

## DNA Hybridization Probe for Use in Determining Restricted Nodulation among *Bradyrhizobium japonicum* Serocluster 123 Field Isolates

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Several soybean plant introduction (PI) genotypes have recently been described which restrict nodulation of *Bradyrhizobium japonicum* serocluster 123 in an apparently serogroup-specific manner. While PI 371607 restricts nodulation of strains in serogroup 123 and some in serogroup 127, those in serogroup 129 are not restricted. When DNA regions within and around the *B. japonicum* I-110 common nodulation genes were used as probes to genomic DNA from the serogroup strains USDA 123, USDA 127, and USDA 129, several of the probes differentially hybridized to the nodulation-restricted and -unrestricted strains. One of the gene regions, cloned in plasmid pMJS12, was subsequently shown to hybridize to 4.6-kilobase *Eco*RI fragments from DNAs from nodulation-restricted strains and to larger fragments in nodulation-unrestricted strains. To determine if the different hybridization patterns could be used to predict nodulation restriction, we hybridized pMJS12 to *Eco*RI-digested genomic DNAs from uncharacterized serocluster 123 field isolates. Of the 36 strains examined, 15 were found to have single, major, 4.6-kilobase hybridizing *Eco*RI fragments. When tested for nodulation, 80% (12 of 15) of the strains were correctly predicted to be restricted for nodulation of the PI genotypes. In addition, hybridization patterns obtained with pMJS12 and nodulation phenotypes on PI 371607 indicated that there are at least three types of serogroup 127 strains. Our results suggest that the pMJS12 gene probe may be useful in selecting compatible host-strain combinations and in determining the suitability of field sites for the placement of soybean genotypes containing restrictive nodulation alleles.

Soybean production fields in the midwestern United States contain relatively high numbers of *Bradyrhizobium japonicum* serocluster 123 strains (6, 11). In addition to their unique competitive ability, some serocluster 123 members are considered to be relatively ineffective for symbiotic nitrogen fixation (4-6). The term serocluster was introduced by Schmidt et al. (21) to describe the serological cross-reactivity of strains in serogroups 123, 127, and 129. We have recently described the identification and usefulness of soybean plant introduction (PI) genotypes which specifically exclude nodulation and reduce competitiveness of *B. japonicum* serocluster 123 strains (4-6). Two of the PI genotypes, PI 377578 and PI 371607, were shown to differentially restrict nodulation by 20 different serocluster isolates. Nodulation by isolates belonging to serogroup 123 was restricted by the PIs, whereas those isolates belonging to serogroup 127 or 129 were only partially restricted or were not restricted at all (11, 19).

While the PI genotypes were initially thought to restrict nodulation in a serogroup-specific manner, recent studies have indicated that the relationship between nodulation restriction and surface somatic antigens is more complex than was previously assumed. For example, in addition to restricting nodulation by serogroup 123 strains, the two PI genotypes also restrict the nodulation of strain USDA 127 (6). Moreover, we have recently identified an additional PI genotype, PI 417566 (5), which not only restricts nodulation and reduces the competitiveness of one of the previously unrestricted isolates, MN1-1c (serogroup 127), but also inhibits nodulation by strains in serogroup 129.

Previous studies (7, 8, 19, 21) have demonstrated that *B. japonicum* serocluster 123 comprises a heterogeneous group of organisms. Moreover, though our initial studies (19) indicated that serocluster 123 strains could be separated into similar groups on the basis of genomic DNA digestion patterns, sodium dodecyl sulfate protein profiles, immunological reaction with serogroup-specific antisera, nodulation characteristics with specific soybean genotypes, and *nifHD* hybridization profiles, the Southern hybridization analyses done with a *nodAB* gene probe were not useful in demonstrating interrelationships among the isolates (19).

Given that serocluster 123 strains have a large amount of genetic diversity and that our ultimate goal is to produce specific, paired, bacterium-host combinations for use in midwestern U.S. fields, it would be important to know if the indigenous *Bradyrhizobium* population would be restricted for nodulation of modified soybean genotypes. In this study, we report that some DNA sequences within and surrounding the *B. japonicum* common nodulation region may be useful as DNA probes to predict nodulation restriction among unclassified serocluster 123 field isolates. Moreover, hybridization patterns obtained with one of the gene probes and nodulation phenotypes on PI 371607 indicated that serogroup 127 comprises genetically diverse organisms.

