***Erwinia pyrifoliae* sp. nov., a novel pathogen that affects Asian pear trees (*Pyrus pyrifolia* Nakai)**

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A novel pathogen from Asian pears (*Pyrus pyrifolia* Nakai) was analysed by sequencing the 16S rDNA and the adjacent intergenic region, and the data were compared to related *Enterobacteriaceae*. The 16S rDNA of the Asian pear pathogen was almost identical with the sequence of *Erwinia amylovora*, in contrast to the 16S–23S rRNA intergenic transcribed spacer region of both species. A dendrogram was deduced from determined sequences of the spacer regions including those of several related species such as *Erwinia amylovora*, *Enterobacter pyrinus*, *Pantoea stewartii* subsp. *stewartii* and *Escherichia coli*. Dendrograms derived from 121 biochemical characteristics including Biotype 100 data placed the Asian pear pathogen close to *Erwinia amylovora* and more distantly to other members of the species *Erwinia* and to the species *Pantoea* and *Enterobacter*. Another DNA relatedness study was performed by DNA hybridizations and estimation of ΔT_m values. The Asian pear strains constituted a tight DNA hybridization group (89–100%) and were barely related to strains of *Erwinia amylovora* (40–50%) with a ΔT_m in the range of 5.2–6.8. The G+C content of DNA from the novel pathogen is 52 mol%. Therefore, it is proposed that strains isolated from Asian pears constitute a new species and the name *Erwinia pyrifoliae* is suggested; the type strain is strain Ep16/96^T (= CFBP 4172^T = DSM 12163^T).

Keywords: *Erwinia pyrifoliae*, taxonomy, DNA–DNA hybridization, rDNA sequencing

INTRODUCTION

A recently observed necrotic disease of Asian pear fruit trees (*Pyrus pyrifolia* cv. ‘Shingo’ and ‘Mansamgil’) near Chuncheon in Korea has resulted in isolation and partial characterization of a bacterial pathogen (Rhim *et al.*, 1999). Symptoms on pear trees in the Korean orchards remotely resembled those of fire blight, but microbiological assays and the absence of the PCR signals expected for the presence of *Erwinia amylovora* distinguished the novel *Erwinia* sp. from the fire blight pathogen. The new pathogen was also different from the recently described Korean pear

pathogen *Enterobacter pyrinus* (Chung *et al.*, 1993) especially by its lack of nitrate reduction and for growth at high temperature, as well as acid production in the presence of aesculin or cellobiose (Rhim *et al.*, 1999).

The purpose of this work was the differentiation of the novel pathogen from *Erwinia amylovora*, *Pantoea* and *Enterobacter* by using DNA sequence analysis of the 16S rDNA and the adjacent intergenic transcribed spacer region, by numerical analysis of phenotypical characteristics, DNA–DNA hybridization and the G+C content. Our findings resulted in the description of a new species: *Erwinia pyrifoliae*.

METHODS

Strains. The strains investigated in the experiments and used for differentiation are listed in Tables 1 and 2. They were used under authorities mentioned in Young *et al.* (1996). We

Abbreviations: ITS, intergenic transcribed spacer region; RCS, reducing compounds from sucrose.

The EMBL accession number for the 16S rRNA sequence of *Erwinia pyrifoliae* strain Ep1/96 is AJ009930.

