Dickeya aquatica sp. nov., isolated from waterways

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Pectinolytic Gram-negative bacteria were isolated from different waterways in the UK and Finland. Three strains (174/2$^T$, 181/2 and Dw054) had the same 16S rRNA gene sequences which shared 99% sequence similarity to species of the genus Dickeya, and a phylogeny of related genera confirmed attribution to this genus. Fatty acid profile analysis of all three strains found a high proportion of C$_{16.1}$t/7c/C$_{16.1}$t/7c and C$_{16.0}$ fatty acids, and library profile searches found closest matches to Dickeya chrysanthemi. Production of a concatenated phylogeny using six loci, recA, gapA, atpD, gyrB, infB and rpoB, provided a high-resolution phylogeny which placed strains 174/2$^T$ and 181/2 as a distinct clade, separated from the other species of the genus Dickeya by a relatively long branch-length. DNA–DNA hybridization analysis with a limited number of reference species also supported the distinctiveness of strains 174/2$^T$ and 181/2 within the genus Dickeya. All three strains could be phenotypically distinguished from other species of the genus by fermentation of melibiose and raffinose but not α-arabinose or mannitol. The name Dickeya aquatica sp. nov. is proposed for the new taxon; the type strain is 174/2$^T$ (=NCPPB 4580$^T$=LMG 27354$^T$).

The genus Dickeya was first described by Samson et al. (2005), and six species were discriminated as D. dadantii, D. dieffenbachiae, D. chrysanthemi, D. paradiisica, D. zeae and D. dianthicola. Recently, D. dieffenbachiae has been reported to belong to the same species as D. dadantii and has been reclassified as D. dadantii subsp. dieffenbachiae (Brady et al., 2012). Furthermore, some isolates from European potatoes belonging to the genus Dickeya (Slawiak et al., 2009) have recently been classified as representing a novel species, Dickeya solani, with IPO 2222$^T$ as the type strain (van der Wolf et al., 2014).

As part of a survey for members of the genus Dickeya, river water was plated onto Crystal Violet Pectate agar (Janse & Ruissen, 1988) and pectolytic colonies were isolated and purified. Dickeya strains were tentatively identified using a real-time PCR test (Nassar et al., 1996). Alignments and production of the neighbour-joining phylogenies used in the study were done using programs within the MEGA version 4 software package (Tamura et al., 2007). Two English strains (174/2$^T$, 181/2) and a third (Dw054) which was isolated from a Finnish river (Laurila et al., 2008), shared identical recA sequences and were placed as a distinct new clade (identified as SLC2) within this phylogeny. To confirm that the SLC2 isolates belonged to the genus Dickeya, 16S rRNA gene sequences were determined using the protocol described by Nhung et al. (2007) and a phylogeny was produced which included all related genera within the family Enterobacteriaceae (Fig. S1, available in the online Supplementary Material). This phylogeny found the three SLC2 strains shared identical sequences and were placed as a distinct clade relative to all the other related taxa. To further confirm the distinctiveness of SLC2 strains within the genus Dickeya, sequence analysis on strains 174/2$^T$ and 181/2, and all species of the genus Dickeya with validly published names was done using six loci: recA, (Parkinson et al., 2009) gapA (Brown et al., 2000), atpD, gyrB, infB and rpoB (Brady et al., 2012). A concatenated phylogeny (Fig. S2) was produced and found to be similar to a previously reported phylogeny for species of the genus Dickeya (Brady et al., 2012). Both SLC2 strains (174/2$^T$ and 181/2) had identical sequences. This high-resolution phylogeny placed the two SLC2 sequences as a distinct clade separated by a long branch-length from all the other species.

Abbreviation: NCPPB, National Collection of Plant Pathogenic Bacteria.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene, dnaJ, recA, gapA, atpD, gyrB, infB and rpoB sequences of Dickeya aquatica sp. nov. 174/2$^T$ are JX273704, JX273684, JX273695, KC238447, KC238451, KC238455 and KC238456, respectively; those for the gyrB, infB and rpoB sequences of Dickeya aquatica sp. nov. 181/2 are KC238452, KC238456 and KC238460, respectively. The GenBank/EMBL/DDBJ accession numbers for the dnaJ and gapA sequences of strains of other species of the genus Dickeya determined in this study are listed in Table S1, available in the online Supplementary Material.

