Erwinia tasmaniensis sp. nov., a non-phytopathogenic bacterium from apple and pear trees

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Bacteria were isolated from flowers and bark of apple and pear trees at three places in Australia. In Victoria, Tasmania and Queensland, strains with white colonies on nutrient agar were screened for dome-shaped colony morphology on agar with sucrose and were found to be closely related by several criteria. The isolates were not pathogenic on apples or pears. They were characterized by a polyphasic approach including microbiological and API assays as well as fatty acid methyl ester analysis, DNA–DNA hybridization and DNA sequencing. For molecular classification, the 16S rRNA cistron and the conserved genes *gpd* and *recA* of these bacteria were investigated. Together with other taxonomic criteria, the results of these studies indicate that the bacteria belong to a novel separate species, which we propose to name *Erwinia tasmaniensis* sp. nov., with the type strain Et1/99^T (=DSM 17950^T=NCPPB 4357^T). From DNA–DNA hybridization kinetics, microbiological characteristics and nucleotide sequence analyses, this species is related to pathogenic *Erwinia* species, but also to the epiphytic species *Erwinia billingiae*.

Colonization of flowers by *Erwinia amylovora* is considered to be a crucial step in establishment of most natural fire blight infections. The artificial increase of beneficial competing bacteria has often been proposed to control fire blight. The non-phytopathogenic epiphytic bacterium *Pseudomonas fluorescens* strain A506 has been used as a fire blight biocontrol agent, as well as *Pantoea agglomerans* (syn. *Erwinia herbicola*) strains for precolonization of flowers against fire blight (Johnson & Stockwell, 2000). Here, we have selected bacteria from apple and pear trees in Australia for a white colony morphology and levan formation with sucrose. These features resemble properties of the fire blight pathogen *E. amylovora* (see review by Vanneste, 2000). A related Asian pear pathogen from Korea, *Erwinia pyrifoliae* (Kim *et al.*, 1999, 2001), does not produce levan. The new isolates were classified by their 16S rRNA gene sequence and by partial nucleotide sequences of two housekeeping genes.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene, *gpd* and *recA* sequences of strain Et1/99^T are AM055716–AM055718.

Details of the fatty acid compositions of three of the novel strains and related strains and extended versions of the trees shown in Figs 1 and 2 are available as supplementary material in IJSEM Online.

Alignments of *gpd* gene sequences of necrogenic *Erwinia* and *Brenneria* species have been described in detail for the clarification of phylogenetic relationships (Brown *et al.*, 2000; *gpd*, encoding glyceraldehyde-3-phosphate dehydrogenase, was referred to as *gapDH* in this earlier study). Due to multiple cellular functions of the RecA protein (Kowalczykowski *et al.*, 1994), the *recA* gene is highly conserved in bacteria at the amino acid sequence level. Nevertheless, several silent changes in the nucleotide sequence can be used for species differentiation (Lloyd & Sharp, 1993). The former genus *Erwinia* has been dissected by microrestriction analysis of amplified *recA* PCR fragments (Waleron *et al.*, 2002). The new isolates may contribute towards limiting the occurrence of fire blight in the Southern hemisphere.

Isolation of strains in Australia

In November 1999, we extracted pear flowers for levan-producing bacteria in the area of Knoxfield, Victoria, apple flowers in orchards near Hobart, Tasmania, and bark from apple trees taken in Applethorpe, Queensland, near Brisbane (Table 1), by suspending the plant tissue in a small amount of sterile water. Aliquots of the supernatants were spread on LB agar plates (with 50 μg cycloheximide ml^{-1} to avoid fungal growth), which were incubated for 2 days at 28 °C.

Table 1. Strains used in these studies

Strain	Source, isolation	Reference
Erwinia amylovora		
Ea1/79 (=DSM 17948=NCPPB 4359)	*Cotoneaster* sp., Germany; isolated by W. Zeller	Falkenstein *et al.* (1988)
CFBP 1232^T (=ATCC 15580^T)	Pear, England; deposited by R. A. Lelliott	–
Erwinia billingiae		
Eb661^T (=NCPPB 661^T)	Pear, England; isolated by E. Billing	Mergaert *et al.* (1999)
Erwinia persicina		
CFBP 3622^T (=ATCC 35998^T)	Tomato, Japan; deposited by D. J. Brenner	Hao *et al.* (1990)
***Erwinia pyrifoliae* from Korea**		
Ep1/96 (=DSM 12162)	Asian pear (*Pyrus pyrifolia*); isolated by S.-L. Rhim	Kim *et al.* (1999)
Ep16/96^T (=DSM 12163^T)	Asian pear (*P. pyrifolia*); isolated by S.-L. Rhim	Kim *et al.* (1999)
Erwinia rhapontici		
CFBP 3618^T (=ATCC 29283^T)	Rhubarb, England; deposited by M. P. Starr	Hauben *et al.* (1998)
***Erwinia tasmaniensis* sp. nov.**		
Et1/99^T (=DSM 17950^T=NCPPB 4357^T)	Apple flowers, Tasmania, Australia; isolated by S. Jock and K. Geider	This work
Et2/99 (=DSM 17949=NCPPB 4358)	Pear flowers, Victoria, Australia; isolated by S. Jock and K. Geider	This work
Et4/99	Apple bark, Queensland, Australia; isolated by S. Jock and K. Geider	This work

