequence for each type strain. The exception was *P. cypripedii*, for which the three available sequences showed discrepancies of phylogeny. Detailed analyses showed that the sequence of *P. cypripedii* LMG 2657^T (GenBank accession no. Z96094) (which appeared to be related to the new sequences) was not the sequence for the authentic strain (likely contamination), as it did not cluster with the two other sequences of type strains available; it was therefore excluded from the final analysis. The final analysis was restricted to a subset of 33 sequences, analysed using three different methods (BIONJ, maximum-likelihood and maximum-parsimony). For the neighbour-joining (BIONJ) analysis, distance matrices were calculated using Kimura's two-parameter correction. BIONJ was performed according to Gascuel (1997); maximum-likelihood and maximum-parsimony were from PHYLIP (Felsenstein, 1995). Because of close relationships, no evident homoplasy was detected and almost the entire sequence corresponding to positions 29–1428 of the *Brenneria alni* sequence was used for this analysis. Phylogenetic trees were drawn using NJPLOT (Perrière & Gouy, 1996). Finally, phylogenies were also investigated with sequences of gyrase and *recA* genes, but too few sequences were available.

RESULTS AND DISCUSSION

DNA–DNA hybridization

Six DNA hybridization groups were delineated and two strains belonging to biovar 3 remained unclustered (Table 2). DNA hybridization group 1 included 20 strains

Table 2. Levels of DNA relatedness among *E. chrysanthemi* complex strains

Strain: 1, *Dickeya zeae* CFBP 2052^T; 2, *Dickeya dadantii* CFBP 1269^T; 3, *Dickeya dianthicola* CFBP 2015; 4, *Dickeya dieffenbachiae* CFBP 2051^T; 5, *Dickeya paradisiaca* CFBP 3477; 6, *Dickeya chrysanthemi* CFBP 2048^T. Values shown are percentages of relative binding with labelled DNA from the relevant strain; ΔT_m values are shown in °C in parentheses. NT, Not tested.

Source of unlabelled DNA	Biovar	1	2	3	4	5	6
Genomic species 1 (*D. zeae*)							
CFBP 2052^T	3	100	38	31	40	21	47
CFBP 4176	3	100	37	NT	NT	NT	NT
CFBP 6466	3	99	NT	NT	NT	NT	NT
CFBP 1533	3	93	NT	NT	NT	NT	NT
CFBP 1277	3	90	NT	NT	NT	NT	NT
CFBP 1496	3	85	NT	NT	NT	NT	NT
CFBP 1884	3	84	NT	NT	NT	NT	NT
CFBP 2268	3	84	NT	NT	NT	NT	NT
CFBP 1871	3	80	34	NT	NT	NT	NT
CFBP 3804	3	80	39	NT	NT	NT	NT
CFBP 4148	3	80	29	NT	NT	NT	NT
CFBP 3805	3	79	NT	NT	NT	NT	NT
CFBP 4150	3	77	NT	NT	NT	NT	NT
CFBP 3707	3	75	NT	NT	NT	NT	NT
CFBP 1502	3	74	NT	NT	NT	NT	NT
CFBP 4149	3	72	28	NT	NT	NT	NT
CFBP 3708	8	83	30	NT	NT	NT	NT
CFBP 1531	8	81	38	NT	NT	NT	NT
CFBP 1447	8	80	NT	37	NT	NT	NT
CFBP 1528	8	76	NT	NT	NT	NT	NT
Mean ± SD...		83·6 ± 8·5	34·1 ± 4·5	34 ± 4·2			
Genomospecies 2 (*D. dadantii*)							
CFBP 1269^T	3	41	100	NT	NT	NT	NT
CFBP 6467	3	38	96	NT	NT	NT	NT
CFBP 3697	3	40	92	NT	NT	NT	NT
CFBP 3780	3	43	90	NT	NT	NT	NT
CFBP 3781	3	43	90	NT	NT	NT	NT
CFBP 3783	3	43	90	NT	NT	NT	NT
CFBP 3695	3	38	89	NT	NT	NT	NT
CFBP 2018	3	41	87	NT	NT	NT	NT
CFBP 1613	3	43	86	NT	NT	NT	NT
CFBP 3782	3	44	85	NT	NT	NT	NT
CFBP 4177	3	41	84	NT	NT	NT	NT
CFBP 3855	3	42	82	NT	NT	NT	NT
CFBP 4151	3	41	73	NT	NT	NT	NT
CFBP 4152	3	42	73	NT	NT	NT	NT
CFBP 4153	3	40	71	NT	NT	NT	NT
CFBP 4180	3	50	69	NT	NT	NT	NT
Mean ± SD...		41·9 ± 2·8	84·8 ± 9·1				
Unclustered strains							
CFBP 1278	3	54	28	NT	NT	NT	NT
CFBP 1537	3	41	39	NT	NT	NT	NT
Mean ± SD...		47·5 ± 9·2	33·5 ± 7·8				
Genomospecies 3 (*D. chrysanthemi*)							
CFBP 1236	6	NT	44	NT	44	20	86
CFBP 1270	6	45	NT	42	NT	18	71
CFBP 2048^T	5	47	NT	43	39	17	100
CFBP 1346	5	NT	NT	NT	NT	NT	93
CFBP 3703	5	NT	NT	NT	NT	NT	92

