Erwinia oleae sp. nov., isolated from olive knots caused by *Pseudomonas savastanoi* pv. *savastanoi*

Chiaraluce Moretti,^{1†} Taha Hosni,^{1†} Katrien Vandemeulebroecke,² Carrie Brady,³ Paul De Vos,² Roberto Buonauro¹ and Ilse Cleenwerck²

Correspondence

Chiaraluce Moretti
chiaraluce.moretti@unipg.it

¹Dipartimento di Scienze Agrarie e Ambientali, University of Perugia, Perugia, Italy

²BCCM/LMG Bacteria Collection, Ghent University, Ghent, Belgium

³LM-UGent, Laboratory of Microbiology, Faculty of Sciences, Ghent University, Ghent, Belgium

Three endophytic bacterial isolates were obtained in Italy from olive knots caused by *Pseudomonas savastanoi* pv. *savastanoi*. Phenotypic tests in combination with 16S rRNA gene sequence analysis indicated a phylogenetic position for these isolates in the genera *Erwinia* or *Pantoea*, and revealed two other strains with highly similar 16S rRNA gene sequences (>99%), CECT 5262 and CECT 5264, obtained in Spain from olive knots. Rep-PCR DNA fingerprinting of the five strains from olive knots with BOX, ERIC and REP primers revealed three groups of profiles that were highly similar to each other. Multilocus sequence analysis (MLSA) based on concatenated partial *atpD*, *gyrB*, *infB* and *rpoB* gene sequences indicated that the strains constituted a single novel species in the genus *Erwinia*. The strains showed general phenotypic characteristics typical of the genus *Erwinia* and whole genome DNA–DNA hybridization data confirmed that they represented a single novel species of the genus *Erwinia*. The strains showed DNA G + C contents ranging from 54.7 to 54.9 mol%. They could be discriminated from phylogenetically related species of the genus *Erwinia* by their ability to utilize potassium gluconate, L-rhamnose and D-arabitol, but not glycerol, inositol or D-sorbitol. The name *Erwinia oleae* sp. nov. (type strain DAPP-PG 531^T=LMG 25322^T=DSM 23398^T) is proposed for this novel taxon.

Knot formation on olive trees (*Olea europaea* L.) is a serious disease found in many olive producing areas. It is caused by *Pseudomonas savastanoi* pv. *savastanoi* and characterized by outgrowth on trunks and branches, and less frequently leaves and fruits (Sisto *et al.*, 2004). Olive knots are ideal niches for bacterial growth, not only of the causal agent of the disease, but also of a number of endophytic members of the class *Gammaproteobacteria* such as *Erwinia toletana* (Rojas *et al.*, 2004), *Pantoea agglomerans* (Marchi *et al.*, 2006; Quesada *et al.*, 2007) and other bacteria from the genera *Burkholderia*, *Hafnia*, *Pseudomonas* and *Stenotrophomonas* (Ouzari *et al.*, 2008). In the last few years, several studies have focussed on the effect of these endophytes in modulating olive knot

disease severity (Marchi *et al.*, 2006; Hosni, 2010). As such, it has been shown that *Pantoea agglomerans*, frequently isolated from olive knots when inoculated in olive plants together with *Pseudomonas savastanoi* pv. *savastanoi*, can either depress growth of the pathogen or produce an increase in knot size (Marchi *et al.*, 2006).

In the present study, five endophytic strains from olive knots (DAPP-PG 531^T, DAPP-PG 537, DAPP-PG 672, CECT 5262 and CECT 5264) were investigated using a polyphasic taxonomic approach.

In September 2003 and May 2007, young knots from branches of diseased olive trees located in orchards at Scanzano in the province of Perugia (Umbria, central Italy) and Valenzano in the province of Bari (Apulia, south Italy) were collected. Small portions of their internal water-soaked tissue were excised with a scalpel and crushed in a few drops of sterile distilled water. Subsequently, a loopful of the suspensions was streaked onto nutrient agar (NA; Oxoid) and the plates incubated at 27 ± 1 °C for 2 days. Along with circular (0.5–2.9 mm in diameter), white to pale yellow colonies resembling a ‘fried egg’, typical for *Pseudomonas savastanoi* pv. *savastanoi*, another bacterial colony type was frequently isolated. The latter type was

†These authors contributed equally to this work.

