Erwinia piriflorinigrans sp. nov., a novel pathogen that causes necrosis of pear blossoms

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Eight *Erwinia* strains, isolated from necrotic pear blossoms in València, Spain, were compared with reference strains of *Erwinia amylovora* and *Erwinia pyrifoliae*, both of which are pathogenic to species of pear tree, and to other species of the family *Enterobacteriaceae* using a polyphasic approach. Phenotypic analyses clustered the novel isolates into one phenon, distinct from other species of the genus *Erwinia*, showing that the novel isolates constituted a homogeneous phenotypic group. Rep-PCR profiles, PCR products obtained with different pairs of primers and plasmid contents determined by restriction analysis showed differences between the novel strains and reference strains of *E. amylovora* and *E. pyrifoliae*. Phylogenetic analysis of 16S rRNA, *gpd* and *recA* gene sequences showed that the eight novel strains could not be assigned to any recognized species. On the basis of DNA–DNA hybridization studies, the novel isolates constituted a single group with relatedness values of 87–100 % to the designated type strain of the group, CFBP 5888^T. Depending on the method used, strain CFBP 5888^T showed DNA–DNA relatedness values of between 22.7 and 50 % to strains of the closely related species *E. amylovora* and *E. tasmaniensis*. The DNA G + C contents of two of the novel strains, CFBP 5888^T and CFBP 5883, were 51.1 and 50.5 mol%, respectively. On the basis of these and previous results, the novel isolates represent a novel species of the genus *Erwinia*, for which the name *Erwinia piriflorinigrans* sp. nov. is proposed. The type strain is CFBP 5888^T (=CECT 7348^T).

Necrotic blossoms were observed in Ercolini (Coscia) and Tendral pear trees growing in Valencia, Spain, from which an unknown bacterium was consistently isolated in 1999 and during the following two years. Infected blossoms were

similar in appearance to those affected by the disease fire blight, caused by *Erwinia amylovora*, but only occurred in spring and the blossoms were the only part of the trees that were affected, unlike the disease caused by *E. amylovora* (Roselló *et al.*, 2002, 2006). The isolated bacterium was subjected to phenotypic and molecular characterization and, although it was recognized as belonging to the genus *Erwinia*, many of its characteristics differed from those of other pathogenic and non-pathogenic species of the genus *Erwinia* that have been isolated from pear trees, including *E. amylovora* (Hauben *et al.*, 1998), *Erwinia billingiae* (Mergaert *et al.*, 1999), *Erwinia pyrifoliae* (Kim *et al.*, 1999) and *Erwinia tasmaniensis* (Geider *et al.*, 2006). The novel isolates provoked a hypersensitivity response when they were allowed to infiltrate tobacco and tomato leaves. Their

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Abbreviations: ERIC, enterobacterial repetitive intergenic consensus; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; REP, repetitive extragenic palindromic; UPGMA, unweighted pair group method with arithmetic mean.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *gpd* and *recA* gene sequences of strain CFBP 5888^T are GQ405202, GQ405201 and GQ421460, respectively.

Four supplementary figures and three supplementary tables are available with the online version of this paper.

pathogenicity was also observed when infecting the same pear cultivars from which they were isolated but not when infecting other hosts of the family Rosaceae (Roselló *et al.*, 2002, 2006).

The purpose of this work was to compare the novel pathogen to other members of the genus *Erwinia* and the family *Enterobacteriaceae*. Its taxonomic status was defined by using phenotypic tests, PCR amplification with several sets of primers (rep-PCR) designed from *E. amylovora* sequences, plasmid content and RFLP analysis after hybridization using pEA29 from *E. amylovora* as a probe, sequence analysis of 16S rRNA, *gpd* and *recA* genes and DNA–DNA hybridization studies.

In this study, we used eight strains that were isolated from necrotic pear blossoms, five strains of *E. amylovora*, two strains of *E. billingiae*, seven strains of *E. pyrifoliae*, three strains of *E. tasmaniensis* (Supplementary Table S1) and 24 type or reference strains of phytopathogenic and saprophytic species of the genera *Brenneria*, *Erwinia*, *Pantoea*, *Pectobacterium*, *Samsonia* and *Enterobacter*. All bacteria were stored at -80°C in 30 % (v/v) glycerol and freeze-dried. Prior to performing the different analyses, the bacteria were streaked on King's medium B (King *et al.*, 1954) and grown for 48 h at 25°C . The GenBank/EMBL/DBJ accession numbers for the 16S rRNA, *gpd* and *recA* gene sequences of the comparison strains are available in Supplementary Table S2, available in IJSEM Online.