Table 1. Strains used for molecular characterization of *Erwinia pyrifoliae*

Strain	No. in collection*	Plant, origin, year of isolation	Reference/source
<i>Erwinia amylovora</i> Ea1/79		<i>Cotoneaster</i> sp., Germany, 1979 (obtained from W. Zeller)	Falkenstein <i>et al.</i> , 1988
<i>Enterobacter pyrinus</i> 90.2 ^T	KCTC 2520 ^T	<i>Pyrus pyrifolia</i> , South Korea (from Korean strain collection)	Chung <i>et al.</i> , 1993
<i>Erwinia pyrifoliae</i> Ep8/95	DSM 12393	<i>Pyrus pyrifolia</i> , South Korea, 1995	S.-L. Rhim, unpublished
Ep1/96	CFBP 4171, DSM 12162	<i>Pyrus pyrifolia</i> , South Korea, 1996	S.-L. Rhim, unpublished
Ep16/96 ^T	CFBP 4172 ^T , DSM 12163 ^T	<i>Pyrus pyrifolia</i> , South Korea, 1996	S.-L. Rhim, unpublished
Ep28/96	CFBP 4173	<i>Pyrus pyrifolia</i> , South Korea, 1996	S.-L. Rhim, unpublished
Ep31/96	CFBP 4174	<i>Pyrus pyrifolia</i> , South Korea, 1996	S.-L. Rhim, unpublished
Ep4/97	DSM 12394	<i>Pyrus pyrifolia</i> , South Korea, 1997	S.-L. Rhim & K. Geider, unpublished
Ep102/98		<i>Pyrus pyrifolia</i> , South Korea, 1998	S.-L. Rhim, unpublished

* CFBP, Collection Française des Bactéries Phytopathogènes; DSM, DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen; KCTC, Korean Collection of Type Cultures.

assayed four strains isolated from *Pyrus pyrifolia*, the type strain of *Erwinia amylovora*, and 20 type strains of phytopathogenic and saprophytic *Erwinia*, *Pantoea* and *Enterobacter* sp. for constructing a dendrogram.

Nucleotide sequencing. The 16S rRNA genes and the 16S–23S rRNA intergenic transcribed spacer region (ITS) were cloned with consensus primers into vector pGEM-T (Promega) and pBluescript KS, respectively. The primers for PCR amplification were for 16S rDNA fd2 (5' AGA GTT TGA TCA TGG CTC AG) and rP1 (5' ACG GTT ACC TTG TTA CGA CTT) (Weisburg *et al.*, 1991) and for the ITS PIGL (5' GCG CGC GTCGAC ACC TGC GGT TGG AAC ACC) and PIGR (5' GCG CGC TCTAGA CAC CGT GTA CGC TTA GTC). They were synthesized with Beckman Oligo 1000M DNA Synthesizer. PCR cloning of ITS was done with *pfu* DNA polymerase and primers with *Sal*I and *Xba*I restriction sites (underlined) and three GC oligonucleotide pairs. PCR was performed in 50 µl containing 30 pmol each primer, 3 U cloned *pfu* DNA polymerase (Stratagene), 10 ng isolated DNA or 10000 cells, and 20 mM dATP, dCTP, dGTP and dTTP (Boehringer Mannheim) in the 10× buffer provided by Stratagene. Amplification was carried out in an Eppendorf mastercycler 5330 in 30 cycles. Denaturation was done at 94 °C (in the first cycle for 45 s and in subsequent cycle for 30 s) and 45 s at 52 °C for annealing followed by 2 min at 72 °C for polymerization. For cloning, the PCR product was eluted from a 1.0% ultrapure agarose gel, digested with *Sal*I and *Xba*I and ligated into the vector pBluescript KS. The insertions were repeatedly sequenced with an automatic sequencer (ALF; Pharmacia).

Biochemical and physiological tests. Assimilation of 99 carbohydrates, organic acids and amino acids was studied using Biotype 100 strips (bioMérieux) which were incubated at 28 °C; the growth was recorded after 4 and 6 d incubation. Twenty-two additional tests were also performed. Acid production from lactose, methyl α -glucoside, melibiose, D(+)–arabitol, sorbitol, D(–)-arabinose, mannitol,

raffinose, D(–)-tartrate and utilization of malonate were tested on the basal medium of Ayers *et al.* (1919) supplemented with 0.1% (w/v) of carbohydrates and organic acid sodium salts. The other tests were classical techniques (Smibert & Krieg, 1981; Lelliott & Stead, 1987; Schroth & Hildebrand, 1988) and included: Simmons citrate, gelatin hydrolysis, lecithinase, casein hydrolysis, indole production, arginine dihydrolase (Møller), reducing compounds from sucrose (RCS), growth on NaCl (5% w/v), growth at 36 °C/39 °C in liquid King's B, liquefaction of calcium polygalacturonate in Sutton medium and utilization of inulin.

