Emended description of the genus *Pantoea*, description of four species from human clinical samples, *Pantoea septica* sp. nov., *Pantoea eucrina* sp. nov., *Pantoea brenneri* sp. nov. and *Pantoea conspicua* sp. nov., and transfer of *Pectobacterium cypripedii* (Hori 1911) Brenner *et al.* 1973 emend. Hauben *et al.* 1998 to the genus as *Pantoea cypripedii* comb. nov.

Carrie L. Brady,¹ Ilse Cleenwerck,² Stephanus N. Venter,¹ Katrien Engelbeen,² Paul De Vos² and Teresa A. Coutinho¹

Correspondence

Teresa A. Coutinho

teresa.coutinho@fab.up.ac.za

¹Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa

²BCCM/LMG Bacteria Collection, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium

Bacterial strains belonging to DNA hybridization groups (HG) II, IV and V, in the *Erwinia herbicola*–*Enterobacter agglomerans* complex, of Brenner *et al.* [Int J Syst Bacteriol **34** (1984), 45–55] were suggested previously to belong to the genus *Pantoea*, but have never been formally described and classified. Additionally, it has been shown in several studies that *Pectobacterium cypripedii* is more closely related to species of *Pantoea* than to those of *Pectobacterium*. In this study, the phylogenetic positions of Brenner's DNA HG II, IV and V and *Pectobacterium cypripedii* were re-examined by both 16S rRNA gene sequencing and multilocus sequence analyses (MLSA) based on the *gyrB*, *rpoB*, *atpD* and *infB* genes. The analyses revealed that DNA HG II, IV and V and *Pectobacterium cypripedii* form five separate branches within the genus *Pantoea* (strains from HG V were split into two branches). DNA–DNA hybridization data further confirmed that DNA HG II, IV and V constitute four separate species. *Pectobacterium cypripedii* was shown to be a close phylogenetic relative of *Pantoea dispersa* and DNA HG IV by both 16S rRNA gene sequence and MLSA analyses. Biochemical analyses performed on strains from DNA HG II, IV and V and *Pectobacterium cypripedii* confirmed their taxonomic position within the genus *Pantoea* and revealed phenotypic characteristics that allow the differentiation of these species from each other and from their closest phylogenetic neighbours. It is proposed to emend the description of the genus *Pantoea* and to describe *Pantoea septica* sp. nov. for DNA HG II (type strain LMG 5345^T = BD 874^T = CDC 3123-70^T), *Pantoea eucrina* sp. nov. for DNA HG IV (type strain LMG 2781^T = BD 872^T = CDC 1741-71^T = LMG 5346^T), *Pantoea brenneri* sp. nov. for strains of DNA HG V excluding LMG 24534 (type strain LMG 5343^T = BD 873^T = CDC 3482-71^T) and *Pantoea conspicua* sp. nov. for the remaining strain of DNA HG V (type strain LMG 24534^T = BD 805^T = CDC 3527-71^T) and to transfer *Pectobacterium cypripedii* to the genus as *Pantoea cypripedii* comb. nov. (type strain LMG 2657^T = ATCC 29267^T = DSM 3873^T = LMG 2655^T).

Abbreviations: HG, hybridization group; MLSA, multilocus sequence analysis.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are EU216734–EU216737 (16S rRNA gene), EU145261–EU145273 and FJ187830–FJ187833 (*gyrB* gene), EU145293–EU145305 and FJ187840–FJ187843 (*rpoB* gene), EU145245–EU145257 and FJ187825–FJ187828 (*atpD* gene) and EU145277–EU145289 and FJ187835–FJ187838 (*infB* gene), as detailed in Supplementary Table S1.

Details of sequence accession numbers and a UPGMA dendrogram based on phenotypic characteristics are available as supplementary material with the online version of this paper.

Since the early 1970s, various attempts have been made to clarify the *Erwinia herbicola*–*Enterobacter agglomerans* complex (Ewing & Fife, 1972; Gardner & Kado, 1972; Young *et al.*, 1978; Dye, 1981; Brenner *et al.*, 1984; Verdonck *et al.*, 1987; Beji *et al.*, 1988). The most comprehensive study to date was performed by Brenner *et al.* (1984), who separated 124 strains belonging to this complex into 13 groups based on their DNA–DNA hybridization relatedness. The majority of strains used in Brenner's study were clinical strains implicated in a nosocomial septicaemia outbreak in 1971 (Maki *et al.*, 1976), although plant-pathogenic strains from the complex were also included. In the years following Brenner's hybridization study, many of the 13 DNA hybridization groups (HG) were further investigated and classified as novel species in existing or novel genera. Gavini *et al.* (1989) transferred DNA HG XIII and DNA HG III to the novel genus *Pantoea* as *Pantoea agglomerans* and *Pantoea dispersa*, respectively. Mergaert *et al.* (1993) proposed the name *Pantoea ananatis* for DNA HG VI, containing strains of *Erwinia ananas* and its synonym *Erwinia uredovora*, whilst DNA HG VII–XII were assigned to species within the genera *Enterobacter*, *Rahnella* and *Leclercia* (Grimont & Grimont, 2005). Beji *et al.* (1988) suggested the inclusion of Brenner's DNA HG V into *Enterobacter agglomerans* (now *Pantoea agglomerans*), although only 62% DNA relatedness was observed between the reference strain (CDC 3482-71 = LMG 5343) and *Pantoea agglomerans* ATCC 27155^T. However, Gavini *et al.* (1989) opposed this recommendation and proposed to unite Brenner's DNA HG V with protein profile group VII (Beji *et al.*, 1988) in a new genus and species, a proposal that has never been implemented.

