Transfer of *Pantoea citrea*, *Pantoea punctata* and *Pantoea terrea* to the genus *Tatumella* emend. as *Tatumella citrea* comb. nov., *Tatumella punctata* comb. nov. and *Tatumella terrea* comb. nov. and description of *Tatumella morbirosei* sp. nov.

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Pantoea citrea, *Pantoea punctata* and *Pantoea terrea* were described for strains isolated from fruit and soil originating in Japan. These three 'Japanese' species have been shown to be phylogenetically distant from other species of the genus *Pantoea*. It has been observed previously that, using multilocus sequence analysis (MLSA), the 'Japanese' species consistently formed a distinct clade with an extended branch length, casting doubt on the inclusion of these species within the genus *Pantoea*. Furthermore, the 'Japanese' species are closely related to *Tatumella ptyseos*, strains of which originate from human clinical specimens. DNA–DNA hybridization and phenotypic tests confirmed the observed phylogenetic distance of *P. citrea*, *P. punctata* and *P. terrea* from the genus *Pantoea* and the affiliation of these species with *Tatumella*. In addition, strains causing pink disease of pineapple, identified previously as *P. citrea*, were shown to represent a separate species by using 16S rRNA gene sequence analysis, and MLSA and DNA–DNA hybridization data. The name *Tatumella morbirosei* sp. nov. with the type strain LMG 23360^T (=BD 878^T=NCPBP 4036^T=CMC6^T) is proposed to accommodate these strains. The new combinations *Tatumella citrea* (Kageyama *et al.* 1992) comb. nov. (type strain, SHS 2003^T=ATCC 31623^T=BD 875^T=CCUG 30156^T=CIP 105599^T=DSM 13699^T=JCM 8882^T=LMG 22049^T), *Tatumella punctata* (Kageyama *et al.* 1992) comb. nov. (type strain, SHS 2006^T=ATCC 31626^T=BD 876^T=CCUG 30159^T=CIP 105598^T=DSM 13700^T=JCM 8885^T=LMG 22050^T) and *Tatumella terrea* (Kageyama *et al.* 1992) comb. nov. (type strain, SHS 2008^T=ATCC 31628^T=BD 877^T=CCUG 30161^T=CIP 105600^T=DSM 13701^T=JCM 8887^T=LMG 22051^T) are proposed for *P. citrea*, *P. punctata* and *P. terrea*, respectively.

Abbreviations: *atpD*, ATP synthase β -subunit; DKGA, 2,5-diketo-D-gluconic acid; *gyrB*, DNA gyrase; *infB*, initiation translation factor 2; MLSA, multilocus sequence analysis; *rpoB*, RNA polymerase β -subunit.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Tatumella ptyseos* LMG 7888^T, *Tatumella punctata* LMG 22050^T, *Tatumella terrea* LMG 22051^T, *Tatumella citrea* LMG 22049^T and *Tatumella morbirosei* LMG 23360^T and LMG 23359 are EU344770, EF688006–EF688008, EU344769 and FJ617235, respectively.

A table showing the signature nucleotides of the 16S rRNA and *atpD* gene sequences for the differentiation of *Tatumella* species from *Pantoea* species is available as supplementary material with the online version of this paper.

Following the proposal of the novel genus *Pantoea* in 1989 (Gavini *et al.*, 1989), but preceding the transfer of *Erwinia ananas* and *Erwinia stewartii* to *Pantoea* (Mergaert *et al.*, 1993), three novel *Pantoea* species from fruit and soil samples in Japan that produced 2,5-diketo-D-gluconic acid (DKGA) from D-glucose were described (Kageyama *et al.*, 1992). *Pantoea citrea*, *Pantoea punctata* and *Pantoea terrea* were included in the genus *Pantoea* based on general phenotypic data and DNA–DNA hybridization values, despite the inability of the original *Pantoea* species to produce DKGA. Until recently, no phylogenetic study had been performed on all *Pantoea* species with validly published names, giving no reason to doubt the inclusion

of *P. citrea*, *P. punctata* and *P. terrea* in the genus *Pantoea*. However, the most recent edition of *Bergey's Manual of Systematic Bacteriology* (Grimont & Grimont, 2005) states that more taxonomic work is required to justify the assignment of these three species to the genus *Pantoea*. A recent phylogenetic study of the *Enterobacteriaceae* revealed an *atpD* (ATP synthase β -subunit) sequence indel that was specific to *Pantoea* and *Tatumella* (Paradis *et al.*, 2005), indicating a close phylogenetic relationship between these two genera. This was in agreement with the initial suggestion of P. Grimont that the 'Japanese' species might be more similar to *Tatumella ptyseos* than *Pantoea* in their nutritional patterns (Kageyama *et al.*, 1992). The single species genus *Tatumella* was created for clinical strains isolated in North and South America between 1960 and 1980 (Hollis *et al.*, 1981). A multilocus sequence analysis (MLSA) scheme based on *gyrB* (DNA gyrase), *rpoB* (RNA polymerase β -subunit), *atpD* and *infB* (initiation translation factor 2) genes was performed recently on 102 *Pantoea* strains including the 'Japanese' species and *T. ptyseos* (Brady *et al.*, 2008). A concatenated tree constructed from the sequences of the four genes was found to be the most robust approach for revealing phylogenetic relationships amongst *Pantoea* strains. The MLSA study indicated a clear phylogenetic divergence between *P. citrea*, *P. punctata* and *P. terrea* and the remaining *Pantoea* species, and confirmed *T. ptyseos* as a close phylogenetic relative of the 'Japanese' species. It was concluded that the 'Japanese' species should be transferred to the genus *Tatumella*. A similar study based on multilocus typing of *Pantoea* species using six protein-coding genes was in agreement regarding the taxonomic position of the 'Japanese' species and also suggested the reclassification of these three species as *Tatumella* (Delétoile *et al.*, 2009).

Isolation of strains

Strains used in this study were obtained from the BCCM/LMG Bacteria Collection (<http://www.belspo.be/bccm>) and the Centers for Disease Control, Atlanta, Georgia, USA, and are listed in Table 1. An alkali extraction method (Niemann *et al.*, 1997) was used to isolate genomic DNA from the strains, which was stored at -20°C .