Twenty-two conventional biochemical and physiological tests were performed and the assimilation of 99 carbon sources was determined using Biotype 100 strips (bioMérieux) on 44 pathogenic strains, excluding strains of *E. tasmaniensis* and *E. billingiae*, following previously described methods (Sutra *et al.*, 2001; Gardan *et al.*, 2003). All tests were performed at 28°C except for determination of growth at different temperatures. For tests using Biotype 100 strips, results were recorded after 4 days of incubation at 28°C . A total of 121 characteristics were included in numerical taxonomic analysis of the strains. The distance matrix was calculated by using the Jaccard's coefficient and cluster analysis was carried out by using the unweighted pair group method with arithmetic mean (UPGMA) (Sneath & Sokal, 1973). A dendrogram of the phenotypic distances among the 44 pathogenic strains was plotted (Fig. 1).

For selecting characteristics that distinguished the novel isolates from other bacterial species (*E. amylovora*, *E. billingiae*, *E. pyrifoliae* and *E. tasmaniensis*) commonly associated with plants of the family Rosaceae, two strains of each species were selected as reference strains for biochemical testing and the results obtained were compared with those obtained from the novel isolates. Tests using API 20 E strips (bioMérieux) were performed following the manufacturer's instructions, except that incubation was at 25°C for 48 h. The API 50 CH strips were inoculated with suspensions prepared in Ayers' medium (Ayers *et al.*, 1919) as described by Donat *et al.* (2007) and incubated at 25°C for 72 h. Conventional tests

were performed as described by Schaad *et al.* (2001). All tests were repeated at least twice.

PCR analyses were performed using several sets of primers to compare the eight novel isolates with the two reference strains of *E. amylovora* and *E. pyrifoliae*. Primers targeting conserved DNA sequences, such as repetitive extragenic palindromic (REP) elements (Louws *et al.*, 1994; Stern *et al.*, 1984), enterobacterial repetitive intergenic consensus (ERIC) sequences (Hulton *et al.*, 1991) and BOX A, a subunit of the BOX element, sequences (Martin *et al.*, 1992), were used as described by the indicated authors. Differences between migration patterns of the PCR products were analyzed by using BioNumerics version 6.0 (Applied Maths). Similarities were calculated using Pearson's coefficient and clustering was achieved using UPGMA. Other PCR analyses were performed using primers *amsBL* and *amsBR*, designed by Bereswill *et al.* (1995) from an *E. amylovora* chromosomal region, and AJ75 and AJ76, designed by McManus & Jones (1995) from the plasmid pEA29 of *E. amylovora*, according to instructions described by the indicated authors. PCR products were visualized after electrophoresis on 1.5 % agarose gels. All tests were repeated at least twice.

The plasmid contents of the eight novel isolates and two reference strains of *E. amylovora* and *E. pyrifoliae* were analyzed following the protocol of Zhou *et al.* (1990) and visualized by electrophoresis on a 0.8 % agarose gel. Restriction analyses were performed using *Bam*HI (Takara), and the plasmid profiles obtained were compared. Plasmid pEA29 was extracted from strain CFBP 1430, excised from the gel, purified, digested with *Eco*RI and labelled using a DIG High Prime DNA labelling kit I (Roche) so that it could be used as a probe with the profiles obtained by restriction analysis. The plasmid extractions and restriction digests were transferred onto nitrocellulose membranes and hybridized at 65°C . Detection was performed using a Dig Wash and Block buffer set (Roche) and the membranes were visualized using a LAS 3000 system version 1.1 imager (Fujifilm).

The 16S rRNA, *gpd* and *recA* gene sequences of two of the novel strains (CFBP 5882 and CFBP 5888^T) were sequenced by using the primer pairs fd2 and rP1 (Weisburg *et al.*, 1991), GAP11 and GAP12c (Geider *et al.*, 2006) and RECA1 and RECA2c (Waleron *et al.*, 2002), respectively, according to instructions described by the indicated authors. Each novel sequence was compared against available sequences in the GenBank/EMBL/DBJ database using BLAST searches (Altschul *et al.*, 1990). Regions common to all these sequences were used for neighbour-joining analysis and tree reconstruction, using Kimura's two-parameter correction with 1000 bootstrap replications. Maximum-parsimony and maximum-likelihood analyses were also performed by using BioNumerics version 6.0 (Applied Maths).