Recently, polyphasic taxonomic studies on strains from Beji's protein profile group VII, *Pantoea* reference strains as well as possible *Pantoea* species isolated from eucalyptus and maize resulted in the description of four novel species (*Pantoea anthophila*, *Pantoea vagans*, *Pantoea eucalypti* and *Pantoea deleyi*) and the transfer of *Pantoea citrea*, *Pantoea punctata* and *Pantoea terrea* to the genus *Tatumella* (Brady *et al.*, 2009, 2010). Multilocus sequence analysis (MLSA) based on *gyrB*, *rpoB*, *atpD* and *infB* sequences was used in these studies as a supporting phylogenetic technique, since these genes were previously shown to be useful phylogenetic markers for *Pantoea* (Brady *et al.*, 2008). In that study, strains from DNA HG II, IV and V (referred to as MLSA groups E, F, H and I) and *Pectobacterium cypripedii* were suggested to be members of the genus *Pantoea*. Previous studies have also questioned the taxonomic position of *Pectobacterium cypripedii*, as it does not cluster with other *Pectobacterium* species and several different 16S rRNA gene sequences are available for the type strain of this species (Spröer *et al.*, 1999; Samson *et al.*, 2005; Young & Park, 2007; Naum *et al.*, 2008; Yarza *et al.*, 2008). In this study, we propose the taxonomic reclassification of strains from Brenner's DNA HG II, IV and V and *Pectobacterium cypripedii* within the genus *Pantoea*.

Strains

Strains used in this study are listed in Table 1. Strains belonging to Brenner's DNA HG II, IV and V were kindly provided by Mrs Caroline Mohr from the Centers for Disease Control (CDC), Atlanta, GA, USA. Strains from Brenner's DNA HG I could not be acquired. Details of individual sequence accession numbers are given in Supplementary Table S1, available in IJSEM Online.

16S rRNA gene sequencing

Genomic DNA was extracted from strains using an alkali extraction method (Niemann *et al.*, 1997) and stored at –20 °C until further use. Almost-complete 16S rRNA gene sequences were determined for representative strains from each DNA HG, LMG 5345^T, LMG 2781^T, LMG 5343^T, LMG 24534^T, and the type strain of *Pectobacterium cypripedii*, LMG 2657^T, using the primers and conditions described by Coenye *et al.* (1999). The 16S rRNA gene sequence of LMG 2657^T was compared to that of *Pectobacterium cypripedii* DSM 3873^T (GenBank accession no. AJ233413), as used in the All Species Living Tree (Yarza *et al.*, 2008), to ascertain whether the sequence was correct, as several different sequences are available for this strain. The sequences were aligned using CLUSTAL_X (Thompson *et al.*, 1997) and the overhangs were trimmed. The MODELTEST 3.7 program (Posada & Crandall, 1998) was then applied to determine the best-fit evolutionary model. Maximum-likelihood and neighbour-joining analyses were performed using Phym1 (Guindon & Gascuel, 2003) and PAUP 4.0b10 (Swofford, 2000), respectively, by applying the models and parameters determined by MODELTEST (only maximum-likelihood phylogenetic trees are shown). Bootstrap analysis with 1000 replicates was performed to assess the support for these clusters.

Strains LMG 5343^T and LMG 24534^T, from Brenner's DNA HG V, demonstrated high 16S rRNA gene sequence similarity to each other (>99%), and to strains of *Pantoea agglomerans*, *Pantoea ananatis*, *Pantoea anthophila*, *Pantoea deleyi*, *Pantoea eucalypti*, *Pantoea stewartii* and *Pantoea vagans* (>98%), which are regarded as the 'core' *Pantoea* species. These strains cluster closely with the type strain of *Pantoea deleyi* (Fig. 1) with a high bootstrap value but on separate branches, suggesting that they belong to different species. Strain LMG 5345^T, from Brenner's DNA HG II, shares more than 98% 16S rRNA gene sequence similarity with LMG 5343^T and the type strains of *Pantoea agglomerans*, *Pantoea vagans* and *Pantoea eucalypti*. This strain is situated at the border of the 'core' *Pantoea* group. Strain LMG 2781^T, from Brenner's DNA HG IV, demonstrated more than 98% 16S rRNA gene sequence similarity to the type strains of *Pantoea agglomerans* and *Pantoea dispersa* and clusters close to the type strain of the latter species but with low bootstrap support. *Pectobacterium cypripedii* DSM 3873^T shows higher 16S rRNA gene sequence similarity to species of *Pantoea* (96.8–98.0%) and *Erwinia* (94.6–97.7%) than to the other species of *Pectobacterium* (95.3–96.4%). This type strain

Table 1. Strains used in this study

Collections: LMG, BCCM/LMG Bacteria Collection, Ghent University, Belgium; CDC, Centers for Disease Control, Atlanta, GA, USA; BD, Plant Pathogenic and Plant Protecting Bacteria (PPPPB) Culture Collection, ARC-PPRI, Pretoria, South Africa.