16S rRNA gene sequencing

Almost-complete 16S rRNA genes were sequenced for the type strains of *P. citrea* (LMG 22049^T), *P. punctata* (LMG 22050^T) and *P. terrea* (LMG 22051^T), and additional *P. citrea* strains (LMG 23359 and LMG 23360^T), which were found to cause pink disease of pineapple (Cha *et al.*, 1997), using the primers and conditions determined by Coenye *et al.* (1999). The sequences were aligned using CLUSTAL_X (Thompson *et al.*, 1997) and the overhangs were trimmed. The MODELLTEST 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 PHYL (Guindon &

Gascuel, 2003) and PAUP 4.0b10 (Swofford, 2000), respectively, by applying the models and parameters determined by MODELLTEST (only maximum-likelihood phylogenetic trees are shown). Bootstrap analysis with 1000 replicates was performed to assess the support for these clusters.

In the 16S rRNA gene sequence tree (Fig. 1) the genus *Pantoea* is split phylogenetically, although this is not unusual as *Pantoea* has been shown to be polyphyletic (Brady *et al.*, 2008). The majority of the *Pantoea* 'core' species are contained in a cluster supported by high bootstrap support, whereas *Pantoea dispersa* and a *Pantoea* species from the Brenner DNA group IV cluster amongst *Erwinia* species. The 'Japanese' species are situated in a distinctly separate clade with a different branch point, also with high bootstrap support. These findings support those of Grimont & Grimont (2005), based on 16S rRNA gene and *rpoB* sequence comparisons, that the 'Japanese' species cluster at a lower level to the *Pantoea* 'core' clade. Interestingly, LMG 7888^T, the type strain of *T. ptyseos*, clustered closely with the type strain of *P. terrea*, within the 'Japanese' species clade, prompting further examination of the relationship of the 'Japanese' species with *T. ptyseos*. The additional *P. citrea* strains LMG 23359 and LMG 23360^T, which cause pink disease of pineapple, do not cluster with the type strain of this species (LMG 22049^T), but are found on a separate branch, suggesting that these strains do not belong to *P. citrea*. The 16S rRNA gene sequence similarity of strain LMG 23360^T was greater than 98 % to the type strains of *P. punctata*, *P. citrea*, *Pantoea* sp. (Brenner DNA group II) and *T. ptyseos*.

gyrB, *rpoB*, *atpD* and *infB* gene sequencing

MLSA was performed on all strains, as described previously (Brady *et al.*, 2008), to provide further support for the reclassification of the 'Japanese' species. Sequence analysis and tree construction were performed as described above. Several *Erwinia* species were included in the MLSA study and housekeeping gene sequences for *Erwinia tasmaniensis*, *Escherichia coli*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Citrobacter rodentium*, *Citrobacter koseri* and *Cronobacter sakazakii* were obtained from genome sequencing databases (<http://www.ncbi.nlm.nih.gov>, <http://www.sanger.ac.uk>, <https://asap.ahabs.wisc.edu/asap/home.php>). In Fig. 2, a MLSA phylogenetic tree based on the concatenated sequences of *gyrB*, *rpoB*, *atpD* and *infB* genes, *Pantoea* strains formed a monophyletic cluster within the *Enterobacteriaceae*. This cluster contained two sublineages that were supported by high bootstrap values of 100 %. The first sublineage contains the *Pantoea* 'core' species including Brenner DNA groups II, IV and V. The second sublineage consists of the 'Japanese' species and the type strain of *T. ptyseos*. The type strain of *T. ptyseos* clustered closely with *P. terrea* strains, specifically with strain LMG 23565 and its duplicate CCUG 30163. This result indicates that LMG 23565 (=CCUG 30163) was erroneously classified and in

Table 1. Strains of *Tatumella* and *Pantoea* used in this study

ATCC, American Type Culture Collection, Rockville, Maryland, USA; BD, Plant Pathogenic and Plant Protecting Bacteria (PPPPB) Culture Collection, ARC-PPRI, Pretoria, South Africa; Br HG, Brenner hybridization group; CCUG, Culture Collection, University of Göteborg, Sweden; LMG, BCCM/LMG Bacteria Collection, Ghent University, Belgium. R, Reference strain.