From pear flowers in Victoria, 25 white colonies on agar plates with approx. 500 colonies were transferred to LB agar with 5 % sucrose and all produced levan. In similar experiments with extracts from apple flowers in Tasmania, 12 of 25 were levan-positive. From extracted bark of apple trees (cultivar 'Fuji') in Queensland, we obtained approx. 200 yellow and 100 white colonies per plate, some of which were levan-positive (2/25). In initial microbiological characterization, the levan-producing isolates did not stain in a Gram treatment and lysed in 3 % KOH (Gregersen, 1978; Suslow *et al.*, 1982), both properties of Gram-negative bacteria. They were also positive in the aminopeptidase reaction (Cerny, 1976; Bascomb & Manafi, 1998) and were facultatively anaerobic, suggesting their classification in the genus *Erwinia*. Three isolated strains, Et1/99^T (isolated in Tasmania), Et2/99 (from Knoxfield, Victoria) and Et4/99 (from the Stanthorpe area in Queensland), were studied in detail. As strains Et1/99^T and Et2/99 were isolated as the dominant bacteria by suspending apple or pear flowers in water, they should exist as epiphytes that colonize plant surfaces.

Virulence assays

For virulence assays on immature pear fruits, strains Et1/99^T, Et2/99 and Et4/99 were inoculated on slices of immature pear with toothpicks dipped into fully grown suspension cultures. In contrast to *E. amylovora* and *E. pyrifoliae*, no ooze production was seen after incubation for 1 week or longer. In assays with apple seedlings (cultivar 'Golden Delicious'), no necrotic symptoms were observed after inoculation with the novel strains and incubation of the seedlings for 2–3 weeks. Consequently, the isolates are non-pathogenic for common fire blight host plants.

16S rRNA gene sequences

For molecular classification of the novel strains, DNA fragments were amplified with three sets of PCR primers (Table 2). DNA bearing 16S rRNA gene sequences was obtained with the primers fd2 and rP1. The 1·4 kb amplification product was cloned into vector pGEM-T (Promega) and sequenced. The nucleotide sequences of the three novel strains were aligned with a series of 16S rRNA gene sequences from other *Erwinia* species and species of *Pectobacterium*, *Pantoea* and *Brenneria* (Fig. 1 and Supplementary Fig. S1 available in IJSEM Online). They were closely related and were separated from *E. amylovora*, *E. pyrifoliae* and *Erwinia billingiae*. Dendrograms were constructed with CLUSTAL X for Windows version 1.81 and MEGA 3.1 (Kumar *et al.*, 2004). Phylogenetic distances were estimated by the method of Jukes & Cantor (1969) and tree topology was inferred by the neighbour-joining method with a bootstrap value of 1000. For reconstruction of phylogeny, the neighbour-joining and maximum-parsimony methods produced similar results. The 16S rRNA gene sequences of strains Et1/99^T, Et2/99 and Et4/99 possessed signature nucleotides identical to those described for the genus *Erwinia* (Hauben *et al.*, 1998) [A, A, C, G, G, C, G, G, G, C, G, C, C, C and G at positions 408, 595, 599, 639, 646, 839, 847, 987, 988, 989, 1216, 1217, 1218, 1308 and 1329, according to the *Escherichia coli* 16S rRNA gene sequence numbering (GenBank accession no. J01695)]. A shift by one position was found for the indicative nucleotides A and C given by Hauben *et al.* (1998) for positions 594 and 598 in the 16S rRNA gene sequences of all *Erwinia* strains included in Fig. 1. Among the 16S rRNA gene sequences, those from *E. amylovora*, *E. pyrifoliae*, *E. billingiae* and *Erwinia persicina* exhibited sequence similarities

Table 2. PCR primers applied in gene amplification

PCR primers GAP11 and GAP12c were used for strains Ea1/79 and Eb661.

Primer	Sequence (5′–3′)	Reference
16S rRNA gene		
fd2	AGAGTTTGATCATGGCTCAG	Weisburg *et al.* (1991)
rP1	ACGGTTACCTTGTTACGACTT	
gpd		
GAP1F	TGAAATATGACTCCACTCACGG	Brown *et al.* (2000)
GAP1R	TAGAGGACGGGATGATGTTCTG	
GAP11	ACGGCACTGTAGAAGTC	This work
GAP12c	CCAGTCTTTGTGAGACG	
recA		
RECA1	GGTAAAGGGTCTATCATGCG	Waleron *et al.* (2002)
RECA2c	CCTTCACCATACATAATTTGGA	

to the novel strains that exceeded 97 %. Consequently, further classification steps were focused on these species.

gpd and *recA* sequences

The housekeeping genes *gpd* and *rec*A have been evaluated previously as phylogenetic tools in discrimination of bacterial species (Brown *et al.*, 2000; Lloyd & Sharp, 1993). Consensus primers from the *gpd* gene (Table 2), applied by Brown *et al.* (2000) for classification of necrogenic *Erwinia* and *Brenneria* species, were used for PCR amplification of genomic DNA from the novel strains and strains of *E. amylovora* and *E. pyrifoliae*. The products were cloned into pGEM-T and the nucleotide sequences were analysed for their relationship to each other and other bacterial species (Fig. 2a and Supplementary Fig. S2a). The three novel strains were highly related and formed a cluster adjacent to several *E. amylovora* strains and to *E. pyrifoliae*. They were separated from those of *E. billingiae*, *Erwinia mallotivora*, *Erwinia papayae*, *E. persicina*, *Erwinia psidii*, *Erwinia rhapontici*, *Erwinia toletana* and *Erwinia tracheiphila*. The genus *Brenneria* and *Dickeya chrysanthemi* were more distantly related, with *Pectobacterium carotovorum* used as outgroup. From the novel strains, *E. amylovora* strains and related bacteria, parts of the *recA* gene were PCR amplified, cloned, sequenced and aligned. According to the alignment, the three novel strains formed a narrow group apart from *E. pyrifoliae* and *E. amylovora* (Fig. 2b and Supplementary Fig. S2b). Interestingly, strains within these species were not completely identical in their *recA* sequences. All three species were well separated from species of the genera *Brenneria* and *Pectobacterium*. Thus, the use of these two housekeeping genes confirmed the results of the previous analysis that classified the novel strains into a new species, separated from *E. amylovora* and *E. pyrifoliae*.