Strain	Source	Location
<i>Pantoea agglomerans</i>		
LMG 1286 ^T	Human	Zimbabwe
LMG 2660	<i>Wisteria floribunda</i>	Japan
<i>Pantoea ananatis</i>		
LMG 2665 ^T	Pineapple	Brazil
LMG 20103	Eucalyptus	South Africa
LMG 24190	Onion	South Africa
<i>Pantoea dispersa</i>		
LMG 2603 ^T	Soil	Japan
LMG 2604	Wild rose	Netherlands
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>		
LMG 2715 ^T	Maize	USA
LMG 2718	Maize	USA
<i>Pantoea stewartii</i> subsp. <i>indologenes</i>		
LMG 2632 ^T	Fox millet	India
LMG 2673	Pineapple	Hawaii, USA
<i>Pantoea vagans</i>		
LMG 24199 ^T	Eucalyptus	Uganda
LMG 24201	Maize	South Africa
<i>Pantoea eucalypti</i>		
LMG 24197 ^T	Eucalyptus	Uruguay
LMG 24198	Eucalyptus	Uruguay
<i>Pantoea deleyi</i>		
LMG 24200 ^T	Eucalyptus	Uganda
<i>Pantoea anthophila</i>		
LMG 2558 ^T	<i>Impatiens balsamina</i>	India
LMG 2560	<i>Tagetes erecta</i>	Unknown
<i>Pantoea cypripedii</i> comb. nov.		
LMG 2655 ^T = LMG 2657 ^T	Orchid	USA
LMG 2654	Orchid	USA
LMG 2658	Orchid	Unknown
<i>Pantoea septica</i> sp. nov. (HG II)		
LMG 5345 ^T = BD 874 ^T = CDC 3123-70 ^T	Human, stool	New Jersey, USA
LMG 24526 = CDC 238-70	Human, blood	New York, USA
LMG 24527 = CDC 1778-70	Human, blood	Oklahoma, USA
LMG 24528 = CDC 217-71	Human, skin	Virginia, USA
<i>Pantoea eucrina</i> sp. nov. (HG IV)		
LMG 2781 ^T = BD 872 ^T = CDC 1741-71 ^T = LMG 5346 ^T	Human, trachea	Connecticut, USA
LMG 24529 = CDC 3638-70	Human, cyst	Georgia, USA
LMG 24530 = CDC 5795-70	Human, urine	Virginia, USA
LMG 24531 = CDC 6148-70	Human, spinal fluid	Hawaii, USA
<i>Pantoea brenneri</i> sp. nov. (HG V)		
LMG 5343 ^T = BD 873 ^T = CDC 3482-71 ^T	Human, urethra	Montana, USA
LMG 24532 = CDC 2928-68	Human, sputum	Wisconsin, USA
LMG 24533 = CDC 2525-70	Unknown	Quebec, Canada
<i>Pantoea conspicua</i> sp. nov. (HG V)		
LMG 24534 ^T = BD 805 ^T = CDC 3527-71 ^T	Human, blood	Paris, France

clusters between the *Erwinia* and *Pantoea* clusters, noticeably distant from the other *Pectobacterium* species. Species of *Erwinia*, *Pectobacterium*, *Brenneria* and *Pantoea* can be characterized to the genus level by 14–21 signature nucleotides in

their 16S rRNA gene sequences according to Hauben & Swings (2005). The 16S rRNA gene sequences of *Pectobacterium cypripedii* and all recognized *Pantoea* species were examined closely for the signature nucleotides using the

Fig. 1. Maximum-likelihood tree based on almost-complete 16S rRNA gene sequences of members of *Pantoea* and phylogenetically related species. Bootstrap values after 1000 replicates are expressed as percentages. *Escherichia coli* ATCC 11775^T was included as an outgroup. Bar, 0.1 substitutions per nucleotide position.

Escherichia coli numbering (Brosius *et al.*, 1978). *Pectobacterium cypripedii* shares ten common signature nucleotides with *Pantoea* species and seven with *Erwinia* species but only four with other *Pectobacterium* species, whilst three

signature nucleotides were shown to be unique to *Pectobacterium cypripedii* (C at positions 847, 1216 and 1217). Furthermore, *Pectobacterium cypripedii* was shown to share 20 of the 23 *atpD* gene signature nucleotides that can be

used to characterize species as belonging to *Pantoea* (Brady *et al.*, 2010).

The genus *Pantoea* is polyphyletic, based on 16S rRNA gene sequence analysis, with *Pantoea dispersa*, *Pectobacterium cypripedii* and strain LMG 2781^T clustering separately from the remaining 'core' species (Brady *et al.*, 2008). MLSA of partial nucleotide sequences of the housekeeping genes *gyrB*, *rpoB*, *atpD* and *infB* is a more useful technique than 16S rRNA gene sequence analysis for examining the phylogeny of *Pantoea* species and strains (Brady *et al.*, 2008).

Analysis of *gyrB*, *rpoB*, *atpD* and *infB* sequences

Partial *gyrB* (742 bp), *rpoB* (637 bp), *atpD* (657 bp) and *infB* (615 bp) gene sequences were determined for all strains (Brady *et al.*, 2008). Sequence analysis and tree construction were performed as described above, and a phylogenetic tree based on concatenated sequences of the four genes is presented in Fig. 2. The phylogenetic tree demonstrates that Brenner's DNA HG II, IV and V and *Pectobacterium cypripedii* form five separate well-supported clusters within the genus, distinct from the existing *Pantoea* species with validly published names. Strains from Brenner's DNA HG V cluster close to *Pantoea agglomerans*, *Pantoea eucalypti*, *Pantoea vagans*, *Pantoea anthophila* and *Pantoea deleyi* with high bootstrap support (100%). LMG 24534^T clusters distant from the other strains within this group, corroborating the existence of two different species within Brenner's DNA HG V. Brenner *et al.* (1984) noted that aerogenic and anaerogenic strains were found simultaneously in two DNA hybridization groups only, HG II and V. These DNA groups consisted of anaerogenic strains, except for LMG 24527 (=CDC 1778-80) in Brenner's DNA HG II and LMG 24534^T (=CDC 3527-71^T) in Brenner's DNA HG V. In Fig. 2, LMG 24527 clusters close to the other strains from Brenner's DNA HG II, whereas LMG 24534^T is separated from the rest of Brenner's DNA HG V. Strains from DNA HG II and IV cluster between *Pantoea ananatis* and *Pantoea dispersa*, whilst *Pectobacterium cypripedii* clusters with DNA HG IV and *Pantoea dispersa* with high bootstrap support. The MLSA data, together with the 16S rRNA gene sequence analysis, suggest that Brenner's DNA HG II, IV and V represent four novel species within the genus *Pantoea* and indicate that *Pectobacterium cypripedii* should be transferred to this genus.

