***Samsonia erythrinae* gen. nov., sp. nov., isolated from bark necrotic lesions of *Erythrina* sp., and discrimination of plant-pathogenic *Enterobacteriaceae* by phenotypic features**

Laurent Sutra,¹ Richard Christen,² Claude Bollet,³ Philippe Simoneau¹ and Louis Gardan¹

¹ UMR de Pathologie Végétale INRA-INH-Université, 42 rue Georges Morel, BP 57, F-49071 Beaucouzé Cedex, France

² ESA 7076, Université Pierre et Marie Curie et CNRS, Observatoire Océanologique, Station Zoologique, F-06230, Villefranche-sur-mer, France

³ INSERM CJF 96.06, Faculté de Médecine, 27 boulevard Jean Moulin, F-13385 Marseille Cedex 05, France

Author for correspondence: Laurent Sutra. Tel: +33 2 41 22 57 29. Fax: +33 2 41 22 57 05. e-mail: sutra@angers.inra.fr

Bacterial strains isolated from diseased erythrina (*Erythrina* sp.) trees in Martinique (French West Indies) were studied using phenotypic tests, 16S rDNA sequence analysis and DNA–DNA hybridization. Numerical analysis of phenotypic characteristics showed that these strains formed an homogeneous phenon among plant-pathogenic *Enterobacteriaceae*, and gave useful and updated information for the identification of these bacteria. Results of DNA–DNA hybridization indicated that strains from erythrina belonged to a discrete genomospecies (89–100 % hybridization) and had low levels of DNA relatedness (2–33 % hybridization) with reference strains of phytopathogenic *Erwinia*, *Brenneria*, *Pectobacterium*, *Pantoea* and *Enterobacter* species. 16S rDNA sequence analysis using three different methods revealed that the position of strain CFBP 5236^T isolated from erythrina was variable in the different trees, so that strains from erythrina could not be assigned to any recognized genus. It is proposed that these strains are included in a new genus, *Samsonia*. The name *Samsonia erythrinae* is proposed for the new species. The G+C content of the DNA of the type strain, CFBP 5236^T (= ICMP 13937^T), is 57.0 mol %.

Keywords: *Samsonia erythrinae* gen. nov., sp. nov., *Erythrina*, plant-pathogenic *Enterobacteriaceae*, 16S rDNA sequence analysis, DNA–DNA hybridization

INTRODUCTION

A bacterial disease of trees of erythrina (*Erythrina* sp.) used as windbreaks around banana plantations was observed in Martinique (French West Indies) in 1995 (Sutra *et al.*, 1999). This disease was characterized by defoliation of the main branches of trees and browning of large areas of the bark surface, which girdled the base of defoliated branches. Removal of the superficial layer of the bark revealed brown, water-soaked necrotic lesions of the deeper layers of the bark and of the cambium, exhaling a nasty smell. All these symptoms suggested that the causal agent of the disease was different from *Xanthomonas axonopodis* pv. *erythrinae* which caused exclusively a leaf spot

disease on erythrina in India (Patel *et al.*, 1952). Bacterial strains were isolated as nearly pure cultures from natural necrotic lesions of different diseased erythrina trees. All isolated strains had the general characteristics of the family *Enterobacteriaceae*. The fact that they were β -galactosidase-positive, arginine-dihydrolase-, lysine- and ornithine-decarboxylase-negative, produced acetoin and were weakly pectolytic (Sutra *et al.*, 1999) suggested that these bacteria belong to the genus *Erwinia* or to one of the related genera recently proposed by Hauben *et al.* (1998). A preliminary study based on a limited number of phenotypic characteristics and on pathogenicity tests showed that strains isolated from erythrina trees were phenotypically related to, but different from, *Pectobacterium carotovorum* subsp. *betavascularum* and subsp. *wasabiae*, and that they were pathogenic when inoculated to erythrina cuttings (Sutra *et al.*, 1999). All these

The GenBank accession number for the 16S rRNA gene sequence of strain CFBP 5236^T is AF273037.

results suggested that bacteria isolated from diseased erythrina trees constitute a novel taxon within the plant-pathogenic *Enterobacteriaceae*.

The aim of the present study was to determine the taxonomic status of these bacteria by comparison with reference strains of previously described plant-pathogenic species of *Enterobacteriaceae* on the basis of numerical analysis of phenotypic characteristics, DNA–DNA hybridizations and sequence analyses of 16S rRNA genes. According to our results, we propose that the strains isolated from diseased erythrina trees are included in a new genus, *Samsonia* gen. nov., and in a new species, *Samsonia erythrinae* sp. nov. Moreover, additional information is given for the identification of plant-pathogenic species of the family *Enterobacteriaceae* based on the phenotypic features of reference and type strains.

METHODS

Bacterial strains. Five strains (CFBP 5236^T–CFBP 5240) were isolated from necrotic lesions of erythrina from two locations in Martinique (French West Indies) (Sutra *et al.*, 1999). Thirty-six type and reference strains of *Brenneria*, *Enterobacter*, *Erwinia*, *Pantoea* and *Pectobacterium* plant-pathogenic species were used for comparisons in numerical analysis of phenotypic data and DNA–DNA hybridizations. All reference strains were obtained from the Collection Française de Bactéries Phytopathogènes (CFBP, Angers, France) and are designated according to the present nomenclature of plant-pathogenic *Enterobacteriaceae* (Young *et al.*, 1996; Hauben *et al.*, 1998) (Fig. 1 and Table 3). Strains were cultured routinely on YBGA (yeast extract, 7 g; bactopectone, 7 g; glucose, 7 g; agar, 15 g; distilled water, 1000 ml; pH 7.2) and incubated at 28 °C.

Phenotypic tests. Twenty-two conventional biochemical and physiological tests were used. Hydrolysis of gelatin, lecithin and casein was tested on gelatin agar, on egg-yolk agar and on skimmed-milk agar, respectively (Smibert & Krieg, 1994). Acid production from inulin in phenol red peptone water, production of reducing substances from sucrose, malonate utilization, indole production from tryptophan, anaerobic degradation of arginine (Moeller medium) and utilization of citrate as sole carbon source (Simmons' medium) were studied using previously described methods (Ngwira & Samson, 1990; Gallois *et al.*, 1992). Liquefaction of calcium polygalacturonate was tested in Sutton's medium modified after Bonnet (1973). Utilization of D-(–)-tartrate and acid production from α -methyl glucoside, D-(+)-arabitol, D-(–)-arabinose, lactose, mannitol, melibiose, raffinose and sorbitol were tested on the basal medium of Ayers *et al.* (1919) supplemented with 0.1 % (w/v) organic acid sodium salt or sugar. All these tests were incubated at 28 °C for 15 d. Strains were also investigated for their ability to grow at 36 and 39 °C in King B broth, and at 25 °C in yeast peptone broth containing 5 % sodium chloride. In addition, strains were tested for the assimilation of 99 organic substrates using commercial Biotype 100 strips (bioMérieux). Strips were inoculated as recommended by the manufacturer and results were read visually after 2, 3 and 4 d incubation at 28 °C.

