Genotypic Diversity among Strains of *Bradyrhizobium japonicum* Belonging to Serogroup 110

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Thirty-three strains of *Bradyrhizobium japonicum* within serogroup 110 were examined for genotypic diversity by using DNA-DNA hybridization analyses. The analysis of the DNA from 15 hydrogen-uptake-negative strains with the bradyrhizobial uptake hydrogenase probe pHU52 showed variation in degree of homology and restriction fragment length polymorphism of EcoRI-restricted DNA. Clustering analysis of the 33 strains on the basis of DNA-DNA hybridization analysis with four restriction enzymes and with the bradyrhizobaial nodulation locus, pRJUT10, as probe indicated the existence of four groups of strains, which were less than 70% similar. Restriction digestion of genomic DNA with BamHI and DNA-DNA hybridization with pRJUT10 permitted classification of each of the strains according to a specific fingerprint pattern.

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*Bradyrhizobium japonicum* strains are soil bacteria which form nitrogen-fixing symbioses with soybean (*Glycine max* (L.) Merr). Phenotypic diversity among strains has been reported for a broad range of traits including nitrogen fixation (2), ex planta nitrogenase activity (3), polysaccharide production (3), rhizobitoxine production (8), hydrogen oxidation capability (24), and intrinsic antibiotic resistance (11). The phenotypic diversity was one of the original criteria used to separate isolates into various groups. Examination of serological reactions and surface antigens identified the existence of major serogroups (4), and serotyping has become one of the most important methods for distinguishing among strains.

With the development of techniques for the genetic analysis of bacteria, there has been an increased popularity of applying these methods for the investigation of diversity among strains. Electrophoresis of enzyme polymorphic forms has been used to determine genetic diversity among strains of *Rhizobium leguminosarum* bv. phaseoli (17), *R. leguminosarum* bv. viciae (26, 27), and *R. meliloti* (5). The existence of at least three groups of *B. japonicum* is based on DNA homology studies (7), and restriction fragment length polymorphism analysis has indicated the presence of wide diversity among the soybean bradyrhizobia (23).

Investigations for diversity within serogroups of *B. japonicum* have received less attention than across serogroups. Nevertheless, serological variation was reported among members of serogroup 123 (6, 20), and Keyser and Cregan (9) showed variability in nodulation of specific soybean host plants among members of serocluster 123. We reported diversity among serogroup 110 strains in effectiveness for nitrogen fixation and diversity in expression of uptake hydrogenase (Hup) activity, which reflects variation in the efficiency of nitrogen fixation. The reported variability in the nitrogen fixation among the serogroup 110 strains (1) is potentially important to agriculture because all the members of this serogroup are assumed to be fully effective for nitrogen fixation and therefore are commonly used in commercial inocula (1). The reported phenotypic variability among strains of serogroup 110 (1) is also of significance to basic science, because USDA 110, or its small-colony derivative I-110 (10), is used by choice in experimentation to elucidate the molecular biology of soybean plant-microorganism interactions because these bacteria are considered to be representative of *B. japonicum*. However, inference made from data obtained in the basic scientific investigation of USDA 110 may be strain specific if serogroup 110 were shown to be genetically diverse. Therefore, the objective of this work was to complement our earlier report of phenotypic variation (1) by examining the serogroup 110 accesses of the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) National Rhizobium Culture Collection for genetic diversity by using DNA-DNA hybridization techniques.

Thirty-three strains within serogroup 110 from the Collection were recovered from storage and verified for their serology by using fluorescent antibodies prepared with USDA 110. The strains were maintained on yeast extract salts mannitol (YEM) agar slants (25) at 4°C for the duration of the investigation. Liquid cultures for the isolation of total DNA were prepared by growing bacteria to approximately mid-log phase in 50 ml of modified arabinose-glucanate (24) broth. DNA extraction was performed as previously described (24), except that a phenol-chloroform extraction followed by ethanol precipitation replaced purification of the DNA by CsCl gradient centrifugation. Genomic DNA was digested with *EcoR*I, *Bam*HI, *Pst*I, or *Hind*III (Bethesda Research Laboratories, Gaithersburg, Md.) and separated by horizontal gel electrophoresis in 0.7% gels. DNA transfer to supported nitrocellulose transfer membranes (Schleicher & Schuell, Keene, N.H.) and hybridization experiments were done as described by Maniatis et al. (13). The membranes were prehybridized for 20 h at 42°C in a buffer containing 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 10× Denhardt’s reagent (13), 1% sodium dodecyl sulfate (SDS), and 0.1 mg of denatured, fragmented salmon sperm DNA per ml. Subsequently, hybridization was carried out for 20 h at 46°C to probe DNA in 6× SSC–1% SDS–50% formamide–5% dextran sulfate–0.1 mg of heat-
variability in analyzed was DNA to compare each strain with each of the other 32 strains produced by HupI homology (the 110 (Hup+ control). Molecular sizes (in kilobases) are indicated on the right.

denatured, fragmented salmon sperm DNA per ml. The membranes were autoradiographed after being washed twice for 30 min at 65°C in a solution containing 0.1x SSC and 0.1% SDS. Whole cosmids used as probes for the hybridization experiments were pHU52 containing bradyrhizobial DNA for uptake hydrogenase (12) and pRJUT10 containing bradyrhizobial DNA for nodulation of legume host plants (19). The cosmids were prepared by a large-scale alkali-lysis method (13) and digested with EcoRI for radiolabeling of denatured DNA with a random prime labeling kit (United States Biochemical, Cleveland, Ohio). The presence or absence of hybridizing restriction fragments with pRJUT10 was scored for each of the 33 strains to produce a rectangular data matrix of 36 comparisons. The rectangular data matrix was analyzed with the Numerical Taxonomy and Multivariate Analysis System program (NTSYS-pc [18]) on an IBM-compatible computer. The rectangular data matrix was used to compare each strain with each of the other 32 strains to generate a matrix of simple matching coefficients, which are the total number of matches between two strains divided by 36 (the total number of comparisons). A phenogram was produced by an unweighted pair group method arithmetic average clustering analysis (21).