DNA–DNA hybridizations

High-quality DNA for DNA–DNA hybridizations was prepared by the method of Wilson (1987), with minor modifications (Cleenwerck *et al.*, 2002). DNA–DNA hybridizations were performed using the microplate method (Ezaki *et al.*, 1989) with some modifications (Cleenwerck *et al.*, 2002) at a hybridization temperature of 45 ± 1 °C. Reciprocal reactions (A × B and B × A) were performed for every DNA pair from all strains and their variation was

within the limits of this method (Goris *et al.*, 1998). The values presented are based on a minimum of four replicates. Strains from each DNA HG were hybridized amongst each group and LMG 24534^T (the aerogenic strain from Brenner's DNA HG V) was hybridized to reference strains from these groups (LMG 5345^T, LMG 2781^T and LMG 5343^T). These four reference strains were also hybridized to the type strains of *Pantoea agglomerans*, *Pantoea ananatis*, *Pantoea anthophila*, *Pantoea deleyi*, *Pantoea dispersa*, *Pantoea eucalypti*, *Pantoea stewartii* and *Pantoea vagans*. The type strain of *Pectobacterium cypripedii* was hybridized to the type strain of *Pantoea dispersa*, the only species with which it shares more than 97% 16S rRNA gene sequence similarity. A summary of the DNA–DNA hybridization results is presented in Table 2 and reveals that the DNA–DNA relatedness within each DNA HG was greater than 70%. Strains LMG 5345^T, LMG 2781^T, LMG 5343^T and LMG 24534^T exhibited less than 70% DNA–DNA relatedness with each other and with the type strains of *Pantoea* species with validly published names. Strain LMG 24534^T, the aerogenic strain from Brenner's DNA HG V, exhibited only 55–60% DNA–DNA relatedness when hybridized with other strains from this DNA HG. This is considerably lower than the 73% observed by Brenner *et al.* (1984). The lower values obtained in our study may be due to the different hybridization method used; however, our DNA–DNA hybridization data are congruent with the MLSA data.

DNA G+C content

The DNA G+C content of the type strains proposed for the four novel species were determined by HPLC (Mesbah *et al.*, 1989) and are as follows: LMG 5345^T, 59.3 mol%; LMG 2781^T, 56.5 mol%; LMG 5343^T, 55.4 mol%; and LMG 24534^T, 55.7 mol%. These values are consistent with the DNA G+C contents of other members of the genus *Pantoea* (Brady *et al.*, 2009; Grimont & Grimont, 2005).

Phenotypic assays

Physiological and biochemical tests were performed on the type strains proposed for the four novel species (LMG 5345^T, LMG 2781^T, LMG 5343^T and LMG 24534^T) using API 50CHB/E and Biotype-100 (bioMérieux) according to the manufacturer's instructions. Strains LMG 5345^T, LMG 2781^T and LMG 5343^T were used as controls to compare results generated in this study to those reported in *Bergey's Manual of Systematic Bacteriology* (Grimont & Grimont, 2005) to ensure that the data agreed with those available in literature. Strain LMG 24534^T was included to provide phenotypic discrimination between the two species in Brenner's DNA HG V. Generally, a good correlation was observed between the two datasets, although some characteristics reported as variable by Grimont & Grimont (2005) were positive or negative for the type strains in our study. A certain level of phenotypic variability within species of the genus *Pantoea* has been observed previously (Delétoile *et al.*, 2009), and may account for these minor differences.

Fig. 2. Maximum-likelihood tree based on concatenated housekeeping gene sequences of *Pantoea* strains. Bootstrap values after 1000 replicates are expressed as percentages. *Escherichia coli* K-12 was included as an outgroup. Gene sequences for *Erwinia tasmaniensis* Et1/99^T, *Escherichia coli* ATCC 11775^T, *Shigella dysenteriae* Sd197, *Klebsiella pneumoniae* 342, *Citrobacter rodentium* ICC168, *Citrobacter koseri* ATCC BAA-895 and *Cronobacter sakazakii* ATCC BAA-894 were obtained from genome sequencing databases (<http://www.ncbi.nlm.nih.gov>, <http://www.sanger.ac.uk>, <http://asap.ahabs.wisc.edu/asap/home.php>). Bar, 0.1 substitutions per nucleotide position.

API 50 CHB/E (bioMérieux) and GN2 MicroPlate (Biolog) tests were performed on all *Pectobacterium cyripedii* strains used in this study, as very little phenotypic information was available for *Pectobacterium cyripedii*. Relevant phenotypic results are given in the species descriptions. A selection of 50 phenotypic characteristics was used to construct a UPGMA dendrogram (Supplementary Fig. S1, available in IJSEM Online) in BioNumerics 5.0 (Applied Maths) to demonstrate the phenotypic relationship between the

strains. The four novel species and *Pectobacterium cyripedii* clustered in the midst of the 'core' *Pantoea* species, showing them to be phenotypically related but clearly distinct taxa of the genus. The four novel species can be differentiated from each other by their reactions to dulcitol, erythritol, raffinose, L-tartrate and meso-tartrate. *Pectobacterium cyripedii* shares multiple phenotypic similarities with *Pantoea* species, including production of glucose dehydrogenase and gluconate dehydrogenase, acid production from L-arabinose,

Table 2. DNA–DNA hybridization amongst strains belonging to the novel species and reference strains of *Pantoea* species with validly published names

