

Genetic Variation in Two Cultures of *Bradyrhizobium japonicum* 110 Differing in Their Ability to Impart Drought Tolerance to Soybean

James N. Mathis,¹ David E. McMillin,² Rebecca A. Champion,³ Patrick G. Hunt⁴

¹Department of Biology, State University of West Georgia, Carrollton, GA 30118, USA

²Department of Biological Sciences, Clark Atlanta University, Atlanta, GA 30314, USA

³Department of Biology, Columbus State University, Columbus, GA 31907, USA

⁴USDA-ARS, Coastal Plains Research Center, 2611 West Lucas Street, Florence, SC 29501-1241, USA

Received: 19 May 1997 / Accepted: 27 June 1997

Abstract. The polymerase chain reaction with arbitrary primers (RAPD) discriminated between two separately maintained cultures of *Bradyrhizobium japonicum* USDA 110 differing in symbiotic performance under drought conditions. Since strain 110 is used in inoculum production, the use of RAPD to monitor inoculum cultures could help to preserve their genetic composition and prevent the loss of important symbiotic properties. The use of RAPD could also be extended to other *B. japonicum* strains currently used in inoculum production.

Bradyrhizobium japonicum USDA 110 was isolated in 1959 from the nodule of a soybean grown in Florida [11]. Symbiotic tests under both field [3] and greenhouse conditions [9, 22] have demonstrated that strain USDA 110 is superior at symbiotic nitrogen fixation compared with other strains. For this reason USDA 110 is commonly used in commercial soybean inoculum and has recently served as the parent strain for an improved inoculum currently in commercial use [8, 13–15].

Two cultures of *B. japonicum* USDA 110 were found to differ in symbiotic performance under drought conditions by Hunt et al. [7]. One of these cultures was obtained from the USDA culture collection (USDA-ARS, Beltsville, MD, USA) while the other was obtained from the NifTAL (Nitrogen Fixation and Tropical Agriculture Laboratory, Paia, HI, USA) collection, where it was renamed NifTAL 102. Hunt et al. [7] found that soybeans inoculated with USDA 110 were more drought tolerant than soybeans inoculated with NifTAL 102. Soybean cultivar Cobb under drought conditions with USDA 110 produced 18% higher seed yield and 9% higher shoot nitrogen content compared with NifTAL 102, whereas soybean cultivar Coker 448 under drought conditions showed a 43% increase in seed yield and a 5% increase in shoot nitrogen content for USDA 110 relative to NifTAL

102. Hunt et al. [7] speculated that variations in the composition of colonial morphology variants, which had been shown to vary in symbiotic nitrogen fixation abilities [17–19], could explain the observed differences between the two cultures.

Phenotypic variation in several different *B. japonicum* USDA 110 cultures was noted previously by Mullen and Wollum [21]. These variations occurred in colony morphology, antibiotic resistance, growth in various media, and competition with other strains of *B. japonicum*. However, statistically significant differences in nitrogen fixation were not detected [21]. In contrast to these results, Hartmann et al. [5] used RS- α (a repeated DNA sequence in *B. japonicum*) as a probe and noted identical hybridization patterns between two USDA 110 cultures maintained in different laboratories. Hartmann et al. [5] concluded from this observation that USDA 110 cultures remain genetically stable when cultured in different laboratories.

In this study, RAPD was used to evaluate genetic variation between NifTAL 102 and USDA 110, which were found to differ in their relative abilities to impart drought tolerance to soybean. Both cultures were also grown in three different carbon sources, and the percentage of mannitol- versus non-mannitol-utilizing colony morphology variants was determined.

Materials and Methods

Bradyrhizobium japonicum strains USDA 122 and USDA 466 were obtained from P. van Berkum (USDA-ARS, Beltsville, MD, USA). *B. japonicum* strains USDA 110 and NifTAL 102 were obtained from A.G. Wollum (Department of Soil Science, North Carolina State University, Raleigh, NC, USA) and were the same cultures used previously to demonstrate a difference in drought tolerance [7].