DNA–DNA hybridization of the selected eight isolates was performed in two experiments. In a first experiment four reference strains of *E. amylovora* and the type strain of *E.*

Fig. 1. Dendrogram based on UPGMA analysis of phenotypic distances among the 44 pathogenic strains studied, including the eight novel strains isolated from necrotic pear blossoms and reference strains of *E. amylovora*, *E. pyrifoliae* and related species. Numbers indicate CFBP accession numbers except for *E. pyrifoliae* strain 11927, which was obtained from the collection of Dr Louis Gardan at INRA, Angers, France. ^{PT}, Pathotype. Distance = $1 - J$, where J is the Jaccard's coefficient.

pyrifoliae were used. The extraction and purification of DNA was performed as described by Brenner *et al.* (1982). Native DNA of strain CFBP 5888^T was labelled *in vitro* by random priming using a Megaprime kit with tritium-labelled nucleotides (Amersham Biosciences). The S1 nuclease-trichloroacetic method (Crosa *et al.*, 1973) was used for DNA–DNA hybridization. DNA reassociation was performed at 65 °C. A second experiment was performed by isolating native DNA from the type strains of *E. amylovora*, *E. pyrifoliae* and *E. tasmaniensis* and the proposed type strain of the novel isolates by using a French pressure cell (Thermo Scientific). The native DNA was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970),

considering the modifications described by Huß *et al.* (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multi-cell changer and a temperature controller with an *in situ* temperature probe (Varian). DNA reassociation was performed in 2 × saline sodium citrate buffer with 5 % formamide at 68 °C.

The thermal stability (T_m) of reassociated DNA was determined by using the method of Crosa *et al.* (1973). The DNA G + C content of strains CFBP 5888^T and CFBP 5883 was determined by the thermal denaturation method of Marmur & Doty (1962) and calculated by using the equation of Owen & Lapage (1976). *Escherichia coli* K-12 CIP 54-117 (DNA G + C content 50.6 mol%) was used as a control.

A dendrogram of phenotypic distances between the 44 strains, excluding those of *E. tasmaniensis* and *E. billingiae*, is shown in Fig. 1. At a distance of 0.24, six phenons and 14 unclustered strains were observed. Phenon 1 corresponded to seven strains of *E. pyrifoliae* pathogenic to Asian pear trees (*Pyrus pyrifolia* Nakai). Phenon 2 clustered five strains of *E. amylovora*. Phenon 3 clustered the eight novel strains isolated from pear blossoms, which constituted a homogeneous phenotypic group. The unclustered strains and those that clustered into three other phenons comprised type or reference strains of the genera *Erwinia*, *Pectobacterium*, *Pantoea*, *Samsonia*, *Brenneria* and *Enterobacter*.

The phenotypic characteristics that differentiated the novel strains from other members of the genus *Erwinia* (*E. amylovora*, *E. billingiae*, *E. pyrifoliae* and *E. tasmaniensis*), isolated from plant hosts of the family Rosaceae, are shown in Table 1. The selected tests also distinguished the novel isolates from *E. amylovora* and *E. pyrifoliae*, both pathogens of rosaceous plants. Moreover, some phenotypic characters of the studied isolates were also different enough (Geider *et al.*, 2006) to differentiate them from non-pathogenic species *E. billingiae* and *E. tasmaniensis* (Table 1). The assimilation of adonitol was positive in the novel strains, negative in *E. amylovora*, *E. pyrifoliae* and *E. tasmaniensis* and variable in *E. billingiae*. In addition, the strong hydrolysis of Tween 20 and acid production from D-raffinose seemed to distinguish the novel strains from other members of the genus *Erwinia*.

The fingerprint profiles generated with ERIC, REP and BOX primer sets showed the same banding patterns between the eight novel isolates but different profiles were seen when these were compared to the patterns obtained with the reference strains of *E. amylovora* and *E. pyrifoliae*.