Two supplementary tables and two supplementary figures are available with the online version of this paper.
of the genus *Dickeya*, which provides further evidence for the genetic distinctiveness of SLC2.

DNA–DNA hybridization analysis was used to compare strains 174/2T and 181/2 to *D. zeae* LMG 2505T, *D. chrysanthemi* LMG 2804T and *D. solani* IPO 2222T. Hybridization experiments were performed using the method of Ezaki *et al.* (1989) including the adaptation given by Willems *et al.* (2001). The hybridization temperature was 46 °C in the presence of 50% formamide. Reciprocal reactions (e.g. A × B and B × A) were performed for every DNA pair and the variation was within the limits of this method (Goris *et al.*, 1998). All data (Table S2) are mean values of four measurements. The DNA–DNA hybridization data confirms high similarity (approx. 90%) between SLC2 strains 174/2T and 181/2. Hybridization values of SLC2 to the other *Dickeya* taxa was 32% or less. The DNA G+C contents were measured by HPLC as described by Mesbah *et al.* (1989). The value of 53.65 mol% determined for the SLC2 strains is lower than the reported range of 56.4–59.5 mol% for members of the genus *Dickeya* (Samson *et al.*, 2005). However, reference to the genome sequence of *Dickeya* strain Ech586 (GenBank accession no.CP001836) provides a direct sequence-based G+C value of 53.64 mol%, which is very similar to our estimate for the SLC2 strains.

Biochemical activities were determined to indicate the phenotypic distinctiveness of SLC2 strains within the genus *Dickeya* using the GN2 MicroPlate (Biolog), which provides 94 test substrates with metabolism to reduced products indicated by a blue to yellow redox dye colour change. Plates were inoculated according to the manufacturer’s recommendations and incubated at 28 °C, development of a yellow colour was recorded. Metabolism of the following reagents (using strains174/2T, 181 and Dw054) was observed: Tween 40, N-acetyl-D-glucosamine, l-fucose, D-psicose, L-rhamnose, trehalose, citric acid, D-galactonic acid lactone, D-glucosaminic acid, z-ketobutyric acid, z-ketovaleric acid, propionic acid, glucuronamide, D-alanine, L-α-aminobutyric acid, l-asparagine, l-leucine, L-phenylalanine, l-2-pyrrol glutamic acid and phenylethylamine. Further tests were completed using bromo-cresol purple pH indicator in 96-well microtitre plates according to the method described by Slawiak *et al.* (2009). The biochemical tests (Table 1) have previously been selected for differential identification of species of the genus *Dickeya* (Samson *et al.*, 2005; Slawiak *et al.*, 2009; van der Wolf *et al.*, 2014). SLC2 biochemical reactions were determined from all three strains. Casein solubilization was determined by streak inoculation onto nutrient agar containing 10% skimmed milk powder. Clear zones were recorded after incubation for 48 h at 28 °C. Additional tests used API 20NE strips (bioMérieux) according to the manufacturer’s instructions. The results of the biochemical analyses indicated that the SLC2 strains were distinct from all species of the genus *Dickeya* with validly published names in their ability to metabolize melibiose and raffinose but not D-arabinose or mannitol. Fatty acid profile analysis (Stead *et al.*, 1992) was done on all three SLC2 strains following incubation for 48 h, using a Hewlett Packard chromatography machine (HP6890), as part of the Sherlock Microbial Identification System (MIDI). Protocols were defined by the supplier. All three SLC2 strains contained high proportions (approx. 63%) of C16 : 1ω7c/C16 : 1ω9c and C16 : 0 fatty acids. Profile library searches of plant pathogens produced by National Collection of Plant Pathogenic Bacteria (NCPBP), which contained representatives of the genera *Erwinia*, *Pectobacterium*, *Brenneria*, *Pantoea*, *Enterobacter* and *Serratia*, found all three SLC2 strains matched most closely to *D. chrysanthemi*, further supporting attribution of SLC2 within the genus *Dickeya* (Weller *et al.*, 2000).