Numerical taxonomy. A total of 121 characteristics (22 conventional tests and assimilation of 99 organic substrates) were included in the numerical taxonomy analysis. The

distance matrix was calculated using the Jaccard coefficient (Sneath & Sokal, 1973). Cluster analysis was done by using the unweighted pair group method with arithmetic averages (UPGMA) (Sneath & Sokal, 1973). At a chosen level of phenotypic distance, the amount of information for each test was measured by calculating the diagnostic ability coefficient (Descamps & Véron, 1981) to determine the discriminating biochemical characteristics of the strains clustered in the different phenons and of unclustered strains.

Sequencing of 16S rDNA and phylogenetic analyses.

Extraction of DNA from strain CFBP 5236^T was carried out by using a QIAmp kit (Qiagen). PCR-mediated amplification of the 16S rDNA of strain CFBP 5236^T and sequence determination were performed as previously described (Weisburg *et al.*, 1991). The 16S rDNA sequence obtained for strain CFBP 5236^T was aligned manually by reference to a database of about 10000 already aligned bacterial 16S rDNA sequences. Phylogenetic trees were constructed using three different methods: neighbour-joining (bioNJ), maximum-likelihood and maximum-parsimony. Only sites that had no obvious homoplasy were used for these analyses (positions 89–451, 475–1001, 1006–1128, 1133–1388 of the 16S rDNA sequence of strain CFBP 5236^T). For the bioNJ analysis, a distance matrix was calculated according to the Kimura two-parameters correction. The results of bioNJ analysis were evaluated with bootstrap using 500 replications. BioNJ analysis was performed according to Gascuel (1997), maximum-likelihood data (options QFYG) were obtained using the fastDNAm1 program of G. J. Olsen (University of Illinois, Urbana, USA) and maximum-parsimony analysis was performed using PHYLIP (Phylogeny Inference Package, version 3.573c, distributed by J. Felsenstein, University of Washington, Seattle, USA). The phylogenetic trees were drawn using NJPLOT (Perrière & Gouy, 1996) and MacDRAW software for the Apple Macintosh.

DNA extraction and DNA–DNA hybridization. Extraction and purification of DNA were performed as described by Brenner *et al.* (1982). Native DNAs of strains CFBP 5236^T and CFBP 5237 were labelled *in vitro* with tritium-labelled nucleotides by random-priming (Feinberg & Vogelstein, 1983) using a Megaprime kit (Amersham). The S1 nuclease/trichloroacetic acid method was used for DNA–DNA hybridizations (Croza *et al.*, 1973; Grimont *et al.*, 1980). The reassociation was performed at 60 °C in 0.42 M NaCl. The strains used in DNA–DNA hybridizations are listed in Table 3.

DNA base composition. The G + C contents of strains CFBP 5236^T and CFBP 5237 were determined by the thermal denaturation temperature (Mamur & Doty, 1962) and were calculated by using the equation of Owen & Lapage (1976). *Escherichia* strain K12 CIP 54–117 (DNA G + C content of 50.6 mol %) was used as control.

RESULTS AND DISCUSSION

Numerical analysis of phenotypic characteristics

A preliminary study based on 48 phenotypic tests suggested that strains isolated from diseased erythrina trees in Martinique (French West Indies) constituted an original phenotypic group within the plant-pathogenic *Enterobacteriaceae* (Sutra *et al.*, 1999). A more complete analysis of 121 phenotypic characteristics performed on these strains in comparison with type

Fig. 1. Dendrogram of phenotypic distances between the 41 strains of plant-pathogenic *Enterobacteriaceae* included in this study. CFBP, Collection Française de Bactéries Phytopathogènes, Angers, France; *P.*, *Pectobacterium*.

Table 1. Biochemical characteristics that differentiate *Samsonia erythrinae* strains (phenon 4) from other plant-pathogenic *Enterobacteriaceae*

+, Positive; –, negative; numbers correspond to the percentage of positive strains.

Characteristic	Phenon no.*							Reference strains†															
	1	2	3	4	5	6	7	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Pectin hydrolysis	+	+	+	w	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Growth at 36 °C	+	+	66	+	75	+	–	+	+	–	–	+	+	+	–	+	+	–	–	+	–	–	–
Growth at 39 °C	80	33	–	+	–	33	–	+	–	–	–	+	+	+	–	+	–	–	–	–	–	–	–
Growth on Simmons' citrate	80	+	66	–	25	–	50	+	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–
Growth in 5% NaCl	80	33	+	+	25	33	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–
Gelatin hydrolysis	+	66	66	40	–	+	–	+	–	+	+	–	–	–	–	+	–	–	–	–	+	–	–
Lecithin hydrolysis	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Production of indole	+	+	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Utilization of malonate	+	+	–	–	+	–	+	+	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–
Acid production from:																							
Arabinose	40	33	33	+	+	+	+	–	–	–	+	–	+	+	+	–	+	+	–	+	–	–	–
Lactose	60	–	+	–	+	+	+	–	–	–	–	+	+	–	–	–	–	–	–	+	–	–	–
Melibiose	+	–	+	–	+	+	+	+	–	–	–	–	–	+	–	+	–	–	+	+	+	–	–
Raffinose	+	–	+	–	–	+	+	–	–	–	–	+	–	–	–	+	–	–	+	+	–	–	–
Assimilation of:																							
D-Alanine	–	–	–	–	+	+	+	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–
L-Alanine	60	–	–	–	–	–	–	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–
L-(+)-Arabinose	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	–	+	–	–	–
D-(+)-Cellobiose	60	66	+	+	50	+	+	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–
<i>cis</i> -Aconitate	60	–	–	–	75	+	+	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	+
Aesculin	+	+	+	+	+	66	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–	–	–
D-Galacturonate	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–
β-Gentobiose	–	33	+	–	25	+	+	+	+	+	–	+	+	+	+	–	+	–	–	–	+	–	–
D-Glucosamine	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	–	–	–
D-Glucuronate	80	+	66	40	+	+	50	+	–	+	–	–	–	–	+	+	+	+	+	+	–	–	–
L-Glutamate	+	66	66	20	+	+	+	+	+	–	+	+	+	+	+	+	+	–	–	+	–	–	–
DL-Glycerate	80	33	+	+	50	66	50	+	–	+	–	–	–	+	–	+	–	–	+	–	–	–	–
L-Histidine	–	–	–	–	+	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
5-Keto-D-gluconate	–	33	–	–	25	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
2-Keto-D-gluconate	20	33	–	–	+	+	+	–	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–
2-Ketoglutarate	20	66	–	40	25	33	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DL-Lactate	80	+	–	+	+	+	+	+	+	+	–	–	+	+	+	–	–	–	–	–	–	–	–
D-Lyxose	–	–	–	–	+	+	50	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D-Malate	+	+	–	–	+	66	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Maltose	–	–	–	–	+	+	+	–	–	–	–	–	+	+	+	–	–	+	–	–	–	–	–
Maltotriose	–	–	–	–	+	+	+	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–
<i>meso</i> -Tartrate	+	+	–	–	+	66	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
1- <i>O</i> -Methyl α-galactoside	60	33	+	–	–	+	+	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–
1- <i>O</i> -Methyl β-galactoside	+	66	+	–	75	+	+	+	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–
Mucate	+	+	+	+	+	+	–	+	+	+	+	–	+	–	–	–	–	–	–	–	–	–	–
<i>myo</i> -Inositol	+	+	+	+	+	+	+	–	+	+	+	–	–	–	+	–	–	–	+	–	+	–	+
L-Proline	–	–	–	–	+	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
Procatechuate	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
Quinate	–	–	–	–	25	+	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
α-L-Rhamnose	40	+	+	+	+	33	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–
D-Saccharate	+	+	+	+	+	+	–	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–
Serine	+	+	+	60	+	+	+	+	–	–	+	–	+	+	+	–	–	–	–	–	–	–	–
Trehalose	–	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–
<i>trans</i> -Aconitate	40	–	–	–	25	+	50	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–