Microbiological assays

The phenotypic characteristics of the Australian isolates were compared with those of various *Erwinia* species listed in Table 1. The Gram-negative, facultatively anaerobic bacteria were motile, straight rods, they metabolized glucose, lacked gas production and were oxidase-negative and catalase-positive. The isolates showed the general characteristics of strains of the genus *Erwinia* in the family *Enterobacteriaceae* (Hauben & Swings, 2005). The isolates from Australia reacted uniformly in most biochemical and physiological tests except for their reactions for galactose, inositol and xylitol. The novel strains shared some metabolic characteristics with other *Erwinia* species, such as acetoin production, utilization of glucose, mannitol and arabinose, the lack of arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase, no production of indole, gelatinase, urease, tryptophan deaminase or H_2S and a lack of nitrate reduction. They differed in a positive reaction of β-galactosidase, they utilized citrate, but did not metabolize sorbitol, rhamnose or amygdalin, they did not reduce nitrate and did not grow at 36 °C (Table 3). Altogether, 79

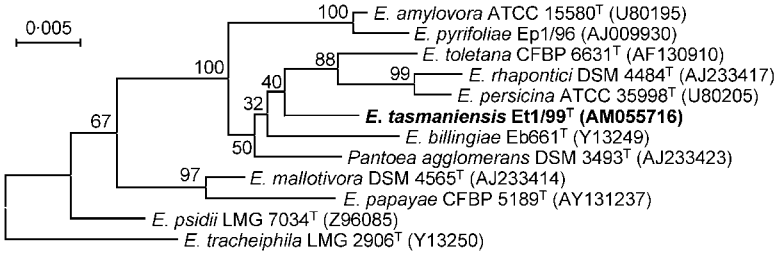

Fig. 1. Dendrogram constructed from 16S rRNA gene sequences for classification of strains Et1/99^T, Et2/99 and Et4/99 isolated in Australia from the apple and pear flora. Bootstrap values, expressed as percentages of 1000 replications, are given at branching points. *Pectobacterium carotovorum* ATCC 15713^T was used as an outgroup (not shown). Accession numbers of the nucleotide sequences are provided in parentheses.

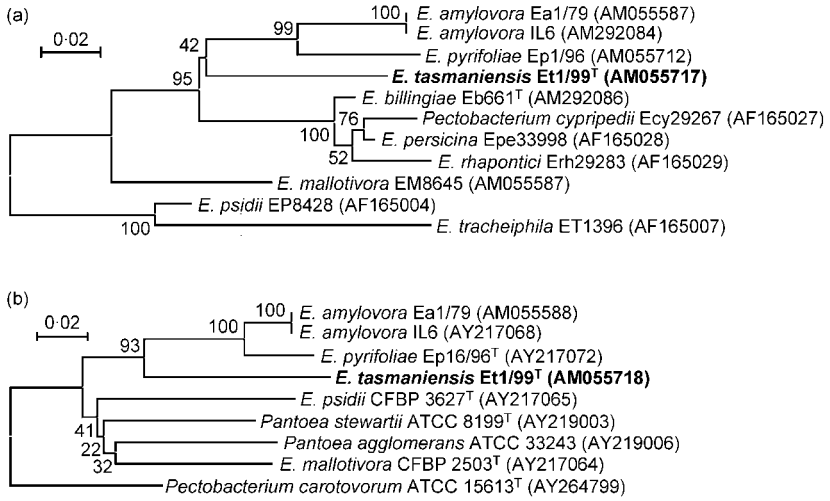

Fig. 2. Dendrograms constructed from *gpd* (a) and *recA* (b) sequences for classification of strains Et1/99^T, Et2/99 and Et4/99. Sequence accession numbers are shown in parentheses. Bootstrap values, expressed as a percentage of 1000 replications, are given at branching points.

characteristics were used to construct a dendrogram showing the phenotypic distance relationships among the strains tested. Isolates Et1/99^T, Et2/99 and Et4/99 clustered together in one group within 80 %. This group is phenotypically related to *E. amylovora* and *E. pyrifoliae*, but clearly different from the other groups (Fig. 3).

Supplementary Table S1 shows the percentage of characteristic fatty acids (>0·5 %) found in the strains tested. Palmitic acid (16 : 0) and palmitoleic acid (16 : 1ω7*c*) dominated, making up 50–65 % of the total fatty acids. The Australian isolates showed profiles that were quantitatively and qualitatively similar, without statistically significant differences. Tridecanoic acid (13 : 0) was completely absent in these isolates. The percentage of 17 : 0 cyclo was low and that of 16 : 1ω7*c* was relatively high compared with other *Erwinia* species. The dendrogram shows that the Australian isolates are significantly separated from the other *Erwinia* species (Fig. 4).