Table 2. cont.

Source of unlabelled DNA	Biovar	1	2	3	4	5	6
CFBP 3262	5	NT	NT	NT	NT	NT	84
CFBP 3701	5	NT	NT	NT	NT	NT	83
Mean ± SD...		46 ± 1·4		42·5 ± 0·7	41·5 ± 3·5	18·3 ± 1·5	87 ± 9·2
Genomospecies 4 (*D. dieffenbachiae*)							
CFBP 2051^T	2	40	NT	53	100	25	38
CFBP 1360	2	NT	NT	NT	96	NT	NT
CFBP 1247	2	NT	NT	NT	93	NT	NT
CFBP 3694	2	NT	NT	NT	75 (2·9)	NT	NT
CFBP 3698	2	NT	NT	NT	73 (1·7)	NT	NT
Mean ± SD...					84·3 ± 11·9		
Genomospecies 5 (*D. dianthicola*)							
CFBP 2015	7	44	NT	100	53	26	43
CFBP 1276	7	NT	NT	82	NT	NT	NT
CFBP 3705	7	NT	NT	80	NT	NT	NT
CFBP 3706	7	NT	NT	80	NT	NT	NT
CFBP 1888	1	NT	NT	97	NT	NT	NT
CFBP 722	1	NT	NT	95	NT	NT	NT
CFBP 3702	1	NT	NT	92	NT	NT	NT
CFBP 1200^T	1	38	NT	90	NT	NT	NT
CFBP 1982	1	NT	NT	81	NT	NT	NT
CFBP 3265	1	NT	NT	71	NT	NT	NT
CFBP 4156	9	37	NT	82	NT	NT	NT
CFBP 1805	9	39	NT	79	NT	NT	NT
CFBP 2598	9	NT	NT	78	NT	NT	NT
CFBP 4155	9	36	NT	77	NT	NT	NT
CFBP 2982	9	NT	NT	75	NT	NT	NT
Mean ± SD...		38·8 ± 3·1		72·7 ± 21·4			
Genomospecies 6 (*D. paradisiaca*)							
CFBP 3477	4	21	NT	23	18	100	19
CFBP 4178^T	4	NT	NT	NT	NT	100	NT
CFBP 1446	4	NT	NT	NT	NT	100	NT
CFBP 1451	4	NT	NT	NT	NT	100	NT
CFBP 3699	4	NT	NT	NT	NT	100	NT
CFBP 1445	4	NT	NT	NT	NT	98	NT
CFBP 3696	4	NT	NT	NT	NT	93	NT
Mean ± SD...						98·9 ± 2·5	

