Erwinia iniecta sp. nov., isolated from Russian wheat aphid (Diuraphis noxia)

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Short, Gram-negative-staining, rod-shaped bacteria were isolated from crushed bodies of Russian wheat aphid [Diuraphis noxia (Kurdjumov)] and artificial diets after Russian wheat aphid feeding. Based on multilocus sequence analysis involving the 16S rRNA, atpD, infB, gyrB and rpoB genes, these bacterial isolates constitute a novel clade in the genus Erwinia, and were most closely related to Erwinia toletana. Representative distinct strains within this clade were used for comparisons with related species of Erwinia. Phenotypic comparisons using four distinct strains and average nucleotide identity (ANI) measurements using two distinct draft genomes revealed that these strains form a novel species within the genus Erwinia. The name Erwinia iniecta sp. nov. is proposed, and strain B120T (=CFBP 8182T=NCCB 100485T) was designated the type strain. Erwinia iniecta sp. nov. was not pathogenic to plants. However, virulence to the Russian wheat aphid was observed.

Abbreviations: ANI, average nucleotide identity; MLSA, multilocus sequence analysis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains B120T, B137, B149 and B150 are KM870781–KM870784, respectively. The accession numbers for the draft genome sequences of B120T and B149 are JRXF00000000 and JRXE00000000, respectively.

Four supplementary tables and a supplementary figure are available with the online Supplementary Material.

Aphids are sap-sucking insects. They penetrate plant tissues using a special mouthpart – the stylet – and they probe between the plant cell layers to find sieve elements. Once stylets penetrate the sieve elements, they alternate between saliva and plant sap (for a review, see Miles, 1999). This process for obtaining nutrients establishes an intimate and long-term interaction between the aphids and the host plant. In a few cases, aphids are proposed to vector or deliver plant-pathogenic bacteria into the plants via their stylets (Plurad et al., 1965; Watanabe et al., 1996; Stavrinides et al., 2009).

In a previous study, we repeatedly and consistently isolated members of the Enterobacteriaceae from sterile artificial diets (15% aqueous sucrose, pH 7.2) fed on by Russian wheat aphids [Diuraphis noxia (Kurdjumov)] (T. Campillo, L. van Eck, E. Luna, N. Lapitan, N. Tisserat and J. E. Leach, unpublished results). The bacteria were isolated only from aphids that had not been exposed to aphids. In addition, bacteria were isolated from crushed aphid bodies and from wheat exposed to D. noxia feeding. Based on 16S rRNA gene sequences of 30 diverse isolates from these various origins, most of the bacteria grouped within the Enterobacteriaceae.

Of the bacteria isolated from aphid bodies or artificial diets or wheat after feeding by aphids, we found a subset of isolates that belong to the Erwinia–Pantoea clade. While members of the genera Erwinia and Pantoea are most commonly
epiphytic or plant-pathogenic bacteria (Martinec & Kocur, 1964; Brady et al., 2010b), various strains of these genera are also known to associate with insects (García-Salazar et al., 2000; Hildebrand et al., 2000; Wells et al., 2002; Gitaïtis et al., 2003; Ellers-Kirk & Fleischer, 2006; Estes et al., 2009; Stavriniðes et al., 2010; Skrodenyte-Arbaicaskienè et al., 2012), and especially aphids (Hemiptera: Aphididae) (Plurad et al., 1965; Harada et al., 1997; Capuzzo et al., 2005; Clark et al., 2012; Bansal et al., 2014). The aim of the present work was to characterize the Erwinia–Pantoea isolates associated with D. noxia and released during feeding.