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
1. <i>P. cyripedii</i> comb. nov. LMG 2657 ^T	100												
<i>P. septica</i> sp. nov. (HG II)													
2. LMG 5345 ^T		100	78	78	92	31				32			27
3. LMG 24526			100	84	77								
4. LMG 24527				100	73								
5. LMG 24528					100								
<i>P. eucrinea</i> sp. nov. (HG IV)													
6. LMG 2781 ^T						100	90	89	93	21			24
7. LMG 24529							100	95	87				
8. LMG 24530								100	95				
9. LMG 24531									100				
<i>P. brenneri</i> sp. nov. (HG V)													
10. LMG 5343 ^T										100	93	91	58
11. LMG 24532											100	97	60
12. LMG 24533												100	55
13. <i>P. conspicua</i> sp. nov. LMG 24534 ^T (HG V)													100
<i>P. agglomerans</i> LMG 1286 ^T		29				24				48			37
<i>P. ananatis</i> LMG 2665 ^T		22				19				29			19
<i>P. stewartii</i> subsp. <i>stewartii</i> LMG 2715 ^T		14				12				14			14
<i>P. dispersa</i> LMG 2603 ^T	26	30				30				26			25
<i>P. vagans</i> LMG 24199 ^T		32				20				37			40
<i>P. eucalypti</i> LMG 24197 ^T		25				31				38			41
<i>P. deleyi</i> LMG 24200 ^T		32				28				43			36
<i>P. anthophila</i> LMG 2558 ^T		29				29				40			34

fructose, maltose and trehalose and the lack of H₂S and pectinase production. The most distinctive phenotypic characteristics that can differentiate between the four novel species, *Pectobacterium cyripedii* and their closest phylogenetic relatives are listed in Table 3. The biochemical profile of *Pantoea conspicua* sp. nov. is currently based on a single strain; as more strains of this species are isolated and tested, the profile may change slightly.

Conclusions

DNA HG II, IV and V from the hybridization study performed on the *Erwinia herbicola*–*Enterobacter agglomerans* complex by Brenner *et al.* (1984) are among the last of the original 13 DNA groups to be classified. This is in part due to the difficulties previously experienced in differentiating *Pantoea* species, both phenotypically and phylogenetically. These difficulties can be overcome by applying MLSA to support data from phenotypic tests and 16S rRNA gene sequencing. The results presented in this study indicate that *Pectobacterium cyripedii* should be transferred to the genus *Pantoea*, of which the description should be emended, as *Pantoea cyripedii* comb. nov. and that Brenner's DNA HG II, IV and V represent four novel species within the genus, *Pantoea septica* sp. nov. (for HG II), *Pantoea eucrinea* sp. nov. (for HG IV), *Pantoea brenneri* sp. nov. (for most strains of HG V) and *Pantoea conspicua* sp. nov. (for strain LMG 24534^T from HG V).

Emended description of the genus *Pantoea* Gavini *et al.* 1989 emend. Mergaert *et al.* 1993

Pantoea [Pan.to'e.a. Gr. adj. *pantoios* of all sorts and sources; N.L. fem. n. *Pantoea* (bacteria) from diverse (geographical and ecological) sources].

The description is based on the data of Gavini *et al.* (1989), Mergaert *et al.* (1993), Grimont & Grimont (2005) and Brady *et al.* (2009, 2010) and data obtained in this study. Straight rods, 0.5–1.3 × 1.0–3.0 µm. Non-encapsulated. Non-spore-forming. Some strains form symplasmata. Most strains are motile by means of peritrichous flagella. Gram-negative. Colonies on nutrient agar are smooth, translucent and convex with entire margins or heterogeneous in consistency and adhering to the agar. Colonies are yellow, beige or non-pigmented. Facultatively anaerobic. Optimum growth temperature between 28 and 30 °C. Oxidase-negative. Glucose dehydrogenase and gluconate dehydrogenase are produced and are active without an added cofactor. Lysine and ornithine are not decarboxylated. Urease-negative. Pectate is not degraded. H₂S is not produced from thiosulphate. Most strains are Voges–Proskauer-positive and indole-negative. Acid is produced from the fermentation of L-arabinose, D-ribose, D-xylose, D-galactose, D-fructose, L-rhamnose, D-mannitol, N-acetylglucosamine, maltose and trehalose. Carbon sources utilized at 28 °C (Biotype-100) are D-glucose, D-fructose, D-galactose, trehalose, D-mannose, cellobiose, 1-O-methyl

Table 3. Phenotypic characteristics that distinguish the novel members of *Pantoea* from each other and from their closest phylogenetic neighbours