Table 1 (cont.)

Characteristic	Phenon no.*							Reference strains†															
	1	2	3	4	5	6	7	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Trigonelline	—	—	—	+	75	+	50	—	—	—	—	—	+	+	+	—	—	—	—	—	—	—	—
D-Xylose	+	+	+	—	+	+	—	+	+	+	—	—	+	—	—	—	—	+	—	+	—	+	—

* Reference strains in each phenon: 1, *Pectobacterium chrysanthemi* bvs 1, 3, 5, 6 and 8; 2, *Pectobacterium chrysanthemi* bvs 2, 7 and 9; 3, *Pectobacterium carotovorum* subsp. *carotovorum*, *atrosepticum* and *odoriferum*; 5, *Pantoea agglomerans*, *Pantoea agglomerans* pv. *millettiae*, *Pectobacterium cypripedii*, *Erwinia herbicola* pv. *gypsophilae*; 6, *Pantoea ananatis* pvs *ananatis* and *uredovora*, *Pantoea stewartii* subsp. *indologenes*; 7, *Erwinia percisina* and *Erwinia rhapontici*. Phenon 4 contains *Samsonia erythrinae* strains CFBP 5236^T, 5237, 5238, 5239 and 5240.

† Reference strains: A, *Pectobacterium chrysanthemi* bv. 4; B, *Pectobacterium carotovorum* subsp. *betavascularum*; C, *Pectobacterium carotovorum* subsp. *wasabiae*; D, *Erwinia psidii*; E, *Pectobacterium cacticida*; F, *Enterobacter cancerogenus*; G, *Enterobacter pyrinus*; H, *Brenneria nigrifluens*; I, *Brenneria quercina*; J, *Brenneria rubrifaciens*; K, *Brenneria alni*; L, *Brenneria salicis*; M, *Pantoea stewartii* subsp. *stewartii*; N, *Erwinia amylovora*; O, *Erwinia mallotivora*; P, *Erwinia tracheiphila*.

and reference stains of plant-pathogenic enterobacterial species is presented in this study. A dendrogram of phenotypic distances among the 41 strains is shown in Fig. 1. At a distance level of 0.28, 7 clusters and 16 strains with a separate position were observed. Phenotypic characteristics that differentiate the strains clustered in the different phenons and the unclustered strains of phytopathogenic *Enterobacteriaceae* are shown in Table 1.

Reference strains of *Pectobacterium chrysanthemi* biovars and type strains of *Pectobacterium carotovorum* subspecies were clustered in phenon 1 and 2, and phenon 3, respectively, except *Pectobacterium chrysanthemi* bv. 4 and *Pectobacterium carotovorum* subsp. *betavascularum* and subsp. *wasabiae*, which were unclustered (Fig. 1). As indicated in Table 2, the two species *Pectobacterium chrysanthemi* and *Pectobacterium carotovorum* are clearly distinguished by hydrolysis of lecithin, production of indole, utilization of malonate and assimilation of trehalose, as previously reported (Dickey & Kelman, 1988; Bradbury, 1986; Leliott & Dickey, 1984; Gallois *et al.*, 1992). The characteristics that discriminate the nine *Pectobacterium chrysanthemi* biovars and the five *Pectobacterium carotovorum* subspecies are also indicated in Table 2.

Pectobacterium chrysanthemi (ex. *Erwinia chrysanthemi*) has been subdivided into biovars using 10 biochemical and physiological tests (Samson *et al.*, 1987; Ngwira & Samson, 1990). Five of these tests (growth at 39 °C, acid production from D-arabinose, melibiose and raffinose, and assimilation of 5-keto-D-gluconate) were confirmed to be pertinent for distinguishing reference strains of *Pectobacterium chrysanthemi* biovars (Table 2). However, numerous additional characteristics that differentiate *Pectobacterium chrysanthemi* biovars were revealed by this study (Table 2). The five *Pectobacterium carotovorum* subspecies can be distinguished by the ability to grow at 36 °C and in the presence of 5% NaCl, utilization of

citrate (Simmons' medium), hydrolysis of gelatin, production of reducing compounds from sucrose, acid production from melibiose and α -methyl glucoside, and assimilation of D-cellobiose and D-galacturonate (Table 2), as previously reported (Dickey & Kelman, 1988; Bradbury, 1986; Leliott & Dickey, 1984; Gallois *et al.*, 1992; Goto & Matsumoto, 1987). As shown in Table 2, additional tests can be used to discriminate the type strains of these subspecies.

The five strains CFBP 5236^T, CFBP 5237, CFBP 5238, CFBP 5239 and CFBP 5240 isolated from erythrina trees were clustered in a single phenon (phenon 4). These strains were weakly pectolytic (Sutton's medium) and were phenotypically related to *Pectobacterium* species (Fig. 1). However, they could be clearly distinguished from *Pectobacterium carotovorum* subspecies and *Pectobacterium chrysanthemi* biovars by numerous characteristics (Table 1).