Previous recA analysis of 188 *Dickeya* strains maintained by the NCPBP (Parkinson *et al.*, 2009), did not find any strains similar to SLC2. Additionally, a Dutch survey of 41

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**Table 1.** Phenotypic differentiation of species of the genus *Dickeya* based on data from this study (TS) and previous studies (PS)

Species (strain numbers for this study and references to previous studies are indicated where applicable): 1, *D. aquatica* sp. nov. SLC2 (174/2T, 181/2 and DwW054); 2, *D. dadantii* subsp. *dadantii* (TS, NCPPB 898T; PS, Samson *et al.* 2005); 3, *D. zeae* (TS, NCPPB 2538T; PS, Samson *et al.* 2005); 4, *D. chrysanthemi* (TS, NCPPB 402T; PS, Samson *et al.* 2005); 5, *D. dadantii* subsp. *dieffenbachiae* (TS, NCPPB 2976T; PS, Samson *et al.* 2005); 6, *D. diastatica* (TS, NCPPB 453T; PS, Samson *et al.* 2005); 7, *D. paradisinca* (TS, NCPPB 2511T; PS, Samson *et al.* 2005); 8, *D. solani* IPO 2222T (van der Wolf *et al.*, 2014; Slawiak *et al.* 2009 W=weak reaction, NT=not tested). +, Positive; −, negative; V, variable; d, different (percentage of positive strains).

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tr>
<td>Growth at 39 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>w+</td>
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<tr>
<td>(−)-D-Arabinose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>(+)-Melibiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>d(44)</td>
<td>+</td>
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<tr>
<td>(+)-Raffinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>d(44)</td>
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<td>Mannitol</td>
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<td>+</td>
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<tr>
<td>β-Gentiobiose</td>
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<td>V</td>
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<td>+</td>
<td>d(80)</td>
<td>d(75)</td>
<td>−</td>
<td>NT</td>
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strains of Erwinia chrysanthemi (Janse & Ruissen, 1988) from potato and ornamental hosts found none with the biochemical profiles matching SLC2. Therefore, waterways are the only known source of SLC2 strains.

On the basis of phylogenetic, phenotypic and chemotaxonomic analyses and DNA–DNA hybridization data, the SLC2 strains represent a novel species of the genus Dickeya for which the name Dickeya aquatica sp. nov. is proposed.

**Description of Dickeya aquatica** sp. nov.

Dickeya aquatica (a.qua’ti.ca. L. fem. adj. aquatica living, growing, or found in the water, aquatic).

Shares the following characteristics of the genus (Samson et al., 2005). Growth occurs in the presence and absence of oxygen. On nutrient agar, cells are Gram-negative rods 0.5–1.0 × 1.0–3.0 mm with rounded ends. Pectinolytic on pectate-layered media. Produces indole and grows at 36 °C. Catalyzes (+)-myo-inositol, δ-mannose, malate and saccharate, but not (+)-trehalose, (+)-δ-arabitol or sorbitol. 16S rRNA gene phylogenies group strains of D. aquatica with other species of the genus Dickeya, distinct from other genera in the family Enterobacteriaceae. Strains of D. aquatica are differentiated phenotypically from other species of the genus Dickeya by catalysis of melibiose and raffinose but not δ-arabinose or mannitol. Casein is solubilized. At 28 °C on potato dextrose agar, colonies are rounded, dry, wrinkled and cream; a diffusible bluish pigment is often present. Sequence comparison of any of the following gene loci: recA, gapA, atpD, gyrB, infB and rpoB can be used to differentiate D. aquatica from other species of the genus.

The type strain, 174/2T (=NCPPB 4580 =LMG 27354T), was isolated from river water. The DNA G+C content of the type strain is 53.65 mol%. Two additional strains of the species are 181/2 and Dw054.

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**References**