Abbreviation: MLSA, multilocus sequence analysis.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain DAPP-PG 531^T is GU810925. The accession numbers for the *atpD*, *gyrB*, *infB* and *rpoB* gene sequences of strains DAPP-PG 531^T, DAPP-PG 672 and CECT 5264 are GU991653–GU991656, HM439616–HM439619 and HM439612–HM439615, respectively.

A supplementary table and four supplementary figures are available with the online version of this paper.

selected for further investigation. Pure cultures of this type were obtained by picking up a single colony and streaking it onto NA plates amended with 5% sucrose. This way, strains DAPP-PG 531^T and DAPP-PG 537 were obtained in 2003 and strain DAPP-PG 672 in 2007. Initial microbiological characterization of these strains revealed that their colonies were not fluorescent when cultivated on King's medium B (20 g peptone l⁻¹, 1.5 g anhydrous K₂HPO₄ l⁻¹, 1.5 g MgSO₄ l⁻¹, 10 ml glycerol l⁻¹, 15 g agar l⁻¹). It also revealed that their cells were Gram-negative (as they lysed in 3% KOH; Suslow *et al.*, 1982), oxidase-negative, catalase-positive and facultatively anaerobic, suggesting that they belonged to the family *Enterobacteriaceae*. Additional strains used in this study were obtained from various biological resource centres and cultivated following the instructions of the provider. All bacterial strains used in this study are listed in Supplementary Table S1 (available in IJSEM Online).

Genomic DNA was extracted from strain DAPP-PG 531^T according to the protocol of Niemann *et al.* (1997). Amplification of the 16S rRNA gene was performed with the conserved primers 16F27 (5'-AGAGTTTGATCCTGGCTCAG-3') and 16R1522 (5'-AAGGAGGTGATCCAGCCGCA-3'). Purification of the amplification product was conducted with the NucleoFast 96 PCR Clean-up kit (Macherey-Nagel). Sequencing reactions were performed with the internal primers listed by Coenye *et al.* (1999) using the BigDye Terminator Cycle Sequencing kit (Applied Biosystems). Purification of the sequencing reaction products was conducted using the BigDye XTerminatorT Purification kit (Applied Biosystems). Sequencing was performed using an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems). Sequence assembly was done using the software package BioNumerics (Applied Maths). A nearly complete 16S rRNA gene sequence (1494 nt) was obtained for strain DAPP-PG 531^T and compared with 16S rRNA gene sequences deposited at NCBI, using BLAST. This analysis indicated that strain DAPP-PG 531^T belonged to the genera *Erwinia* or *Pantoea*, and it revealed two strains with very similar 16S rRNA gene sequences (>99% pairwise similarity), '*Pantoea oleae*' CECT 5262 and CECT 5264, that were obtained in Spain from olive knots. Using the software package BioNumerics (Applied Maths), the nearly complete 16S rRNA gene sequences of strains DAPP-PG 531^T, CECT 5262 and CECT 5264 were compared with those of reference strains of species of the genera *Erwinia*, *Pantoea* and related taxa collected from GenBank. Pairwise similarities were calculated using an open gap penalty of 100% and a unit gap penalty of 0%. A neighbour-joining phylogenetic tree (Fig. 1) was constructed using BioNumerics, and the robustness of the branches was evaluated by bootstrap analysis (Felsenstein, 1985). A maximum-likelihood phylogenetic tree was constructed (see Supplementary Fig. S1 in IJSEM Online) as described previously (Brady *et al.*, 2008). The three strains from olive knots showed more than 99% 16S rRNA gene sequence similarity with each other and less than 97% with recognized species of the genera *Erwinia* and *Pantoea*. As strains showing less than 97% 16S rRNA gene

sequence similarity are not likely to have more than 60–70% DNA–DNA relatedness (Stackebrandt & Goebel, 1994), these similarity values strongly suggested that the strains from olive knots represented at least one novel species in the family *Enterobacteriaceae*.