Reference strains of *Erwinia herbicola*, *Pantoea ananatis* and *Pantoea stewartii* subsp. *indologenes* were clustered in two closely related phenons, 5 and 6 (Fig. 1); this is in accordance with the close relatedness or the synonymy of these taxa (Mergaert *et al.*, 1984; Verdonck *et al.*, 1987; Beji *et al.*, 1988). The *Pectobacterium cypripedii* type strain was also included in phenon 5. Although belonging to the genus *Pectobacterium* (Hauben *et al.*, 1998), *Pectobacterium cypripedii* is phenotypically more related to *Pantoea* species than to other *Pectobacterium* species, as previously observed (Mergaert *et al.*, 1984; Verdonck *et al.*, 1987). However, *Pectobacterium cypripedii* can be distinguished from the reference strains of *Pantoea* species and *Erwinia herbicola* included in phenon 5 by the assimilation of 5-keto-D-gluconate and quinate (positive for *Pectobacterium cypripedii*), and 1-O-methyl β -galactopyranoside and *trans*-aconitate (negative for *Pectobacterium cypripedii*) (data not shown). Type strains of *Pantoea ananatis* pv. *ananatis* and pv. *uredovora* can be differentiated by aesculin hydrolysis, assimilation of DL-glycerate and growth in

Table 2. Biochemical characteristics that differentiate reference strains of *Pectobacterium chrysanthemi* bvs 1–9 and type strains of *Pectobacterium carotovorum* subspecies

The characteristics that differentiate *Pectobacterium chrysanthemi* biovars are in bold face. The characteristics that differentiate *Pectobacterium carotovorum* subspecies are underlined.

Characteristic	<i>Pectobacterium chrysanthemi</i> biovars									<i>Pectobacterium carotovorum</i> subspecies*				
	1	2	3	4	5	6	7	8	9	<u>caro.</u>	<u>atro.</u>	<u>odori.</u>	<u>beta.</u>	<u>was.</u>
ADH (Moeller)	+	–	+	–	+	–	+	–	–	–	–	–	–	–
Growth at 39 °C	–	+	+	+	+	+	–	+	–	–	–	–	–	–
Growth at 36 °C	+	+	+	+	+	+	+	+	+	+	–	+	+	–
Growth on Simmons' citrate	–	+	+	+	+	+	+	+	+	–	+	+	–	+
Growth in 5% NaCl	+	+	+	–	+	–	–	+	–	+	+	+	+	+
Gelatin hydrolysis	+	–	+	–	+	+	+	+	+	+	–	+	–	+
Lecithin hydrolysis	+	+	+	+	+	+	+	+	+	–	–	–	–	–
<u>Reducing substances from sucrose</u>	–	–	–	–	–	–	–	–	–	–	+	+	+	–
Production of indole	+	+	+	+	+	+	+	+	+	–	–	–	–	–
Utilization of malonate	+	+	+	+	+	+	+	+	+	–	–	–	–	–
Utilization of inulin	+	–	–	–	+	–	+	–	+	–	–	–	+	–
Acid production from:														
<u>D-Arabinose</u>	–	+	+	+	–	–	–	+	–	–	–	+	–	–
<u>Lactose</u>	–	–	+	–	+	–	–	+	–	+	+	+	–	–
<u>Melibiose</u>	+	–	+	+	+	+	–	+	–	+	+	+	+	–
<u>α-Methyl glucoside</u>	–	–	–	–	–	–	+	–	–	–	+	+	+	–
<u>Raffinose</u>	+	–	+	+	+	+	–	+	–	+	+	+	+	–
Assimilation of:														
<u>L-Alanine</u>	–	–	+	+	+	+	–	–	–	–	–	–	–	–
<u>D-(+)-Cellobiose</u>	–	+	+	–	+	–	+	–	–	–	+	+	+	+
<u>cis-Aconitate</u>	–	–	+	–	+	–	–	+	–	–	–	–	–	–
<u>trans-Aconitate</u>	–	–	+	–	–	–	–	+	–	–	–	–	–	–
<u>D-Galacturonate</u>	+	+	+	+	+	+	+	+	+	+	+	+	–	+
<u>β-Gentobiose</u>	–	+	–	+	–	–	–	–	–	+	+	+	+	+
<u>D-Glucuronate</u>	+	+	+	+	+	–	+	+	+	+	+	–	–	+
<u>L-Glutamate</u>	+	+	+	+	+	+	+	+	–	+	–	+	+	–
<u>DL-Glycerate</u>	+	–	+	+	+	–	+	+	–	+	+	+	–	+
<u>5-Keto-D-gluconate</u>	–	+	–	+	–	–	–	–	–	–	–	–	–	–
<u>2-Keto-D-gluconate</u>	–	+	+	–	–	–	–	–	–	–	–	–	+	–
<u>2-Ketoglutarate</u>	–	+	–	–	+	–	+	–	–	–	–	–	–	–
<u>DL-Lactate</u>	+	+	+	+	+	–	+	+	+	–	–	–	+	+
<u>Lactulose</u>	–	–	–	–	–	–	–	–	–	+	+	+	–	–
<u>D-Malate</u>	+	+	+	+	–	+	+	+	+	–	–	–	–	+
<u>meso-Tartrate</u>	+	+	+	+	–	+	+	+	+	–	–	–	–	+
<u>1-O-Methyl α-galactopyranoside</u>	+	–	–	+	+	+	+	–	–	+	+	+	–	–
<u>1-O-Methyl β-galactopyranoside</u>	+	+	+	+	+	+	+	+	–	–	+	+	+	–
<u>Palatinose</u>	–	–	–	–	–	–	–	–	–	–	+	+	+	–
<u>α-L-Rhamnose</u>	+	+	+	+	+	–	+	–	+	+	+	+	+	+
<u>L-(+)-Tartrate</u>	–	+	+	+	–	–	–	+	–	–	–	–	–	–
<u>D-(–)-Tartrate</u>	+	+	–	+	–	–	+	–	+	–	–	–	–	–
<u>Trehalose</u>	–	–	–	–	–	–	–	–	–	+	+	+	+	+

* *caro.*, *carotovorum*; *atro.*, *atrosepticum*; *odori.*, *odoriferum*; *beta.*, *betavascularum*; *was.*, *wasabiae*.