belonging to biovars 3 and 8 that demonstrated 72–100 % relatedness to strain CFBP 2052^T. Strains of the other groups were 21–54 % related to strain CFBP 2052^T. Thus, these 20 strains constituted genomic species 1. DNA hybridization group 2 included 16 strains out of 34 belonging to biovar 3 that demonstrated 69–100 % relatedness to strain CFBP 1269^T. Strains of the other groups were 28–44 % related to strain CFBP 1269^T. Thus, these 16 strains constituted genomospecies 2. Two strains of biovar 3 (CFBP 1278 and CFBP 1537) remained unclustered, since they were only loosely related to strain CFBP 2052^T or strain CFBP 1269^T. DNA hybridization group 3 included seven strains (biovars 5 and 6) that demonstrated 71–100 % relatedness to the type strain of *P. chrysanthemi* (CFBP 2048^T). Strains of the other groups were 19–47 % related to strain CFBP 2048^T. Thus, these seven strains constituted genomospecies 3. DNA hybridization group 4 included five strains of biovar 2 that demonstrated 73–100 % relatedness to strain CFBP 2051^T, with ΔT_m values of 1·7 and 2·9 °C for the lowest values, 73 and 75 %, respectively. Strains of the other groups were 18–53 % related to strain CFBP 2051^T. Thus, these strains constituted genomospecies 4. DNA hybridization group 5 included 15 strains belonging to biovar 1 (six strains), biovar 7 (four strains) and biovar 9 (five strains) that demonstrated 71–100 % relatedness to strain CFBP 2015. Strains of the other groups were 23–53 % related to strain CFBP 2015. Thus, these 15 strains constituted genomospecies 5. DNA hybridization group 6 included six strains that were 93–100 % related to the strain CFBP 3477. Strains of the other groups were 17–26 % related to strain CFBP 3477. Thus, these six strains constituted genomospecies 6, which corresponded to *B. paradisiaca* as delineated by Hauben *et al.*

(1998). DNA hybridization tests performed between *P. cypripedii* CFBP 3613^T and *B. paradisiaca* CFBP 3477 gave a mean of only 6·3 % relatedness (data not shown in Table 2), showing that *P. cypripedii* was very distantly related to the six genomic species described here.

Three of the six genomic species delineated in the present study were shown to correspond to three DNA hybridization groups reported by Brenner *et al.* (1977), because of common strains present in the two studies. Genomic species 3, corresponding to Brenner's group 1, contained the type strain CFBP 2048^T along with other *Chrysanthemum* sp. and *Parthenium* sp. isolates. Genomospecies 4, corresponding to Brenner's group 2, contained two strains in common (CFBP 2051^T and CFBP 1247). Genomospecies 1, corresponding to Brenner's group 3, contained several strains in common (CFBP 1277, CFBP 1528 and CFBP 1533). Two other results obtained by Brenner *et al.* (1977) showed the consistency of the two DNA hybridization classifications. CFBP 1276 (a *Dahlia* isolate) belonged to our genomospecies 5 and was excluded from Brenner's hybridization groups. The fourth hybridization group of Brenner *et al.* (1977) was defined by CFBP 1537. We found that CFBP 1537 was excluded from all six of our genomic species. CFBP 1537 could therefore constitute a candidate for a seventh genomic species of the *E. chrysanthemi* complex. Thus, the study of Brenner *et al.* (1977) and the present one fit perfectly. In addition to Brenner's groups, we delineated two new hybridization groups, genomospecies 6 for *B. paradisiaca*, which, although a described species, has not yet been confirmed as a species according to the criteria of Wayne *et al.* (1987), and genomospecies 2, whose type strain CFBP 1269^T (=Hayward B374^T), isolated from *Pelargonium capitatum* (Rasolofo & Dadant, 1962), is often used for fundamental studies. It is remarkable that only two genomic species (genomospecies 1 and genomospecies 2) harboured 20 and 16 strains, respectively, out of 38 strains that were isolated from 16 different plant species in 17 countries.

Phenotypic characteristics

The dendrogram of phenotypic distances among 70 strains (68 strains of *P. chrysanthemi* and the type strains of *B. paradisiaca* and *P. cypripedii*) is shown in Fig. 1. At a distance of 0·2, six phena and one unclustered strain (*P. cypripedii*) were observed. The phenotypic characteristics that differentiate the six phena and *P. cypripedii* were deduced from the numerical taxonomic analysis (Table 3). The phena matched the genotypic groups, with the exception of phenon 1.

Phenon 1 included 33 strains that all belonged to former biovars 3 or 8. At a distance of 0·115, the first 18 strains constituted a subphenon (not shown on the dendrogram) which grouped together 79 % of the strains of genomospecies 1. Actually, phenon 1 clustered four genomic entities: *Dickeya zeae* sp. nov., *Dickeya dadantii* sp. nov. and two genomically distinct strains, CFBP 1278 and CFBP 1537. Four carbon sources [α-lactose, 1-*o*-methyl β-galactopyranoside, β-gentiobiose and (+)-L-tartrate] yielded two exclusive patterns, ++−+ versus −−+−, for almost half of the strains of the two species (Table 4). Nevertheless, variations in one or two reactions of the other half of the strains required the use of two more carbon sources to allocate all strains to the four entities of phenon 1.