Genomic DNA was extracted from strains DAPP-PG 531^T, DAPP-PG 537, DAPP-PG 672, CECT 5262 and CECT 5264 and from *Erwinia toletana* CFBP 6631^T with the GenElute Bacterial Genomic DNA kit (Sigma Aldrich). Rep-PCR fingerprinting was performed with the BOX (Versalovic *et al.*, 1994), ERIC (Hulton *et al.*, 1991) and REP (Higgins *et al.*, 1982; Versalovic *et al.*, 1991) primers, according to the method described by Rademaker & de Bruijn (1997). Repeat experiments were performed and identical results were obtained. The rep-PCR profiles are shown in Supplementary Fig. S2. Irrespective of the primers used, strains DAPP-PG 531^T and DAPP-PG 537 generated the same fingerprints as well as strains CECT 5262 and CECT 5264. The Dice coefficient between the two groups of strains was 0.88. Strain DAPP-PG 672 had a similarity index of 0.88 with strains DAPP-PG 531^T and DAPP-PG 537 and 0.94 with strains CECT 5262 and CECT 5264. *E. toletana* CFBP 6631^T generated different fingerprints and with low similarity (0.36) in comparison with the other tested strains. Based on previous studies (Gevers *et al.*, 2001; De Vuyst *et al.*, 2008), the rep-PCR data suggested that strains DAPP-PG 531^T, DAPP-PG 537, DAPP-PG 672, CECT 5262 and CECT 5264 probably constituted a single species.

Multilocus sequence analysis (MLSA) of concatenated partial *atpD*, *gyrB*, *infB* and *rpoB* gene sequences enables the phylogenetically related genera *Erwinia*, *Pantoea* and *Tatumella* to be differentiated from each other (Brady *et al.*, 2008, 2009, 2010a, b, c). To refine the taxonomic position of the five strains from olive knots, partial sequences of the above-mentioned housekeeping genes were determined for three representative strains, DAPP-PG 531^T, DAPP-PG 672 and CECT 5264, and 12 reference strains from recognized species of the genus *Erwinia* (see Fig. 2 and Supplementary Fig. S3; gene sequences from GenBank accession nos HM439612–HM439619, GU991653–GU991656, HQ393588–HQ393635 were determined in this study). Partial fragments of the *atpD*, *gyrB*, *infB* and *rpoB* genes for strains DAPP-PG 531^T, DAPP-PG 672 and CECT 5264 were amplified and sequenced using the protocol of Brady *et al.* (2008) with the following modifications: (i) primer *atpD* 08-R (5'-CCGAGCAGCGCGGAGACTTC-3') was used instead of *atpD* 04-R; (ii) primer *infB* 05-F (5'-ACGGBATGRTBACSTTCCTKG-3') was used instead of *infB* 03-F. The modifications were needed because good technical sequences could not be obtained with primers *atpD* 04-R and *infB* 03-F, probably because they could not bind efficiently. Primers *atpD* 08-R and *infB* 05-F were designed based on sequences obtained with the primers *atpD* 03-F and *infB* 04-R, respectively. Sequence assembly was performed using the BioNumerics software package (Applied Maths), and partial nucleotide *atpD*, *gyrB*, *infB* and *rpoB* gene sequences were concatenated and aligned with concatenated partial *atpD*,

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic relationship between *Erwinia oleae* sp. nov. and related taxa within the family Enterobacteriaceae. *Brenneria alni* ICMP 12481^T was used as the outgroup. Bar, 1 % nucleotide substitutions. Numbers at branching points are bootstrap percentage values based on 1000 replications. Only values >50% are shown.

gyrB, *infB* and *rpoB* gene sequences of reference strains of species of the genera *Erwinia*, *Pantoea* and *Tatumella* taken from GenBank. The BioNumerics software package (Applied Maths) was used for this analysis and neighbour-joining

and maximum-likelihood phylogenetic trees (Fig. 2 and Supplementary Fig. S3) were constructed as described for the 16S rRNA gene. MLSA revealed that strains DAPP-PG 531^T, DAPP-PG 672 and CECT 5264 belonged to the genus

Fig. 2. Neighbour-joining tree based on concatenated partial *atpD*, *gyrB*, *infB* and *rpoB* gene sequences showing the phylogenetic relationship between *Erwinia oleae* sp. nov. and related taxa of the genera *Erwinia*, *Pantoea* and *Tatumella*. *Cronobacter sakazakii* ATCC BAA-894 was included as the outgroup. Bar, 10% nucleotide substitutions. Numbers at branching points are bootstrap percentage values based on 1000 replications. Only values >50% are shown.