Table 1. Phenotypic characteristics that differentiate eight novel strains isolated from necrotic pear blossoms from strains of related species of the genus *Erwinia*

Taxa: 1, *E. piriflorinigrans* sp. nov.; 2, *E. amylovora*; 3, *E. billingiae*; 4, *E. pyrifoliae*; 5, *E. tasmaniensis*. Two strains were tested for all taxa. +, Positive; –, negative; w, weakly positive; v, variable.

Characteristic	1	2	3	4	5
Gelatinase	–	+	+	v	–
Nitrate reduction	–	–	+	–	–
Tween 20 hydrolysis	+	–	w	–	–
API 20 E:					
Arabinose	+	–	+	–	–
β-Galactosidase	+	–	+	–	+
API 50 CH:					
D-Adonitol	+	–	v	–	–
D-Arabitol	–	–	+	–	–
Arbutin	–	–	+	–	–
L-Fucose	–	–	+	–	–
Gentiobiose	+	–	+	–	–
D-Raffinose	+	–	–	–	–

Resulting dendrograms clearly grouped the eight novel isolates in a cluster distinct from those formed by reference strains of *E. amylovora* and *E. pyrifoliae*. A combined dendrogram for rep-PCR (ERIC, BOX and REP-PCR) is provided as Supplementary Fig. S1, available in IJSEM Online. No amplification was obtained from plasmid pEA29 sequences using the primers AJ75 and AJ76 (McManus & Jones, 1995). However, results were positive when using the primers for the amplification of chromosomal *amsBL* and *amsBR* gene sequences (Bereswill *et al.*, 1995).

The plasmid pattern of each of the eight novel isolates provided only one band, similar in size to the band obtained from the 29 kb plasmid of *E. amylovora*, except for one strain (CFBP 5887) that harboured two plasmids, one of ~29 kb and another of ~5 kb. Restriction analyses with *Bam*HI showed that the profiles obtained with the plasmids from the eight novel isolates were identical to each other but were different to those of reference strains of *E. amylovora* and *E. pyrifoliae*. Nevertheless, hybridizations using pEA29 as probe on these isolates were positive for some of the plasmid fragments as well as for some fragments of two reference strains of *E. pyrifoliae* (Supplementary Fig. S2).

According to the phylogenetic analysis of 16S rRNA, *gpd* and *recA* gene sequences of two of the novel strains, strains CFBP 5882 and CFBP 5888^T represent a novel bacterial species within the genus *Erwinia* (Fig. 2 and Supplementary Figs S3 and S4). The 16S rRNA gene sequence similarities between strain CFBP 5888^T and type strains of the genus *Erwinia* were as follows: 97.40 % with *E. tasmaniensis* Et1/99^T, 97.25 % with *E. amylovora* NCPPB 683^T, 97.18 % with *Erwinia toletana* A37^T, 97.09 % with *E. pyrifoliae* CFBP 4172^T, 97.03 % with *Erwinia rhapontici* ATCC 29283^T, 96.59 % with *Erwinia persicina* ATCC 35998^T and 96.36 % with *E. billingiae* LMG 2613^T. A more detailed analysis showed that these sequences form a very robust clade that is distinct from described species of the genus *Erwinia*. Accordingly, the phylogenetic analysis of the 16S rRNA, *gpd* and *recA* gene sequences strongly suggests that these two strains represent a novel species within the genus *Erwinia*.

Results of DNA–DNA hybridizations are shown in Supplementary Table S3. The eight necrotic pear-blossom isolates were 87–100 % related to strain CFBP 5888^T. The five strains of *E. amylovora* and *E. pyrifoliae* were 32–50 % related to strain CFBP 5888^T, with ΔT_m values for the type strains of these species of 8.5 and 6.0 °C, respectively, in the first experiment. In a second experiment, using a different methodology, type strains of *E. amylovora*, *E. pyrifoliae* and *E. tasmaniensis* were 22.7, 39.0 and 38.4 % related, respectively, to strain CFBP 5888^T. Thus, based on DNA hybridization values, the necrotic pear-blossom isolates constituted a single group and, consequently, represent a novel species of the genus *Erwinia*. Strains CFBP 5888^T and CFBP 5883 had DNA G+C contents of 51.1 and 50.5 mol%, respectively.

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences of strains CFBP 5888^T and CFBP 5882, isolated from necrotic pear blossoms, and closely related species and reconstructed using the neighbour-joining method. Solid and open circles indicate branches of the tree that were also recovered in trees reconstructed using the maximum-likelihood and maximum-parsimony methods, respectively. Bootstrap percentages >50% (based on 1000 replications) are indicated at branch points. Bar, 0.01 substitutions per nucleotide position. *Escherichia coli* ATCC 11775^T was used as an outgroup.