The D. noxia–associated bacteria characterized in this study were isolated by inoculation on eosin methylene blue agar (EMBa) medium (Holt-Harris & Teague, 1916) and incubation for 48 h at 28 °C. One strain (B137) was isolated from whole bodies of D. noxia (biotype 1), previously decontaminated for 5 min in 70 % ethanol, and crushed into 100 µl Carlson’s solution (Harada et al., 1996). The solution was used to inoculate EMBa plates. Three additional strains (B120T, B149 and B150) were obtained from sterile artificial diets (15 % sucrose aqueous solution, pH 7.2) presented to approximately 500 adult aphids under a Parafilm layer as described previously (Cooper et al., 2010). After 48 h of feeding, the diet solution was centrifuged and the resuspended pellet was used to inoculate EMBa. No bacteria were isolated from artificial diets that were not exposed to aphid probing, showing that the bacteria were released into the diets by aphid feeding. All bacterial strains were purified three times on EMBa medium to obtain pure cultures. The origin of each strain is detailed in Table 1.

Whole-genome sequencing was performed on DNA from two selected isolates (B120T and B149). Genomic DNA was extracted using an Easy DNA kit (Invitrogen) according to the manufacturer’s instructions, and DNA sequencing was performed on an Illumina MiSeq (2 × 250 bp run format on a full MiSeq v2 flowcell) by the Research Technology Support Facility of Michigan State University (East Lansing, MI, USA). After trimming and high-quality sequence-read selection using Trimmomatic (version 0.27), a total of 1 697 366 reads with a mean read length of 214 bp were obtained for B120T, and 2 721 395 reads with a mean read length of 218 bp were obtained for B149. The mean coverage for B120T and B149 was equivalent to 75.9 × and 124.4 ×, respectively. De novo assembly was performed with Velvet (version 1.2.08). Manually optimized parameters allowed us to obtain 101 contigs (maximum length 259 650 bp; N50 139 128 bp) totalling 4 790 664 bp for B120T and 142 contigs (maximum length 292 162 bp; N50 123 532 bp) totalling 4 781 836 bp for B149.

Sequences of the housekeeping genes atpD, gyrB, infB and rpoB and the 16S rRNA gene of strains B137 and B150 were obtained from PCR fragments. PCR was performed using 5 × PCR Phusion HF buffer (Finnzymes), 0.2 mM dNTP, 0.5 mM forward and reverse primers (listed in Table S1, available in the online Supplementary Material), 25 ng extracted genomic DNA, 3 % DMSO and 0.5 U Phusion Taq polymerase (Finnzymes) in 20 µl aqueous