Species	Strain	Source	Location	<i>gyrB</i> *	<i>rpoB</i> *	<i>atpD</i> *	<i>infB</i> *
<i>Tatumella citrea</i>	LMG 22049 ^T =BD 875 ^T	Mandarin orange	Japan	EF988802	EF988974	EF988715	EF988888
<i>Tatumella morbirosei</i>	LMG 23360 ^T =BD 878 ^T	Pineapple	Philippines	EU344760	EU344768	EU344756	EU344764
	LMG 23359=NCPPB 4035	Pineapple	Philippines	EU344759	EU344767	EU344755	EU344763
<i>Tatumella punctata</i>	LMG 22050 ^T =BD 876 ^T	Mandarin orange	Japan	EF988803	EF988975	EF988716	EF988889
	LMG 22097	Mandarin orange	Japan	EF988805	EF988977	EF988718	EF988891
	LMG 22098	Persimmon	Japan	EF988806	EF988978	EF988719	EF988892
	LMG 23562	Mandarin orange	Japan	EF988807	EF988979	EF988720	EF988893
	LMG 23563	Mandarin orange	Japan	EF988808	EF988980	EF988721	EF988894
	CCUG 30157	Mandarin orange	Japan	EF988795	EF988967	EF988708	EF988881
	CCUG 30160	Mandarin orange	Japan	EF988796	EF988968	EF988709	EF988882
<i>Tatumella terrea</i>	LMG 22051 ^T =BD 877 ^T	Soil	Japan	EF988804	EF988976	EF988717	EF988890
	LMG 23564	Soil	Japan	EF988809	EF988981	EF988722	EF988895
	CCUG 30162	Soil	Japan	EF988797	EF988969	EF988710	EF988883
<i>Tatumella ptyseos</i>	LMG 7888 ^T =ATCC 33301 ^T	Human	USA	EU145260	EU145292	EU145244	EU145276
	LMG 23565	Soil	Japan	EU344758	EU344766	EU344754	EU344762
	CCUG 30163	Soil	Japan	EU344757	EU344765	EU244753	EU344761
<i>Pantoea agglomerans</i>	LMG 1286 ^T	Human	Zimbabwe	EF988798	EF988970	EF988711	EF988884
	LMG 2660	<i>Wisteria floribunda</i>	Japan	EF988823	EF988995	EF988736	EF988909
<i>Pantoea ananatis</i>	LMG 2665 ^T	Pineapple	Brazil	EF988824	EF988996	EF988737	EF988910
	LMG 20103	Eucalyptus	South Africa	EF988799	EF988971	EF988712	EF988885
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	LMG 2715 ^T	Maize	USA	EF988831	EF989003	EF988744	EF988917
	LMG 2718	Maize	USA	EF988832	EF989004	EF988745	EF988918
<i>Pantoea stewartii</i> subsp. <i>indologenes</i>	LMG 2632 ^T	Fox millet	India	EF988822	EF988994	EF988735	EF988908
	LMG 2673	Pineapple	Hawaii	EF988827	EF988999	EF988740	EF988914
<i>Pantoea dispersa</i>	LMG 2603 ^T	Soil	Japan	EF988818	EF988990	EF988731	EF988904
	LMG 2604	Wild rose	Netherlands	EF988819	EF988991	EF988732	EF988905
<i>Pantoea anthophila</i>	LMG 2558 ^T	<i>Impatiens balsamina</i>	India	EF988812	EF988984	EF988725	EF988898
	LMG 2560	<i>Tagetes erecta</i>	Unknown	EF988813	EF988985	EF988726	EF988899
<i>Pantoea vagans</i>	LMG 24199 ^T	Eucalyptus	Uganda	EF988768	EF988940	EF988715	EF988854
	LMG 24201	Maize	South Africa	EF988792	EF988964	EF988705	EF988878
<i>Pantoea eucalypti</i>	LMG 24197 ^T	Eucalyptus	Uruguay	EF988762	EF988934	EF988675	EF988848
	LMG 24198	Eucalyptus	Uruguay	EF988763	EF988935	EF988676	EF988849
<i>Pantoea deleyi</i>	LMG 24200 ^T	Eucalyptus	Uganda	EF988770	EF988950	EF988683	EF988856
<i>Pantoea</i> sp. (Br HG II)	LMG 5345 ^R	Human, stool	New Jersey, USA	EU145272	EU145304	EU145256	EU145288
	LMG 24526	Human, blood	New York, USA	EU145261	EU145293	EU145245	EU145277
<i>Pantoea</i> sp. (Br HG IV)	LMG 2781 ^R	Human, trachea	Connecticut, USA	EU145271	EU145303	EU145255	EU145287
	LMG 24529	Human, cyst	Georgia, USA	EU145264	EU145296	EU145248	EU145280
<i>Pantoea</i> sp. (Br HG V)	LMG 5343 ^R	Human, urethra	Montana, USA	EU145270	EU145302	EU145254	EU145286
	LMG 24532	Human, sputum	Wisconsin, USA	EU145267	EU145299	EU145251	EU145283
<i>Pantoea</i> sp. (Br HG V)	LMG 24534 ^R	Human, blood	Paris, France	EU145269	EU145301	EU145253	EU145285
<i>Erwinia billingiae</i>	LMG 2613 ^T	Pear	UK	EU145275	EU145307	EU145259	EU145291
<i>Erwinia psidii</i>	LMG 7034	Guava	Brazil	FJ187834	FJ187844	FJ187829	FJ187839
<i>Erwinia rhapontici</i>	LMG 2688 ^T	Rhubarb	UK	EF988838	EF989010	EF988751	EF988924
<i>Erwinia toletana</i>	LMG 24162 ^T	Olive tree	Spain	EU145274	EU145306	EU145258	EU145290

*GenBank accession numbers.

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences of *Tatumella* species. Percentage bootstrap values based on 1000 replicates are given at nodes. The sequence of *Escherichia coli* ATCC 11775^T was included as an outgroup. R, Reference strain. Bar, 0.1 substitutions per nucleotide position.

fact belongs to *T. tyseos*. LMG 23359 and LMG 23360^T, two supposed *P. citrea* strains causing pink disease of pineapple referred to as MLSA group J (Brady *et al.*, 2008), grouped with LMG 22049^T, the type strain of *P. citrea* but were situated on a separate branch with a long branch length. This supports the probability that these two strains do not belong to *P. citrea*, but to a novel species. All species clustered within both sublineages of *Pantoea* were supported with 100 % bootstrap values. All four house-keeping- and 16S rRNA gene sequences were examined for heterogeneous nucleotides that could be used as signature nucleotide positions to differentiate between *Tatumella* and *Pantoea*. Eight signature nucleotides were identified in the 16S rRNA gene sequences that differ between *Tatumella* and *Pantoea*. The gene sequences of *atpD* revealed the most heterogeneity with 23 signature nucleotides, which are conserved in *Tatumella* species and differ from all *Pantoea* species. The signature nucleotides for the 16S rRNA and *atpD* genes are listed in Supplementary Table S1 (available in IJSEM Online). The *Escherichia coli* numbering positions were used to designate the nucleotide

positions (Brosius *et al.*, 1978; <http://www.ncbi.nlm.nih.gov>).