Phenon 2 (including all four studied strains of former biovar 6) and phenon 4 (including all five studied strains of former biovar 5 including the type strain of *P. chrysanthemi*, CFBP 2048^T) belonged to the same genomic species (*Dickeya chrysanthemi* sp. nov.), which could be distinguished from the other phena on the basis of (−)-D-arabinose assimilation and growth at 39 °C (Table 3). The existence of two clearly distinct phena in the latter species led to the creation of two biovars named after the pathovars they came from: bv. *parthenii* for phenon 2 and bv. *chrysanthemi* for phenon 4. These two biovars differed from each other by inulin utilization and Moeller's arginine alkalinization (Table 3).

Phenon 3 included all five studied strains of former biovar 2 and corresponded to the genomic species *Dickeya dieffenbachiae* sp. nov. Phenon 5 included all 16 studied strains of former biovars 1, 7 and 9 and corresponded to the genomic species *Dickeya dianthicola* sp. nov. Phenon 6 included all six studied strains of former biovar 4 with *B. paradisiaca* CFBP 4178^T and corresponded to the genomic species *Dickeya paradisiaca* sp. nov. Table 3 shows characteristics useful for differentiating the six above-mentioned genomic species.

As expected, the phenotypic study yielded the summing-up of all differential criteria reported previously and in this study for the *E. chrysanthemi* complex (Dickey, 1979; Dickey & Victoria, 1980; Thomson *et al.*, 1981; Verdonck *et al.*, 1987; Samson *et al.*, 1987). The novelty of this study consisted of the help of the TAXONUM software to select the traits that contributed most to the cut realized on the dendrogram at a distance (0·2) chosen on the basis of the DNA relatedness of the strains. A surprising result was provided by strains of former biovars 3 and 8, which presented phenotypic features rather similar to each other although they belonged to two main genomic species: *Dickeya zeae* and *Dickeya dadantii*. Table 4 provides identification criteria for the two species but we admit that, in order to identify large numbers of isolates of phenon 1 and to get more clear-cut characteristics, alternative phenotypic criteria must be looked for. Notice that all *Zea mays* isolates of phenon 1 (originating from five countries) belonged to *Dickeya zeae*, but seven other sources are reported for this bacterial species (Table 1).

Serological typing

The majority of the studied strains (43 out of 76) belonged to O-serogroup 1 (Table 1), which was present in all biovars and genomic species, with the exception of biovar 4 (genomospecies 6 including *B. paradisiaca*), whose seven