### Table 1. Strains used in the present study and their origins

<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolation</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td><strong>Erwinia inicta</strong> sp. nov.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B112</td>
<td>Surface-decontaminated wheat leaf fed by <em>D. noxia</em> biotype 2</td>
<td>This study</td>
</tr>
<tr>
<td>B115</td>
<td>Surface-decontaminated wheat leaf fed by <em>D. noxia</em> biotype 1</td>
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<tr>
<td>B118</td>
<td>Surface-decontaminated crushed <em>D. noxia</em> biotype 2</td>
<td>This study</td>
</tr>
<tr>
<td>B120T (=CFBP 8182T=NCCB 100485T)</td>
<td>Artificial diet fed by <em>D. noxia</em> biotype 2</td>
<td>This study</td>
</tr>
<tr>
<td>B137 (=CFBP 8183=NCCB 100486)</td>
<td>Surface-decontaminated crushed <em>D. noxia</em> biotype 1</td>
<td>This study</td>
</tr>
<tr>
<td>B138</td>
<td>Surface-decontaminated crushed <em>D. noxia</em> biotype 1</td>
<td>This study</td>
</tr>
<tr>
<td>B139</td>
<td>Surface-decontaminated crushed <em>D. noxia</em> biotype 1</td>
<td>This study</td>
</tr>
<tr>
<td>B140</td>
<td>Surface-decontaminated crushed <em>D. noxia</em> biotype 1</td>
<td>This study</td>
</tr>
<tr>
<td>B144</td>
<td>Artificial diet fed by <em>D. noxia</em> biotype 2</td>
<td>This study</td>
</tr>
<tr>
<td>B149 (=CFBP 8184=NCCB 100487)</td>
<td>Artificial diet fed by <em>D. noxia</em> biotype 2</td>
<td>This study</td>
</tr>
<tr>
<td>B150 (=CFBP 8185=NCCB 100488)</td>
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<td>This study</td>
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<td>Wilt disease on Rosaceae</td>
<td>Hauben et al. (1998)</td>
</tr>
<tr>
<td><strong>Erwinia aphidicola</strong> CFBP 6829T</td>
<td>Pea aphid (<em>Acrithosiphon pisum</em>) gut</td>
<td>Harada et al. (1997)</td>
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<tr>
<td><strong>Erwinia iberica</strong> CFBP 8201T</td>
<td>Olive knots</td>
<td>Moretti et al. (2011)</td>
</tr>
<tr>
<td><strong>Erwinia iberica</strong> CFBP 8201T</td>
<td>Olive knots</td>
<td>Rojas et al. (2004)</td>
</tr>
<tr>
<td><strong>Erwinia iberica</strong> CFBP 6631T</td>
<td>Bark beetle (<em>Ips typographus</em>) gut</td>
<td>Skrodenyte-Arbaicaskienè et al. (2012)</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong> K-12 MG1655</td>
<td>Human faeces</td>
<td>Demerec et al. (1945)</td>
</tr>
<tr>
<td><strong>Pseudomonas syringae</strong> pv. <em>syringae</em> B106</td>
<td>Turkish filbert</td>
<td>Ibarra et al. (2012)</td>
</tr>
</tbody>
</table>
solution. An initial denaturation step was performed at 98 °C for 1 min followed by 35 cycles of 10 s at 98 °C, 30 s at the primer annealing temperature and 1 min at 72 °C. The reaction ended with a final extension step of 4 min at 72 °C. Amplified genes were sequenced in both directions by the Colorado State University Proteomics and Metabolomics Facility (Fort Collins, CO, USA) after a purification step using the Wizard SV Gel and PCR Clean-Up System (Promega).

The 16S rRNA gene sequences of strains B120T and B149 were obtained from the genome sequence (by BLAST to the 16S rRNA gene sequence of Erwinia amylovora CFBP 1232T), and were each 1497 nt. Sequences of overlapping regions obtained from B120T, B149, B137 (1360 nt) and B150 (1382 nt) were identical. Comparison of these sequences to the GenBank/EMBL/DDJB database using BLAST searches showed that they shared the highest percentage identity to 16S rRNA gene sequences from the type strains Erwinia toletana LMG 24162T (99 %), E. persicina LMG 11254T (98 %), E. billingiae LMG 2613T (98 %), E. aphidicola LMG 24877T (98 %) and Pantoea calida 1400/07T (98 %).

Using CLUSTAL W and the software MEGA version 5 (Thompson et al., 1994; Tamura et al., 2011), the 16S rRNA gene sequences of isolates B120T, B137, B149 and B150 were aligned with the available sequences of the type strains of 16 species of Erwinia, five species of Pantoea, three species of Tatunella and Escherichia coli (strains and accession numbers are detailed in Table S2).

This alignment allowed the reconstruction of two phylogenetic trees based on the maximum-likelihood (Fig. 1) and neighbour-joining (data not shown) methods (Saitou & Nei, 1987; Tamura & Nei, 1993). In both trees, the four new isolates clustered together into the clade Erwinia–Pantoea. This affiliation was confirmed by the 15 Erwinia-specific nucleotide positions in the 16S rRNA gene sequences, as described by Hauben et al. (1998). Positions of these nucleotide signatures are indicated in the species description.