Numerical taxonomy. A total of 121 characteristics were included in the numerical taxonomy analysis. The matrix of distances was calculated by using the Jaccard coefficient (Sneath & Sokal, 1973) and cluster analysis was done with the unweighted pair group method with averages (UPGMA) (Sneath & Sokal, 1973). ITS sequence were aligned with the program ALIGN version 2.0 (Scientific & Educational Software) using a mismatch penalty of 2, an open gap penalty of 4 and an extend gap penalty of 1. Phylogenetic interferences were derived from multiple alignments of the ITS region using programs CLUSTREE and CLUSTAL W with the neighbour-joining method of Saitou & Nei (1987).

DNA–DNA hybridization and thermal stability. Extraction and purification of DNA were performed with previously described methods (Brenner *et al.*, 1982). Native DNAs were labelled *in vitro* by using the Megaprime DNA-labelling system (Amersham International). DNA–DNA hybridization experiments were done with labelled DNA from *Erwinia pyrifoliae* Ep16/96^T (CFBP 4172^T) at a temperature of 65 °C for reassociation. The S1 nuclease–TCA method was used for hybridization (Brenner *et al.*, 1982; Crosa *et al.*, 1973).

The temperature at which 50% of reassociated DNA became hydrolysable by enzyme S₁ (T_m) was determined by the method of Crosa *et al.* (1973). The ΔT_m is the difference

between the T_m of the homoduplex and the T_m of the heteroduplex.

DNA base composition. The G+C content of *Erwinia pyrifoliae* CFBP 4172^T was determined by a thermal denaturation temperature method (Marmur & Doty, 1962) and was calculated with the equation of Owen & Lapage (1976).

RESULTS

Characterization of *Erwinia pyrifoliae* by sequencing of the 16S rDNA and the adjacent ITS

Analogous to characterization of the 16S rDNA sequence of *Erwinia amylovora* (Bereswill *et al.*, 1995), we amplified the corresponding sequence of *Erwinia pyrifoliae* with consensus primers. Cleavage of the amplification product with restriction enzyme *Hae*III produced identical DNA fragments for both organisms, but different fragments in comparison to

Enterobacter pyrinus and *Pantoea stewartii* subsp. *stewartii* (data not shown). To further analyse the 16S rDNA sequences of *Erwinia pyrifoliae*, *Enterobacter pyrinus* and *Pantoea stewartii* subsp. *stewartii* on the nucleotide level, the amplified fragments were cloned into vector pGEM-T. The 16S rDNA of *Erwinia amylovora* was previously sequenced (Bereswill *et al.*, 1995; Kwon *et al.*, 1997), but for confirmation the 1.4 kb DNA fragment was cloned again from strain Ea1/79, and sequenced several times to confirm the corrected nucleotide sequence. When the 16S rDNA sequence of Ep1/96 and those of related bacteria were compared, the *Erwinia pyrifoliae* sequence was more than 99% homologous to *Erwinia amylovora* Ea1/79 and 97% homologous to *Pantoea stewartii* subsp. *stewartii* DC283 and decreased for other erwiniae (Table 2). This correlation of identical residues assigned *Erwinia pyrifoliae* to be closely related to *Erwinia amylovora*, but more distinct from *Enterobacter pyrinus* and *Pantoea stewartii* subsp. *stewartii*.

Table 2. Differences in the 16S rRNA nucleotide sequence of several bacterial species in comparison to *Erwinia pyrifoliae*

^TType strain; ATCC, American Type Culture Collection; DSM, DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen; KCTC, Korean Collection of Type Cultures; LMG, Laboratorium voor Microbiologie, Gent.