DNA–DNA hybridization

High quality DNA for DNA–DNA hybridization of strains was prepared by using 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). The hybridization temperature was $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$, reciprocal reactions ($A \times B$ and $B \times A$) were performed for each DNA pair from all strains and their variation was within the limits of this method (Goris *et al.*, 1998). The values presented were based on a minimum of four replicates. Representative strains from each ‘Japanese’ *Pantoea* species were selected for hybridization based on the 16S rRNA gene and MLSA phylogenetic trees. The type strains of the ‘Japanese’ species were hybridized with each other, and with the type strain of *T. tyseos*, their closest phylogenetic neighbour, as

Fig. 2. Maximum-likelihood tree based on the concatenated nucleotide sequences of *gyrB*, *rpoB*, *atpD* and *infB* genes of *Tatumella* and *Pantoea* strains. Percentage bootstrap values based on 1000 replicates are given at nodes. The sequence of *Cronobacter sakazakii* was included as an outgroup. Sequences for *Erwinia tasmaniensis*, *Escherichia coli*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Citrobacter rodentium*, *Citrobacter koseri* and *Cronobacter sakazakii* were obtained from genome sequencing databases (<http://www.ncbi.nlm.nih.gov>, <http://www.sanger.ac.uk>, <https://asap.ahabs.wisc.edu/asap/home.php>). R, Reference strain. Bar, 0.1 substitutions per nucleotide position.

well as with the type strains of *Pantoea agglomerans*, *Pantoea ananatis*, *Pantoea vagans*, *Pantoea stewartii* subsp. *stewartii* and *P. dispersa*. A summary of the hybridization results is presented in Table 2. The DNA–DNA relatedness between *P. citrea* LMG 22049^T, *P. punctata* LMG 22050^T and *P. terrea* LMG 22051^T ranged from 13 to 21 %, which was considerably lower than the 28–43 % reported by Kageyama *et al.* (1992) and could be due to different hybridization methods used. LMG 23359 and LMG 23360^T, the strains that can cause pink disease of pineapple (Cha *et al.*, 1997) shared 96 % DNA–DNA relatedness when hybridized against each other, whereas strain LMG 23360^T displayed only 42 % DNA–DNA relatedness with *P. citrea* LMG 22049^T. These results together with the 16S rRNA gene sequence and MLSA data proved that strains LMG 23360^T and LMG 23359 do not belong to *P. citrea*.

P. citrea LMG 22049^T and *P. punctata* LMG 22050^T displayed 14 and 21 % DNA–DNA relatedness when hybridized with *T. tyseos* LMG 7888^T, respectively. In contrast, the *T. tyseos* type strain shared 66 % DNA–DNA

relatedness with *P. terrea* LMG 22051^T and 87 % with LMG 23565, originally considered to be *P. terrea*. Strain LMG 23565 demonstrated only 55 % DNA–DNA relatedness, when hybridized with *P. terrea* LMG 22051^T. These results are in favour of the reclassification of strain LMG 23565 (=CCUG 30163) as *T. tyseos* and confirm the close phylogenetic relationship between *P. terrea* and *T. tyseos*, observed in both the 16S rRNA gene and MLSA phylogenetic trees (Figs 1 and 2). The DNA–DNA relatedness between the type strains of the ‘Japanese’ *Pantoea* species and the type strains of *P. agglomerans*, *P. ananatis*, *P. vagans*, *P. stewartii* and *P. dispersa* was below 10 %. These low hybridization values were also reflected in the phylogenetic distance between the ‘Japanese’ species and the *Pantoea* ‘core’ species in both Figs 1 and 2.

DNA G+C content

The DNA G + C content of the type strains, determined by HPLC (Mesbah *et al.*, 1989), were as follows: *P. citrea*

Table 2. DNA–DNA hybridization values between *T. citrea* comb. nov., *T. morbirosei* sp. nov., *T. punctata* comb. nov., *T. terrea* comb. nov., *T. pyseos* and selected species belonging to *Pantoea*

Strain	Hybridization (%) with ³ H-labelled DNA from:													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. <i>Tatumella citrea</i> LMG 22049 ^T	100													
2. <i>Tatumella morbirosei</i> LMG 23360 ^T	42	100												
3. <i>Tatumella morbirosei</i> LMG 23359		96	100											
4. <i>Tatumella punctata</i> LMG 22050 ^T	21	17		100										
5. <i>Tatumella punctata</i> LMG 22098	16			93	100									
6. <i>Tatumella terrea</i> LMG 22051 ^T	13	11		17	18	100								
7. <i>Tatumella terrea</i> LMG 23564						82	100							
8. <i>Tatumella pyseos</i> LMG 7888 ^T	14	15		21		66		100						
9. <i>Tatumella pyseos</i> LMG 23565	15	13		20		55	54	87	100					
10. <i>Pantoea agglomerans</i> LMG 1286 ^T	6			8		8				100				
11. <i>Pantoea ananatis</i> LMG 2665 ^T	5			6		6				21	100			
12. <i>Pantoea vagans</i> LMG 24199 ^T	5			7		8				65	20	100		
13. <i>Pantoea stewartii</i> subsp. <i>stewartii</i> LMG 2715 ^T	2			3		3				6	20	9	100	
14. <i>Pantoea dispersa</i> LMG 2603 ^T	7			7	7	8				24	20	19	22	100

(LMG 22049^T), 49.8 mol%; MLSA group J (LMG 23360^T), 50.2 mol%; *P. punctata* (LMG 22050^T, LMG 22098), 50.7 mol%; *P. terrea* (LMG 22051^T, LMG 23564), 52.6–52.8 mol%; and *T. pyseos* (LMG 7888^T, LMG 23565), 51.7–52.1 mol%.

Phenotypic assays

Differences between the *Pantoea* ‘core’ species and the ‘Japanese’ species have been noted previously, not only phylogenetically but also phenotypically (Grimont & Grimont, 2005; Delétoile *et al.*, 2009). API 20E, API 50 CHB/E, Biotype 100 (bioMérieux) and GN2 MicroPlate (Biolog) tests were performed on all *Tatumella* and *Pantoea* strains used in this study, to verify that the phenotypic data agree with those available in the literature. Cell suspensions were prepared from strains grown on tryptone soya agar (Oxoid) for 12 h. All phenotypic test strips and plates were incubated for 24–48 h at 28 °C, except the Biotype 100 strips which were incubated for up to 6 days. Scoring was performed according to the manufacturer’s instructions. Phenylalanine deaminase activity was determined by adding 10% aqueous FeCl₃ to 24 h cultures grown on (D)-phenylalanine agar (Merck). The development of a green colour indicated a positive reaction. A high correlation was observed between the phenotypic results generated in this study, the phenotypic data presented in *Bergey’s Manual of Systematic Bacteriology* (Grimont & Grimont, 2005) and the original species descriptions of *P. citrea*, *P. punctata* and *P. terrea* (Kageyama *et al.*, 1992) and *T. pyseos* (Hollis *et al.*, 1981). *T. pyseos* and the ‘Japanese’ species could be distinguished from the genus *Pantoea* by means of a positive reaction for arginine dihydrolase (except *P. terrea* and *T. pyseos*) and 2-ketogluconate dehydrogenase, their inability to produce acid from arbutin, *myo*-inositol and L-rhamnose and their inability to utilize D-galacturonic acid,

D-glucuronic acid, *myo*-inositol, methyl β-D-glucoside, L-rhamnose, D-saccharic acid and *meso*-tartrate (except *T. pyseos*). A summary of the most useful phenotypic and biochemical characteristics for differentiating *T. pyseos* and the ‘Japanese’ species from *Pantoea* species is presented in Table 3. Table 3 was assembled using data generated in this study (except for DKGA), of which the majority agreed with that published previously.