Table 3. Selected physiological and biochemical characteristics of the novel strains in comparison with other *Erwinia* species

Strains: 1, *E. persicina* CFBP 3622^T; 2, *E. rhapontici* CFBP 3618^T; 3, *E. billingiae* Eb661^T; 4, *E. amylovora* Ea1/79; 5, *E. pyrifoliae* Ep16/96^T; 6, strains Et1/99^T, Et2/99 and Et4/99. The methods have been described by Fahy & Hayward (1983).

Test	1	2	3	4	5	6
β-Galactosidase	+	+	+	−	−	+
Citrate utilization	+	+	−	−	−	+
Utilization of:						
Inositol	+	+	+	−	−	d*
Sorbitol	+	−	+	+	+	−
Rhamnose	+	+	+	−	−	−
Sucrose	+	+	−	+	+	+
Melibiose	+	+	+	−	−	+
Amygdalin	+	+	+	−	−	−
Nitrate reduction	+	+	+	−	−	−
Growth at 36 °C	+	−	−	−	−	−
Growth in 5 % NaCl	+	+	+	+	−	+

*Negative for Et1/99^T and Et4/99 and positive for Et2/99.

Polyamine and protein patterns

By using the method of Busse & Auling (1988), diaminopropane was found to be the major polyamine compound of isolates Et1/99^T, Et2/99 and Et4/99 (data not shown), grouping them close to *E. amylovora* (Zherebilo *et al.*, 2001) by their polyamine pattern. The three strains displayed identical protein patterns when extracts of whole-cell proteins were assayed by SDS-PAGE (Fig. 5). The similarity

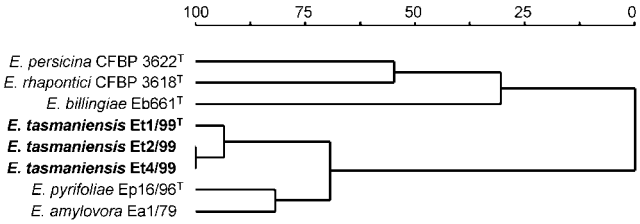

Fig. 3. Dendrogram constructed from 79 phenotypic characteristics for classification of strains Et1/99^T, Et2/99 and Et4/99 in comparison with several other *Erwinia* species. In addition to physiological characteristics (Table 3), the strains were examined at 28 °C in the standardized identification systems API 20E (bioMérieux), containing 22 miniaturized biochemical tests, and API 50CHE (bioMérieux), containing 49 carbon compounds, read visually after 24 and 48 h. Inocula for the tests were prepared from 24–48 h cultures grown on Stl agar (Merck) at 28 °C for *E. amylovora*, *E. billingiae*, *E. persicina*, *E. rhapontici*, *E. pyrifoliae* and the novel strains. The distance matrix was calculated by using Euclidean distances and cluster analysis was done with the nearest-neighbour method (single linkage) (PC-ORD, version 4.14; MjM software).

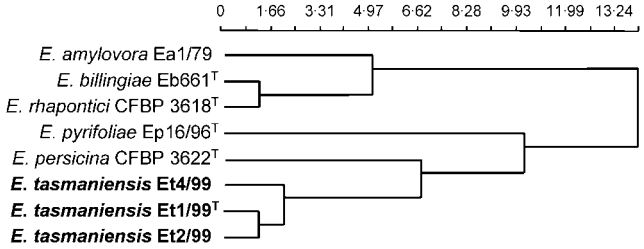

Fig. 4. Dendrogram (UPGMA clustering method, Euclidian distance) constructed from whole-cell fatty acids for classification of strains Et1/99^T, Et2/99 and Et4/99.

to reference strains of the species *E. amylovora* and *E. pyrifoliae* was lower.

DNA–DNA hybridizations

The DNA base composition of the isolates was in a narrow range between 50·5 and 52·4 mol% G+C. The results of DNA–DNA hybridizations confirmed that the three isolates Et1/99^T, Et2/99 and Et4/99 were highly related (Table 4). DNA–DNA relatedness provides a single species definition that can be applied equally to the genomes of all bacteria without limitation to single genes. A phylogenetic definition of a species (genomospecies) was formulated as strains with approximately 70 % or greater DNA–DNA relatedness by Wayne *et al.* (1987). The degree of binding in reassociation experiments with DNA from reference strains of *E. amylovora*, *E. billingiae*, *E. persicina*, *E. pyrifoliae* and *E. rhapontici* was well below 70 % (Table 4), indicating that the *Erwinia* isolates from Australia belong to a species different from the other *Erwinia* species.

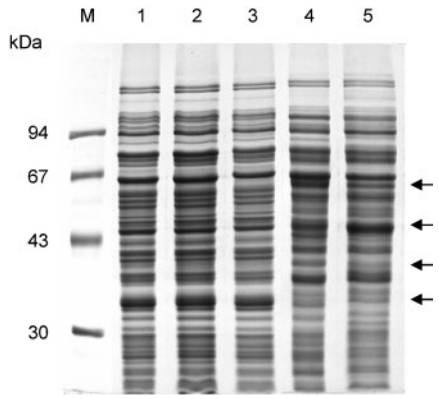

Fig. 5. Protein gel from cell extracts of several *Erwinia* strains. Lanes: M, protein size markers; 1, *E. tasmaniensis* Et4/99; 2, *E. tasmaniensis* Et1/99^T; 3, *E. tasmaniensis* Et2/99; 4, *E. pyrifoliae* Ep1/96; 5, *E. amylovora* Ea1/79. Some characteristic bands for *E. tasmaniensis* strains in comparison with the other erwinias are marked with arrows. The protocol of Busse *et al.* (1989) was used for SDS-PAGE of soluble proteins.