5% NaCl (positive for *Pantoea ananatis* pv. *ananatis*), and acid production from raffinose and assimilation of *meso*-tartrate and D-(+)-malate (positive for *Pantoea ananatis* pv. *uredovora*) (data not shown). *Erwinia persicina* and *Erwinia rhapontici* type strains clustered in phenon 7 at a high distance level (0.26) and

can be differentiated by numerous characteristics: assimilation of adonitol, erythritol, D-glucuronate, lactulose, D-sorbitol, D-tartrate, *trans*-aconitate (positive for *Erwinia persicina*), and growth on Simmons' citrate medium and assimilation of dulcitol, DL-glycerate, D-lyxose, maltitol, D-(+)-melezitose, 1-O-

Fig. 2. For legend see page 1299.

methyl α -D-glucoside, palatinose, D-turanose, xylitol (positive for *Erwinia rhapontici*) (data not shown). *Erwinia* species which have been recently transferred to the new genus *Brenneria* as *B. nigrifluens*, *B. quercina*, *B. rubrifaciens*, *B. alni* and *B. salicis* were related to the other strains included in this study at a high level of phenotypic distance (> 0.5), together with *Erwinia amylovora*, *Erwinia mallotivora* and *Erwinia tracheiphila*, and *Pantoea stewartii* subsp. *stewartii* (Fig. 1). All these strains could be clearly

distinguished by numerous phenotypic characteristics (Table 1).

Numerical analysis of phenotypic data from *Erwinia* species has been previously reported (Mergaert *et al.*, 1984; Verdonck *et al.*, 1987). Mergaert *et al.* (1984) studied 123 *Erwinia* strains using commercially available biochemical strips API20E and API50CH (acid production of 49 organic substrates) and 9 API enzyme systems, whereas Verdonck *et al.* (1987) analysed the

pathovars and biovars of plant-pathogenic *Enterobacteriaceae*, and thus could be useful for their identification.

Moreover, our phenotypic data showed that strains isolated from diseased erythrina trees formed a homogeneous phenon different from other plant-pathogenic *Enterobacteriaceae*, as suggested by a previous preliminary study (Sutra *et al.*, 1999).

An estimated 97.5% of the primary sequence of the 16S rDNA gene was determined for strain CFBP 5236^T, corresponding to 1503 bases from positions 6 to 1507 according to the numbering of the *E. coli* 16S rRNA gene sequence (Brosius *et al.*, 1981). The 16S

Fig. 2. Phylogenetic analyses of the 16S rDNA sequence of strain CFBP 5236^T and reference strains of plant-pathogenic *Enterobacteriaceae*. Three methods were used to analyse the phylogenetic position of strain CFBP 5236^T, as detailed in Methods: (a) a neighbour-joining algorithm, including bootstrap values as indicated along branches; (b) a maximum-parsimony method; (c) a maximum-likelihood method (branches with an asterisk were positive at $P < 0.01$).

rDNA sequence of strain CFBP 5236^T had 93.8–94.7% similarity with the 16S rDNA of *Erwinia* species, 94.3–96.0% similarity with the 16S rDNA of *Brenneria* species, 92.7–96.5% similarity with the 16S rDNA of *Pectobacterium* species or subspecies, and 93.3–93.7% similarity with the 16S rDNA of *Pantoea* species (positions 28–1469). In the description of the genera *Erwinia*, *Pectobacterium* and *Brenneria*, Hauben *et al.* (1998) have proposed nucleotide signature positions of

the 16S rDNA that allow the differentiation of these three genera from each other. Discrepancies were found between the nucleotide signatures reported by these authors and those deduced from our own sequence comparison: (i) positions 593, 594, 598 and 599 correspond to positions 594, 595, 599 and 600, respectively, in our alignment by reference to *E. coli* 16S rDNA numbering; (ii) some positions that were considered characteristic (Hauben *et al.*, 1998) are not:

positions 593, 594 and 1219 (594, 595 and 1219 according to our alignment), which correspond respectively to T or C/T, A and A for all three genera. These three positions being excluded, the 16S rDNA sequence of strain CFBP 5236^T possessed 8 of the 10 signature nucleotides defined for the genus *Brenneria*, 10 of the 17 signature nucleotides defined for the genus *Pectobacterium* and 3 of the 14 signature nucleotides defined for the genus *Erwinia* (Hauben *et al.*, 1998). The 16S rDNA sequence of strain CFBP 5236^T can be differentiated from the sequences of *Brenneria* species at positions 379 and 384 (C and G for strain CFBP 5236^T instead of G and C for *Brenneria* species), from the sequences of *Pectobacterium* species at positions 599, 600, 638, 639, 646, 839 and 847 (respectively C, A, T, G, G, G and C for strain CFBP 5236^T instead of T, G, C, A, A, C and G for *Pectobacterium* species) and from the sequences of *Erwinia* species at positions 408, 839, 847, 987, 988, 989, 1216, 1217, 1218, 1308 and 1329 (respectively G, G, C, A, C, T, A, G, T, T and A for strain CFBP 5236^T instead of A, C, G, G, G, C, G, C, C, C and G for *Erwinia* species) (Hauben *et al.*, 1998). Nucleotide signatures that are specific, i.e. not shared by the other genera, were found for *Brenneria* species and *Erwinia* species: G, A, C and G at positions 379, 381, 384 and 839, respectively, for *Brenneria* species (C, C, G and C for *Erwinia* and *Pectobacterium* species) as reported by Hauben *et al.* (1998), and G, C, G, C and C at positions 988, 989, 1216, 1217 and 1218, respectively, for *Erwinia* species (C, T, A, G/A or G, and T for *Brenneria* and *Pectobacterium* species). Only one specific nucleotide signature was found for strain CFBP 5336, A at position 1047. No specific signature was found for *Pectobacterium* species.

All three methods of sequence analysis (bioNJ, maximum-likelihood and maximum-parsimony) showed that strain CFBP 5236^T always clustered within the γ -03 division of the *Proteobacteria*, with species of the genera *Pectobacterium*, *Brenneria* and *Erwinia* (data not shown). More detailed analyses were then conducted based on 16S rDNA sequences of strains including type strains of species of these three genera as well as representatives of outgroups selected within the *Enterobacteriaceae* (Fig. 2). Results of bioNJ analysis showed that *Pectobacterium* and *Brenneria* species and subspecies formed two different clusters, with strain CFBP 5236^T being included in the *Brenneria* cluster (Fig. 2a). However, bootstrap analyses based on 500 samplings showed no support for the genus *Pectobacterium* and low support (35%) for the genus *Brenneria*. Analyses of 16S rDNA sequences by both maximum-parsimony (Fig. 2b) and maximum-likelihood (Fig. 2c) showed that *Brenneria* and *Pectobacterium* species consisted of intermixed clusters. Strain CFBP 5236^T was grouped with subsets of *Pectobacterium* species according to maximum-parsimony (Fig. 2b) and with *Yersinia enterocolitica* and *Leminorella grimontii* according to maximum-likelihood (Fig. 2c). Whatever the method of analysis used, *Erwinia* species formed a unique cluster (Fig. 2a–c).