A summary of biochemical and phenotypic characteristics that can be used to differentiate between *T. pyseos*, the ‘Japanese’ species and the pink disease-causing strains LMG 23360^T and LMG 23359 is given in Table 4. The strains causing pink disease of pineapple could be differentiated from *P. citrea* by means of a positive phenylalanine deaminase reaction, acid production from starch, their ability to utilize adonitol and trigonelline, and their inability to utilize gentiobiose and lactulose. *P. terrea* could be differentiated from *T. pyseos*, its closest phylogenetic neighbour, by means of a negative phenylalanine deaminase reaction, the ability to utilize formic acid and pyruvic acid methyl ester, and the inability to utilize quinic acid and L-tartrate.

A selection of 50 phenotypic characteristics was used to construct a UPGMA dendrogram (Fig. 3) in PAUP 4.0b10 (Swofford, 2000). The dendrogram showed a clear distinction of *T. pyseos* and the ‘Japanese’ species from the *Pantoea* ‘core’ species, lending further support for the reclassification of these three species in the genus *Tatumella*, and the description of a novel species for the strains causing pink disease of pineapple.

Conclusions

The many previous rearrangements observed in the ‘*Erwinia herbicola*–*Enterobacter agglomerans* complex’ were,

Table 3. Selected phenotypic characteristics useful for distinguishing *Tatumella* species from *Pantoea* species

Taxa: 1, *T. ptyseos* (1 strain); 2, *T. citrea* comb. nov. (1 strain); 3, *T. morbirosei* sp. nov. (2 strains); 4, *T. punctata* comb. nov. (7 strains); 5, *T. terrea* comb. nov. (3 strains); 6, *P. agglomerans* (3 strains); 7, *P. ananatis* (4 strains); 8, *P. anthophila* (2 strains); 9, *P. deleyi* (1 strain); 10, *P. dispersa* (2 strains); 11, *P. eucalypti* (2 strains); 12, *P. stewartii* subsp. *stewartii* (1 strain); 13, *P. stewartii* subsp. *indologenes* (2 strains); 14, *P. vagans* (7 strains). All data presented were generated during this study, except for 2-ketogluconate dehydrogenase for which the results were taken from Kageyama *et al.* (1992) and Bouvet *et al.* (1989). +, Positive; –, negative; (+), weakly positive; v, variable reaction; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Motility (36 °C)*	–	–	–	–	+	+	+	–	–	+	–	–	+	–
Arginine dihydrolase	–	+	(+)	+	–	–	–	–	–	–	–	–	–	–
2-Ketogluconate dehydrogenase	+	+	+	+	+	–	–	ND	ND	–	ND	–	–	ND
Acid from:†														
Arbutin	–	–	–	–	–	+	+	+	–	+	+	+	+	+
<i>myo</i> -Inositol	–	–	–	–	–	(+)	+	+	–	+	+	–	+	+
L-Rhamnose	–	–	–	–	–	+	+	+	+	+	+	–	+	+
Utilization of:‡														
<i>cis</i> -Aconitic acid	–	+	–	–	–	+	+	+	+	+	+	+	+	+
D-Alanine	–	–	–	–	–	+	+	+	+	+	+	+	+	+
L-Alanine	–	–	–	–	–	+	+	+	+	+	+	+	+	+
Cellobiose	–	–	–	v	–	+	+	+	+	+	+	+	+	+
D-Galacturonic acid	–	–	–	–	–	+	+	+	+	+	+	+	+	+
D-Glucuronic acid	–	–	–	–	–	+	+	+	+	+	+	+	+	+
L-Histidine	–	–	–	–	–	+	+	+	+	+	+	+	+	+
<i>myo</i> -Inositol	–	–	–	–	–	+	+	+	+	+	+	+	+	+
DL-Lactic acid	–	–	–	–	–	+	+	+	+	+	+	+	+	+
Maltose	–	+	+	–	–	+	+	+	+	+	+	–	+	+
Methyl β -D-glucoside	–	–	–	–	–	+	+	+	+	+	+	+	+	+
L-Proline	–	–	–	–	–	+	+	+	+	+	+	+	+	+
L-Rhamnose	–	–	–	–	–	+	+	+	+	+	+	+	+	+
D-Saccharic acid	–	–	–	–	–	+	+	+	+	+	+	+	+	+
L-Serine	–	–	–	–	–	+	+	+	+	+	+	+	+	+
<i>meso</i> -Tartrate	(+)	–	–	–	–	+	+	+	+	+	+	+	+	+

**T. ptyseos* is non-motile at 36 °C but many strains are motile at 25 °C. Flagella of *Tatumella* species are polar, subpolar or lateral rather than peritrichous like *Pantoea* species.

†API 50 CHB/E.

‡Biotype 100, GN2 MicroPlate.

for the most part, based on DNA–DNA hybridization data, biochemical characterization and protein profiling. When *P. citrea*, *P. punctata* and *P. terrea* were described as belonging to the genus *Pantoea*, molecular techniques such as 16S rRNA gene sequencing and MLSA were not readily accessible. With rapid gene sequencing now a reality, it is possible to observe true phylogenetic relationships and to resolve evident taxonomic issues.