Species/subspecies: 1, *Pantoea agglomerans*; 2, *Pantoea ananatis*; 3, *Pantoea dispersa*; 4, *Pantoea stewartii* subsp. *stewartii*; 5, *Pantoea stewartii* subsp. *indologenes*; 6, *Pantoea vagans*; 7, *Pantoea eucalypti*; 8, *Pantoea deleyi*; 9, *Pantoea anthophila*; 10, *Pantoea cypripedii* comb. nov.; 11, *Pantoea septica* sp. nov.; 12, *Pantoea eucrina* sp. nov.; 13, *Pantoea brenneri* sp. nov.; 14, *Pantoea conspicua* sp. nov. +, 90–100% strains positive in 1–2 days; (+), 90–100% strains positive in 1–4 days; d, 11–89% strains positive in 1–4 days; (d), 11–89% strains positive in 3–4 days; –, negative; ND, not determined. Data were taken from the following sources: columns 1–5, Grimont & Grimont (2005) and Brady *et al.* (2009); 6–9, Brady *et al.* (2009); 10, 14, this study; 11–13, Grimont & Grimont (2005) and this study.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Indole production	–	+	–	–	+	–	–	–	–	–	–	–	–	–
Phenylalanine deaminase	d	–	–	–	–	+	+	+	+	+	+	d	d	+
Acid production from:*														
Glycerol	(d)	+	(d)	–	d	d	d	+	+	+	d	–	d	–
Dulcitol	–	–	–	–	–	–	–	–	–	–	–	–	d	+
Inositol	(d)	+	d	–	+	+	+	–	+	+	d	d	d	+
Cellobiose	d	+	d	–	+	–	d	–	+	+	d	d	d	+
Lactose	d	+	d	–	+	d	d	–	–	–	d	d	d	+
Raffinose	(d)	+	–	+	+	–	–	–	–	–	–	–	d	–
Utilization of:†														
Melibiose	–	+	–	+	+	–	–	–	–	+	d	–	–	–
Raffinose	–	+	–	+	+	–	–	–	–	–	d	–	d	–
Maltose	(+)	+	+	–	+	+	+	+	+	+	d	+	+	+
Lactose	–	+	–	d	+	–	+	–	–	+	d	–	(d)	+
Lactulose	–	+	–	d	+	–	–	–	–	–	d	–	d	–
Gentiobiose	–	+	+	–	d	d	–	+	–	+	d	d	–	+
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	–	+	+
L-Rhamnose	+	d	+	+	d	+	+	+	+	+	+	d	+	+
Dulcitol	–	–	(d)	–	–	–	–	–	–	–	(d)	–	–	+
D-Sorbitol	–	d	–	–	–	–	–	–	–	+	(d)	–	–	–
Adonitol	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Erythritol	–	–	+	–	–	–	–	–	–	–	–	d	–	–
L-Tartrate	–	–	d	–	–	+	+	+	–	ND	d	–	d	+
meso-Tartrate	(+)	d	d	+	+	+	+	+	+	ND	d	–	(+)	–

*Data from API 50 CHB/E system.

†Data from Biotype-100, GN2 MicroPlate.

β -D-glucopyranoside, L-arabinose, glycerol, inositol, D-saccharate, *cis*-aconitate, D-glucuronate, D-galacturonate, N-acetylglucosamine, D-gluconate, DL-lactate, L-histidine, L-aspartate, L-glutamate, L-alanine and L-serine. Carbon sources not utilized at 28 °C (Biotype-100) are L-sorbose, palatinose, melezitose, maltitol, turanose, tricarballoylate, 4-hydroxybenzoate, gentisate, 3-hydroxybenzoate, benzoate, 3-phenylpropionate, *m*-coumarate, histamine, caprate, caprylate, glutarate, 5-aminovalerate, ethanolamine,

tryptamine, itaconate, 3-hydroxybutyrate, propionate and L-tyrosine. Strains have been isolated from plants, seeds, fruits, soils and water and from humans (urine, blood, wounds, internal organs) and other animals. Strains of several species are phytopathogenic on a wide range of plant and agricultural hosts. The G+C content of the DNA ranges from 52.7 to 60.6 mol%. The type species is *Pantoea agglomerans* (Ewing and Fife 1972) Gavini *et al.* 1989.

Description of *Pantoea septica* sp. nov.

Pantoea septica (sep'ti.ca. N.L. fem. adj. *septica* from Gr. adj. *septikos* putrefying, decaying or septic, referring to the septicaemia outbreak associated with these strains).

Displays the following properties in addition to those in the emended genus description. Cells are motile, 0.9×1.5 – $3.0 \mu\text{m}$, occurring singly or in pairs. Colonies are beige, round, convex and smooth with entire margins. Indole is not produced. Phenylalanine deaminase reaction is weakly positive. Acid is produced from the substrates listed in the genus description; some strains can also produce acid from glycerol, inositol, cellobiose and lactose. Carbon sources utilized at 28 °C are as listed in the genus description and D-xylose, L-rhamnose, D-mannitol and D-malate. Some strains can utilize melibiose, sucrose, raffinose, maltotriose, maltose, lactose, lactulose, gentiobiose, L-fucose, D-arabitol, xylitol, dulcitol (weakly), D-sorbitol (weakly), L-tartrate, *meso*-tartrate and citrate. Carbon sources not utilized at 28 °C are as listed in the genus description and L-arabitol, D-tagatose, adonitol, erythritol, 3-O-methyl D-glucopyranose, D-tartrate, quinate and betaine. The DNA G+C content of the type strain is 59.3 mol%. Strains belonging to this species were implicated in a nationwide septicaemia outbreak in the USA in 1971.

The type strain is LMG 5345^T (=BD 874^T =CDC 3123-70^T), isolated from a human stool sample in New Jersey, USA.

Description of *Pantoea eucrina* sp. nov.

Pantoea eucrina (eu.cri'na. N.L. fem. adj. *eucrina* from Gr. adj. *eukrines* well-separated, referring to the clear separation of the strains from other species within the genus).

Displays the following properties in addition to those in the emended genus description. Cells are motile, 0.9×1.5 – $3.0 \mu\text{m}$, occurring singly or in pairs. Colonies are beige, round, convex and smooth with entire margins. Indole is not produced. Phenylalanine deaminase reaction is weakly positive. Acid is produced from the substrates listed in the genus description; some strains can also produce acid from inositol, cellobiose and lactose. Carbon sources utilized at 28 °C are as listed in the genus description and sucrose, maltotriose, maltose, D-arabitol, L-arabitol, xylitol, D-mannitol, adonitol and citrate. Some strains can utilize gentiobiose, L-rhamnose and erythritol. Carbon sources not utilized at 28 °C are as listed in the genus description

and melibiose, raffinose, lactose, lactulose, D-xylose, L-fucose, dulcitol, D-tagatose, D-sorbitol, 3-O-methyl D-glucopyranose, L-, D- and *meso*-tartrate, D-malate, quinate and betaine. The DNA G+C content of the type strain is 56.5 mol%. Strains belonging to this species were implicated in a nationwide septicaemia outbreak in the USA in 1971.