Table 4. Levels of DNA–DNA reassociation among strains Et1/99^T, Et2/99 and Et4/99 and strains of *E. amylovora* and *E. pyrifoliae*

Hybridization studies with labelled DNA of strain Et1/99^T were done as described by Auling *et al.* (1986) using the optical method of De Ley *et al.* (1970) for measurement of initial renaturation rates. The concentrations of DNA were determined chemically according to Richards (1974).

Strain	Relative DNA binding (%)
E. tasmaniensis sp. nov.	
Et1/99^T	100
Et2/99	100
Et4/99	100
E. amylovora	
Ea1/79	43
CFBP 1232^T	51
E. billingiae Eb661^T	56
E. persicina CFBP 3622^T	54
E. pyrifoliae	
Ep1/96	58
Ep16/96^T	58
E. rhapontici CFBP 3618^T	44

Conclusions

According to the microbiological and molecular results, we propose to classify the described levan-producing strains from apple and pear into a novel species, named *Erwinia tasmaniensis* sp. nov., with Et1/99^T as the type strain. The isolated bacteria are distinct from *E. amylovora* or the Asian-pear-pathogenic species *E. pyrifoliae* according to several criteria, including their inability to cause disease symptoms on immature pears or necrosis on apple seedlings. API tests, DNA–DNA hybridization and nucleotide sequence analysis allow them to be classified into the novel species *E. tasmaniensis*. Two other isolates, Et2/99 and Et4/99, were highly related in many properties. The classification of the levan-producing bacteria into the genus *Erwinia* has added a novel species into a group of mostly necrogenic bacteria. After transfer of the *Erwinia herbicola* group to the genus *Pantoea*, *E. tasmaniensis* is a second non-pathogenic species in the genus *Erwinia* in addition to *E. billingiae* (Mergaert *et al.*, 1999). Similarities above 97 % for 16S rRNA gene sequences may indicate a high relationship of strains and additional parameters may be needed for species classification (Gevers *et al.*, 2005). Nevertheless, the 16S rRNA gene sequence trees that we constructed showed the same monophyletic groups as the trees from *gpd* and *recA* genes. Minor differences that were observed in the positions of the groups relative to each other are in agreement with previous phylogenetic analysis of these two and several other housekeeping genes (Wertz *et al.*, 2003). Thus, we concluded that the *gpd* and *recA* genes can also be used to discriminate between *E. amylovora* and related species.

Description of *Erwinia tasmaniensis* sp. nov.

Erwinia tasmaniensis (tas.ma.ni.en′sis. N.L. fem. adj. *tasmaniensis* pertaining to Tasmania, where the type strain was isolated).

Cells are Gram-negative, non-spore-forming, motile, straight rods, measuring 0·5–1 by 1·5–2 μm. Facultatively anaerobic, oxidase-negative and catalase-positive. The species has all of the characteristics of the genus *Erwinia*. Colonies on Standard I agar (StI; Merck) are light-beige, circular, smooth, translucent, flat to slightly convex and about 3 mm in diameter after 24–48 h at 28 °C. No diffusible or fluorescent pigment is observed. Strains grow well on StI agar and nutrient agar at 28 °C, but not at 36 °C. They are able to grow in 5 % NaCl. Glucose is fermented without gas production. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, gelatinase and tryptophan deaminase are not produced, but β-galactosidase is present. Indole and H_2S are not produced. There is acetoin production. Nitrates are not reduced. The following carbon sources are utilized at 28 °C within 3 days: L-arabinose, ribose, glucose, fructose, galactose (except strain Et4/99), mannitol, *N*-acetylglucosamine, sucrose, trehalose, melibiose (weak) and citrate. The following carbon sources are not utilized at 28 °C within 3 days: sorbitol, rhamnose, amygdalin, glycerol, erythritol, D-arabinose, D-xylose, L-xylose, adonitol, methyl β-D-xyloside, mannose, sorbose, dulcitol, inositol (except strain Et2/99), methyl α-D-mannoside, methyl α-D-glucoside, arbutin, aesculin, salicin, cellobiose, maltose, lactose, inulin, melezitose, raffinose, starch, glycogen, xylitol (except strain Et2/99), gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate. The G+C contents of strains Et1/99^T, Et2/99 and Et4/99 are 50·5–52·4 mol% as determined by the T_m method. The known strains were isolated from the phyllosphere, in particular apple flowers, pear flowers and bark of apple trees. The species may also occur on other plant surfaces and may also be found outside Australia.

The type strain, Et1/99^T (=DSM 17950^T=NCPPB 4357^T), was collected in Tasmania from apple flowers. Strain Et2/99 (=DSM 17949=NCPPB 4358) was isolated from pear flowers in Victoria and strain Et4/99 was isolated from apple bark in Queensland.

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Supplementary Table S1. Fatty acid profiles of selected *Erwinia* species and from strains of *Erwinia tasmaniensis* sp. nov.