The 16S rRNA gene and MLSA trees showed a clear division of the *Pantoea* ‘core’ species from the ‘Japanese’ species and *T. ptyseos*. Furthermore, MLSA group J (strains causing pink disease of pineapple) was shown to cluster independently from the type strain of *P. citrea* in both phylogenetic trees. These results were supported equally by DNA–DNA hybridization values, which shared a high correlation with MLSA data (Brady *et al.*, 2008), and phenotypic data. Although the genus *Tatumella* is reported to have few distinguishing phenotypic properties (Farmer, 2005), a number of biochemical characteristics were identified that

are shared by the ‘Japanese’ species and can be used to differentiate these species from *Pantoea* (see Table 3).

An important characteristic that distinguishes *P. citrea*, *P. punctata*, *P. terrea*, *T. ptyseos* and MLSA group J from *Pantoea* species is their ability to produce 2-ketogluconate dehydrogenase, which oxidizes 2-ketogluconate to DKGA. The production of DKGA is responsible for the discolouration of fruit tissue typical of pink disease of pineapple (Pujol & Kado, 2000), and therefore plays an important role in disease expression. Noticeably, not one *Pantoea* ‘core’ species has this ability (Bouvet *et al.*, 1989). Several objections for not including the ‘Japanese’ species in the genus *Tatumella* were stated in the original description, despite a personal communication from P. Grimont suggesting that the ‘Japanese’ species were phenotypically similar to *Tatumella* (cited by Kageyama *et al.*, 1992). These included acid production from D-xylose and L-arabinose, arginine dihydrolase and phenylalanine deaminase activity, methyl red and Voges–Proskauer reactions,

Table 4. Phenotypic characteristics that distinguish *T. citrea* comb. nov., *T. morbirosei* sp. nov., *T. punctata* comb. nov. and *T. terrea* comb. nov. from each other and from *T. ptyseos*

Strains: 1, *T. ptyseos* LMG 7888^T and LMG 23565; 2, *T. citrea* LMG 22049^T; 3, *T. morbirosei* LMG 23360^T and LMG 23359; 4, *T. punctata* LMG 22050^T, LMG 22097, LMG 22098 and LMG 23563; 5, *T. terrea* LMG 22051^T and LMG 23564. +, Positive; (+), weakly positive; –, negative. All data were generated in this study.

Characteristic	1	2	3	4	5
Arginine dihydrolase	–	+	(+)	+	–
Phenylalanine deaminase	+	–	+	–	–
Acid from:					
Starch	–	–	+	–	–
D-Mannitol	–	+	+	–	–
Raffinose	–	–	–	+	–
Sucrose	+	–	–	+	+
Utilization of:					
Adonitol	–	–	+	–	–
L-Arabinose	–	+	+	–	–
Dextrin	–	+	+	–	–
Erythritol	–	+	+	–	–
Formic acid	–	+	(+)	–	+
Gentiobiose	–	(+)	–	+	–
Lactulose	–	+	–	–	–
Pyruvic acid methyl ester	–	(+)	(+)	–	+
Quinic acid	+	–	–	–	–
L-Tartrate	+	–	–	–	–
Trigonelline	–	–	+	–	–

aesculin hydrolase activity, citrate utilization and lower DNA G+C content. However, closer examination of all available data revealed that all taxa were positive for acid production from D-xylose and L-arabinose, methyl red and Voges–Proskauer reactions and citrate utilization. *T. ptyseos* and *P. terrea* are both negative for arginine dihydrolase activity, whereas *P. citrea* and *P. punctata* are both positive. The DNA G+C content of *T. ptyseos* was 51.7–52.1 mol%, which is slightly higher than those of the ‘Japanese’ species, which range from 49.8 to 52.8 mol%. However, the DNA G+C contents of *Pantoea* ‘core’ species ranged from 53 to 61 mol%, which is considerably higher. Therefore the current data, both phylogenetic and phenotypic, support the transfer of the ‘Japanese’ species from the genus *Pantoea* to the genus *Tatumella*.

We propose to transfer *P. citrea*, *P. punctata* and *P. terrea* to the genus *Tatumella* as *Tatumella citrea* comb. nov., *Tatumella punctata* comb. nov. and *Tatumella terrea* comb. nov. We further propose *Tatumella morbirosei* sp. nov. for strains LMG 23359 and LMG 23360^T, the causal agent of pink disease of pineapple.

Emended description of the genus *Tatumella* Hollis, Hickman & Fanning 1982

Tatumella (Ta.tum.el'la. N.L. fem. dim. n. *Tatumella* named to honour Harvey Tatum, an American bacteriolo-

gist who made many contributions to our understanding of the classification and identification of fermentative and nonfermentative bacteria of medical importance.) The description below is based on the data of Hollis *et al.* (1981), Kageyama *et al.* (1992) and this paper.

Gram-negative, non-capsulated, non-spore-forming small rods that are 0.6–1.2 × 0.9–3.0 µm in size. Cells are motile by means of polar, subpolar or lateral flagella or non-motile at 36 °C. Facultatively anaerobic, fermentative, catalase-positive (weak and slow) and oxidase-negative. Non-pigmented, or pale beige to pale orange. Glucose dehydrogenase, gluconate dehydrogenase and 2-ketogluconate dehydrogenase are produced. Reduce nitrate to nitrite. Indole, urease and gelatin tests are negative. Positive for Voges–Proskauer (Coblenz), methyl red and citrate (Simmons); phenylalanine, arginine dihydrolase and ONPG tests are variable. Negative for H₂S (TSI), lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, KCN test, lipase and DNase. Acid is produced from L-arabinose, D-galactose, D-glucose, glycerol, D-mannitol, D-mannose, melibiose, D-ribose, trehalose and D-xylose (variable reaction for *T. morbirosei*), but not from amygdalin, dulcitol, erythritol, glutarate, glycogen, histamine, *myo*-inositol, methyl α-D-glucoside, propionate, L-rhamnose, sorbitol or L-sorbose. *N*-Acetyl-D-glucosamine, 4-aminobutyrate, L-asparagine, L-aspartic acid, bromosuccinic acid, D-fructose, fumarate, β-galactopyranoside, D-gluconic acid, α-D-glucose 1-phosphate, α-D-glucose 6-phosphate, L-glutamic acid, glycerol, inosine, 5-ketogluconate, D-mannose, L-proline, D-psicose, D-ribose, succinic acid, trehalose, thymidine and uridine are utilized as sole sources of carbon and energy. Susceptible to many antibiotics. Isolated from human clinical samples, fruit and soil. The G+C content of the DNA ranges from 49.8 to 53 mol%. The type species is *Tatumella ptyseos* Hollis, Hickman & Fanning 1982.