B. japonicum strains were grown to stationary phase in a yeast extract basal medium supplemented with either 1% mannitol (YEM), 1% arabinose (YEA), or 1% gluconate (YEG) [18]. Cells used for DNA isolation were grown in YEM. Following cell growth, DNA was isolated and PCR amplified as described by Mathis and McMillin [16]. The following arbitrary primers were used in PCR amplification (RAPD): OPE-01–OPE-10 and OPO-1–OPO-10 (Operon Company, Alameda, CA, USA), ARP-2, ARP-5, ARP-6 [20], ARP-7 (GTACGTG-GCG), SPH-1 [4], MICROSAT [(T or A)GAGGGTGG], CRL-7, and CRL-13 [12].

Results and Discussion

B. japonicum strains USDA 110, NifTAL 102, 110-serogroup strain USDA 466 (95% homology to USDA 110 under stringent hybridization conditions, [6]) and USDA 122 (2% homology to USDA 110 under stringent hybridization conditions, [6]) were analyzed with RAPD. Six of the 28 primers tested (21%) were found to give distinct DNA banding patterns for each of the strains studied, OPE-01, OPO-07, OPO-09, SPH-1, ARP-7 (data not shown), and CRL-7 (Fig. 1). Six primers (21%) failed to amplify DNA from any of the strains (OPE-05, OPE-09, OPO-01, OPO-02, OPO-08, and ARP-6, data not shown) and three others (11%) amplified only USDA 122 DNA (OPE-06, OPE-08, and OPE-10, data not shown). None of the 13 remaining primers (46%) could clearly distinguish all four of the strains tested from each other (OPE-02–OPE-04, OPE-07, OPO-03–OPE-06, OPO-10, MICROSAT, CRL-13, ARP-2, and ARP-5, data not shown).

RAPD analyses provided genetic markers separating USDA 110 from NifTAL 102 (Fig. 1), whereas other DNA analyses performed in our laboratories such as RFLP studies [1,2] with five random probes from a *B. japonicum* USDA 110 library, REP-PCR [10, 23] and ERIC-PCR [10], were unable to separate USDA 110 from NifTAL 102 (J.N. Mathis and D.E. McMillin, unpublished data). RAPD analyses clearly demonstrate that genetic divergence has occurred in the *B. japonicum* 110 cultures, which differed in symbiotic performance under drought conditions. However, further research is required to demonstrate that specific variations in RAPD banding pattern directly correlate with differences in drought tolerance.

The percentage of non-mannitol-utilizing variants present in NifTAL 102 was less than the percentage found in USDA 110 for three different carbon sources studied

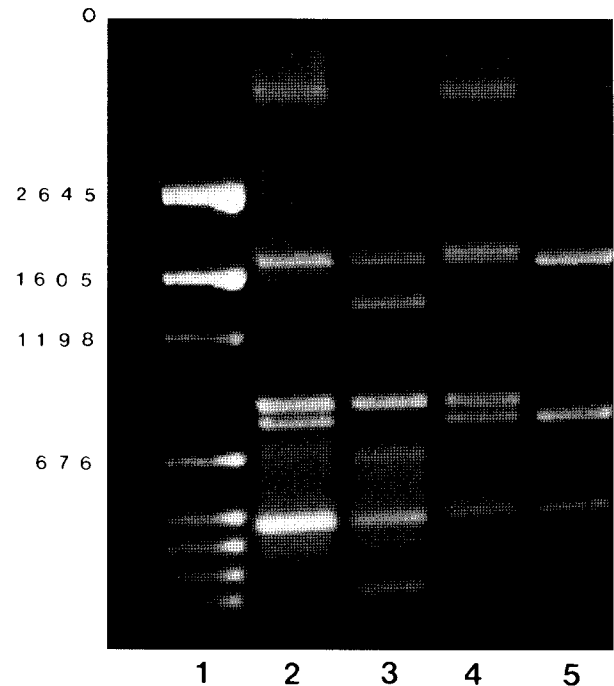


Fig. 1. DNA fingerprints of *B. japonicum* USDA 110, NifTAL 102, and control strains USDA 466 and USDA 122 generated by arbitrary PCR primer CRL-7 (GCCCCGCC). DNAs were separated on a 2% agarose gel: lane 1, molecular weight marker, pGEM, fragment sizes in nucleotides are denoted; lane 2, USDA 110; lane 3, NifTAL 102; lane 4, USDA 122; lane 5, USDA 466. All PCR patterns are representative of at least three separate amplification runs in which different cultures of a given strain were used. Within each of these separate runs two to four replicate reactions were performed which gave the same patterns of amplification, thus eliminating the possibility of position effects within the thermocycler. Each reaction for a designate strain was found to give the same pattern for each culture of that strain, thus indicating the reliability of the technique.