To delimit the clade constituted by isolates B120T, B137, B149 and B150 clearly, a multilocus sequence analysis (MLSA) was performed with a concatenate of the 16S rRNA gene plus four housekeeping genes (atpD, gyrB, infB and rpoB) that were used previously to derive the taxonomy of Erwinia (Brady et al., 2010a, b, 2012; Popp et al., 2010). Using either the maximum-likelihood method (Fig. 2) or the neighbour-joining method (data not shown), the MLSA clearly separated the three genera Erwinia, Pantoea and Tatunella, as shown previously (Brady et al., 2010a, b; Popp et al., 2010). The novel monophyletic group composed of B120T, B137, B149 and B150 was supported by high bootstrap values, and it belongs unambiguously to the genus Erwinia. The cluster was clearly distinct from its closest relative, E. toletana, suggesting that it represents a novel species. The MLSA also showed diversity among the new isolates in the analysed housekeeping genes. B120T and B149 were distinct from B137 and B150 in at least five polymorphic sites and B137 was distinct from B150 in one polymorphic site. This confirms that our analysis was based on at least three distinct strains.

The DNA G+C contents of strains B120T and B149 were 52.21 and 51.08 %, respectively (from whole-genome sequences). Genome comparison with related strains allowed measurement of the average nucleotide identity (ANI), a tool for bacterial species delineation that can replace DNA–DNA hybridization (Goris et al., 2007; Richter & Rosselló-Móra, 2009). The genome sequence of B120T (used as query) was compared with nine genome sequences of members of the genus Erwinia, including members of seven different known species, using the software Ispecies set on default parameters for BLAST-based analysis (ANiB). Our previous MLSA study showed that E. amylovora ATCC 49946, E. amylovora CFBP 1430, E. toletana DAPP-PG 735 and E. tracheiphila PSU 1 were close to the type strains of their species (less than 0.002 base substitutions per site; Fig. 2). Therefore, these strains were used for the ANI evaluation, even though they were not type strains (sequences of the type strains of E. toletana and E. tracheiphila or other species of the genus Erwinia were not available). The ANI between B120T and the reference strains was between 76.16 and 77.38 % (Table 2). The accepted ANI cut-off that distinguishes two bacterial species (corresponding to the threshold of 70 % DNA–DNA hybridization) is 95 % (Richter & Rosselló-Móra, 2009). To explore this cut-off within the genus Erwinia, ANI values were calculated among E. amylovora CFBP 1232T, E. billingiae CFBP 6830T, E. piriflorinigrans CFBP 5888T, E. pyrifoliae CFBP 4172T and E. tasmaniensis Et1/99T (Table S3). The ANI values calculated among these type strains ranged between 75.59 and 90.20 %, and at least six pairs of type strains had ANI values higher than the value obtained between B120T and its closest neighbour E. toletana DAPP-PG 735 (77.38 %). Taken together, these results suggested that B120T does not belong to the species E. toletana or to any other tested species.

The draft genomes of B120T and B149 were uploaded on the SEED server and genes were delimited and annotated using the Rapid Annotation using Subsystem Technology (RAST) service (Aziz et al., 2008). About 4324 and 4329 coding sequences were predicted for B120T and B149, respectively, allowing prediction of functions commonly found in genomes of members of the genus Erwinia. Both strains harboured three different type-III secretion systems that were similar to the well-described Erwinia type-III secretion systems encoded by the pathogenicity islands PAI-1 (hrp genes), PAI-2 and PAI-3 (inv/spa genes) (Smits et al., 2010). Genes encoding type-III secretion systems clustered with known chaperone and translocator protein-encoding genes like dspEF, suggesting that these secretion systems are involved in pathogenicity (Bogdanove et al., 1998). Automatic annotation suggested the presence of genes necessary for flagellar synthesis
(flgABCDEFGHIJKLMN, fliACDEFGHIJKLMNOPQRSTZ and flhCD), antibiotic resistance (genes bcr, uppP, pmrHFIJKLM and sanA conferring, respectively, resistance to bicyclomycin, bacitracin, polymixin and vancomycin), arabitol and acetoin/butandiol degradation (budRABC) and quorum sensing (sdiA, phzI and luxS).