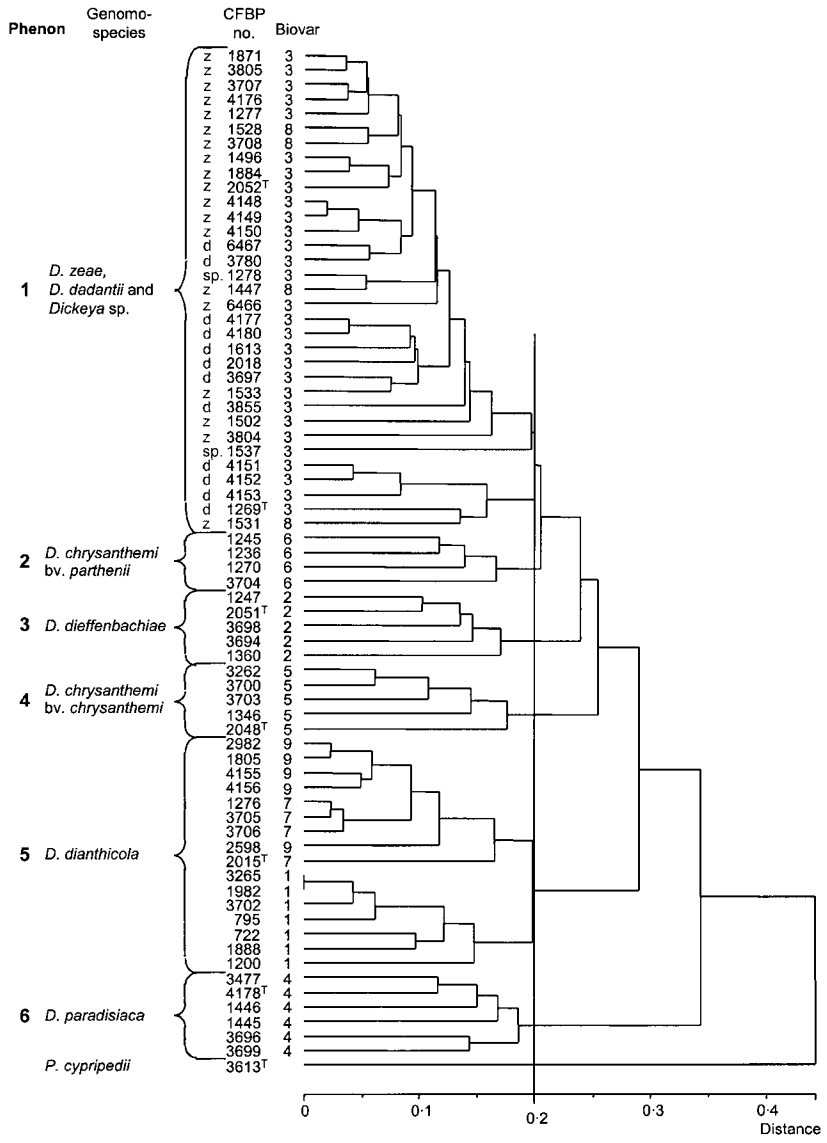

Fig. 1. Dendrogram of phenotypic characteristics of 70 strains belonging to the *E. chrysanthemi* complex (*Pectobacterium chrysanthemi* and *Brenneria paradisiaca*), based on UPGMA. Distance is 1−Jaccard coefficient. Within phenon 1, strains are identified as z (*D. zeae*), d (*D. dadantii*) or sp. (*Dickeya* sp.).

strains belonged specifically to O-serogroup 3. The other O-serogroups were represented by one to four strains, and six strains did not belong to any of the 12 described O-serogroups. H : a-type flagella were predominant, as they were borne by 39 strains. Five strains were not H-typed because they were non-motile and 17 strains displayed flagella that were not recognized in any of the five H-serotypes. Genomospecies 4 and genomospecies 5 contained 14 of 16 strains with the serological formula O : 1, H : a. Genomospecies 3 displayed the serogroups O : 1 and O : 2 independently of the two phena (bv. *chrysanthemi* or bv. *parthenii*). Within genomospecies 2, 14 strains fell in serogroup O : 1 against two strains only in serogroup O : 6. The greatest diversity was observed in genomospecies 1, whose strains were either distributed into nine O-serogroups or non-typed (Table 4). Surprisingly, the two strains (CFBP 1278 and CFBP 1537) of phenon 1 that did not hybridize with any of the genomic species both reacted in a particular serogroup, O : 7.

Phylogenetic analyses

Results of the phylogenetic analyses of 16S rRNA gene sequences (Fig. 2) showed that (i) all strains of *P. chrysanthemi* and *B. paradisiaca* were grouped within a robust clade, identified by all methods and 89 % of bootstrap replications, thus suggesting that they could be grouped within a single taxon, which we propose to name genus *Dickeya*, and (ii) *Pectobacterium carotovorum* formed a robust clade that was distant from the aforementioned clade, and the depths of the internal branches suggest that several species could be recognized, as published by Gardan *et al.* (2003). The sequences for *P. cypripedii* DSM 3873^T (GenBank accession numbers Z96094 and AJ233413) and the type strains of *Brenneria salicis*, *Brenneria nigrifluens* and *B. rubrifaciens* were included in the tree.

The transfer of *B. paradisiaca* to a novel genus should not raise questions, as all *Brenneria* species, including *B. salicis*,

Table 3. Characteristics that differentiate phena 1 to 6 (*Dickeya* species) and *Pectobacterium cypripedii* as delineated in Fig. 1

+, 90–100 % of strains positive; −, 90–100 % of strains negative; d (*n*), percentage of positive strains.