Description of *Tatumella citrea* (Kageyama *et al.* 1992) comb. nov.

Tatumella citrea (ci'tre.a. L. fem. adj. *citrea* belonging to the citrus tree).

Basonym: *Pantoea citrea* Kageyama *et al.* 1992.

Cells are Gram-negative, short rods (0.8–1.2 × 1.0–3.0 µm) occurring singly or in pairs, non-motile and non-spore-forming. Colonies are pale beige to pale orange, round, convex and smooth with entire margins. Nicotinic acid or nicotinamide are required for growth. Facultatively anaerobic, oxidase-negative, catalase-positive and glucose dehydrogenase, gluconate dehydrogenase and 2-ketogluconate dehydrogenase are produced. Indole, urease and phenylalanine deaminase are negative, whereas arginine dihydrolase is positive. Reduces nitrate to nitrite. Acid is produced from L-arabinose, D-fucose, D-galactose, D-glucose, glycerol, maltose, D-mannose, D-mannitol, D-tagatose and trehalose, but not from starch, raffinose or sucrose. The following carbon sources are utilized at 28 °C: *N*-acetyl-D-glucos-

Fig. 3. UPGMA dendrogram constructed from 50 phenotypic characteristics useful for the differentiation of the 'core' *Pantoea* species from species of the genus *Tatumella*.

amine, *cis*-aconitic acid, L-arabinose, L-asparagine, L-aspartic acid, citrate, dextrin, erythritol, formic acid, D-fructose, D-galactose, gentiobiose (weak), D-gluconic acid, D-glucose, 5-ketogluconate, lactose, lactulose, D-malate, maltose, maltotriose, D-mannitol, pyruvic acid methyl ester, succinic acid and D-tagatose. The following carbon sources are not utilized at 28 °C: D-adonitol, L-alanine, D-arabinose, betaine, dulcitol, L-fucose, methyl α -D-glucoside, methyl β -D-glucoside, D-galacturonic acid, D-glucuronic acid, glutarate, histidine, *myo*-inositol, melibiose, L-proline, propionate, quinic acid, raffinose, L-rhamnose, D-sorbitol, L-serine, sucrose, L-tartrate, *meso*-tartrate and trigonelline. The DNA G+C content of the type strain is 49.8 mol%.

The type strain, SHS 2003^T (=ATCC 31623^T=BD 875^T=CCUG 30156^T=CIP 105599^T=DSM 13699^T=JCM 8882^T=LMG 22049^T), was isolated from mandarin orange in Japan.

Description of *Tatumella punctata* (Kageyama et al. 1992) comb. nov.

Tatumella punctata (punc.ta'ta. L. n. *punctum* a point; N.L. fem. adj. *punctata* full of points).

Basonym: *Pantoea punctata* Kageyama et al. 1992.

Cells are Gram-negative, short rods (1.1–1.2 × 1.3–2.3 µm) occurring singly or in pairs, non-motile and non-spore-

forming. Colonies are pale beige to pale orange, round, convex and smooth with entire margins. Nicotinic acid or nicotinamide are required for growth. Facultatively anaerobic, oxidase-negative, catalase-positive and glucose dehydrogenase, gluconate dehydrogenase and 2-ketogluconate dehydrogenase are produced. Indole, urease and phenylalanine deaminase are negative, whereas arginine dihydrolyase is positive. Reduces nitrate to nitrite. Acid is produced from L-arabinose, D-fructose, D-fucose, D-galactose, D-glucose, glycerol, D-mannose, raffinose, sucrose and trehalose, but not from starch or D-mannitol. The following carbon sources are utilized at 28 °C: *N*-acetyl-D-glucosamine, D-arabinose, L-asparagine, L-aspartic acid, cellobiose (variable), citrate, D-fructose, D-galactose, gentiobiose, D-gluconic acid, D-glucose, 5-ketogluconate, D-mannitol, D-mannose, succinic acid, sucrose and trehalose. The following carbon sources are not utilized at 28 °C within 3–6 days: *cis*-aconitic acid, D-adonitol, L-alanine, L-arabinose, D-arabitol, L-arabitol, betaine, dextrin, dulcitol, erythritol, formic acid, L-fucose, D-galacturonic acid, D-glucuronic acid, methyl α -D-glucoside, methyl β -D-glucoside, glutarate, histidine, *myo*-inositol, lactose, lactulose, maltose, maltotriose, melibiose, L-proline, propionate, pyruvic acid methyl ester, quinic acid, raffinose, L-rhamnose, D-sorbitol, L-serine, L-tartrate, *meso*-tartrate and trigonelline. The DNA G+C content of the type strain is 50.7 mol%.

The type strain, SHS 2006^T (=ATCC 31626^T=BD 876^T=CCUG 30159^T=CIP 105598^T=DSM 13700^T=JCM 8885^T=LMG 22050^T), was isolated from mandarin orange in Japan.

Description of *Tatumella terre* (Kageyama *et al.* 1992) comb. nov.

Tatumella terre (ter're.a. L. fem. adj. *terre* of the earth).

Basionym: *Panteoa terre* Kageyama *et al.* 1992.