All the comparative analyses support the conclusion that the eight isolates constitute a novel species of the genus *Erwinia*. On the basis of phenotypic analysis, the novel isolates seemed to be metabolically more active than strains of *E. amylovora* and *E. pyrifoliae* and could be differentiated from these species, as well as from *E. billingiae* and *E. tasmaniensis*, by the results of a set of eleven tests. The novel isolates could also be differentiated from *E. amylovora* and *E. pyrifoliae* on the basis of other features, including serological properties and fatty acid profiles as previously described (Roselló *et al.*, 2006). As it is generally accepted that for an isolate to belong to a given species there must be at least 80–85% sequence similarity based on analysis with UPGMA (Wayne *et al.*, 1987; Janda & Abbott, 2002), the isolates under study could not be assigned to another phenon.

The results obtained from different molecular analyses mostly showed differences between the novel strains and strains of *E. amylovora* and *E. pyrifoliae*, but there were also some similarities. Patterns obtained from the amplification of sequences that are repeated along the genome (rep-PCR) demonstrated these differences and supported the conclusion that the novel isolates from necrotic pear blossoms were significantly different from strains of *E. amylovora* and *E. pyrifoliae*.

PCR analyses from this study and from a previous study (Roselló *et al.*, 2006) showed negative results for the novel isolates with the primers targeting plasmid pEA29 of *E. amylovora* (Bereswill *et al.*, 1992; Llop *et al.*, 2000; McManus & Jones, 1995) and with some primers based on chromosomal sequences of *E. amylovora*, such as primers Ea71/72 (Guilford *et al.*, 1996). Nevertheless, with primers such as *amsBL* and *amsBR* (Bereswill *et al.*, 1995) and EaF and EaR (Maes *et al.*, 1996) targeting other chromosomal sequences, the amplification was positive, indicating similarities in some chromosomal sequences. Moreover, when the strains of *Erwinia* isolated in Japan and those of *E. pyrifoliae* were analyzed by PCR using primers based on sequences of the amylovoran synthesis gene (*ams*) region of *E. amylovora* (Bereswill *et al.*, 1995),

no amplified products were obtained (Kim *et al.*, 2001), thus supporting the differences between these groups of strains.

Analysis of the plasmid content and subsequent hybridizations have shown the presence of one plasmid in the novel isolates, which is of a similar size, not only to pEA29, but also to one of the plasmids present in strains of *E. pyrifoliae*. Restriction profiles of the plasmids analyzed using *Bam*HI were different to those of pEA29 but hybridization using pEA29 as a probe provided similar results to those described in previous studies (Kim *et al.*, 2001; McGhee *et al.*, 2002; Maxson-Stein *et al.*, 2003), in which related sequences were found in plasmids from strains of *E. pyrifoliae* and *E. amylovora* and the strains of *Erwinia* isolated in Japan. This suggests that some common plasmid DNA sequences can be found in these pathogenic species from different and distant locations.

Finally, 16S rRNA, *gpd* and *recA* gene sequencing and DNA–DNA hybridization studies on the novel isolates confirmed the identification of these isolates as a novel species, the results being in agreement with the recommended standards of quantitative differentiation (Wayne *et al.*, 1987; Janda & Abbott, 2002).

The polyphasic approach used to identify these isolates, in this and in a previous study (Roselló *et al.*, 2006), included a combination of phenotypic methods (biochemical tests, fatty acid profiles, serological properties and numerical analysis) and genotypic methods (rep-PCR profiles, PCR products with several primers, plasmid content and RFLP, 16S rRNA, *gpd* and *recA* gene sequencing, analysis of DNA G+C content and DNA–DNA hybridization), the results of which fulfilled the recommended criteria reported as necessary for the definition of a novel species (Stackebrandt *et al.*, 2002). On the basis of these and previous results, the novel isolates represent members of a novel species of the genus *Erwinia*, for which the name *Erwinia piriflorinigrans* sp. nov. is proposed.

Description of *Erwinia piriflorinigrans* sp. nov.

Erwinia piriflorinigrans (pi.ri.flo.ri.ni'grans. L. n. *pirus* pear tree; L. n. *flos* -*floris* flower; L. part. adj. *nigrans* blackened, darkened; N.L. part. adj. *piriflorinigrans* blackened pear blossom, from which the type strain was isolated).

