Rhizobium giardinii is the microsymbiont of Illinois bundleflower (*Desmanthus illinoensis* (Michx.) Macmillan) in midwestern prairies

Elena Beyhaut, Becki Tlusty, Peter van Berkum, and Peter H. Graham

**Abstract:** Illinois bundleflower (*Desmanthus illinoensis* (Michx.) Macmillan) has potential as a grain and forage legume for the American Midwest. Inoculant-quality rhizobia for this legume have been identified but not previously characterized. Rhizobia trapped from 20 soils in the natural range of the Illinois bundleflower had characteristics that placed them overwhelmingly within the species *Rhizobium giardinii*, one of the few occasions this species has been recovered from legumes, raising questions on the biogeography and spread of midwestern prairie rhizobia.

**Key words:** *Rhizobium* taxonomy, biogeography, diversity, prairie legumes.

Illinois bundleflower (*Desmanthus illinoensis* (Michx.) Macmillan) is a native self-pollinated warm-season perennial legume with potential as both a grain and forage crop (Posler et al. 1993; DeHaan et al. 2003; Fischbach et al. 2005). Information on its potential for nodulation and nitrogen fixation has been limited. Byun et al. (2004) noted lower nitrogen fixation with a commercial peat-based inoculant than when indigenous rhizobia dominated nodule formation. Beyhaut et al. (2006) sought inoculant-quality rhizobia for this legume among isolates collected from the natural range of this legume in the American Midwest. When the strains selected in that study were not readily identifiable to species, we undertook additional studies to characterize the rhizobia associated with Illinois bundleflower. This note describes studies in which the predominant microsymbiont recovered from nodules on *Desmanthus illinoensis* was unexpectedly placed within the species *Rhizobium giardinii*, previously reported to occur only at low frequency in nodules of *Phaseolus vulgaris* (Amarger et al. 1997; Herrera-Cervera et al. 1999; Mhamdi et al. 2002), *Arachis hypogaea* (Taurian et al. 2002), and *Kummerowia stipulacea* and *Aeschynomene indica* (Kwon et al. 2005), and not previously recovered from legumes in the USA.

**Rhizobium strain isolation**

The rhizobia used in this study were recovered from nodules produced on *Desmanthus illinoensis* grown from surface-sterilized seed in magenta units and inoculated with soil collected from 20 sites within the natural range of this legume (Byun 2003). Methods used for strain isolation are described by Beyhaut et al. (2006). The sites from which the strains originated are shown in Table 1. A total of 231 rhizobia were collected, with single colony isolates from crushed nodules transferred into 1:1 glycerol – arabinose gluconate medium, and maintained at –70 °C (Gherna 1994; Somasegaran and Hoben 1994) until needed. Strains were routinely maintained on yeast extract – mannitol agar medium at 28 °C (Vincent 1970).

**Characterization of rhizobia, using BOXA1R PCR**

Strains of rhizobia recovered from *Desmanthus illinoensis* were subject to rep polymerase chain reaction (PCR) with
BOXA1R primer (5'CTACGGCAAGGCGACGCTGACG-3'; Versalovic et al. 1994; Schneider and De Bruijn 1996) in a PTC-200 thermocycler (MJ Research, Waltham, Mass.). Subsamples (10 μL) of PCR product were then separated on 20 cm × 25 cm 1.5% agarose gels (Sambrook et al. 1989), and subject to qualitative analysis using Bionumerics version 3.0 (Applied Biosystems, Biosystematica, Devon, UK). The methods used are detailed by Tlusty et al. (2005).

16S rRNA gene sequence analysis

Based on the results of BOXA1R PCR, 20 representative strains, including the inoculant quality strains 56.6, 35.10, 30.8, and 31.7 identified by Beyhaut et al. (2006), were chosen for 16S rRNA gene sequence analysis. Methods of 16S rRNA gene sequence analysis were as described by Tlusty et al. (2005). Following analysis, 16S rRNA partial gene sequences for the strains 3.5, 4.1, 4.11, 7.7, 7.10, 8.3, 8.6, 24.8, 24.10, 25.1, 25.13, 35.15, 41.2, 47.1, 47.4, 48.8, 50.12, and 50.15 were submitted to GenBank, where they were assigned the accession Nos. DQ499513–DQ499530.

Host range of strains 56.6 and 35.10 with 10 species of legume

Host range for two of the strains identified as being of inoculant quality (56.6 and 35.10) was evaluated using *Dalea purpurea*, *Desmanthus illinoensis*, *Desmanthus leptolobus*, *Desmanthus virginatus*, *Leucaena leucocephala*, *Macroptilium atropurpureum*, *Onobrychis vicifolia*, *Phaseolus vulgaris*, *Prosopis juliflora*, and *Psoralea esculenta* as potential hosts, according to the methods of Tlusty et al. (2004). The host species used included those reported as nodulated by *R. giardinii* in the studies by Amarger et al. (1997) plus others that occur together with *Desmanthus illinoensis* in natural prairie areas of the American Midwest.

Carbohydrate utilization patterns of strains 35.10, 30.8, and 31.7

Carbohydrate utilization patterns were determined for strains 35.10, 30.8, and 31.7 using GN2 Biolog microplates (Biolog, Hayward, Calif.). Again, this was done to compare results for representative strains from *Desmanthus illinoensis* with results for *R. giardinii* from the studies of Amarger et al. (1997). The methodology used was that recommended by the manufacturer.

The 231 strains isolated from *Desmanthus illinoensis* clustered into 11 major groups at the 70% similarity level (Fig. 1), with *R. giardinii* H152 (= UMR6917) (Amarger et al. 1997) the only reference strain to cluster with strains of *Rhzobium* from *Desmanthus illinoensis*. As with other studies reported recently (McInnes et al. 2004; Tlusty et al. 2005), strains in the same cluster tended to be from the same sites or from very few sites, with only clusters 3 and 4 including strains from a number of different sites.

