For many years, the genus *Agrobacterium* contained species of bacteria that caused tumours or hairy roots in over 800 plant species. A few years ago, however, the genus *Agrobacterium* was merged with the genus *Rhizobium*. At the time of writing, *41 Rhizobium* species have been described (http://www.bacterio.cict.fr), including five plant-pathogenic species: *Rhizobium radiobacter, Rhizobium rhizogenes, Rhizobium rubi, Rhizobium vitis* and *Rhizobium larrymoorei* (Young et al., 2004).

In this study, a polyphasic approach was used for the taxonomic characterization of three bacterial strains: two (Ch11<sup>T</sup> and Ch12) isolated from tumours on chrysanthemum plants (*Chrysanthemum L.*) and one (AL9.3) isolated from a gall on cherry plum (*Prunus cerasifera var. divaricata*). The media used to recover strains Ch11<sup>T</sup>, Ch12 and AL9.3, with incubation at 27°C for 3 days, were Patel medium (Patel, 1926), King B medium (King et al., 1954) and tellurite-amended mannitol-glutamate medium (Mougel et al., 2001), respectively. All three strains were pathogenic and caused crown galls on various plants, including sunflower, chrysanthemum, *Cerasus* species and *Rosa* species (unpublished results).

Almost the entire 16S rRNA gene sequence of each of the three novel isolates was amplified by PCR and sequenced using primers fD1 and rP2 (Weisburg et al., 1991). The sequences were then aligned using the *CLUSTAL W* algorithm (Thompson et al., 1994), with overhangs trimmed to give a final length of 1285 bp. The genetic distances between the sequences were estimated by using the Kimura two-parameter correction method (Kimura, 1980), before neighbour-joining and maximum-parsimony trees were generated in MEGA4.0 (Tamura et al., 2007). The significance of each internal branch of the phylogenetic trees was estimated with 1000 bootstrap replicates. (Fig. 1; Supplementary Fig. S1, available in IJSEM Online, shows extended trees containing a larger number of reference sequences). In terms of their 16S rRNA gene sequences were then aligned using the CLUSTAL W algorithm (Thompson et al., 1994). The media used to recover strains Ch11<sup>T</sup>, Ch12 and AL9.3, with incubation at 27°C for 3 days, were Patel medium (Patel, 1926), King B medium (King et al., 1954) and tellurite-amended mannitol-glutamate medium (Mougel et al., 2001), respectively. All three strains were pathogenic and caused crown galls on various plants, including sunflower, chrysanthemum, *Cerasus* species and *Rosa* species (unpublished results).
sequences, strains Ch11T, Ch12 and AL9.3 were identical (100% sequence similarity) and appeared most closely related to \textit{R. rubi} LMG 156T (99.6%) and \textit{R. radiobacter} LMG 140T (98.7%).

The \textit{glnA} gene sequences of the novel isolates and some reference strains were amplified with the primers described by Martens \textit{et al.} (2007). The \textit{glnA} gene sequence of \textit{R. vitis} LMG 8750T was, however, amplified with the universal primers \textit{glnA}.vitisF (5'-GTCATGTTGACGGGYTCYTCT-3') and \textit{glnA}.vitisR (5'-TGGAKCTTGTCTTGTATGCCA-3'). The \textit{gyrB} gene sequences of strains Ch11T, Ch12 and AL9.3 were amplified by using the universal primers UP-1 and UP-2r (Yamamoto & Harayama, 1995). Since the \textit{parE} gene coding for topoisomerase IV was also probably amplified in the PCR based on these primers (Yamamoto & Harayama, 1995), the amplicons were cloned into pGEM-T vectors, to separate the \textit{parE} inserts from the \textit{gyrB} inserts, before sequencing of the \textit{gyrB} gene. The \textit{gyrB} sequences of the reference strains and the \textit{rpoB} gene sequences of the novel isolates and reference strains were determined as described previously (Martens \textit{et al.}, 2008). Phylogenetic analyses were performed on the individual gene sequences (896, 699 and 930 bp for \textit{glnA}, \textit{gyrB} and \textit{rpoB}, respectively) as well as on the concatenated dataset of 2525 bp, with neighbour-joining and maximum-parsimony trees generated as for the 16S rRNA gene sequences. The novel isolates Ch11T, Ch12 and AL9.3 possessed identical \textit{glnA}, \textit{gyrB} and \textit{rpoB} gene sequences and formed a phylogenetic lineage that was separate from established species (Fig. 2; Supplementary Figs S2, S3 and S4, available in IJSEM Online). In terms of their \textit{glnA}, \textit{gyrB} and \textit{rpoB} gene sequences, the novel isolates appeared most closely related to \textit{R. rubi}, with sequence similarities of 91.2, 91.4 and 89.0%, respectively. These values are lower than the between-strain levels of \textit{gyrB} gene sequence similarity seen in \textit{R. rubi}, which were all found to exceed 94.9% (J. Puławska & M. Kaluzna, unpublished results).