Two phylogenetic analyses of *Erwinia* species based on 16S rDNA sequences have been recently reported (Kwon *et al.*, 1997; Hauben *et al.*, 1998). The study of Kwon *et al.* (1997) showed that the genus *Erwinia* was phylogenetically heterogeneous and that *Erwinia* species were distributed into four different clusters (I–IV) that were intermixed with members of other genera of the family *Enterobacteriaceae*. Using a similar but enlarged approach, Hauben *et al.* (1998) analysed the 16S rDNA sequences of the type strains of all phytopathogenic *Enterobacteriaceae*. They also obtained four clusters, confirming the heterogeneity of the genus *Erwinia*. Cluster I represents the so-called ‘true’ erwinias and contained *Erwinia amylovora*, *Erwinia mallotivora*, *Erwinia persicina*, *Erwinia psidii*, *Erwinia rhapontici* and *Erwinia tracheiphila*. Pectolytic *Erwinia* species (*Erwinia carotovora* and *Erwinia chrysanthemi*) and *Erwinia cypripedii* included in cluster II were united in the genus *Pectobacterium* as *Pectobacterium carotovorum*, *Pectobacterium chrysanthemi* and *Pectobacterium cypripedii*, respectively. *Erwinia* species of cluster III were classified in the new genus *Brenneria* as *B. alni*, *B. nigrifluens*, *B. paradisiaca*, *B. quercina*, *B. rubrifaciens* and *B. salicis*, whereas cluster IV contained plant-pathogenic *Pantoea* species. Moreover, Hauben *et al.* (1998) defined nucleotide signature positions of the 16S rDNA that differentiate the four genera *Erwinia*, *Brenneria*, *Pectobacterium* and *Pantoea*. Although the number of sequences analysed was different, results obtained in these two studies were in agreement since clusters I, II, III and IV defined by Hauben *et al.* (1998) corresponded to clusters II, III, IV and I obtained by Kwon *et al.* (1997), respectively. However, it must be pointed out that in both studies, the only method used for analysing 16S rDNA sequences was the neighbour-joining method and that the clusters obtained were supported by low bootstrap values. The basis of modern bacterial taxonomy relies mostly on a cladistic model: bacterial species grouped within a single genus descend from a common ancestor from which no other species that is not within the genus also descends. Although gene trees cannot always be equated to species trees (Slowinski & Page, 1999), gene trees derived from 16S rDNA sequences have so far been considered as good indicators of species trees and used accordingly in bacterial systematics. An important rule for analysing gene trees is to estimate the robustness of a topology, but also to ascertain whether the tree obtained is the ‘true’ tree. The consistency of any given method is only valid within limits (Felsenstein, 1998; Huelsenbeck & Hillis, 1993). A given method may produce a robust tree, attested by high bootstrap values, that is not the true tree. One way to circumvent this problem is to compare different methods that rely on different models (Kim, 1993). The basis of this approach is that it is unlikely that different methods, if they all produce an incorrect tree, will produce the same tree. In conclusion, if all methods indicate the same topology, there is a good chance that the true tree has been obtained; in contrast, when

different trees are obtained, the true tree cannot be inferred from the data.

In this paper, results of 16S rDNA sequence analysis by neighbour-joining (bioNJ) showed that the genera *Pectobacterium*, *Brenneria* and *Erwinia* formed three separate clusters and were in full agreement with previous publications that used only this method of analysis (Kwon *et al.*, 1997; Hauben *et al.*, 1998). However, the tree obtained by neighbour-joining was in contradiction to the trees obtained by maximum-parsimony and maximum-likelihood, in which *Pectobacterium* and *Brenneria* species are distributed in several intermixed clusters, indicating that the genera *Pectobacterium* and *Brenneria* cannot be described as clades on the basis of 16S rDNA sequence analysis only. These data are in agreement with recent results obtained by Spröer *et al.* (1999) who showed that *Pectobacterium* and *Brenneria* genera were not clearly phylogenetically separated on the basis of 16S rDNA sequence analyses using three different treeing algorithms. Results presented in this study could be used as a basis for separating the different species (now grouped in only the two genera *Pectobacterium* and *Brenneria*) into several new genera, each of which supported by all methods at the phylogenetic level. This is probably not the proper approach, because the smallest clades common to the three methods may not reflect the true existence of a larger genus (if, for example, only the neighbour-joining tree is incorrect). Consequently, it is now necessary to examine all phenotypic and molecular data in a polyphasic approach, or perhaps to acquire more sequence data, to resolve this taxonomic problem.

The 16S rDNA sequence of strain CFBP 5236^T, which is representative of strains isolated from erythrina, did not contain all the nucleotide signatures defined for the genera *Erwinia*, *Pectobacterium* or *Brenneria* (Hauben *et al.*, 1998). Moreover, the position of strain CFBP 5236^T in the different trees (Fig. 2) was so variable that it cannot be attributed to any recognized genus, no matter what final decision is taken about the taxonomic status of the genera *Pectobacterium* and *Brenneria*.

DNA–DNA hybridizations and DNA base composition

The results of DNA–DNA hybridizations are shown in Table 3. The labelled DNAs from strains CFBP 5236^T and CFBP 5237 exhibited high levels of reassociation (89–100% hybridization) with DNAs from other strains isolated from erythrina. In contrast, low levels of reassociation were observed with DNAs from reference strains of phytopathogenic *Enterobacteriaceae*. The percentages of hybridization of DNAs from strains CFBP 5236^T and CFBP 5237 ranged from 7 to 29%, from 14 to 20%, from 4 to 33%, from 7 to 30% and from 5 to 11% with DNA from reference strains of *Pectobacterium* species, *Brenneria* species, *Erwinia* species, *Pantoea* species and *Enterobacter* species, respectively (Table 3). These

results indicated that strains isolated from erythrina formed a homogeneous genomic group with a low level of DNA relatedness with all other plant-pathogenic *Enterobacteriaceae*.

The DNA G + C content of both strains CFBP 5236^T and CFBP 5237 was 57.0 mol%. This value is slightly more than the G + C values previously reported for the genera *Erwinia* (49.8–54.1 mol%), *Brenneria* (51.3–56.1 mol%) and *Pectobacterium* (50.5–56.1 mol%) (Leliott & Dickey, 1984; Hauben *et al.*, 1998).

On the basis of phenotypic data, 16S rDNA sequence analyses and results of DNA–DNA hybridizations, we conclude that strains isolated from diseased erythrina trees should be recognized as a new genus, *Samsonia*, and as a new species, *Samsonia erythrinae* gen. nov., sp. nov.

Description of *Samsonia* gen. nov.

Samsonia (Sam.so'ni.a. M.L. fem. n. *Samsonia* after the French phytobacteriologist Régine Samson, INRA, Angers, France, who works on pectolytic *Erwinia*).