Initially the uptake hydrogenase probe pHU52 was used in DNA-DNA hybridization analyses with 15 Hup− strains of serogroup 110. The rationale for using cloned uptake hydrogenase DNA was that van Berkum (24) reported variability in homology and restriction fragment patterns among Hup− strains across several serogroups. Similarly, our results showed variability in the presence of homologous DNA among the 110 serogroup strains (Fig. 1). Strains USDA 452 and USDA 456 lacked homology with the DNA cloned in pHU52, similar to the serogroup 122 strain DE1 5a, which lacked homology with probe pHU1 (24). Homology with pHU52 was observed in the DNA of the other 13 strains, but variability in restriction fragment patterns was evident. The analysis with pHU52 indicated genetic variability among the Hup− serogroup 110 strains.

Results of DNA hybridization analyses with pRJUT10 and four different enzyme digests of the bradyrhizobial DNA also indicated genetic variability among the 33 strains (Fig. 2). Divergence in similarity was at 65%, and from the phenogram four major groups of strains were evident, each with less than 70% similarity to the others. The four groups were represented by 6, 1, 24, and 2 strains. The three smaller groups represented strains which were recently added to the USDA-ARS National Rhizobium Culture Collection (strain accession numbers USDA 443 and above), indicating that a wider diversity of serogroup 110 soybean bradyrhizobia were preserved. USDA 468 (6A50), USDA 467 (8A), USDA 466 (5631), and USDA 110, used by Hollis et al. (7) to determine DNA homology among the soybean bradyrhizobia, were part of the group with 24 strains. Therefore, our data indicate that the serogroup 110 strains used in the DNA homology determinations do not represent the widest diversity present within this serogroup. Hollis et al. (7) described three DNA homology groups within the soybean bradyrhizobia and placed the serogroup 110 strains they tested within a DNA homology group they termed 1a. Whether the more divergent serogroup 110 strains identified in our work also can be classified as belonging to DNA homology group 1a is unknown.

Phenotypic variability within the USDA 110 culture has also been reported. Kuykendall and Elkan (10) reported the presence of sublines within USDA 110 differing in nitrogen fixation capability and carbohydrate utilization. Subsequently, additional sublines occurring within USDA 110 were discovered (14, 15). Southern hybridization analysis with nifDK and nifH as probes with I-110 and L1-110 failed to reveal rearrangement of the nif genes in these two sublines of USDA 110 (16). Similarly, our data obtained with pRJUT10 as a probe indicated no apparent genetic difference between L1-110 and USDA 110.
FIG. 3. DNA-DNA hybridization patterns of BamHI-digested DNA from representative serogroup 110 strains by using the probe pRJUT10. Lanes: A, USDA 455; B, USDA 456; C, USDA 457; D, USDA 458; E, USDA 459; F, USDA 451; G, USDA 450; H, USDA 449. Molecular sizes (in kilobases) are indicated on the right.

The restriction fragment length polymorphism analysis indicated that a DNA-DNA hybridization with pRJUT10 as the probe in combination with a BamHI digestion of genomic DNA could be used to classify the serogroup 110 strains according to specific banding patterns (Fig. 3). The BamHI digestion of the genomic DNA resulted in the largest variability of the banding patterns among the strains. Eight different patterns among the 33 strains were observed (Fig. 3). The number of strains with banding patterns represented by lanes A, B, C, D, E, F, G, and H were 7, 2, 4, 1, 12, 2, 3, and 2, respectively. Both USDA 110 and L1-110 possessed the fingerprint pattern represented by USDA 455 in lane A. The fingerprint patterns of the serogroup 110 strains were also related to the Hup phenotype. All the strains with the patterns represented by USDA 455 (lane A), USDA 451 (lane F), and USDA 450 (lane G) are of the Hup+ phenotype, whereas all the strains with the remaining patterns are Hup-. Therefore, the fingerprint analysis of serogroup 110 strains should be useful to predict the Hup phenotype similarly to the reported use of DNA-DNA hybridization analyses with pMJS12 to select compatible host-strain combinations with soybean genotypes containing restrictive nodulation alleles (22).

Abdel Basit et al. (1) reported the existence of six phage reaction types among the serogroup 110 strains. The representative fingerprint patterns of the serogroup 110 strains (Fig. 3) were not as strongly related to the phage susceptibility type as to the Hup phenotype. Strains with phage types III and IV were observed only in strains represented by USDA 455 (lane A), whereas phage type V was present only in strains represented by USDA 459 (lane E). Only one phage type VI was reported (1) to be associated with USDA 456, with a fingerprint pattern represented by lane B. Phage susceptibility type I (no reaction) was present in strains belonging to all the fingerprint types except for those of lane F (USDA 451), whereas phage type II strains were present in patterns represented by USDA 459 (lane E), USDA 451 (lane F), USDA 450 (lane G), and USDA 449 (lane H).

Two colony morphology types were reported to be present among the serogroup 110 strains (1). However, no relationships between colony morphology and DNA fingerprint patterns were evident.

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REFERENCES