Erwinia and also suggested that they probably constituted a single novel species.

Strains DAPP-PG 531^T, DAPP-PG 537, DAPP-PG 672, CECT 5262 and CECT 5264 were subjected to API 20E and API 50CHE systems (bioMérieux), according to the manufacturer's instructions. The results are presented in the species description below. API 50CHE tests were also carried out on type and reference strains of the 12 recognized species of the genus *Erwinia*. The strains studied are presented in

Supplementary Fig. S4, and the data obtained were analysed numerically to reveal the phenotypic relationship between the five strains from olive knots and the recognized species of the genus *Erwinia*. A distance matrix was calculated from similarity matrices generated using the Dice coefficient (Dice, 1945) and subjected to the unweighted pair-group method with arithmetic mean (UPGMA) clustering algorithm using NTSYSpc software (Exeter Software) version 2.1. A cophenetic value of 0.86 was determined for this matrix, which indicated a high goodness-of-fit. The dendrogram in

Supplementary Fig. S4 revealed that strains DAPP-PG 531^T, DAPP-PG 537, DAPP-PG 672, CECT 5262 and CECT 5264 formed a very homogeneous cluster with an overall similarity of about 93 % that was well discriminated from the 12 recognized species of the genus *Erwinia* that each formed a separate cluster. It also showed that the five strains from olive knots had phenotypic features common to the genus *Erwinia*. Table 1 lists a selected number of phenotypic features that enable strains DAPP-PG 531^T, DAPP-PG 537, DAPP-PG 672, CECT 5262 and CECT 5264 to be differentiated from recognized species of the genus *Erwinia*, including *Erwinia toletana*, the phylogenetically most closely related species of the genus also isolated from olive knots. Table 1 also reveals that the strains can be discriminated from each recognized species of the genus *Erwinia*, including *Erwinia piriflorinigra* (López *et al.*, 2011), by at least two characteristics.

To confirm whether or not strains DAPP-PG 531^T, DAPP-PG 537, DAPP-PG 672, CECT 5262 and CECT 5264 truly constituted a single novel species of the genus *Erwinia*, DNA–DNA hybridizations were performed. High-molecular mass DNA for DNA–DNA hybridization studies and DNA base composition determination was extracted using the method of Wilson (1987), with minor modifications (Cleenwerck *et al.*, 2002). DNA quantity and quality were determined by measuring the absorptions at 260, 280 and 234 nm and only high quality DNA with A₂₆₀/A₂₈₀ and A₂₃₄/A₂₆₀ ratios of 1.8–2.0 and 0.40–0.60, respectively, was

selected for further use. The size of the DNA was estimated by agarose gel electrophoresis. DNA–DNA hybridizations were performed with strains DAPP-PG 531^T, DAPP-PG 672 and CECT 5264 and *E. toletana* LMG 24162^T using a modification (Goris *et al.*, 1998; Cleenwerck *et al.*, 2002) of the microplate method described by Ezaki *et al.* (1989). The hybridization temperature was 44 °C. Reciprocal reactions (A × B and B × A) were performed and their variation was generally taken to be within the limits of this method (Goris *et al.*, 1998). The DNA–DNA relatedness values reported are the mean of minimum six hybridizations. Strains DAPP-PG 531^T, DAPP-PG 672 and CECT 5264 exhibited high levels of DNA–DNA relatedness (>80 %) and showed low levels (<25 %) with *E. toletana* LMG 24162^T (Table 2). As levels of 60–70 % DNA–DNA relatedness are generally accepted as the limit for species delineation (Wayne *et al.*, 1987), the DNA–DNA hybridization results confirmed that the strains from olive knots represented a single novel species of the genus *Erwinia*.