(Table 1), thus indicating that USDA 110 and NifTAL 102 are also phenotypically distinct from each other. Twenty individual mannitol-utilizing and 20 individual non-mannitol-utilizing colonies were screened from USDA 110 and NifTAL 102 for nitrogen fixation ability (Mathis and Champion, unpublished data). Each of these single colony isolates was found to have similar symbiotic nitrogen fixation abilities, as determined by the nitrogen contents of inoculated plants. Furthermore, replicate greenhouse studies showed similar levels of symbiotic nitrogen fixation for plants inoculated with either NifTAL 102 or USDA 110 (Mathis and Champion, unpublished data). Since both cultures contain only symbiotically competent (Fix⁺) colony morphology variants and have similar symbiotic nitrogen fixation abilities under greenhouse conditions, it would appear that nitrogen fixation ability is not directly involved in the observed differences in symbiotic performance between USDA 110 and NifTAL 102 under drought conditions.

Table 1. Colony morphology variant composition of *Bradyrhizobium japonicum* NifTAL 102 and USDA 110 when grown in different carbon sources

Carbon source	% non-mannitol-utilizing variants in each <i>Bradyrhizobium japonicum</i> USDA 110 culture grown in each carbon source ^a	
	USDA 110	NifTAL 102
arabinose (YEA)	10	<1
mannitol (YEM)	8	3
gluconate (YEG)	12	<1

^a Serial dilutions were plated onto YEM-plates from each of the media YEM, YEA and YEG [18] from five replicate stationary phase cultures (grown for 72 h and containing $1-4 \times 10^9$ CFU/ml). Duplicate plates were scored for dilutions that plated between 50 and 150 colonies for each of the five replicate cultures. Non-mannitol-utilizing versus mannitol-utilizing colony morphology variants were distinguished by their appearance on YEM plates. Mannitol utilizing are large and mucoid, whereas non-mannitol utilizing are small and non-mucoid.

Interestingly, in the analysis of three different USDA 110 colony morphology variants (differing in mannitol utilization and/or symbiotic nitrogen fixation ability) with the same set of 28 primers, only two of the primers were found to give differences in pattern (CRL7 and ARP7, [16]). These differences were found to correlate with differences in nitrogen fixation rather than mannitol utilization. Since loss of symbiotic nitrogen fixation was not detected in any of the variants observed in this current study, and differences in RAPD pattern were not previously found to correlate with differences in mannitol utilization, further analyses of colony morphology variants by RAPD were not conducted [16].

Since *B. japonicum* USDA 110 is used extensively for the preparation of soybean inoculum, changes in cultures of this strain that result in alteration of symbiotic performance are of agricultural significance [8, 13, 14, 15]. While occurrences of phenotypic variation have been documented previously for cultures of *B. japonicum* USDA 110 [21], other investigators have reported evidence for the genetic stability of USDA 110 cultures maintained in different laboratories [5]. Our data document the occurrence of both phenotypic and genetic variation between USDA 110 cultures. Development of DNA fingerprints and their use in monitoring strains used for inoculum preparation may therefore be a useful practice and should help in the preservation of important symbiotic properties.

ACKNOWLEDGMENTS

We thank Tony Griffin and Julie Baggett for excellent technical assistance and Tom Beggs for assistance with photography. This investigation was partially supported by an RCMI award, G12RR03062, from the Division of Research Resources, National Institutes of Health

to D.E. McMillin and by State University of West Georgia Faculty Research Grants awarded by the Learning Resources Committee to J.N. Mathis.