Pathogenicity of B120T, B137, B149 and B150 to wheat TAM 107 (Triticum aestivum L.) and tobacco (Nicotiana benthamiana) was tested using three different inoculation methods (leaf infiltration, leaf clipping and needle injection), and was assayed in three independent experiments. After 4 h of incubation in Luria–Bertani (LB) broth at 28°C (exponential growth phase), strains were suspended in 0.8% NaCl at an OD600 of 0.1 (5 × 10⁷ c.f.u. ml⁻¹). Bacterial suspensions were infiltrated directly into 7-day-old wheat leaves or 30-day-old tobacco leaves using a 1 ml needleless syringe. Inoculations by leaf clipping with scissors dipped in bacterial suspensions and by injection of the bacterial suspension into the first node using a syringe with a needle were also performed on wheat. After inoculation, plants were incubated at 24°C with a 16 : 8 h day/night photoperiod and 85% humidity for 21 days. No aberrant phenotypes suggestive of disease were caused by any of the four isolates in inoculated 7-day-old wheat leaves after 15 days of incubation (data not shown). In addition, 30-day-old tobacco leaves inoculated by infiltration did not show any symptoms.
Erwinia tracheiphila
LMG 5020

Erwinia tracheiphila
PSU 1

Erwinia psidii
LMG 7035

Erwinia psidii
LMG 7039T

Erwinia mallotivora
LMG 1270

Erwinia mallotivora
LMG 2708T

Erwinia papayae
CFBP 8300

Erwinia papayae
CFBP 11606T

Erwinia bilineae
LMG 2619

Erwinia bilineae
LMG 2613T

Erwinia typographi
DSM 24223

Erwinia typographi
DSM 22678T

Erwinia oleae
LMG 25321

Erwinia oleae
DAPP-PG 531T

Erwinia persicina
ICMP 15602

Erwinia persicina
LMG 11254T

Erwinia rhapontici
LMG 2648

Erwinia rhapontici
LMG 2888T

Erwinia aphidicola
LMG 26028

Erwinia aphidicola
LMG 24877T

Erwinia piriflorinigrans
CFBP 5888T

Erwinia piriflorinigrans
CFBP 5885

Erwinia tasmaniensis
NCPPB 4358

Erwinia tasmaniensis
ET1/99T

Erwinia pyrifoliae
ICMP 13250

Erwinia pyrifoliae
DSM 12163T

Erwinia amylovora
LMG 2085

Erwinia amylovora
CFBP 1232T

Erwinia amylovora
CFBP 1430

Erwinia amylovora
ATCC 49946

Erwinia iniecta
B120T

Erwinia iniecta
B149

Erwinia iniecta
B137

Erwinia iniecta
B150

Erwinia toletana
CFBP 6630

Erwinia toletana
CFBP 6640

Erwinia toletana
CFBP 6644

Erwinia toletana
LMG 24162T

Erwinia toletana
DAPP-PG 735

Tatumella punctata
LMG 22050T

Tatumella morbirosei
LMG 23360T

Tatumella terrea
LMG 22051T

Pantoea calida
1400/07T

Pantoea gaviniae
LMG 25382T

Pantoea cypripedii
LMG 2657T

Pantoea eucrina
LMG 2781T

Pantoea dispersa
LMG 2603T

Escherichia coli
K-12 MG1655

Erwinia iniecta sp. nov., isolated from D. noxia
hypsersensitive response, as induced by *Pseudomonas syringae*) after 21 days. These data suggest that strains of the novel species of the genus *Erwinia* are not pathogenic to plants.