The type strain is LMG 2781^T (=BD 872^T =CDC 1741-71^T =LMG 5346^T), isolated from a human trachea in Connecticut, USA.

Description of *Pantoea brenneri* sp. nov.

Pantoea brenneri (bren'ne.ri. N.L. gen. n. *brenneri* of Brenner, named after Don J. Brenner, in recognition of his contribution in resolving the *Erwinia herbicola*-*Enterobacter agglomerans* complex).

Displays the following properties in addition to those in the emended genus description. Cells are motile, 0.9 × 1.5–3.0 µm, occurring singly or in pairs. Colonies are beige, round, convex and smooth with entire margins. Indole is not produced. Phenylalanine deaminase reaction is weakly positive. Acid is produced from the substrates listed in the genus description; some strains can also produce acid from glycerol, dulcitol, inositol, D-sorbitol, cellobiose, lactose and raffinose. Carbon sources utilized at 28 °C are as listed in the genus description and sucrose, maltotriose, maltose, D-xylose, L-rhamnose, D-mannitol, *meso*-tartrate, D-malate and citrate. Some strains can utilize raffinose, lactose (weakly), lactulose, L-fucose, D-arabitol and L-tartrate. Carbon sources not utilized at 28 °C are as listed in the genus description as well as melibiose, gentiobiose, L-arabitol, xylitol, dulcitol, D-tagatose, D-sorbitol, adonitol, erythritol, 3-O-methyl D-glucopyranose, D-tartrate, quinate and betaine. The DNA G+C content of the type strain is 55.4 mol%. Strains belonging to this species were implicated in a nationwide septicaemia outbreak in the USA in 1971.

The type strain is LMG 5343^T (=BD 873^T =CDC 3482-71^T), isolated from a human urethra in Montana, USA.

Description of *Pantoea conspicua* sp. nov.

Pantoea conspicua (con.sp'i'cu.a. L. fem. adj. *conspicua* conspicuous, referring to the conspicuous separation from other strains within DNA HG V).

Displays the following properties in addition to those in the emended genus description. Cells are motile, 0.9 × 1.5–3.0 µm, occurring singly or in pairs. Colonies are beige, round, convex and smooth with entire margins. Aerogenic. Indole is not produced. Phenylalanine deaminase reaction is weakly positive. Acid is produced from the substrates listed in the genus description and from dulcitol, inositol, cellobiose and lactose. Carbon sources utilized at 28 °C are as listed in the genus description as well as maltotriose, maltose, lactose, gentiobiose, D-ribose, D-xylose, L-rhamnose, L-fucose, D-arabitol, dulcitol, D-mannitol, L-tartrate,

D-malate and citrate. Carbon sources not utilized at 28 °C are as listed in the genus description as well as melibiose, sucrose, raffinose, lactulose, L-arabitol, xylitol, D-tagatose, D-sorbitol, adonitol, erythritol, 3-O-methyl D-glucopyranose, D-tartrate, *meso*-tartrate, quinate and betaine. The DNA G+C content of the type strain is 55.7 mol%.

The type strain is LMG 24534^T (=BD 805^T =CDC 3527-71^T), isolated from a human blood sample in Paris, France.

Description of *Pantoea cypripedii* (Hori 1911) comb. nov.

Pantoea cypripedii (cyp.ri.pe'di.i. N.L. n. *Cypripedium* botanical name for a genus of orchid; N.L. gen. n. *cypripedii* of cypripedium orchids).

Basonym: *Pectobacterium cypripedii* (Hori 1911) Brenner *et al.* 1973.

Other synonyms: '*Bacillus cypripedii*' Hori 1911, '*Erwinia carotovora* var. *cypripedii*' (Hori 1911) Dye 1969, *Erwinia cypripedii* (Hori 1911) Bergey *et al.* 1923.

Displays the following properties in addition to those in the emended genus description. Cells are motile, 0.5–1.0 × 1.0–3.0 µm, occurring singly or in pairs. Colonies are beige, round, convex and smooth with entire margins. Indole is not produced. Phenylalanine deaminase reaction is weakly positive. Acid is produced from the substrates listed in the genus description and from glycerol (weakly), inositol (weakly) and cellobiose. Carbon sources utilized at 28 °C are as listed in the genus description as well as melibiose, sucrose, maltose, lactose, gentiobiose, D-ribose, D-xylose, L-rhamnose, D-arabitol, D-mannitol, D-sorbitol (weakly), D-tartrate, D-malate, citrate and quinate. Carbon sources not utilized at 28 °C are as listed in the genus description as well as raffinose, lactulose, L-fucose, L-arabitol, xylitol, dulcitol, D-tagatose, adonitol, erythritol and betaine. The DNA G+C content is 54.1–54.6 mol%.

The type strain, LMG 2657^T (=ATCC 29267^T =DSM 3873^T =LMG 2655^T), was isolated from a cypripedium orchid in California, USA.

Acknowledgements

This study was partially supported by the South African-Flemish Bilateral Agreement, the National Research Foundation (NRF), the Tree Protection Co-operative Programme (TPCP) and the THRIP support programme of the Department of Trade and Industry, South Africa. The BCCM/LMG Bacteria collection is supported by the Federal Public Planning Service-Science Policy, Belgium. The authors wish to acknowledge Katrien Vandemeulebroecke for technical assistance and Mrs Caroline Mohr for providing us with the CDC strains.