The results of 16S rRNA gene sequence analysis with representative strains from the PCR analysis are shown in Fig. 2; 18 of the 20 strains tested were placed with *R. giardinii*, and two strains from site 50 were more closely similar to *R. etli, R. leguminosarum*, and *R. tropici*. The 16S rRNA gene sequence for strain 3.5 was the same as that found for strains 7.7, 7.10, 8.3, 8.6, 24.8, 24.10, 35.15, 41.2, 47.4, and 48.8, and the sequences of 25.1, 47.4, and 4.1 were the same as for 25.13, 47.1, and 4.11, respectively. It is interesting that the four inoculant-quality strains identified by Beyhaut et al. (2006) were each derived from a different collection

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Table 1. Collection site information for Illinois bundleflower rhizobia evaluated in this study.
Fig. 1. Dendrogram resulting from BOXA1R banding pattern analysis of 231 rhizobial strains recovered from *Desmanthus illinoensis* originating from 20 sites. The 70% similarity level is indicated by a dotted vertical line. Eleven major groups were identified, with site(s) of collection of the rhizobia represented shown to the right of the figure. Seven reference strains included in the analysis failed to group with any major cluster.

site but possessed a very similar 16S rRNA gene sequence. Four subgroups were evident among the strains clustering with *R. giardinii* and, as with the biovars in this species noted by Amarger et al. (1997), warrant further study.

Amarger et al. (1997) characterized *R. giardinii* bv. *giardinii* as infective on *Phaseolus vulgaris*, *Leucaena leucocephala*, and *Macroptilium atropurpureum*, noninfective on *Onobrychis viciifolia*, and ineffective in symbiosis with *Phaseolus vulgaris*. Studies undertaken with *Desmanthus virgatus* in Australia have emphasized strains of rhizobia isolated from *Leucaena leucocephala* (Date 1991; Bahnisch et al. 1998). Host range and effectiveness data for the inoculant strains 56.6 and 35.10 are, in the main, consistent with that provided by Amarger et al. (1997). Both isolates nodulated *Phaseolus vulgaris* and *Macroptilium atropurpureum* but were ineffective in symbiosis with these hosts, and both nodulated and fixed nitrogen with *Leucaena leucocephala*. We can also extend the host range for this species to include nodulation and nitrogen fixation with *Desmanthus leptolobus*, *Dalea purpurea*, *Psoralea esculenta*, and *Prosopis juliflora*, but neither strain nodulated *Desmanthus virgatus*. The ability of strains 35.10, 30.8, and 56.6 to grow on specific carbon compounds and not others was also consistent with the data provided by Amarger et al. (1997). All three strains grew on L-arabinose, D-fructose, D-galactose, D-glucose, D-glucosamine, D-glucuronate, lactose, maltose, mannitol, D-mannose, raffinose, L-rhamnose, D-sorbitol, trehalose, and glycerol as carbon sources but failed to grow on citrate or erythritol.

The placement of most representative rhizobia isolated from nodules of the prairie legume *Desmanthus illinoensis* (including four inoculant quality strains identified by Beyhaut et al. (2006)) with *R. giardinii* is noteworthy. Prior to this study, only 34 of the many different strains of *Rhizobium* studied had been identified as belonging to this species (Amarger et al. 1997; Herrera-Cervera et al. 1999; Mhamdi et al. 2002; Taurian et al. 2002; Kwon et al. 2005), with none of these obtained from US soils. Amarger et al. (1997) first identified *R. gallicum* and *R. giardinii* as micro-
Fig. 2. Similarity of 16S rRNA gene sequences among isolates representing eight clusters of *Desmanthus illinoensis* rhizobia, including four inoculant strains identified by Beyhaut et al. (2006). The number of nucleotide differences was derived from the aligned sequences to construct an unrooted tree using unweighted pair group method with arithmetic mean (UPGMA). Sequences were aligned using Pileup in the Wisconsin software package of the Genetics Computer Group (Madison, Wis.), and with the Molecular Evolutionary Genetics Analysis (MEGA) software version 1.02 (Kumar et al. 2001) used to derive the nucleotide differences and to construct the tree. Levels of support for the presence of nodes were obtained from bootstrap analysis using 500 permutations of the data set.
symbionts associated with bean plants in France. Rhizobia with the characteristics of these two species have now been identified as the major microsymbiont of the prairie legumes *Dalea purpurea* (Tlusty et al. 2005; M. Martir, personal communication) and *Desmanthus illinoensis*, respectively. This raises questions on the biogeography and possible transatlantic movement of these bacteria. *Dalea purpurea* was first grown in Europe in the early 1800s (Locklear and Vickerman 1997), but we have no parallel information for *Desmanthus*.

Also in this study, two strains of *Rhizobium* from *Desmanthus* were shown to share characteristics with the closely related species *R. etli*, *R. leguminosarum*, and *R. tropici*. These isolates were obtained from a single location, but that organisms from these species should nodulate native American legumes is not without precedent (Graham et al. 1999; Bernal et al. 2004; Tlusty et al. 2005). It justifies further study of host range, plasmid transfer, and possible genetic rearrangement among a group of bacteria that with the exception of *R. tropici*, has generally been considered to be specific in host nodulation.

Finally, it is noteworthy that the strains tested in this study should be effective on *Desmanthus illinoensis* but noninfective on *Desmanthus virgatus*. This parallels the situation with *Dalea purpurea* and *Dalea leporina* (Tlusty et al. 2004, 2005) and also warrants further study on the host range of each of these species and on host range determinants.

References