The DNA G + C content of strains DAPP-PG 531^T, DAPP-PG 537, DAPP-PG 672, CECT 5262 and CECT 5264 was determined by HPLC according to the method of Mesbah *et al.* (1989), and varied from 54.7 to 54.9 mol%, which was within the range reported previously for members of the genus *Erwinia* (Hauben *et al.*, 1998; Mergaert *et al.*, 1999; Kim *et al.*, 1999; Gardan *et al.*, 2004; Rojas *et al.*, 2004; Geider *et al.*, 2006). The DNA G + C content range was also less than 2 %, the generally accepted range within a species.

Table 1. Phenotypic characteristics differentiating strains of *Erwinia oleae* sp. nov. from other species of the genus *Erwinia*

Taxa: 1, *Erwinia oleae* sp. nov. (five strains); 2, *E. amylovora* LMG 2024^T; 3, *E. aphidicola* LMG 24877^T; 4, *E. billingiae* LMG 2613^T; 5, *E. mallotivora* LMG 2708^T; 6, *E. papayae* CFBP 5189^T; 7, *E. persicina* LMG 11254^T; 8, *E. psidii* LMG 7034^T; 9, *E. piriflorinigra* CECT 7348^T; 10, *E. pyrifoliae* ICMP 14143^T; 11, *E. rhapontici* LMG 2688^T; 12, *E. tasmaniensis* LMG 25318^T; 13, *E. toletana* CFBP 6631^T; 14, *E. tracheiphila* LMG 2707^T. +, Positive; –, negative; ND, not determined.

Characteristic*	1	2	3	4	5	6	7	8	9†	10	11	12	13	14
Nitrate reduction‡	+	–	+	+	–	–	+	–	ND	–	+	–	–	–
Growth at 36 °C in yeast salt and liquid 523 medium‡	+	–	+	–	–	–	+	–	ND	–	–	–	+	–
Fermentation of (API 50-CHE):														
Aesculin	+	–	+	+	–	+	+	+	–	–	+	–	+	–
L-Arabinose	+	+	+	+	–	+	+	+	+	+	+	+	+	+
D-Arabitol	+	–	–	+	–	–	–	–	–	–	–	–	+	+
Gluconate	+	–	+	–	–	+	–	–	+	–	–	–	–	–
Glycerol	–	–	+	+	–	–	+	+	+	+	+	+	+	+
Inositol	–	+	+	+	–	–	+	–	+	+	+	+	+	–
2-Ketogluconate	+	–	+	–	–	–	–	–	–	–	–	–	–	–
D-Mannose	+	–	+	+	+	+	+	+	–	–	+	–	+	+
L-Rhamnose	+	–	+	+	–	–	+	+	–	–	+	–	–	–
D-Sorbitol	–	+	–	+	–	–	+	–	–	+	–	–	–	–
Sucrose	–	+	+	–	+	+	+	+	+	+	+	+	–	+
Xylitol	–	–	+	–	–	–	–	–	–	–	+	+	–	–

*The strains tested for each *Erwinia* species are given in Supplementary Table S1.

†Data from López *et al.* (2011).

‡Tests performed according to Schaad *et al.* (2001).

Table 2. DNA–DNA relatedness (%) between strains DAPP-PG 531^T, DAPP-PG 672 and CECT 5264 of *Erwinia oleae* sp. nov. and *E. toletana* LMG 24162^T

Strain	DNA–DNA relatedness (%) with strain*:			
	1	2	3	4
1. <i>E. oleae</i> DAPP-PG 672	100			
2. <i>E. oleae</i> DAPP-PG 531 ^T	88 ± 6	100		
3. <i>E. oleae</i> CECT 5264	88 ± 15	89 ± 16	100	
4. <i>E. toletana</i> LMG 24162 ^T	17 ± 4	14 ± 5	20 ± 5	100

*Each value is the mean of a minimum of six hybridizations ± SD.

In conclusion, based on the genotypic data (from 16S rRNA gene sequence analysis, rep-PCR DNA fingerprinting, MLSA and DNA–DNA hybridizations) and phenotypic data obtained in this study, the five endophytic bacterial strains DAPP-PG 531^T, DAPP-PG 537, DAPP-PG 672, CECT 5262 and CECT 5264 from olive knots, caused by *Pseudomonas savastanoi* pv. *savastanoi*, represent a novel species for which the name *Erwinia oleae* sp. nov. is proposed. Strain DAPP-PG 531^T (=LMG 25322^T=DSM 23398^T) is the type strain.