For fatty acid analysis, strains were grown for 24 h on trypticase soy agar (LMG medium 185) at 28°C. Harvesting of cells, extraction and analysis were performed by following the recommendations of the manufacturer of the MIDI identification system (Microbial Identification System) and peaks were identified using version 5.0 of the Sherlock MIDI Library. The fatty acid profiles of the novel isolates were very similar to that of \textit{R. rubi}, with \textit{C18:1\(\Delta7\)c} as predominant fatty acid; other fatty acids present in large amounts in the cells of the novel isolates and \textit{R. rubi} were \textit{C16:0}, \textit{C16:0 3-OH}, summed feature 2 (comprising \textit{C12:0} aldehyde, iso-\textit{C16:1} \(I\) and/or \textit{C14:0} 3-OH) and summed feature 3 (comprising \textit{C16:1\(\Delta7\)c} and/or iso-\textit{C15:0} 2-OH), whereas 11-methyl-\textit{C18:1\(\Delta7\)c} and 10-methyl-\textit{C19:0} are minor components (Supplementary Table S1, available in IJSEM Online). The presence of \textit{C10:0 3-OH} and 11-methyl-\textit{C18:1\(\Delta7\)c} in the novel isolates distinguishes them from their close phylogenetic relatives \textit{R. larrymoorei} and \textit{R. radiobacter}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Neighbour-joining tree based on 16S rRNA gene sequences, showing the relationships between strains Ch11\(^T\), Ch12 and AL9.3 and members of related species. Bootstrap values (expressed as percentages of 1000 replications) are given at the nodes. Bar, 0.01 substitutions per nucleotide position.}
\end{figure}
DNA–DNA hybridizations were performed at 42 °C with photobiotin-labelled probes in microplate wells, as described by Goris et al. (1998), using an HTS7000 bioassay reader (PE Applied Biosystems) for the fluorescent measurements. The level of DNA–DNA relatedness between Ch11T and *R. rubi* LMG 156T was 48 %. The genomic DNA G+C content of strain Ch11T, which was determined by HPLC as described previously (Mesbah et al., 1989), was 57.2 mol%. Physiological and biochemical tests were performed using API 50CH strips (bioMérieux) and GN2 MicroPlates (Biolog) according to the manufacturers’ instructions. Production of 3-ketolactose, citrate utilization, growth and pigmentation in ferric ammonium citrate broth, acid production from erythritol and (*+*)-D-melezitose, action on litmus milk, ability to grow in the presence of 2 % (w/v) NaCl, and oxidase reaction were also investigated (Moore et al., 2001). The GN2 plates were read 24 h after inoculation. Other tests were observed for 6 days, although most reactions occurred within 3 days. The three novel isolates could be differentiated from *R. larrymoorei* LMG 21410T, *R. vitis* LMG 8750T, *R. radiobacter* LMG 1403, *R. rhizogenes* ATCC 11325T and *R. rubi* LMG 156T by their inability to utilize L-fucose and ability to utilize β-hydroxybutyric acid. The main discriminatory phenotypic characteristics are summarized in Table 1.

Based on the phylogenetic and phenotypic evidence, strains Ch11T, Ch12 and AL9.3 belong to the same novel species of *Rhizobium*, for which the name *Rhizobium skierniewicense* sp. nov. is proposed.

**Description of Rhizobium skierniewicense**

*Rhizobium skierniewicense* (ski.er.nie.wi.cen’se. N.L. neut. adj. skierniewicense of or belonging to Skierniewice, the town in Poland where the type strain was isolated).

Cells are Gram-negative, oxidase-positive, strictly aerobic, non-spore-forming rods (0.5 × 2.5–3.0 μm). Good growth occurs on King B medium, nutrient agar and mannitol-glutamate medium with and without tellurite (at 70 mg 1−1; Mougel et al., 2001). After 48–72 h of growth at 26 °C, colonies on King B medium are small, beige and round, with a diameter of approximately 1 mm. Gives alkaline reaction, with proteolysis, in litmus milk. Does not produce 3-ketolactose from lactose or utilize citrate. Grows in the presence of 2 % NaCl and, with pigmentation, in ferric ammonium citrate broth. Able to utilize adonitol,
**Table 1. Phenotypic characters that differentiate Rhizobium skinniewicense sp. nov. from other closely related members of the genus Rhizobium**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Erythritol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Acetoin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid from (±)-D-melezitose</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Acid from (+)-D-melezitose</td>
<td>-</td>
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</table>

Poland. The DNA G+C content of the type strain is 57.2 mol%. The two reference strains, Ch12 and AL9.3, were isolated from galls on chrysanthemum and cherry plum, respectively.

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**References**