Cells are Gram-reaction-negative, facultatively anaerobic, non-spore-forming, motile, straight rods. Glucose is oxidized and fermented without producing gas in Hugh-Leifson's medium (Hugh & Leifson, 1953). Catalase-positive and oxidase-negative. Nitrates are not reduced. Grows on King's medium B (King *et al.*, 1954) producing non-fluorescent, circular, white, slightly convex, mucoid colonies, 2–3 mm in diameter with regular margins after 48 h at 25 °C. Produces convex colonies on Sucrose Nutrient agar and CCT media (Ishimaru & Klos, 1984) and produces levan. Does not hydrolyse arginine in anaerobiosis. Urease-negative. Does not use calcium galacturonate in Sutton's medium (Sutton, 1957), or show ice nucleation activity. Hydrolyses Tween 20 but not Tween 80 or gelatin. Reduces sugars from sucrose and produces acetoin. Grows in nutrient broth at 25 and 30 °C but not at 37 °C or higher. In the API 20E (bioMérieux) system, tests are positive for β -galactosidase activity, citrate and acetoin production and acidification of glucose, mannitol, *myo*-inositol, sucrose, melibiose and arabinose but negative for arginine dihydrolase and lysine and ornithine decarboxylase activities, acid production from sodium thiosulfate, urease, tryptophan deaminase, indole, gelatin and acidification of sorbitol, rhamnose and amygdalin. In the API 50 CH (bioMérieux) (Roselló *et al.*, 2006) and Biotype 100 systems (bioMérieux) (this study), glycerol, L-arabinose, ribose, D-xylose, adonitol, galactose, D-glucose, D-fructose, *myo*-inositol, mannitol, *N*-acetylglucosamine, melibiose, sucrose, trehalose, raffinose, β -gentiobiose methyl- α -glucoside, D-fucose, D- and L-alanine, L-serine, L-proline, fumarate, D-gluconate, L-glutamate, L-malate and succinate are utilized. The predominant fatty acids are (in decreasing order): C_{16:0}, summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH), summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} 1), C_{18:1} ω 7c, C_{14:0} and C_{12:0}, C_{17:0} cyclo, unknown fatty acids of equivalent chain-lengths (ECL) 14 502 and 13 597, C_{18:0}, C_{15:0}, C_{17:0}, and, as a minor component, C_{18:1} ω 5c (Roselló *et al.*, 2006). Does not react with a mixture of commercially available mAbs (IVIA 3B + IVIA 5H; PlantPrint Diagnostics) designed for the detection of *E. amylovora* in a DASi-ELISA protocol (Roselló *et al.*, 2006).

The type strain, CFBP 5888^T (=CECT 7348^T), was isolated from, and causes necrosis of, pear blossoms of Ercolini (Coscia) and Tendral pear trees and elicits a hypersensitive reaction when inoculated in tobacco and tomato leaves.

Strains show identical rep-PCR profiles, which are different from the profiles obtained with *E. amylovora* or *E. pyrifoliae*. Amplification is observed when using the primers EaF-EaR (Maes *et al.*, 1996), whose targets are 23S rRNA gene sequences, and primers from other chromosomal sequences such as *amsBL*-*amsBR* (Bereswill

et al., 1995). PCR analyses give negative results using the *E. amylovora* pEA29 primers (Bereswill *et al.*, 1992; Llop *et al.*, 2000; McManus & Jones, 1995) and using other primers based on *E. amylovora* chromosomal sequences, such as the Ea71 primers (Guilford *et al.*, 1996). The isolates contain a plasmid of similar size to the pEA29 of *E. amylovora*. The DNA G + C content is 50–51 mol%.

Acknowledgements

We are grateful to M^a Luisa Palazón and Javier Martínez Calvo for their help in naming the novel species, to Clara Morente, Julia Romaguera, Lourdes Rius and Roland Chartier for technical assistance and to Jean-Pierre Paulin for helpful discussions. This work was supported by grants from projects CICYT AGL2001-2349-C03-02 and CICYT AGL2005-06982/AGR. P.L. had a contract from INIA-CCAA (Ministry of Education and Science, Spain) with funding from the European Union (Social European Funding). The work was performed in the framework of COST 864.

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