Erwinia uzenensis sp. nov., a novel pathogen that affects European pear trees (Pyrus communis L.)

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Bacteria were isolated from black lesions on shoots of European pear trees (Pyrus communis L.) in an orchard in Japan. Previous characterization of this novel pathogen by phenotypic and genotypic methods suggested that it should belong to the genus Erwinia but might not correspond to either Erwinia amylovora or Erwinia pyrifoliae. Here, phylogenetic analyses of the 16S rRNA gene, gyrB, and rpoD gene sequences indicated that it could not be assigned to any recognized species of the genus Erwinia. DNA–DNA hybridization confirmed that the bacterial strains represented a novel species. The DNA G+C contents, the fatty acid profile and phenotypic characteristics resembled those previously reported for members of the genus Erwinia. On the basis of these and previous results, the pathogen represents a novel species of the genus Erwinia, for which the name Erwinia uzenensis sp. nov. (type strain: YPPS 951T = LMG 25843T = NCPPB 4475T) is proposed.

In late May 2007, blackening of young shoots was found on European pear trees (Pyrus communis cv. La France) in an orchard in Kaminoyama City, Yamagata Prefecture, Japan. Although the symptoms of this new disease, which was named the bacterial black shoot disease of European pear (Mizuno et al., 2010), generally resembled those of fire blight of pear caused by Erwinia amylovora (Van der Zwet & Keil, 1979) or bacterial shoot blight of pear caused by Erwinia amylovora bv. 4 (Mizuno et al., 2000), some typical symptoms of the latter two diseases, i.e. blossom blight, fruitlet blight, and shoot tip wilting (shepherd’s crook), were not observed in the case of black shoot disease. The symptoms of black shoot disease also differed from those of necrotic disease of Asian pear trees caused by Erwinia pyrifoliae (Kim et al., 1999; Rhim et al., 1999) in that necrotic symptoms on blossoms and fruitlets were not observed in this case. In addition, the symptoms were different from necrotic pear blossoms caused by Erwinia pirihoflorigrana (Roselló et al., 2006; López et al., 2011) in that the necrotic symptoms were limited to blossoms. We isolated 18 pathogenic bacteria (YPPS 950–YPPS 967) from lesions of black shoot disease, and previously completed a preliminary characterization of them (Mizuno et al., 2010). The purpose of the present study was to define their taxonomic status more precisely.

All bacteria used in this study were stored at −40 °C in dispersion medium [10% (w/v) skimmed milk, 1% (w/v) monosodium glutamate]. Prior to each analysis, the bacteria were streaked on King’s medium B (King et al., 1954) or PSA (Wakimoto, 1955) and grown for 48 h at 26 °C. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene, gyrB and rpoD sequences of the reference strains used and the pathogenic strains we isolated are listed in Table S1, available in IJSEM Online.

The phenotypic features were determined using the method described by Dye (1968). All tests were performed at 26 °C except for determination of growth at different
temperatures. Additional biochemical tests were performed with API 20 NE strips (bioMérieux) according to the manufacturer’s instructions. The API strips were incubated at 26 °C for 72 h and the results were recorded.

Fatty acid analysis was carried out by TechnoSuruga Laboratory Co., Ltd (Japan). For the fatty acid analysis, strain YPPS 951T was grown in trypticase soy broth agar at 28 °C for 48 h. The cellular fatty acid profile of isolate YPPS 951T was determined using a microbial ID system equipped with a GC according to a standard protocol (Paisley, 1996).

The nearly complete 16S rRNA gene sequences (1467 bp) and partial gyrB and rpoD gene sequences of the 18 strains were amplified and sequenced according to previously described procedures (Matsuura et al., 2007; Yamamoto & Harayama, 1995, 1998), except that alternative primers were used for 16S rRNA gene sequencing (Sawada et al., 1995, 1998), except that alternative primers were used for 16S rRNA gene sequencing (Sawada et al., 2011) (Table S2). The sequences were determined and compared with available sequences in the GenBank/EMBL/ DDBJ database using BLAST searches (Altschul et al., 1990). Sequence similarity values were calculated using GENETYX version 7 (Genetyx Corporation). The sequences were aligned using CLUSTAL W (Larkin et al., 2007). Positions with gaps or ambiguous bases were excluded from the sequences, and evolutionary distances were estimated using the HKY 85 model (Hasegawa et al., 1985) with gamma distributed rate heterogeneity. A phylogenetic tree was constructed employing the neighbour-joining method (Saitou & Nei, 1987) using PAUP* version 4.0b10 (Sinauer Associates). The strength of the internal branches of the resulting tree was tested by bootstrap analysis with 1000 replications (Felsenstein, 1985). The tree was drawn and edited using DendroMaker for Macintosh version 4.1 (http://www.cib.nig.ac.jp/dda/timanish/dendromaker/home.html). Maximum-likelihood analyses were also performed using PAUP* version 4.0b10 and Treefinder version October 2008 (Jobb et al., 2004).

The HPLC determination of the DNA G + C content of isolate YPPS 951T was performed by TechnoSuruga Laboratory Co., Ltd according to the method of Katayama-Fujimura et al. (1984). DNA–DNA hybridization was carried out according to the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and microplates. The hybridization temperature was 42 °C.