Literature Cited

1. Beckmann J, Soller M (1983) Restriction fragment length polymorphisms in genetic improvements: methodologies, mapping and costs. *Theor Appl Genet* 67:35-43
2. Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. *Genetics* 112:887-898
3. Caldwell BE (1969) Initial competition of root-nodule bacteria on soybean in a field environment. *Agron J* 61:813-815
4. Harrison SP, Mytton LR, Skot L, Malcolm D, Cresswell A (1992) Characterization of *Rhizobium* isolates by amplification of DNA polymorphisms using random primers. *Can J Microbiol* 38:1009-1015
5. Hartmann A, Catroux G, Amarger N (1992) *Bradyrhizobium japonicum* strain identification by RFLP analysis using the repeated sequence RS α . *Lett Appl Microbiol* 15:15-19
6. Hollis AB, Kloos WE, Elkan GH (1981) DNA:DNA hybridization studies of *Rhizobium japonicum* and related *Rhizobiaceae*. *J Gen Microbiol* 123:215-222
7. Hunt PG, Matheny TA, Wollum AG II (1988) Yield and N accumulation response of late-season determinant soybean to irrigation and inoculation with various strains of *Bradyrhizobium japonicum*. *Commun Soil Sci Plant Anal* 19:1601-1612
8. Hunter WJ, Kuykendall LD (1990) Enhanced nodulation and nitrogen fixation by a revertant of a nodulation-defective *Bradyrhizobium japonicum* tryptophan auxotroph. *Appl Environ Microbiol* 56:2399-2403
9. Israel DW (1981) Cultivar and *Rhizobium* strain effects on nitrogen fixation and remobilization by soybeans. *Agron J* 73:509-516
10. Judd AK, Schneider M, Sadowski MJ, De Bruijn F (1993) Use of repetitive sequences and the polymerase chain reaction technique to classify genetically related *Bradyrhizobium japonicum* serocluster 123 strains. *Appl Environ Microbiol* 59:1702-1708
11. Keyser HH, Griffin RF (1987) Beltsville *Rhizobium* culture collection catalog. USDA-ARS, ARS-60, Beltsville, MD
12. Kubelik AR, Szabo LJ (1995) High-GC primers are useful in RAPD analysis of fungi. *Curr Genet* 28:384-389
13. Kuykendall LD, Hunter WJ (1991) Enhancement of nitrogen fixation with *Bradyrhizobium japonicum* mutants. US Patent 5,021,076
14. Kuykendall LD, Hahn M, Hennecke H, Hunter WJ (1992) Genetically improved rhizobia and their use in tropical agriculture. In: Mulongoy K, Gueye M, Spencer DSC (eds) *Biological nitrogen fixation and sustainability of tropical agriculture*, London: John Wiley and Sons, pp 211-218
15. Kuykendall LD, Hashem FM, Hunter WJ (1996) Enhanced competitiveness by a *Bradyrhizobium japonicum* strain improved for nodulation and nitrogen fixation. *Plant Soil* 186:121-125
16. Mathis JN, McMillin DE (1996) Detection of genetic variation in *Bradyrhizobium japonicum* USDA 110 variants using DNA fingerprints generated with GC rich arbitrary PCR primers. *Plant Soil* 186:81-85
17. Mathis JN, Kuykendall LD, Elkan GH (1986) Restriction endonuclease and *nif* homology patterns of *Bradyrhizobium japonicum* USDA 110 derivatives with and without nitrogen fixation competence. *Appl Environ Microbiol* 51:477-480
18. Mathis JN, Israel DW, Barbour WM, Jarvis BDW, Elkan GH (1986) Analysis of the symbiotic performance of *Bradyrhizobium japonicum* USDA 110 and its derivative I-110 and discovery of a

- new mannitol-utilizing, nitrogen-fixing USDA 110 derivative. *Appl Environ Microbiol* 52:75–80
19. Mathis JN, Barbour WM, Miller TB, Israel DW, Elkan GH (1986) Characterization of a mannitol utilizing, nitrogen-fixing *Bradyrhizobium japonicum* USDA 110 derivative. *Appl Environ Microbiol* 52:81–85
 20. McMillin DE, Muldrow LL (1992) Typing of toxic strains of *Clostridium difficile* using DNA fingerprints generated with arbitrary polymerase chain reaction primers. *FEMS Microbiol Lett* 92:5–10
 21. Mullen MD, Wollum AG II (1989) Variation among different cultures of *Bradyrhizobium japonicum* strain USDA 110 and 122. *Can J Microbiol* 35:583–588
 22. Schubert KR, Jennings KT, Evans HJ (1978) Hydrogen reactions of nodulated leguminous plants. II. Effects on dry matter accumulation and nitrogen fixation. *Plant Physiol* 61:398–401
 23. Woods CR, Versalovic J, Koeuth T, Lupski JR (1993) Whole-cell repetitive element sequence-based polymerase chain reaction allows rapid assessment of clonal relationships of bacterial isolates. *J Clin Microbiol* 31:1927–1931