The description below is based on data from this study and those obtained by Sutra *et al.* (1999). Cells are Gram-negative, oxidase-negative and catalase-positive. They are rod-shaped with rounded ends, and are motile by means of peritrichous flagella. The strains are β -galactosidase-positive, produce acetoin but not hydrogen sulfide, and do not possess arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease or tryptophan desaminase. Glucose is fermented without gas production. Nitrate is reduced. Strains hydrolyse aesculin but not gelatin, Tween 80, casein or lecithin. They do not utilize citrate and malonate as carbon source and do not produce indole from tryptophan. The type species is *Samsonia erythrinae*.

Description of *Samsonia erythrinae* sp. nov.

Samsonia erythrinae (e.ry.thri'nae. L. gen. n. *erythrinae* of *Erythrina*, referring to plants of the genus *Erythrina*, family *Fabaceae*, from which the organism was isolated).

The description of *Samsonia erythrinae* is the same as that of the genus. Colonies on YBGA medium are white to light beige, circular, convex and glistening, with regular edges and a diameter of 2–5 mm after 48 h at 28 °C. The organism grows at 28, 36 and 39 °C. Strains produce acid from amygdaline, arabinose, inositol, mannitol, rhamnose and saccharose but not from D-(+)-arabitol, erythritol, inulin, lactose, melibiose, α -methyl glucoside, raffinose and sorbitol. Acid production from mannitol is variable. They do not produce reducing compounds from sucrose. No hypersensitivity reaction is produced on tobacco leaves (Sutra *et al.*, 1999). *Samsonia erythrinae* strains as-

Table 3. Results of DNA–DNA hybridization experiments

Strain	Percentage of reassociation with labelled DNA from strains:	
	CFBP 5236 ^T	CFBP 5237
<i>Samsonia erythrinae</i>		
CFBP 5236 ^T	100	95
CFBP 5237	100	100
CFBP 5238	100	89
CFBP 5239	95	ND
CFBP 5240	93	ND
<i>Pectobacterium carotovorum</i>		
subsp. <i>carotovorum</i> CFBP 2046 ^T	29	ND
subsp. <i>atrosepticum</i> CFBP 1526 ^T	25	ND
subsp. <i>odoriferum</i> CFBP 1878 ^T	10	5
subsp. <i>betavascularum</i> CFBP 2122 ^T	27	28
subsp. <i>wasabiae</i> CFBP 3304 ^T	29	ND
<i>Pectobacterium chrysanthemi</i>		
bv. 1 CFBP 1200	29	ND
bv. 2 CFBP 2051	12	ND
bv. 3 CFBP 1447	9	ND
bv. 4 CFBP 3477	16	ND
bv. 5 CFBP 2048 ^T	8	ND
bv. 6 CFBP 1270	16	12
bv. 7 CFBP 2015	21	ND
bv. 8 CFBP 2052	9	ND
bv. 9 CFBP 1805	10	ND
<i>Pectobacterium cacticida</i> CFBP 3628 ^T	20	ND
<i>Pectobacterium cypripedii</i> CFBP 3613 ^T	7	ND
<i>Erwinia amylovora</i> CFBP 1232 ^T	2	8
<i>Erwinia percisina</i> CFBP 3622 ^T	6	ND
<i>Erwinia rhapontici</i> CFBP 3618 ^T	5	ND
<i>Erwinia mallotivora</i> CFBP 2503 ^T	ND	4
<i>Erwinia tracheiphila</i> CFBP 2355 ^T	ND	33
<i>Erwinia psidii</i> CFBP 3627 ^T	ND	7
<i>Brenneria nigrifluens</i> CFBP 3616 ^T	ND	20
<i>Brenneria rubrifaciens</i> CFBP 3619 ^T	ND	19
<i>Brenneria quercina</i> CFBP 3617 ^T	8	ND
<i>Brenneria alni</i> CFBP 3923 ^T	ND	14
<i>Brenneria salicis</i> CFBP 802 ^T	ND	20
<i>Erwinia herbicola</i> pv. <i>gypsophillae</i> CFBP 11141	5	ND
<i>Pantoea agglomerans</i> CFBP 2240	5	ND
<i>Pantoea agglomerans</i> pv. <i>milletiae</i> CFBP 3615	8	ND
<i>Pantoea ananatis</i> pv. <i>ananatis</i> CFBP 3612 ^T	7	8
<i>Pantoea ananatis</i> pv. <i>uredovora</i> CFBP 3621	8	ND
<i>Pantoea stewartii</i> subsp. <i>stewartii</i> CFBP 3620 ^T	30	ND
<i>Enterobacter cancerogenus</i> CFBP 4167 ^T	ND	11
<i>Enterobacter pyrinus</i> CFBP 4168 ^T	ND	10

ND, Not determined.

simulate the following substrates (Biotype 100 strips): *N*-acetylglucosamine, L-(+)-arabinose, L-aspartate, D-(+)-cellobiose, D-(+)-fructose, fumarate, D-(+)-galactose, D-gluconate, D-glucosamine, D-(+)-glucose, glycerate, glycerol, 2-keto-D-gluconate, DL-lactate, L-

(–)-malate, D-mannitol, mucate, *myo*-inositol, L-rhamnose, D-saccharate, succinate, sucrose, D-(+)-trehalose and trigonelline. They do not assimilate D-alanine, L-alanine, adonitol, 4-aminobutyrate, 5-aminovalerate, D-(+)-arabitol, L-(–)-arabitol, ben-

zoate, betaine, caprate, caprylate, *cis*-aconitate, citrate, *m*-coumarate, dulcitol, erythritol, ethanolamine, α -L-fucose, gentisate, β -gentiobiose, β -glucuronide, histamine, histidine, *m*- and *p*-hydroxybenzoate, DL- β -hydroxybutyrate, hydroxyquinoleine, itaconate, 5-keto-D-glucuronate, lactose, lactulose, lyxose, D-(+)-malate, malonate, maltitol, maltose, maltotriose, D-(+)-melezitose, D-(+)-melibiose, *meso*-tartrate, 1-*O*-methyl α -galactopyranoside, 1-*O*-methyl β -galactopyranoside, 1-*O*-methyl α -glucopyranoside, 1-*O*-methyl β -glucopyranoside, 3-*O*-methyl D-glucose, palatinose, phenylacetate, 3-phenylpropionate, L-proline, propionate, protocatechuate, putrescine, quinate, D-(+)-raffinose, D-sorbitol, L-(+)-sorbose, D-tagatose, D-(−)-tartrate, L-(+)-tartrate, *trans*-aconitate, tricarballoylate, tryptamine, L-tryptophan, L-tyrosine, D-(+)-turandose, xylitol or D-(+)-xylose. The assimilation of D-galacturonate, D-glucuronate, L-glutamate, L-serine and 2-oxoglutarate is variable. DNA G+C content of strains CFBP 5236^T and 5237 is 57.0 mol%. Strains of *Samsonia erythrinae* cause bark necrotic lesions on *Erythrina* sp. trees. The type strain is CFBP 5236^T (= ICMP 13937^T).