Description of *Erwinia oleae* sp. nov.

Erwinia oleae [o'le.ae. L. gen. fem. n. *oleae* of olive (*Olea europaea*), the plant from which the bacterium was isolated].

Strains have all the characteristics of members of the family *Enterobacteriaceae*. Cells are Gram-negative rods, measuring 0.9 × 1.5–3.0 µm, and occur as single and pairs. Cells are motile and non-spore-forming. After growth for 24–48 h on nutrient agar at 27 ± 1 °C, colonies are light beige, circular (1–1.2 mm in diameter) and convex and with entire margins. Does not produce fluorescent pigment on King's medium B. Growth in yeast salt and liquid 523 medium (Schaad *et al.*, 2001) occurs at 36 °C, but not at 39 °C. Able to grow in 5% (w/v) NaCl. Facultatively anaerobic. Oxidase is not produced. Results obtained with API20E tests (bioMérieux) give a positive result for β-galactosidase activity, but a negative result for activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, phenylalanine deaminase and gelatinase (except strain DAPP-PG 672). Citrate is not utilized; hydrogen sulfide, indole and acetoin (except strains CECT 5262 and CECT 5264) are not produced. Nitrate is reduced to nitrite. Results obtained with API 50CHE tests (bioMérieux) indicate that strains utilize the following substrates as sole carbon sources at 27 ± 1 °C within 2 days: L-arabinose, D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, N-acetylglucosamine, aesculin, trehalose, D-arabitol, potassium gluconate and potassium 2-ketogluconate, and arbutin and salicin (except strains DAPP-PG 531^T and DAPP-PG 537). The following carbon sources are not utilized at 27 ± 1 °C within 2 days: glycerol, erythritol, D-arabinose, D-xylose, L-xylose,

D-adonitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, cellobiose, maltose, lactose, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol and potassium 5-ketogluconate. The DNA G + C content of the five strains ranges from 54.7 to 54.9 mol% as determined by the method of Mesbah *et al.* (1989).

The type strain, DAPP-PG 531^T (=LMG 25322^T=DSM 23398^T) and DAPP-PG 537 (=LMG 25323=DSM 23412), were isolated in Umbria (Italy) from olive knots caused by *Pseudomonas savastanoi* pv. *savastanoi*. Additional strains were isolated in Apulia (Italy) (DAPP-PG 672=LMG 25321=DSM 23411) and Spain (CECT 5262=LMG 25327, CECT 5264=LMG 25328) also from olive knots caused by *Pseudomonas savastanoi* pv. *savastanoi*.

Acknowledgements

This research was supported by 'Fondazione Cassa di Risparmio di Perugia', Italy, project 'Indagini sui batteri endofiti associati a piante di olivo affette da rogna, volte all'individuazione di nuove strategie di lotta alla malattia' to R. B. The BCCM/LMG Bacteria Collection is supported by the Federal Public Planning Service – Science Policy, Belgium. C. B. is the beneficiary of a fellowship granted by the Federal Science Policy Office, Belgium. The authors wish to acknowledge Katrien Engelbeen and Luca Bonciarelli for their technical support.