Species	Strain	EMBL no.	Length (bp)	Homology (%)	Non-matching nucleotides
<i>Erwinia pyrifoliae</i>	Ep1/96	AJ009930	1435	100	0
<i>Erwinia amylovora</i>	Ea1/79	AJ010485	1437	99	8
<i>Erwinia amylovora</i>	ATCC 15580 ^T	U80195	1439	99	12
<i>Erwinia persicina</i>	LMG 2691	AJ001190	1440	98	38
<i>Erwinia rhapontici</i>	LMG 2688	Z96087	1439	97	42
<i>Pantoea stewartii</i> subsp. <i>stewartii</i> *	DC283†	Unpublished	1439	97	45
<i>Erwinia psidii</i>	LMG 7034	Z96085	1438	97	53
<i>Pantoea ananatis</i> pv. <i>ananatis</i>	ATCC 33244 ^T	U80196	1438	97	55
<i>Klebsiella pneumoniae</i>	DSM 30104	X87276	1439	97	56
<i>Pantoea ananatis</i> pv. <i>uredovora</i>	ATCC 19321 ^T	U80209	1438	97	57
<i>Pantoea agglomerans</i> ‡	ATCC 33243 ^T	U80202	1437	97	58
<i>Escherichia coli</i>	K-12	J01695	1440	97	58
<i>Erwinia mallotivora</i>	LMG 2708	Z96084	1431	96	59
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i> §	ATCC 15713 ^T	U80197	1440	96	63
<i>Pectobacterium carotovorum</i> subsp. <i>betavascularum</i>	ATCC 43762 ^T	U80198	1441	96	65
<i>Erwinia chrysanthemi</i>	ATCC 11663 ^T	U80200	1441	96	68
<i>Enterobacter pyrinus</i>	KCTC 2520 ^T	AJ010486	1439	96	72
<i>Erwinia tracheiphila</i>	LMG 2906 ^T	Y13250	1428	94	89
<i>Brenneria salicis</i> ¶	ATCC 15721 ^T	U80210	1438	94	91

* Previous name: *Erwinia stewartii*.

† NaI^r mutant of SS104 (Z05, Dye's isolate; related to the type strain SS11, Dye's isolate Z010, ATCC 8199^T).

‡ Previous name: *Erwinia herbicola*.

§ Previous name: *Erwinia carotovora* subsp. *carotovora*.

|| Previous name: *Erwinia carotovora* subsp. *betavascularum*.

¶ Previous name: *Erwinia salicis*.

Fig. 1. Scheme aligning the 16S–23S spacer region from *Erwinia pyrifoliae* (Ep1/96; EMBL accession no. AJ009930), *Erwinia amylovora* (Ea1/79; AJ010485) and *Enterobacter pyrinus* (Enp90.2^T; AJ010486). The Glu-tRNA gene is marked. A similar profile was obtained in the alignment of the related nucleotide sequence from the ITS of strain Ep16/96 (AJ132969).

Additional nucleotide sequences were obtained from published data, which were also used for comparison with the 16S rDNA of *Erwinia pyrifoliae*. A gradient of relatedness is shown in Table 2 based on the number of nucleotide differences.

Comparison of the intergenic regions

We have also investigated the DNA sequence of the 16S rDNA–23S rDNA intergenic transcribed spacer (ITS) region. This sequence is more variable among bacteria and has widely been used for differentiation (Zavaleta *et al.*, 1996). The consensus primers PIGL and PIGR were used to amplify the intergenic region from *Erwinia pyrifoliae* strain Ep1/96, *Erwinia amylovora* strain Ea1/79, *Enterobacter pyrinus* strain 90.2^T, *Pantoea stewartii* subsp. *stewartii* strain DC283 and the *Escherichia coli* K-12 strain DH5 α . The DNA sequences of these organisms were significantly divergent, not only between *Erwinia pyrifoliae*, *Erwinia amylovora* and *Enterobacter pyrinus* (Fig. 1), but also to *Pantoea stewartii* subsp. *stewartii* and *Escherichia coli* with 86, 74, 81 and 75% identical residues, respectively. All ITSs in the sequence alignment showed a single copy of the gene for tRNA^{Glu}. The DNA adjacent to the tRNA^{Glu} genes of *Erwinia pyrifoliae* and *Erwinia amylovora* was most divergent (Fig. 1). When the determined ITS sequences and additional sequences from nucleotide databases were applied to calculate species relatedness, the dendrogram (Fig. 2) showed a remarkable evolutionary distance of *Erwinia pyrifoliae* and *Erwinia amylovora*.