Strains B120<sup>T</sup> and B137 were tested for virulence to *D. noxia*. Viability of *D. noxia* feeding on artificial diets supplemented with *Escherichia coli* K-12, B120<sup>T</sup> or B137 individually at a final concentration of 1 × 10<sup>8</sup> c.f.u. ml<sup>−1</sup>, or no bacteria, was monitored daily by counting living and dead aphids over 11 days. Artificial diets with aphids feeding on them were incubated at room temperature under fluorescent light for 12 h per day. Three independent replications (each containing three technical repetitions) were performed. *D. noxia* populations that were fed on *Escherichia coli* K-12, B120<sup>T</sup> or B137 decreased from 50 to five aphids in 6 days or less (Fig. S1). In contrast, all of 50 *D. noxia* fed on a diet without any bacteria remained alive over the 11 days. During the first 2 days, 90, 50 and 30 % of the *D. noxia* populations died after feeding B137, B120<sup>T</sup> and *Escherichia coli* K-12, respectively. These experiments confirm that *Escherichia coli* K-12 is an aphid pathogen when ingested (Altincicek *et al.*, 2011) and show that B137 and B120<sup>T</sup> are more virulent than *Escherichia coli* K-12.

Using API strips 20E, 20NE and 50CH (bioMérieux), 64 different phenotypic traits of the novel strains B120<sup>T</sup>, B137, B149 and B150 and the strains *E. toletana* CFBP 6631<sup>T</sup>, *E. oleae* CFBP 8201<sup>T</sup>, *E. amylovora* CFBP 1232<sup>T</sup>, *E. aphidicola* LMG 24877<sup>T</sup> and *E. typographi* CFBP 8202<sup>T</sup> were analysed. Tests were read after 48 and 72 h of incubation at 25 °C. Complementary tests were performed using the Biolog GN microplate system (GEN III plate) at the Colorado State University Veterinary Diagnostic Laboratories (http://csu-cvmbs.colostate.edu/vdl/). Carbon-source utilization was observed in each well by five OD<sub>590</sub> measurements taken between 15 and 48 h after bacterial strains had been incubated in the plate at 28 °C (Table S4). The data we obtained for the type strains of *E. toletana*, *E. oleae*, *E. amylovora*, *E. aphidicola* and *E. typographi* (Table 3) matched their original descriptions (Harada *et al.*, 1997; Hauben *et al.*, 1998; Rojas *et al.*, 2004; Moretti *et al.*, 2011; Skrodenyte-Arbaciauskiene *et al.*, 2012). Three of the four novel strains (including the type strain B120<sup>T</sup>) exhibited the same profile for all tests. One strain, B149, differed for only two tests; this strain was the only strain to degrade potassium gluconate and potassium 5-ketogluconate. These novel strains were differentiated from their closest relative *E. toletana* by their ability to degrade D-arabitol, cellobiose, raffinose and gentiobiose, their lack of ability to degrade citrate, their ability to produce acetoin and their ability to reduce nitrate (Table 3). Phenotypic characteristics of the novel species of the genus *Erwinia* are provided in the species description.

Fatty acid methyl ester profile was performed by Microbial ID (Newark, DE, USA). Strain B120<sup>T</sup> was grown on LB agar at 28 °C for 48 h, and its fatty acid profile was determined using the MIDI Sherlock Microbial Identification System (http://www.microbialid.com/). The four most abundant fatty acids were C<sub>16</sub>:<sub>0</sub> (32.0 %), C<sub>18</sub>:<sub>1</sub>ω<sub>7</sub>c (18.8 %), C<sub>16</sub>:<sub>1</sub>ω<sub>6</sub>c (17.5 %) and C<sub>17</sub>:<sub>0</sub> cyclo (10.3 %), accounting for around 78 % of the total fatty acids.