For fatty acis profiles, the bacteria were grown on trypticase soy broth agar (Difco) at 28 °C for 48 h. Approximately 40 mg wet weight of cells was harvested from the most dilute quadrant showing confluent growth (cells in the late exponential phase). Whole fatty acids were analysed at the Hans-Knöll-Institut für Naturstofforschung e.V. (Jena) as described in the Microbial Identification System Manual (Sasser, 1990) using the MIDI Microbial Identification. Statistical analysis of the fatty acid profiles was performed using the software programs of the MIDI system. Quantities are expressed as percentages of the total peak area (only fatty acids making up >0·5 % are listed). For each strain, fatty acid profiles from three extractions were applied to analytical runs. The mean (standard deviation) for each fatty acid are given. ECL, Equivalent chain length of unknown fatty acid.

Fatty acid	***E. persicina*** **CFBP 3622^T**	***E. rhapontici*** **CFBP 3618^T**	***E. billingiae*** **Eb661^T**	***E. amylovora*** **Ea1/79**	***E. pyrifoliae*** **Ep16/96^T**	***E. tasmaniensis***		
						Et1/99^T	**Et2/99**	**Et4/99**
12:0	4·34 (0·39)	3·47 (0·13)	3·68 (0·33)	5·48 (0·15)	4·58 (0·17)	4·34 (0·04)	4·33 (0·07)	4·27 (0·14)
13:0		0·61 (0·13)	0·54 (0·15)	0·96 (0·06)	0·73 (0·14)			
14:0	5·99 (0·52)	5·33 (0·30)	5·81 (0·62)	4·95 (0·15)	4·95 (0·14)	6·1 (0·37)	6·15 (0·27)	6·91 (0·21)
15:1 iso/13:0 3-OH			0·70 (0·19)	1·22 (0·16)				
ECL 14·502	1·32 (0·19)	1·13 (0·35)	0·94 (0·08)	0·92 (0·02)	1·04 (0·37)	1·11 (0·27)	1·07 (0·01)	0·97 (0·07)
15:0	1·30 (0·33)	2·86 (0·21)	2·57 (0·52)	1·87 (0·23)	2·60 (0·93)	1·73 (0·20)	1·16 (0·15)	1·04 (0·11)
16:1 iso/14:0 3-OH	10·86 (0·26)	9·82 (0·18)	10·03 (0·57)	10·32 (0·80)	9·32 (0·51)	9·19 (0·17)	9·67 (0·12)	9·23 (0·7)
16:1*ω7c*/15:0 iso 2-OH	24·81 (3·35)	19·73 (0·93)	22·15 (2·81)	18·13 (0·51)	36·20 (0·96)	29·66 (0·25)	30·29 (0·77)	30·11 (0·53)
16:0	30·61 (0·08)	31·64 (0·35)	31·26 (1·99)	29·46 (0·62)	31·66 (0·98)	34·34 (0·95)	34·49 (0·93)	35·38 (0·46)
17:0 cyclo	9·15 (2·14)	12·43 (0·85)	8·92 (2·23)	10·64 (0·67)		3·79 (0·12)	3·66 (0·17)	3·82 (0·17)
17:0	1·97 (0·29)	3·28 (0·31)	3·18 (0·99)	5·22 (0·6)	2·21 (0·75)	1·42 (0·17)	0·92 (0·15)	0·80 (0·20)
18:1*ω7c*		8·47 (0·23)	9·61 (1·63)	9·76 (0·35)	6·42 (0·53)	8·17 (0·46)	8·45 (0·30)	7·50 (0·29)