Oligonucleotides were designed from the ITS and the 16S rDNA region of *Erwinia pyrifoliae*, which specifically detected the pathogen extracted from wood samples of Asian pear trees (W.-S. Kim & K. Geider, unpublished). Strains Ep8/95, isolated in 1995, Ep4/97 (1997) and a series of strains isolated in 1998 (represented by strain Ep102/98) gave rise to a positive signal with these primers in contrast to PCR assays with lysates from other bacteria such as *Pseudomonas syringae* pv. *syringae*, *Pantoea agglomerans* (*Erwinia*

Fig. 2. Dendrogram of *Erwinia pyrifoliae* and related bacteria based on the 16S–23S spacer region. Bootstrap values presented at the nodes were calculated with a frequency of a particular group to appear among 1000 bootstrapping samples. Strains Ep1/96 and Ep16/96^T were used for the input of nucleotide sequence information.

herbicola) or *Erwinia amylovora*. Strains Ep8/95 and Ep4/97 were also tested with additional procedures and produced the same responses as found for *Erwinia pyrifoliae* Ep16/96^T. These investigations confirm the isolation of *Erwinia pyrifoliae* in four subsequent years from necrotic branches of *Pyrus pyrifolia*.

Numerical analysis of phenotypic characteristics

Biotype 100 assays were applied to describe a dendrogram displaying the distance relationships among 25 strains included in this study. At a distance of 0–31, five phenotypes clustering at least two strains and 10

Fig. 3. Dendrogram for *Erwinia pyrifoliae* strains and *Erwinia*, *Enterobacter* and *Pantoea* type strains. The graph was calculated from data with Biotype 100 strips and 22 additional tests by the unweighted pair group method with averages (UPGMA) of the distances of the Jaccard coefficient. See footnotes to Table 2 concerning recent name changes.

isolated strains were observed. The distribution of strains in the different phenons and isolated strains is shown in Fig. 3. Phenon 1, containing the four strains of *Erwinia pyrifoliae*, is closely related to the type strain CFBP 1232 of *Erwinia amylovora* and to *Erwinia mallotivora*. The four other phenons contained two to four type strains of phytopathogenic *Erwinia*, *Pantoea* and *Enterobacter*. Biochemical and physiological characteristics that differentiate the five phenons and the 10 isolated strains are shown in Table 3. Six tests, i.e. growth in the presence of L-proline, melibiose, gelatinase, β -gentiobiose, L-glutamate or L(+)-arabinose differentiated *Erwinia pyrifoliae* from *Erwinia amylovora*. The 17 biochemical and physiological tests were selected to differentiate the five phenons and

isolated strains from each other on the basis of at least three characteristics.

DNA base composition and relatedness

Erwinia pyrifoliae Ep16/96^T (CFBP 4172^T) had a DNA G+C content of 52 mol%, *Erwinia amylovora* 54 mol% and *Erwinia mallotivora* 50 mol%. The strains of *Erwinia pyrifoliae* were 89–100% related to the strain CFBP 4172^T. The three strains of *Erwinia amylovora* including the type strain CFBP 1232 were 40–52% related to *Erwinia pyrifoliae* strain CFBP 4172^T. The ΔT_m values were in the range of 5.2–6.8. Thus, the strains isolated from *Pyrus pyrifolia* constituted a single DNA hybridization group.

Table 3. Phenotypic characteristics that differentiate *Erwinia pyrifoliae* from phenon 1, 2, 3, 4, 5 and other bacterial species

Strains assigned to a phenon are given in Fig. 3. 1, *Erwinia pyrifoliae*; A, *Erwinia amylovora*; B, *Erwinia mallotivora*; C, *Erwinia psidii*; D, *Pectobacterium cacticida*; E, *Erwinia salicis*; F, *Erwinia tracheiphila*; G, *Pantoea stewartii* subsp. *stewartii*; H, *Enterobacter cancerogenus*; I, *Enterobacter pyrinus*; J, *Brenneria nigrifluens*. Values are percentage of positive strains.