In conclusion, based on 16S rRNA gene sequence comparison, MLSA and phenotypic tests, the four isolates B120<sup>T</sup>, B137, B149 and B150 are distinct from each other, but they belong to the genus *Erwinia*, in the family *Enterobacteriaceae*. Combined with the ANI analysis, we propose that

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**Table 2.** ANI between strain B120<sup>T</sup> and available genome sequences of other members of the genus *Erwinia*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Accession number</th>
<th>ANI with B120&lt;sup&gt;T&lt;/sup&gt; (%)</th>
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<tr>
<td><em>Erwinia amylovora</em></td>
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<tr>
<td>CFBP 1232&lt;sup&gt;T&lt;/sup&gt;</td>
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<tr>
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<td><em>Erwinia inicta</em> sp. nov.</td>
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<td>99.8</td>
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Table 3. Selected phenotypic characteristics that differentiate E. inicta sp. nov. from type strains of other species of the genus Erwinia

<table>
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<td>Amygdalin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Arbutin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>d-Adonitol</td>
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<td>-</td>
<td>-</td>
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<td>d-Arabinose</td>
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<td>D-Fucose</td>
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<tr>
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*aData taken from: a, Moretti et al. (2011); b, Rojas et al. (2004); c, Harada et al. (1997).

these strains represent a novel species of the genus Erwinia, for which we propose the name Erwinia inicta sp. nov.

Description of Erwinia inicta sp. nov.

Erwinia inicta (in.iecta. L. fem. part. adj. inicta thrust in, injected, referring to the fact that the first strains isolated from artificial media were introduced or ‘thrust in’ via the aphid stylet).

Cells are rod-shaped (0.5–0.7 × 1.5–2 μm), Gram-negative, aerobic, motile and non-spore-forming. After 24 h at 28 °C on nutrient agar, cells form light-beige, circular, small (1–2 mm diameter), translucent colonies with entire margins. No specific pigment (diffusible or fluorescent) is observed. The optimal growth temperature is 28 °C. Growth occurs at 37 °C but not at 42 °C. Strains grow in nutrient broth in the presence of 1 % NaCl, but not 4 %. They are negative for gelatin liquefaction and hydrogen sulfide production but positive for acetoin production. They exhibit catalase, tryptophan deaminase and nitrate reductase but do not exhibit β-galactosidase, oxidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, nitrite reductase or tryptophanase.

Strains degrade the following carbon sources: sucrose, melibiose, amygdalin, d-xylose, d-mannose, arbutin, aesculin, salicin, cellobiose, malto7e, gentibo7e, raffino7e, lactose, d-arabitol, glycerol and d-fucuco. However, up to 48 h they are unable to degrade citrate, d-aden07e, d-arabin07e, lactose, erythrito7e and xylito7e. Strains B120, B137, B149 and B150 exhibit the same profile for all tests except for degradation of potassium gluconate and potassium 5-ketoglucono7e; only strain B149 is able to degrade these two compounds. Strains are not pathogenic on wheat or tobacco. Strains are pathogenic to Russian wheat aphid (D. noxia) when ingested.

Harbours the following Erwinia-specific signature nucleotides in the 16S rRNA gene sequence (Hauben et al., 1998): A408, A594, C598, G639, G646, C839, G847, G987, G988, C989, G1216, C1217, C1218, C1308 and G1329, according to the Escherichia coli 16S rRNA gene sequence numbering (Brosius et al., 1981).

The DNA G+C content is 52.21 and 51.08 % for B120 and B149, respectively (determined from the whole-genome sequence). The predominant fatty acids are C16:0, C18:1ω7c, C16:1ω6c and/or C16:1ω7c, C17:0 cyclo, C14:0 3-OH and/or C16:1ω7c iso I, C14:0 3:OH and C12:0-0.

The type strain, B120 (=CFBP 8182T=NCCB 100485T), and strains B137 (=CFBP 8183=NCCB 100486), B149 (=CFBP 8184=NCCB 100487) and B150 (=CFBP 8185=NCCB 100488) were isolated in Colorado, USA, from surface-decontaminated bodies of D. noxia and artificial diets fed on by D. noxia, biotype 1 or 2. Additional strains of the species were also isolated from ground, surface-decontaminated wheat leaves.

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fire blight pathogen *Erwinia amylovora* CFBP 1430 and comparison to other *Erwinia* spp. *Mol Plant Microbe Interact* 23, 384–393.