References

- Beji, A., Mergaert, J., Gavini, F., Izard, D., Kersters, K., Leclerc, H. & De Ley, J. (1988). Subjective synonymy of *Erwinia herbicola*, *Erwinia*

- millietiae*, and *Enterobacter agglomerans* and redefinition of the taxon by genotypic and phenotypic data. *Int J Syst Bacteriol* **38**, 77–88.
- Brady, C. L., Cleenwerck, I., Venter, S. N., Vancanneyt, M., Swings, J. & Coutinho, T. A. (2008). Phylogeny and identification of *Pantoea* species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). *Syst Appl Microbiol* **31**, 447–460.
- Brady, C. L., Venter, S. N., Cleenwerck, I., Engelbeen, K., Vancanneyt, M., Swings, J. & Coutinho, T. A. (2009). *Pantoea vagans* sp. nov., *Pantoea eucalypti* sp. nov., *Pantoea deleyi* sp. nov. and *Pantoea anthophila* sp. nov. *Int J Syst Evol Microbiol* **59**, 2339–2345.
- Brady, C. L., Venter, S. N., Cleenwerck, I., Vandemeulebroecke, K., De Vos, P. & Coutinho, T. A. (2010). Transfer of *Pantoea citrea*, *Pantoea punctata* and *Pantoea terre* to the genus *Tatumella* emend. as *Tatumella citrea* comb. nov., *Tatumella punctata* comb. nov. and *Tatumella terre* comb. nov. and description of *Tatumella morbiroesei* sp. nov. *Int J Syst Evol Microbiol* **60**, 484–494.
- Brenner, D. J., Fanning, G. R., Leete Knutson, J. K., Steigerwalt, A. G. & Krichevsky, M. I. (1984). Attempts to classify herbicola group-*Enterobacter agglomerans* strains by deoxyribonucleic acid hybridization and phenotypic tests. *Int J Syst Bacteriol* **34**, 45–55.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Cleenwerck, I., Vandemeulebroecke, K., Janssens, D. & Swings, J. (2002). Re-examination of the genus *Acetobacter*, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov. *Int J Syst Evol Microbiol* **52**, 1551–1558.
- Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J. R. W., Kersters, K. & Vandamme, P. (1999). Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* **49**, 405–413.
- Delétoile, A., Decré, D., Courant, S., Passet, V., Audou, J., Grimont, P., Arlet, G. & Brisse, S. (2009). Phylogeny and identification of *Pantoea* species and typing of *Pantoea agglomerans* strains by multilocus gene sequencing. *J Clin Microbiol* **47**, 300–310.
- Dye, D. W. (1981). A numerical taxonomic study of the genus *Erwinia*. *N Z J Agric Res* **24**, 223–229.
- Ewing, W. H. & Fife, M. A. (1972). *Enterobacter agglomerans* (Beijerinck) comb. nov. (the herbicola-lathyri bacteria). *Int J Syst Bacteriol* **22**, 4–11.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Gardner, J. M. & Kado, C. I. (1972). Comparative base sequence homologies of the deoxyribonucleic acids of *Erwinia* species and other *Enterobacteriaceae*. *Int J Syst Bacteriol* **22**, 201–209.
- Gavini, F., Mergaert, J., Beji, A., Mielcarek, C., Izard, D., Kersters, K. & De Ley, J. (1989). Transfer of *Enterobacter agglomerans* (Beijerinck 1988) 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.
- Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.
- Grimont, P. A. D. & Grimont, F. (2005). Genus XXIII. *Pantoea* Gavini, Mergaert, Beji, Mielcarek, Izard, Kersters and De Ley 1989b, 343^{VP} emend. Mergaert, Verdonck and Kersters 1993, 171. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2B, pp. 713–720. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**, 696–704.
- Hauben, L. & Swings, J. (2005). Genus XIII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209^{AL} emend. Hauben, Moore, Vauterin, Steenackers, Mergaert, Verdonck and Swings 1999a, 1. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2B, pp. 670–679. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Maki, D. G., Rhame, F. S., Mackel, D. C. & Bennett, J. V. (1976). Nationwide epidemic of septicemia caused by contaminated intravenous products. *Am J Med* **60**, 471–485.
- 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.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Naum, M., Brown, E. W. & Mason-Gamer, R. (2008). Is 16S rDNA a reliable phylogenetic marker to characterize relationships below the family level in the *Enterobacteriaceae*? *J Mol Evol* **66**, 630–642.
- Niemann, S., Pühler, A., Tichy, H.-V., Simon, R. & Selbitschka, W. (1997). Evaluation of the resolving power of three different DNA fingerprinting methods to discriminate among isolates of a natural *Rhizobium meliloti* population. *J Appl Microbiol* **82**, 477–484.
- Posada, D. & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.
- Samson, R., Legendre, J. B., Christen, R., Achouak, W. & Gardan, L. (2005). 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. *Int J Syst Evol Microbiol* **55**, 1415–1427.
- Spröer, C., Mendrock, U., Swiderski, J., Lang, E. & Stackebrandt, E. (1999). The phylogenetic position of *Serratia*, *Buttiauxella* and some other genera of the family *Enterobacteriaceae*. *Int J Syst Bacteriol* **49**, 1433–1438.
- Swofford, D. L. (2000). PAUP*: Phylogenetic Analysis Using Parsimony and other methods. Sunderland, MA: Sinauer Associates.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- 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.
- Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.5. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Green Publishing and Wiley-Interscience.
- Yarza, P., Richter, M., Peplies, J., Euzéby, J., Amann, R., Schleifer, K.-H., Ludwig, W., Glöckner, F. O. & Rosselló-Móra, R. (2008). The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Syst Appl Microbiol* **31**, 241–250.

Young, J. M. & Park, D.-C. (2007). Relationships of plant pathogenic enterobacteria based on partial *atpD*, *carA*, and *recA* as individual and concatenated nucleotide and peptide sequences. *Syst Appl Microbiol* **30**, 343–354.

Young, J. M., Dye, D. W., Bradbury, J. F., Panagopoulos, C. G. & Robbs, C. R. (1978). A proposed nomenclature and classification for plant pathogenic bacteria. *N Z J Agric Res* **21**, 153–177.