Character	Phenon					Other strains									
	1	2	3	4	5	A	B	C	D	E	F	G	H	I	J
Growth in presence of:															
L-Proline	+	—	+	+	+	—	—	—	—	—	—	—	—	+	+
Gelatin	—	—	—	+	—	+	—	+	—	—	—	—	—	—	+
β -Gentiobiose	—	50	25	+	+	+	—	—	+	—	—	—	+	+	+
L-Glutamate	+	+	+	+	+	—	—	+	—	—	—	+	+	+	+
L(+)-Arabinose	+	+	+	+	+	—	—	+	—	—	—	+	+	+	+
Utilization of:															
Maltotriose	—	—	+	+	+	—	—	—	—	—	—	—	+	+	+
α -Lactose	—	—	+	+	+	—	—	—	—	—	—	—	+	+	+
D-Saccharate	—	—	+	+	—	—	+	+	—	—	+	—	+	+	—
RCS	+	+	—	—	—	+	+	—	—	+	+	—	—	—	—
D(+)-Xylose	—	—	+	+	—	—	+	—	—	—	—	+	+	—	—
Mucate	—	—	+	+	—	—	—	+	—	—	+	—	+	—	—
L-Histidine	—	—	+	+	+	—	—	—	—	—	—	—	—	—	+
Malonate	—	—	+	—	+	—	—	—	+	—	—	—	+	+	—
D(+)-Melibiose	—	—	—	+	+	—	—	—	—	+	—	+	—	—	+
D(+)-Raffinose	—	—	—	+	+	—	—	—	—	+	—	+	—	—	+
D(+)-Mannose	—	+	+	+	+	—	+	+	+	—	—	+	+	+	+

DISCUSSION

Strains of the Asian pear pathogen *Erwinia pyrifoliae* are related to each other but distinct from *Erwinia amylovora*. A high similarity to the fire blight pathogen was based on the 16S rRNA sequences. The sequence of the ITS showed discordance for the two species. This result matches that of Leblond-Bourget *et al.* (1996), whereby evolutionary divergence of the ITS is much greater than that of 16S rRNA. In a sequence comparison of the ITS from eight *Erwinia amylovora* strains, which were isolated from different geographic origins, all strains showed a complete sequence homology (W.-S. Kim & K. Geider, unpublished). On the other hand, *Erwinia pyrifoliae* is quite divergent from *Erwinia amylovora* in its ITS sequence, including nucleotide changes in the tRNA^{Glu} ORF. This tRNA gene between the 16S and 23S rRNA has also been used for differentiation (Loughney *et al.*, 1982). All species investigated here had one tRNA^{Glu} gene. *Erwinia pyrifoliae* differs in that region from *Erwinia amylovora* and from *Enterobacter pyrinus* by one additional and nine different nucleotides (Fig. 1).

This ambiguous relation of *Erwinia pyrifoliae* and *Erwinia amylovora* was already observed by the first characterization of the Asian pear pathogen (Rhim *et al.*, 1999). It produces necrotic symptoms resembling fire blight, although the bark tissue is still greenish below the black-brown surface in contrast to the

red-brown colour in case of fire blight. Plating on MM2Cu agar (Bereswill *et al.*, 1997) gave a mucoid colony morphology as for *Erwinia amylovora*, but of a slightly yellow colour. Microbiological assays were overlapping in part or divergent for others. PCR assays with plasmid primers (Bereswill *et al.*, 1992) or from the *ams* region (Bereswill *et al.*, 1995) were negative for *Erwinia pyrifoliae*, which lacks the plasmid of 29 kb common to *Erwinia amylovora* (Falkenstein *et al.*, 1988).

Strains isolated from necrotic branches of *Pyrus pyrifolia* constituted a discrete DNA genomic group (genomospecies) with the type strain Ep16/96^T (CFBP 4172^T), since they were 89–100% related to each other. The strains of *Erwinia amylovora*, including the type strain CFBP 1232, are 40–52% related to the type strain of *Erwinia pyrifoliae* with a ΔT_m above 5 °C (5.2–6.8 °C). Presently, the molecular definition of bacterial species is applied to strains which are about 70% related, with ΔT_m values that do not exceed 5 °C. Thus, the bacteria associated with necrosis of Asian pear trees (Nashi) are four members of one new species.