Characteristic	Phenon 1	Phenon 2	Phenon 4	Phenon 3	Phenon 5	Phenon 6	*P. cypripedii*
(−)-D-Arabinose	+	−	−	+	−	+	+
(−)-D-Tartrate	−	d (25)	−	−	+	+	+
Inulin	−	−	+	−	d (88)	−	−
Lactose	+	d (75)	d (20)	−	−	d (17)	+
Growth at 39 °C	+	+	+	+	−	d (83)	−
cis-Aconitate	+	−	d (20)	d (80)	−	−	+
(+)-D-Melibiose, (+)-D-raffinose	+	+	+	−	d (44)	d (83)	−
5-Keto-D-gluconate	−	−	−	d (20)	−	+	+
Mannitol	+	+	+	+	+	−	+
Lecithin	+	+	+	+	+	−	−
ADH Moeller	d (15)	−	+	−	d (69)	−	−
meso-Tartrate	+	d (75)	−	+	+	+	+
myo-Inositol	+	+	d (80)	+	+	−	+
Casein	+	d (75)	+	d (80)	d (75)	−	−
Novel species	*D. dadantii* + *D. zeae*	*D. chrysanthemi* bv. *parthenii*	*D. chrysanthemi* bv. *chrysanthemi*	*D. dieffenbachiae*	*D. dianthicola*	*D. paradisiaca*	–

the type species of the genus, are phylogenetically distantly related to the novel genus *Dickeya*.

Taxonomic conclusions

Based on the above-mentioned phylogenetic analyses, which show that eight 16S rRNA gene sequences of the *E. chrysanthemi* complex form a clade distinct from the genera *Pectobacterium* and *Brenneria*, we propose that *P. chrysanthemi* be reclassified as the first species of a novel genus, *Dickeya*, as *Dickeya chrysanthemi*. Six DNA–DNA hybridization groups were delineated within the *E. chrysanthemi* complex corresponding to the six genomic species described herein that we propose to identify as six species. Biochemical characteristics and serological reactions presented above allowed identification of these six species. Therefore, the six genomic species being in accordance with the phylogenetic definition of bacterial species of Wayne *et al.* (1987), we propose for them the following names: *Dickeya chrysanthemi*, *Dickeya dadantii*, *Dickeya dianthicola*, *Dickeya dieffenbachiae*, *Dickeya paradisiaca* and *Dickeya zeae*.

Considering the discrepancies observed between four 16S rRNA gene sequences of *P. cypripedii* deposited in the databases, the phylogenetic position of this species remains uncertain. The strain giving the nearest sequence (GenBank accession no. Z96094) showed such low DNA relatedness with *Dickeya* species that it might be supposed not to be related to this genus.

Description of *Dickeya* gen. nov.

Dickeya (Dic.ke′ya. N.L. fem. n. *Dickeya* after the American phytopathologist Robert S. Dickey, for his contribution to research on the *Erwinia chrysanthemi* complex).

The genus description is based on our data and those of Waldee (1945) and Hauben *et al.* (1998). Cells are Gram-negative rods, 0·5–1·0 × 1·0–3·0 μm with rounded ends. They occur mostly alone or in pairs, but sometimes in chains. Cells are usually motile by means of peritrichous flagella. Facultatively aero-/anaerobic bacteria that catabolize glucose by a fermentative pathway and reduce nitrates to nitrites. Pectinolytic, produce indole and grow at 36 °C. Catabolize (+)-L-arabinose, *myo*-inositol, (+)-D-malate, malonate, D-mannose, mucate, saccharate and *meso*-tartrate, but do not catabolize (+)-D-trehalose, methyl α-glucoside, (+)-D-arabitol or sorbitol. Cause vascular wilts or soft rots on a range of host plants. Members of the genus *Dickeya* form a clade as determined by 16S rRNA gene sequence analyses. G+C contents of the genus range from 56·4 to 59·5 mol%. The type species is *Dickeya chrysanthemi* (Burkholder *et al.* 1953) Samson *et al.*

Description of *Dickeya chrysanthemi* comb. nov.

Dickeya chrysanthemi (chrys.an′the.mi. N.L. gen. n. *chrysanthemi* of the plant genus *Chrysanthemum*).

Basonym: *Pectobacterium chrysanthemi* (Burkholder *et al.* 1953) Brenner *et al.* 1973 emend. Hauben *et al.* 1998.