References

- Brady, C. L., Cleenwerck, I., Venter, S., Vancanneyt, M., Swings, J. & Coutinho, T. A. (2008). Phylogeny and identification of *Pantoea* species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). *Syst Appl Microbiol* **31**, 447–460.
- Brady, C. L., Venter, S. N., Cleenwerck, I., Engelbeen, K., Vancanneyt, M., Swings, J. & Coutinho, T. A. (2009). *Pantoea vagans* sp. nov., *Pantoea eucalypti* sp. nov., *Pantoea deleyi* sp. nov. and *Pantoea anthophila* sp. nov. *Int J Syst Evol Microbiol* **59**, 2339–2345.
- Brady, C. L., Cleenwerck, I., Venter, S. N., Engelbeen, K., De Vos, P. & Coutinho, T. A. (2010a). Emended description of the genus *Pantoea*, description of four species from human clinical samples, *Pantoea septica* sp. nov., *Pantoea eucrinea* sp. nov., *Pantoea brenneri* sp. nov. and *Pantoea conspicua* sp. nov., and transfer of *Pectobacterium cyripedii* (Hori 1911) Brenner *et al.* 1973 emend. Hauben *et al.* 1998 to the genus as *Pantoea cyripedii* comb. nov. *Int J Syst Evol Microbiol* **60**, 2430–2440.
- Brady, C. L., Venter, S. N., Cleenwerck, I., Vandemeulebroecke, K., De Vos, P. & Coutinho, T. A. (2010b). Transfer of *Pantoea citrea*, *Pantoea punctata* and *Pantoea terrea* to the genus *Tatumella* emend. as *Tatumella citrea* comb. nov., *Tatumella punctata* comb. nov. and *Tatumella terrea* comb. nov. and description of *Tatumella morbiose* sp. nov. *Int J Syst Evol Microbiol* **60**, 484–494.
- Brady, C. L., Goszczynska, T., Venter, S. N., Cleenwerck, I., De Vos, P., Gitaitis, R. D. & Coutinho, T. A. (2010c). *Pantoea allii* sp. nov., a novel species isolated from onion and onion seed. *Int J Syst Evol Microbiol* **60**, 932–937.
- Cleenwerck, I., Vandemeulebroecke, K., Janssens, D. & Swings, J. (2002). Re-examination of the genus *Acetobacter*, with descriptions of

- Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov. *Int J Syst Evol Microbiol* **52**, 1551–1558.
- Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J. R. W., Kersters, K. & Vandamme, P. (1999). Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* **49**, 405–413.
- De Vuyst, L., Camu, N., De Winter, T., Vandemeulebroecke, K., Van de Perre, V., Vancanneyt, M., De Vos, P. & Cleenwerck, I. (2008). Validation of the (GTG)(5)-rep-PCR fingerprinting technique for rapid classification and identification of acetic acid bacteria, with a focus on isolates from Ghanaian fermented cocoa beans. *Int J Food Microbiol* **125**, 79–90.
- Dice, L. (1945). Measurement of the amount of ecological association between species. *Ecology* **26**, 297–302.
- Dye, D. W. (1968). A taxonomic study of the genus *Erwinia*. I. The “amylovora” group. *N Z J Sci* **11**, 590–607.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gardan, L., Christen, R., Achouak, W. & Prior, P. (2004). *Erwinia papayae* sp. nov., a pathogen of papaya (*Carica papaya*). *Int J Syst Evol Microbiol* **54**, 107–113.
- Geider, K., Auling, G., Du, Z., Jakovljevic, V., Jock, S. & Völksch, B. (2006). *Erwinia tasmaniensis* sp. nov., a non-phytopathogenic bacterium from apple and pear trees. *Int J Syst Evol Microbiol* **56**, 2937–2943.
- Gevers, D., Huys, G. & Swings, J. (2001). Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiol Lett* **205**, 31–36.
- Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.
- Goto, M. (1976). *Erwinia mallotovora* sp. nov., the causal organism of bacterial leaf spot of *Mallotus japonicus*. *Int J Syst Bacteriol* **26**, 467–473.
- Hao, M. V., Brenner, D. J., Steigerwalt, A. G., Kosako, Y. & Komagata, K. (1990). *Erwinia persicinus*, a new species isolated from plants. *Int J Syst Bacteriol* **40**, 379–383.
- Harada, H., Oyaizu, H., Kosako, Y. & Ishikawa, H. (1997). *Erwinia aphidicola*, a new species isolated from pea aphid, *Acyrtosiphon pisum*. *J Gen Appl Microbiol* **43**, 349–354.
- 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.
- Higgins, C. F., Ames, G. F. L., Barnes, W. M., Clement, J. M. & Hofnung, M. (1982). A novel intercistronic regulatory element of prokaryotic operons. *Nature* **298**, 760–762.
- Hosni, T. (2010). Interaction between *Pseudomonas savastanoi* pv. *savastanoi*, the causal agent of olive knot, and the endophytic bacterial species associated with the knot. PhD thesis, University of Perugia, Italy, pp. 108.
- Hulton, C. S. J., Higgins, C. F. & Sharp, P. M. (1991). ERIC sequences: a novel family of repetitive elements in the genomes of *Escherichia coli*, *Salmonella typhimurium* and other enterobacteria. *Mol Microbiol* **5**, 825–834.
- Kim, W.-S., Gardan, L., Rhim, S.-L. & Geider, K. (1999). *Erwinia pyrifoliae* sp. nov., a novel pathogen that affects Asian pear trees (*Pyrus pyrifolia* Nakai). *Int J Syst Bacteriol* **49**, 899–906.
- López, M. M., Roselló, M., Llop, P., Ferrer, S., Christen, R. & Gardan, L. (2011). *Erwinia piriflorinigra* sp. nov., a novel pathogen that causes necrosis of pear blossoms. *Int J Syst Evol Microbiol* **61**, 561–567.
- Marchi, G., Sisto, A., Cimmino, A., Andolfi, A., Cipriani, M. G., Evidente, A. & Surico, G. (2006). Interaction between *Pseudomonas savastanoi* pv. *savastanoi* and *Pantoea agglomerans* in olive knots. *Plant Pathol* **55**, 614–624.
- Mergaert, J., Hauben, L., Cnockaert, M. C. & Swings, J. (1999). Reclassification of non-pigmented *Erwinia herbicola* strains from trees as *Erwinia billingiae* sp. nov. *Int J Syst Bacteriol* **49**, 377–383.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Neto, J. R., Robbs, C. F. & Yamashiro, T. (1987). A bacterial disease of guava (*Psidium guajava*) caused by *Erwinia psidii* sp. nov. *Fitopatol Bras* **12**, 345–350.
- Niemann, S., Pühler, A., Tichy, H.-V., Simon, R. & Selbitschka, W. (1997). Evaluation of the resolving power of three different DNA fingerprinting methods to discriminate among isolates of a natural *Rhizobium meliloti* population. *J Appl Microbiol* **82**, 477–484.
- Ouzari, H., Khsairi, A., Raddadi, N., Jaoua, L., Hassen, A., Zarrouk, M., Daffonchio, D. & Boudabous, A. (2008). Diversity of auxin-producing bacteria associated to *Pseudomonas savastanoi*-induced olive knots. *J Basic Microbiol* **48**, 370–377.
- Quesada, J. M., García, A., Bertolini, E., López, M. M. & Penyalver, R. (2007). Recovery of *Pseudomonas savastanoi* pv. *savastanoi* from symptomless shoots of naturally infected olive trees. *Int Microbiol* **10**, 77–84.
- Rademaker, J. L. W. & de Bruijn, F. J. (1997). Characterization and classification of microbes by rep-PCR genomic fingerprinting and computer-assisted pattern analysis. In *Protocols, Application and Overviews*, pp. 151–171. Edited by G. Caetano-Anolles & P. Gresshoff. New York: J. Wiley and Sons.
- Roberts, P. (1974). *Erwinia rhapontici* (Millard) Burkholder associated with pink grain of wheat. *J Appl Bacteriol* **37**, 353–358.
- Rojas, A. M., de los Rios, J. E., Fischer-Le Saux, M., Jimenez, P., Roche, P., Bonneau, S., Sutra, L., Mathieu-Daudé, F. & McClelland, M. (2004). *Erwinia toletana* sp. nov., associated with *Pseudomonas savastanoi*-induced tree knots. *Int J Syst Evol Microbiol* **54**, 2217–2222.
- Schaad, N. W., Jones, J. B. & Chun, W. (2001). Initial identification of common genera. In *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 3rd edn, pp. 84–120. Edited by N. W. Schaad, J. B. Jones & W. Chun. St Paul, MN: APS.
- Sisto, A., Cipriani, M. G. & Morea, M. (2004). Knot formation caused by *Pseudomonas savastanoi* subsp. *savastanoi* on olive plants is *hrp*-dependent. *Phytopathology* **94**, 484–489.
- Stackebrandt, E. & Goebel, B. M. (1994). A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Suslow, T. V., Schroth, M. N. & Isaka, M. (1982). Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology* **72**, 917–918.

Versalovic, J., Koeuth, T. & Lupski, J. R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* **19**, 6823–6831.

Versalovic, J., Schneider, M., de Bruijn, F. P. & Lupski, J. R. (1994). Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Methods Mol Cell Biol* **5**, 25–40.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other

authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.5. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Green Publishing and Wiley-Interscience.