ACKNOWLEDGEMENTS

The authors are grateful to Solange Belouin, Karine Perlemoine and Alain Huard for technical assistance.

REFERENCES

- Ayers, S. H., Rupp, P. & Johnson, W. T. (1919). *A Study of Alkali-forming Bacteria in Milk*. Bulletin no. 782. Washington, DC: US Department of Agriculture.
- Beji, A., Mergaert, J., Gavini, F., Izard, D., Kersters, K., Leclerc, H. & De Ley, J. (1988). Subjective synonymy of *Erwinia herbicola*, *Erwinia milletiae*, and *Enterobacter agglomerans* and redefinition of the taxon by genotypic and phenotypic data. *Int J Syst Bacteriol* **38**, 77–88.
- Bonnet, P. (1973). Les *Erwinia* pectinolytiques. I. Diagnostic biochimique rapide. *Ann Phytopathol* **5**, 355–376.
- Bradbury, J. F. (1986). *Guide to Plant Pathogenic Bacteria*. Kew: CAB International Mycological Institute.
- Brenner, D. J., McWorter, A. C., Leete Knutson, J. K. & Steigerwalt, A. G. (1982). *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. *J Clin Microbiol* **15**, 1133–1140.
- Brosius, J., Dull, T. J., Sleeter, D. D. & Noller, H. F. (1981). Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* **148**, 107–127.
- Croza, J. M., Brenner, D. J. & Falkow, S. (1973). Use of a single-stranded specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *J Bacteriol* **115**, 904–911.
- Descamps, P. & Véron, M. (1981). Une méthode de choix des caractères d'identification basée sur le théorème de Bayes et la mesure de l'information. *Ann Microbiol* **132B**, 157–170.
- Dickey, R. S. & Kelman, A. (1988). *Erwinia* "Carotovora" or soft rot group. In *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 2nd edn, pp. 44–46. Edited by N. W. Schaad. St Paul, MN: American Phytopathological Society.
- Feinberg, A. P. & Vogelstein, B. (1983). A technique for radio-labeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* **132**, 6.
- Felsenstein, J. (1998). Phylogenies from molecular sequences: inference and reliability. *Annu Rev Genet* **22**, 521–565.
- Gallois, A., Samson, R., Ageron, E. & Grimont, P. A. D. (1992). *Erwinia carotovora* subsp. *odorifera* subsp. nov., associated with odorous soft rot of chicory (*Cichorium intybus* L.). *Int J Syst Bacteriol* **42**, 582–588.
- Gascuel, O. (1997). BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Mol Biol Evol* **14**, 685–695.
- Goto, M. & Matsumoto, K. (1987). *Erwinia carotovora* subsp. *wasabiae* subsp. nov. isolated from diseased rhizomes and fibrous roots of Japanese horseradish (*Eutrema wasabi* Maxim.). *Int J Syst Bacteriol* **37**, 130–137.
- Grimont, P. A. D., Popoff, M. Y., Grimont, F., Coynault, C. & Lemelin, M. (1980). Reproducibility and correlation study of three deoxynucleic acid hybridization procedures. *Curr Microbiol* **4**, 325–330.
- Hauben, L., Moore, E. R. B., Vauterin, L., Steenackers, M., Mergaert, J., Verdonck, L. & Swings, J. (1998). Phylogenetic position of phytopathogens within the *Enterobacteriaceae*. *Syst Appl Microbiol* **21**, 384–397.
- Huelsenbeck, J. P. & Hillis, D. M. (1993). Success of phylogenetic methods in the four-taxon case. *Syst Biol* **42**, 247–264.
- Kim, J. (1993). Improving the accuracy of phylogenetic estimation by combining different methods. *Syst Biol* **42**, 331–334.
- Kwon, S.-W., Go, S.-J., Kang, H.-W., Ryu, J.-C. & Jo, J.-K. (1997). Phylogenetic analysis of *Erwinia* species based on 16S rRNA gene sequences. *Int J Syst Bacteriol* **47**, 1061–1067.
- Leliott, R. A. & Dickey, R. S. (1984). Genus VII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 469–476. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Mergaert, J., Verdonck, L., Kersters, K., Swings, J., Boeufgras, J. M. & De Ley, J. (1984). Numerical taxonomy of *Erwinia* species using API systems. *J Gen Microbiol* **130**, 1893–1910.
- Ngwira, N. & Samson, R. (1990). *Erwinia chrysanthemi*: description of two new biovars (bv 8 and 9) isolated from kalanchoe and maize host plants. *Agronomie* **10**, 341–345.
- Owen, R. J. & Lapage, S. P. (1976). The thermal denaturation of partly purified bacterial deoxyribonucleic acid and its taxonomic implications. *J Appl Bacteriol* **41**, 335–340.
- Patel, M. K., Kulkarni, Y. S. & Dhande, G. W. (1952). Some new bacterial diseases of plants. *Curr Sci* **12**, 345–346.
- Perrière, G. & Gouy, M. (1996). WWW-Query: an on-line retrieval system for biological sequence banks. *Biochimie* **78**, 364–369.
- Samson, R., Poutier, F., Saily, M. & Jouan, B. (1987). Caractérisation des *Erwinia chrysanthemi* isolées de *Solanum tuberosum* et d'autres plantes hôtes selon les biovars et sérogroupes. *EPPO Bull* **17**, 11–16.
- Slowinski, J. B. & Page, R. D. (1999). How should species phylogenies be inferred from sequence data. *Syst Biol* **48**, 814–825.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization.

In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Sneath, P. H. A. & Sokal, R. R. (1973). *Numerical Taxonomy: the Principles and Practice of Numerical Classification*. San Francisco: Freeman.

Spröer, C., Mendrock, U., Swiderski, J., Lang, E. & Stackebrandt, E. (1999). The phylogenetic position of *Serratia*, *Buttauxiella* and some other genera of the family *Enterobacteriaceae*. *Int J Syst Bacteriol* **49**, 1433–1438.

Sutra, L., Prior, P., Perlemoine, K., Risède, J. M., Cao-Van, P. & Gardan, L. (1999). Description of a new disease on *Erythrina* sp.

in Martinique (French West Indies) and preliminary characterization of the causal agent as a novel *Erwinia* species. *Plant Pathol* **48**, 253–259.

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

Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.

Young, J. M., Saddler, G. S., Takikawa, Y., De Boer, S. H., Vauterin, L., Gardan, L., Gvozdyak, R. I. & Stead, D. (1996). Names of plant pathogenic bacteria 1864–1995. *Rev Plant Pathol* **75**, 721–863.