Cells are Gram-negative, short rods (0.8–0.9 × 1.2–2.0 µm) occurring singly or in pairs, motile by means of one or two lateral flagella and non-spore-forming. Colonies are pale beige to pale orange, round, convex and smooth with entire margins. Nicotinic acid or nicotinamide is required for growth. Facultatively anaerobic, oxidase-negative, catalase-positive and glucose dehydrogenase, gluconate dehydrogenase and 2-ketogluconate dehydrogenase are produced. Indole, urease, phenylalanine deaminase and arginine dihydro-lase are negative. Reduces nitrate to nitrite. Acid is produced from L-arabinose, D-fructose, D-galactose, D-glucose, glycerol, D-mannose, sucrose and trehalose, but not from starch, D-mannitol or raffinose. The following carbon sources are utilized at 28 °C: *N*-acetyl-D-glucosamine, L-asparagine, L-aspartic acid, citrate, D-fructose, formic acid, D-galactose, D-gluconic acid, D-glucose, 5-ketogluconate, D-mannose, pyruvic acid methyl ester, succinic acid and trehalose. The following carbon sources are not utilized at 28 °C: *cis*-aconitic acid, D-adonitol, L-alanine, D-arabinose, L-arabinose, D-arabitol, L-arabitol, betaine, cellobiose, dextrin, dulcitol, erythritol, L-fucose, D-galacturonic acid, gentiobiose, D-glucuronic acid, methyl α-D-glucoside, methyl β-D-glucoside, glutarate, histidine, *myo*-inositol, lactose, lactulose, maltose, maltotriose, melibiose, L-proline, propionate, quinic acid, raffinose, L-rhamnose, D-sorbitol, L-serine, sucrose, L-tartrate and trigonelline. The DNA G+C content of the type strain is 52.8 mol%.

The type strain, SHS 2008^T (=ATCC 31628^T=BD 877^T=CCUG 30161^T=CIP 105600^T=DSM 13701^T=JCM 8887^T=LMG 22051^T), was isolated from soil in Japan.

Description of *Tatumella morbirosei* sp. nov.

Tatumella morbirosei (mor.bi.ró.se.i. L. n. *morbus* disease; L. adj. *roseus* rosy pink; N.L. gen. n. *morbirosei* of the pink disease, referring to the causal agent of pink disease of pineapple).

Cells are Gram-negative, short rods (0.8–1.2 × 1.0–3.0 µm) occurring singly or in pairs, non-motile and non-spore-forming. Colonies are pale beige, round, convex and smooth with entire margins. Facultatively anaerobic, oxidase-negative, catalase-positive and glucose dehydrogenase, gluconate dehydrogenase and 2-ketogluconate dehydrogenase are produced. Indole and urease are

negative, whereas phenylalanine deaminase is positive and arginine dihydro-lase is weakly positive. Reduces nitrate to nitrite. Acid is produced from starch, L-arabinose, D-fucose, D-fructose, D-galactose, D-glucose, glycerol, 5-ketogluconate, melibiose, D-mannitol, D-mannose, D-tagatose and trehalose, but not from raffinose or sucrose. The following carbon sources are utilized at 28 °C: *N*-acetyl-D-glucosamine, D-adonitol, D-arabinose, L-arabinose, L-asparagine, L-aspartic acid, citrate, dextrin, erythritol, formic acid (weak), D-fructose, D-galactose, D-gluconic acid, D-glucose, 5-ketogluconate, D-malate, maltose, maltotriose, D-mannitol, D-mannose, pyruvic acid methyl ester (weak), succinic acid, D-tagatose, trehalose and trigonelline. The following carbon sources are not utilized at 28 °C: *cis*-aconitic acid, L-alanine, L-arabitol, betaine, cellobiose, dulcitol, L-fucose, gentiobiose, methyl α-D-glucoside, methyl β-D-glucoside, D-galacturonic acid, D-glucuronic acid, glutarate, histidine, *myo*-inositol, lactose, lactulose, melibiose, L-proline, propionate, quinic acid, raffinose, L-rhamnose, D-sorbitol, L-serine, L-tartrate and *meso*-tartrate. The DNA G+C content of the type strain is 50.2 mol%.

The type strain, LMG 23360^T (=BD 878^T=NCPBB 4036^T=CMC6^T), was isolated from pineapple in the Philippines.

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References

- Bouvet, O. M. M., Lenormand, P. & Grimont, P. A. D. (1989). Taxonomic diversity of the D-glucose oxidation pathway in the *Enterobacteriaceae*. *Int J Syst Bacteriol* 39, 61–67.
- 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.
- 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.
- Cha, J.-S., Pujol, C., Ducusin, A. R., Macion, E. A., Hubbard, C. H. & Kado, C. I. (1997). Studies on *Pantoea citrea*, the causal agent of pink disease of pineapple. *J Phytopathol* 145, 313–319.
- 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., Audo, 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.

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.

Farmer, J. J., III (2005). Genus XXXVII. *Tatumella* Hollis, Hickman and Fanning 1982, 267^{VP} (Effective publication: Hollis, Hickman, Fanning, Farmer, Weaver and Brenner 1981, 86) (Group EF-9 Hollis, Hickman, Fanning, Brenner and Weaver 1980). In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, The *Proteobacteria*, part B, The *Gammaproteobacteria*, pp. 825–826. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

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. 2, The *Proteobacteria*, part B, The *Gammaproteobacteria*, 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.

Hollis, D. G., Hickman, F. W., Fanning, G. R., Farmer, J. J., III, Weaver, R. E. & Brenner, D. J. (1981). *Tatumella ptyseos* gen. nov., sp. nov., a member of the family *Enterobacteriaceae* found in clinical samples. *J Clin Microbiol* **14**, 79–88.

Kageyama, B., Nakae, M., Yagi, S. & Sonoyama, T. (1992). *Pantoea punctata* sp. nov., *Pantoea citrea* sp. nov., and *Pantoea terrea* sp. nov. isolated from fruit and soil samples. *Int J Syst Bacteriol* **42**, 203–210.

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.

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.

Paradis, S., Biossinot, M., Paquette, N., Bélanger, S. D., Martel, E. A., Boudreau, D. K., Picard, F. J., Ouellette, M., Roy, P. H. & Bergeron, M. G. (2005). Phylogeny of the *Enterobacteriaceae* based on genes encoding elongation factor Tu and F-ATPase β -subunit. *Int J Syst Evol Microbiol* **55**, 2013–2025.

Posada, D. & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Pujol, C. J. & Kado, C. I. (2000). Genetic and biochemical characterization of the pathway in *Pantoea citrea* leading to pink disease of pineapple. *J Bacteriol* **182**, 2230–2237.

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.

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 & Wiley-Interscience.