Reference

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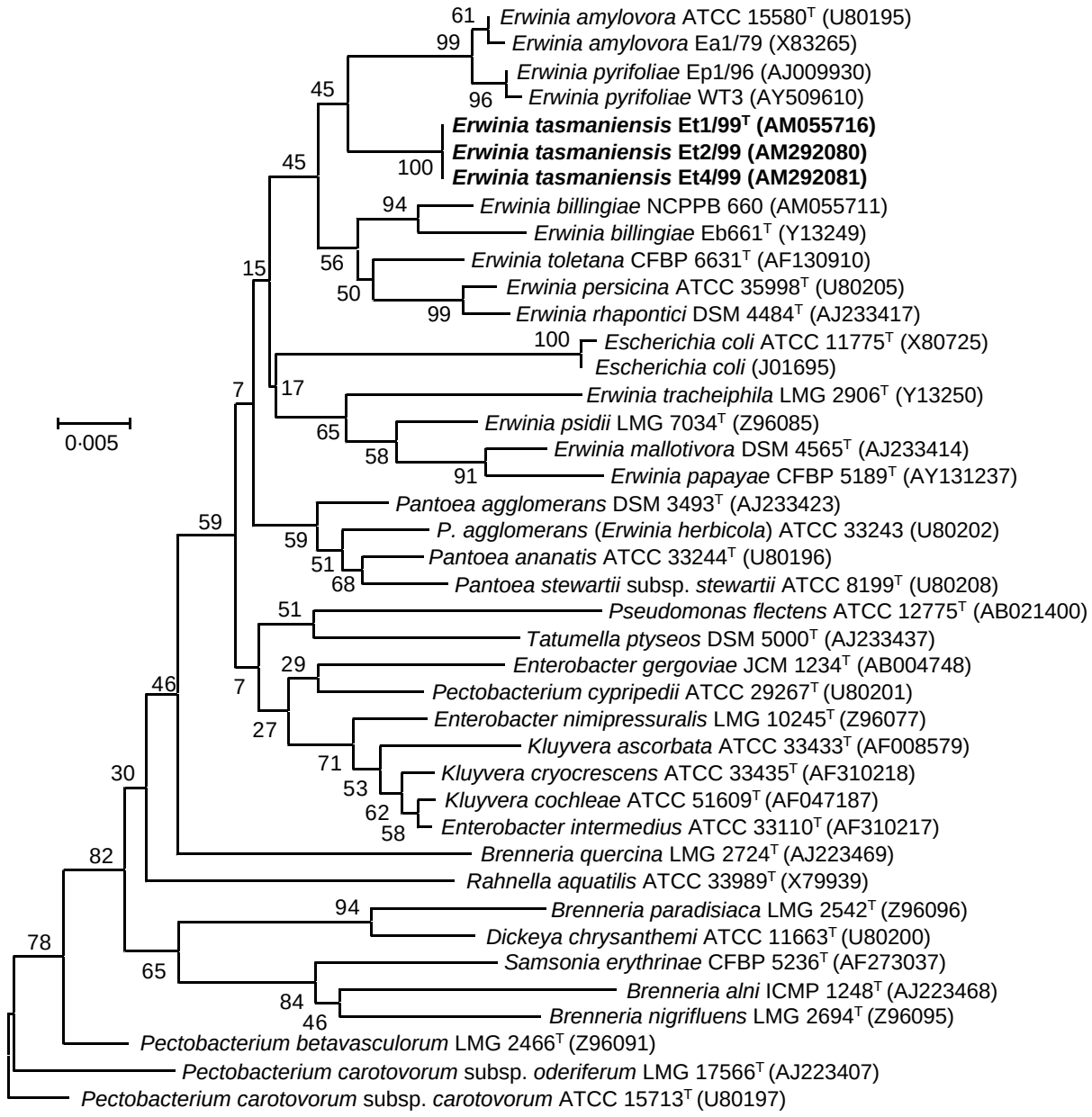
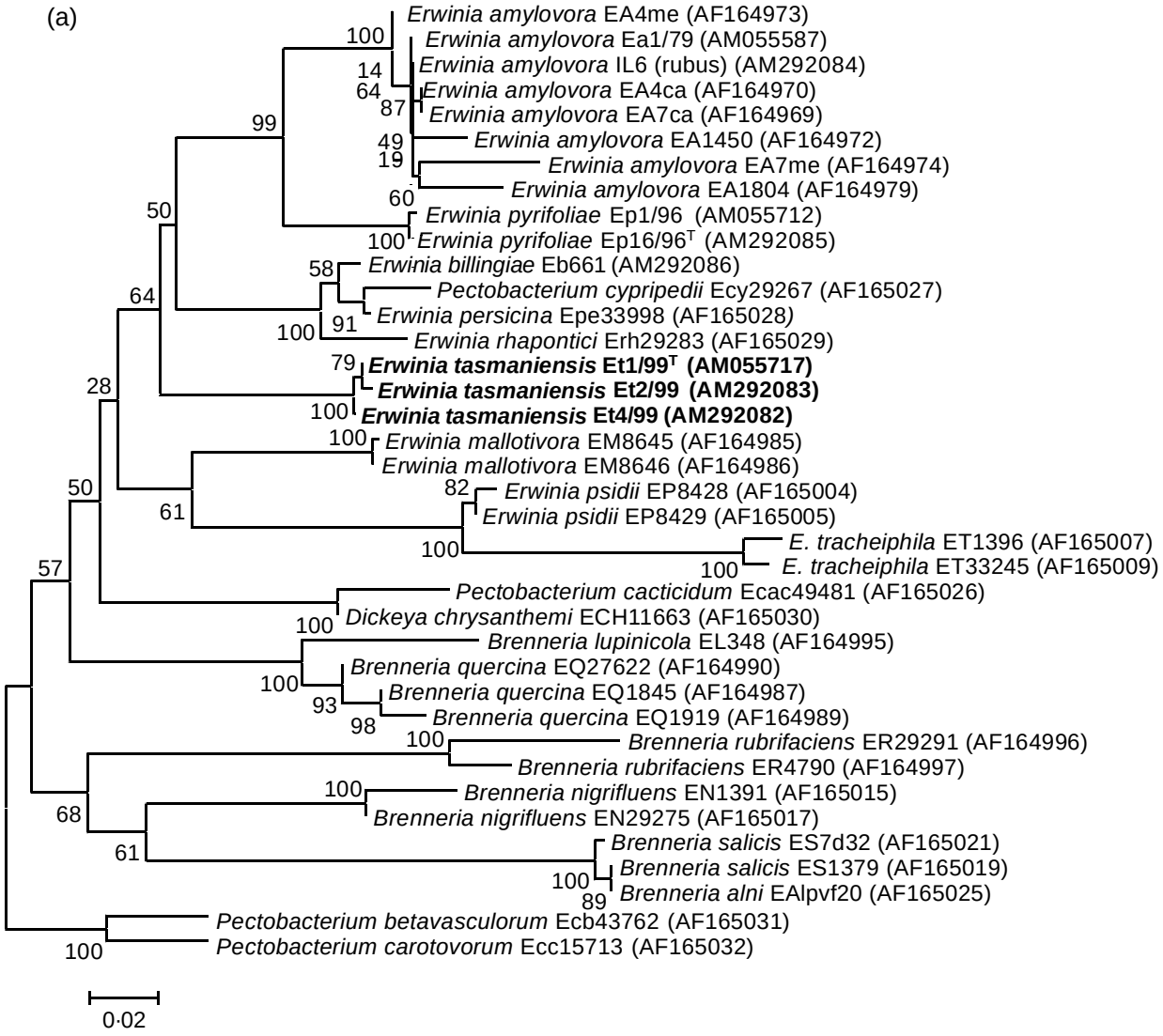