Has the characteristics of the genus. Additional characteristics are listed in Table 3. Strains belong to two biovars: *chrysanthemi* (from pv. *chrysanthemi* pathogenic to *Chrysanthemum morifolium*; Burkholder *et al.*, 1953) and *parthenii* (from pv. *parthenii* described on gayule, *Parthenium argentatum*; Campbell, 1947). Isolated from soft rot and wilt of various plants, such as *Chrysanthemum* spp., *Cynara scolymus*, *Cichorium intybus*, *Helianthus annuus*,

Table 4. Differential phenotypic patterns of strains belonging to phenon 1

Strain (CFBP no.)	α-Lactose	1-o-Methyl β-galactopyranoside	β-Gentiobiose	(+)-L-Tartrate	L-Alanine	Cellobiose	O-serogroup
Dickeya dadantii							
1269^T	−	−	+	−	−	−	1
4151	−	−	+	−	−	−	6
4152	−	−	+	−	−	−	6
4180	−	−	+	−	−	+	1
4177	−	−	+	−	−	+	1
4153	−	−	+	−	−	+	1
2018	−	−	−	−	−	−	1
3697	+	−	−	−	−	−	1
1613	−	+	−	−	−	+	1
3780	+	+	+	−	−	+	1
3855	−	+	−	−	−	+	1
6467	+	+	−	−	−	+	1
Dickeya zeae							
2052^T	+	+	−	+	−	−	6
1528	+	+	−	+	−	−	10
1884	+	+	−	+	−	−	11
3708	+	+	−	+	−	+	?
4176	+	+	−	+	−	+	6
1871	+	+	−	+	+	+	?
3707	+	+	−	+	+	+	1
6466	+	+	−	+	+	+	10
1496	+	+	−	−	−	−	5
4149	+	+	−	−	−	+	?
4148	+	+	−	−	+	+	?
4150	+	+	−	−	−	+	?
1502	−	+	−	+	−	−	4
1277	−	+	−	+	+	−	9
1447	−	+	−	+	+	+	?
1531	−	−	−	+	−	−	8
3805	−	+	−	−	+	−	11
3804	+	−	−	−	+	−	12
1533	+	−	+	−	−	+	?
***Dickeya* sp.**							
1278	−	+	−	+	+	+	7
1537	+	+	+	−	−	−	7

Lycopersicon esculentum, *Parthenium argentatum* and *Philodendron* spp. Serogroups O:1 and O:2.

The type strain is CFBP 2048^T (=NCPPB 402^T=ICMP 5703^T=LMG 2804^T); its G+C content is 58·8 mol%.

Description of *Dickeya dadantii* sp. nov.

Dickeya dadantii (da.dan.ti′i. N.L. gen. masc. n. *dadantii* of Dadant, in honour of the phytopathologist R. Dadant, for his description of the bacterium isolated from diseased *Pelargonium capitatum*).

Has the characteristics of the genus. Additional characteristics are listed in Table 3. Part of *ex Pectobacterium chrysanthemi* biovar 3. Isolated from soft rot and wilt of a range of plants, such as *Pelargonium capitatum*, *Ananas comosus*, *Dianthus* spp., *Euphorbia pulcherrima*, *Ipomoea batatas*, *Musa* spp., *Philodendron* spp., *Saintpaulia ionantha* and *Zea mays*. Serogroups O:1 and O:6.

The type strain is CFBP 1269^T (=NCPPB 898^T=ICMP 1544^T=Hayward B374^T); its G+C content is 59·5 mol%.

Description of *Dickeya dianthicola* sp. nov.

Dickeya dianthicola (di.an.thi.co′la. N.L. n. *dianthicola* the dweller of *Dianthus* sp.).

The description of the species is after '*Pectobacterium parthenii-dianthicola*' described on *Dianthus* sp. (Hellmers, 1955). Has the characteristics of the genus. Additional