The phenotypic characteristics that differentiated the novel strains from other members of the genus Erwinia (E. amylovora, E. amylovora bv. 4, E. pyrifoliae and Erwinia tasmaniensis) are shown in Table 1. The selected tests also distinguished the novel isolates from the other species of the genus Erwinia used in this study. Acid production from methyl α-D-glucoside was positive except for two of the novel strains (YPPS 950 and YPPS 954). The predominant fatty acid content of strain YPPS 951T was: C16:0;97c and/or iso-C15:0 2-OH (summed feature 3), 33.37%; C16:0 28.97%; C18:1ω7c 9.97%; and iso C16:1 I and/or C14:0 3-OH, 8.36% (summed feature 2). This profile resembled those previously reported for other species of the genus Erwinia (Geider et al., 2006).

16S rRNA gene sequences of the 18 strains determined in this study were divided into three types (GenBank accession nos. AB546197, AB546198 and AB546199) whose mutual similarity was more than 99.8% and three representative strains (YPPS 950, YPPS 951T and YPPS 952, one of each 16S rRNA gene sequence type) were selected for further comparisons. The 16S rRNA gene sequence similarities between strain YPPS 951T and strains of species of the genus Erwinia were as follows: 99.18% with E. amylovora bv. 4 YPPS 200; 98.51% with E. piriformis CFMB 5888T; 98.50% with E. amylovora DSM 30165T; 98.16% with E. pyrifoliae EP1/96; 98.16% with E. tasmaniensis Et1/99T; 97.21% with E. rhapontici DSM 4484T; 96.87% with E. billingsiae Eb661T; 95.71% with E. toletana CFMB 6639. The phylogenetic tree based on 16S rRNA gene sequences (Fig. 1) showed that the novel strains formed a separate clade within the genus Erwinia. The phylogenetic trees based on the partial gyrB and rpoD gene sequences (Figs 2 and S1) also showed that the novel strains formed an independent monophyletic cluster on the periphery of the genus Erwinia. The topologies of the maximum-likelihood phylogenetic trees were similar (data not shown). These results differentiated the novel strains from all recognized species of the genus Erwinia.

The DNA G + C content of strain YPPS 951T was 53.4 mol%. This value was within the G + C content range of 49.8–54.1 mol% previously observed for members of the

Table 1. Phenotypic characteristics that differentiate the 18 novel strains, isolated from blackened lesions of young shoots of Pyrus communis cv. La France, from strains of related species of the genus Erwinia

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Taxa: 1. E. uzensis sp. nov.; 2. E. amylovora NCPPB 683T; 3. E. amylovora bv. 4 YPPS 200; 4. E. pyrifoliae DSM 12163T; 5. E. tasmaniensis NCPPB 4357T. Data except for API 20 NE results are from Mizuno et al. (2010). +, 90–100% positive in 1–2 days; −, negative; d, 11–89% strains positive in 1–4 days; w+, weakly positive. |
genus *Erwinia* (Hauben & Swings, 2005). DNA–DNA hybridizations revealed 81–100 % DNA–DNA binding among six selected novel strains (YPPS 950, YPPS 951T, YPPS 954, YPPS 955, YPPS 957 and YPPS 960). We previously found that these strains showed relatively low DNA–DNA relatedness values to *E. amylovora* NCPPB 683T (27–41 %), *E. amylovora* bv. 4 YPPS 200 (48–58 %), *E. pyrifoliae* DSM 12163T (36–49 %), and *E. tasmaniensis* NCPPB 4357T (11–23 %) (Mizuno et al., 2010). On the basis of the above results, the novel strains are considered to represent a novel species.

All the data obtained in this study agree with our previous studies, and clearly show that the 18 strains used in this study, isolated as the causative agent of bacterial black shoot disease of European pear which occurred in Japan, should constitute a novel species of the genus *Erwinia*, for which the name *Erwinia uzenensis* sp. nov. is proposed.

**Description of *Erwinia uzenensis* sp. nov.**

*Erwinia uzenensis* (u.zen.en sis. N.L. fem. adj. uzenensis pertaining to Uzen, which is the old name for Yamagata prefecture, where the strain was isolated).
fermentation of glucose, β-galactosidase (4-nitrophenyl-β-D-galactopyranoside) and assimilation of glucose, arabinose, mannitol, N-acetylglucosamine, maltose (except strains YPPS 950 and 954), potassium gluconate, and malic acid, but negative results for nitrate reduction, indole production, arginine dihydrolase, urease, β-glucosidase, gelatin hydrolysis and assimilation of mannose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Predominant fatty acids are C₁₆ : ₁v<sub>7</sub>c and iso-C<sub>₁₅ : ₀</sub>2-OH (summed feature 3); C<sub>₁₆ : ₀</sub>; C₁₈ : ₁v<sub>7</sub>c; and iso-C₁₆ : ₁ and C₁₄ : ₀ 3-OH (summed feature 2).

The type strain is YPPS 951<sup>T</sup> (=LMG 25843<sup>T</sup> =NCPPB 4475<sup>T</sup>). The pathogen was isolated from black lesions of young shoots of Pyrus communis cv. La France in an orchard in Japan. The DNA G+C content of the type strain is 53.4 mol%.

References