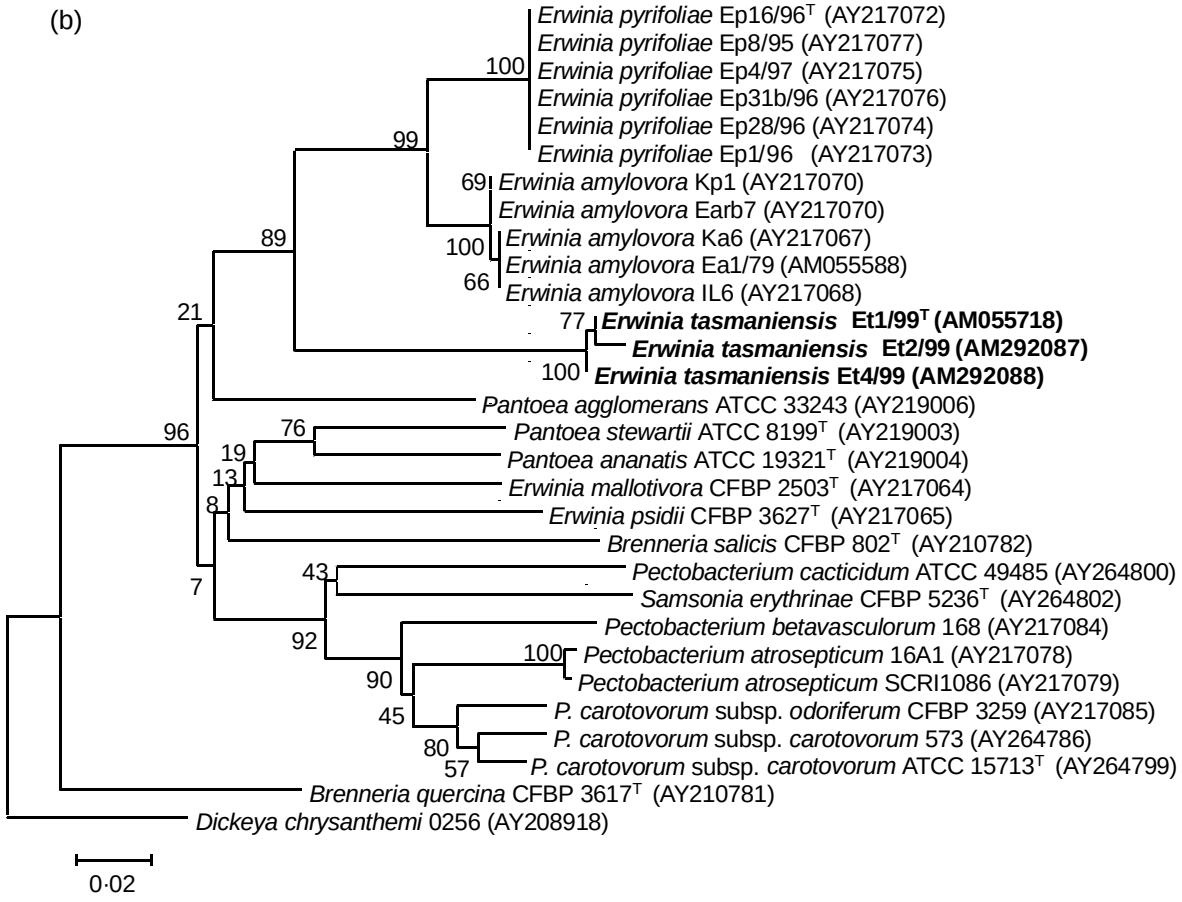
Supplementary Fig. S1. Dendrogram from 16S rRNA gene sequences for classification of bacteria isolated in Australia from the apple and pear flora as *Erwinia tasmaniensis* sp. nov. Bootstrap values, expressed as percentags of 1000 replications, are given at branching points. *Pectobacterium carotovorum* subsp. *carotovorum* ATCC 15713^T was used as an outgroup.

Geider, K., Auling, G., Du, Z., Jakovljevic, V., Jock, S. & Völksch, B. (2006). *Erwinia tasmaniensis* sp. nov., a non-phytopathogenic bacterium from apple and pear trees. *Int J Syst Evol Microbiol* **56**, 2937–2943.



Supplementary Fig. S2(a). Dendrogram from *gpd* sequences for classification of three *E. tasmaniensis* strains isolated in Australia. Sequence accession numbers are shown in parentheses. Bootstrap values, expressed as percentages of 1000 replications, are given at branching points.

Geider, K., Auling, G., Du, Z., Jakovljevic, V., Jock, S. & Völksch, B. (2006). *Erwinia tasmaniensis* sp. nov., a non-phytopathogenic bacterium from apple and pear trees. *Int J Syst Evol Microbiol* **56**, 2937–2943.



Supplementary Fig. S2(b). Dendrogram from *recA* sequences for classification of three *E. tasmaniensis* strains isolated in Australia. Sequence accession numbers are shown in parentheses. Bootstrap values, expressed as percentages of 1000 replications, are given at branching points

Geider, K., Auling, G., Du, Z., Jakovljevic, V., Jock, S. & Völksch, B. (2006). *Erwinia tasmaniensis* sp. nov., a non-phytopathogenic bacterium from apple and pear trees. *Int J Syst Evol Microbiol* **56**, 2937–2943.