A numerical taxonomy study of the Asian pear pathogen was related to 25 phytopathogenic *Erwinia*, *Pantoea* and *Enterobacter* strains including type strain of each species and the four strains pathogenic to *Pyrus pyrifolia* that were characterized by using Bio-type 100 strips and 22 additional tests. The Asian pear

pathogen constituted the phenon 1, which was closely related but distinct from *Erwinia amylovora*. Since these strains constitute a close DNA group that can be identified by using phenotypic tests, we propose that this new phytopathogenic organism should be recognized as a new species, *Erwinia pyrifoliae* sp. nov.

Description of *Erwinia pyrifoliae* sp. nov.

Erwinia pyrifoliae (py.ri.fo'liae. L. gen. fem. n. *pyrifoliae* of *pyrifolia*, the species name of the host plant, the Nashi pear, *Pyrus pyrifolia*).

Cells are Gram-negative, non-spore-forming, peritrichous, straight rods. The strains are facultatively anaerobic, oxidase is not produced. Nitrates are not reduced. The species conforms to the definition of the family *Enterobacteriaceae*. Strains grow on YPDA medium, producing colonies that are 2 mm after 48 h at 28 °C. Colonies are circular white, well-domed and opaque. Glucose (dextrose) is fermented without gas production. Voges-Proskauer test is (weakly) positive. Polypectate gel is not acidified or liquefied. Arginine dihydrolase, lysin decarboxylase and ornithine decarboxylase are not present. Indole is not produced from tryptophan. RCS are produced. Acid is not produced from inulin, lactose, methyl α -glucoside, melibiose, D-arabitol, D-arabinose or raffinose. Acid is produced from mannitol, sorbitol, saccharose. There is no alkalization of malonate, citrate or D-tartrate. β -Galactosidase is not produced. The following substrates are utilized as sole sources of carbon and energy: D-fructose, D-galactose, D-trehalose, saccharose, methyl D-glucopyranoside, D-ribose, L-arabinose, glycerol, myo-inositol, D-mannitol, D-sorbitol, L-malate, N-acetyl-D-glucosamine, D-gluconate, succinate, fumarate, L-glutamate, L-proline. The following substances are not utilized: D-mannose, D-sorbose, α -D-melibiose, D-raffinose, maltotriose, maltose, α -lactose, lactulose, methyl β -galactopyranoside, D-cellobiose, β -gentiobiose, aesculin, D-xylose, palatinose, α -L-rhamnose, α -L-fructose, D-melibiose, D-arabitol, L-arabitol, xylitol, dulcitol, D-tagatose, maltitol, D-turanose, adonitol, hydroxyquinoline β -glucoside, 3-methyl D-glucopyranoside, D-saccharate, mucate, L-tartrate, D-tartrate, meso-tartrate, D-malate, trans-aconitate, tricarballic acid, D-glucuronate, D-galacturonate, keto-D-gluconate, L-tryptophan, phenylacetate, protocatechuate, p-hydroxybenzoate, quinate, m-hydroxybenzoate, benzoate, phenylpropionate, m-coumarate, trigonelline, betaine, putrescine, aminobutyrate, histamine, DL-lactate, caprate, caprylate, L-histidine, glutarate, DL-glycerate, aminovalerate, ethanalamine, tryptamine, itaconate, hydroxybutyrate, D-alanine, malonate, propionate, L-tyrosine, ketoglutarate.

The pathogen was isolated from necrotic symptoms of leaves and branches of *Pyrus pyrifolia* cv. 'Shingo'. It is closely related to the fire blight pathogen *Erwinia amylovora* for the DNA sequence of the 16S rDNA,

but distantly for its intergenic transcribed spacer region (ITS). It does not contain plasmid pEA29 (Rhim *et al.*, 1999). The G + C content of the genomic DNA is 52 mol%. The type strain (Ep16/96^T) has been deposited in the Collection Française des Bactéries Phytopathogènes as CFBP 4172^T and the Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 12163^T. Additional strains deposited are Ep8/95 (= DSM 12393), Ep1/96 (= CFBP 4171 = DSM 12162), Ep28/96 (= CFBP 4173), Ep31/96 (= CFBP 4174) and Ep4/97 (= DSM 12394).

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