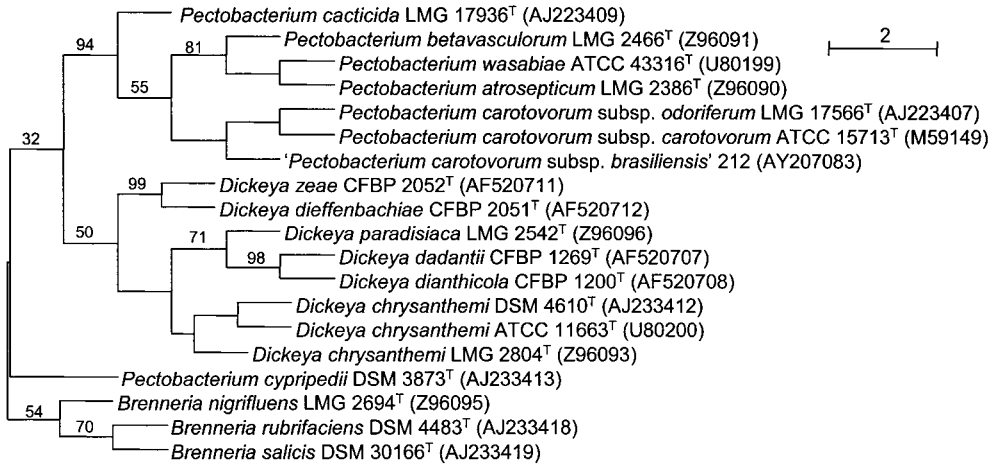

Fig. 2. Unrooted tree, the result of a phylogenetic analysis (parsimony shown) of 16S rRNA gene sequences of type strains of *Dickeya*, *Pectobacterium* and *Brenneria* species. Bootstrap values (expressed as percentages of 1000 replications, BIONJ+Kimura two-parameter) are indicated only for branches also retrieved by BIONJ and maximum-likelihood analyses ($P<0{\cdot}01$).

characteristics are listed in Table 3. Strains belong to *ex Pectobacterium chrysanthemi* biovars 1, 7 and 9. Isolated from soft rot and wilt of various plants, such as *Dianthus* spp., *Cichorium intybus*, *Cynara scolymus*, *Dahlia variabilis*, *Kalanchoe blossfeldiana*, *Lycopersicon esculentum* and *Solanum tuberosum*. Serogroup O : 1.

The type strain is CFBP 1200^T (=NCPPB 453^T=ICMP 6427^T=LMG 2485^T); its G+C content is 59·5 mol%.

Description of *Dickeya dieffenbachiae* sp. nov.

Dickeya dieffenbachiae (dief.fen.ba′chi.ae. N.L. gen. n. *dieffenbachiae* of the plant genus *Dieffenbachia*).

The description (after '*Erwinia dieffenbachiae*' described on *Dieffenbachia* sp.; McFadden, 1961) is the same as for the genus. Additional characteristics are listed in Table 3. Strains belong to *ex Pectobacterium chrysanthemi* biovar 2. Isolated from soft rot and wilt of *Dieffenbachia* spp., *Lycopersicon esculentum* and *Musa* spp. Serogroup O : 1.

The type strain is CFBP 2051^T (=NCPPB 2976^T=ICMP 1568^T); its G+C content is 57·9 mol%.

Description of *Dickeya paradisiaca* comb. nov.

Dickeya paradisiaca (pa.ra.di.si.a′ca. L. fem. adj. *paradisiaca* of or belonging to paradise, referring to the isolation of the organism from *Musa paradisiaca*).

Basonym: *Erwinia paradisiaca* Fernandez-Borrero and Lopez-Duque 1970.

Other synonyms: *Pectobacterium chrysanthemi* biovar 4; *Brenneria paradisiaca*.

The description (after *Erwinia paradisiaca* described on *Musa paradisiaca*; Fernandez-Borrero & Lopez-Duque, 1970) is the same as for the genus. Additional characteristics are listed in Table 3. Strains have been isolated from soft rot and wilt of *Musa* spp. and *Zea mays*. Serogroup O : 3.

The type strain is CFBP 4178^T (=NCPPB 2511^T=LMG 2542^T); its G+C content is 58·0 mol%.

Description of *Dickeya zeae* sp. nov.

Dickeya zeae (ze′ae. N.L. gen. n. *zeae* of the plant genus *Zea*).

The description (after *Erwinia carotovora* f. sp. *zeae* described on maize; Sabet, 1954) is the same as for the genus. Additional characteristics are listed in Table 3. Strains are part of *ex Pectobacterium chrysanthemi* biovars 3 and 8. Isolated from soft rot and wilt of a various range of plants, such as *Zea mays*, *Ananas comosus*, *Brachiaria ruziziensis*, *Chrysanthemum morifolium*, *Musa* spp., *Nicotiana tabacum*, *Oryza sativa* and *Solanum tuberosum*, and from water. More than nine O-serogroups (O : 1, O : 4, O : 5, O : 6, O : 8, O : 9, O : 10, O : 11, O : 12, and untyped strains).

The type strain is CFBP 2052^T (=NCPPB 2538^T=ICMP 5704^T=LMG 2505^T); its G+C content is 56·4 mol%.

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