### MATERIALS AND METHODS

**Bacterial strains, media, and growth conditions.** The *B. japonicum* serocluster 123 strains, designated by two-letter postal code abbreviations (IA-4, IA-5, IA-12, IA-23, IA-25, IA-35, IA-37, IA-39, IA-44, IA-49, IA-51, IA-56, IA-67, IN-2, IN-9, IN-34, IN-55, IN-56, IN-64, IN-77, IN-78, IN-79, IL-25, IL-68, OH-1, OH-2, OH-3, OH-5, OH-6,

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OH-9, OH-13, MN-3, MN-8, MN-9, MN-11, and MN-12), were isolated from soybean root nodules obtained from field soils collected from the following five midwestern U.S. states: Illinois, Iowa, Ohio, Minnesota, and Indiana. *Glycine max* cv. Williams was the "trap" host, and plants were grown in pots as described previously (6). *B. japonicum* was isolated from nodules as previously described (24), and single-colony nodule isolates were purified by streaking, twice, on yeast extract-mannitol agar medium (24). Isolates reacting with fluorescent antibodies prepared against strain USDA 123 were used for further study. The fluorescent antibodies, prepared by the method described by Schmidt et al. (20), identify strains belonging to serogroup 123, 127, or 129 (21). Serological relationships among these isolates and strains USDA 123, USDA 127, and USDA 129 were determined by using serogroup-specific, cross-adsorbed antisera (21) and a spot-blot assay (indirect enzyme-linked immunosorbent assay) (1, 6). The *B. japonicum* strains USDA 123, USDA 127, and USDA 129 were from the culture collection of the U.S. Department of Agriculture, Beltsville, Md. The *B. japonicum* strains USDA 162, USDA 171, USDA 185, and USDA 228 were isolated in 1979 from soybean nodules obtained from the People's Republic of China (11, 19). The *B. japonicum* serogroup 127 strains, Webster 48 and Becker 4-N18, were obtained from E. L. Schmidt (University of Minnesota, St. Paul, Minn.), and strains WI 3058 and WI 3105 were obtained from B. Kamicker (University of Wisconsin, Madison, Wis.). The *B. japonicum* serocluster 123 strains, which were previously grouped into high-, medium-, and low-nodulation classes (11, 19), AR1-3a, AR9-3b, DE3-1a, IA3H2-8, IA3H2-17, KS5-2c, KS6-3b, MN1-1c, MN5-4a, MN6-1b, NC3-1a, NJ1-4c, NJ2-1a, SC2-3c, and SD6-1c, were obtained from the culture collection of the U.S. Department of Agriculture as accession numbers USDA 422, USDA 423, USDA 424, USDA 426, USDA 427, USDA 428, USDA 429, USDA 430, USDA 431, USDA 432, USDA 434, USDA 435, USDA 436, USDA 437, and USDA 438, respectively. Strain PA3 (serogroup 127) was previously isolated from Pennsylvania as described previously (12). All *Bradyrhizobium* strains were grown at 28°C. The *B. japonicum* strains were grown in yeast extract-mannitol liquid medium to determine serological reactions and in arabinose-gluconate medium (19) for DNA preparations. The *Escherichia coli* strains were grown at 37°C on Luria-Bertani medium (14). When appropriate, the Luria-Bertani medium was supplemented with 30 µg of tetracycline per ml or 25 µg of kanamycin per ml.

**DNA manipulations.** Cosmid pR32, a pVK102 genomic DNA clone which overlaps the 37-kilobase (kb) nodulation gene-containing cosmid pRJUT10 (17), was digested to completion with *Hind*III, and the fragment mixture was ligated to *Hind*III-digested pVK102 DNA (13). *E. coli* HB101 was transformed with the ligation mixture, and Tc<sup>r</sup> Km<sup>s</sup> colonies were isolated by replica plating on Luria-Bertani medium containing tetracycline or tetracycline plus kanamycin. Cosmids that contained fragments corresponding to the *nodDYABC*, *nodIJ*, *hsn* (*Macropitilium*), *nodD2*, and other gene regions (2, 9, 15, 17, 18; M. J. Sadowsky, M. Gottfert, P. B. Cregan, F. Rodriguez-Quinones, D. Gerhold, H. Hennecke, H. H. Keyser, and G. Stacey, submitted for publication) were identified by agarose gel electrophoresis and were designated pMJS18, pMJS24, pMJS22, pMJS9, and pMJS12, respectively (Fig. 1). These subclones served as DNA probes. The 2.3-kb gene region contained in cosmid pMJS12 has been shown to be involved in the genotype-specific nodulation of soybean. That is, pMJS12 can com-

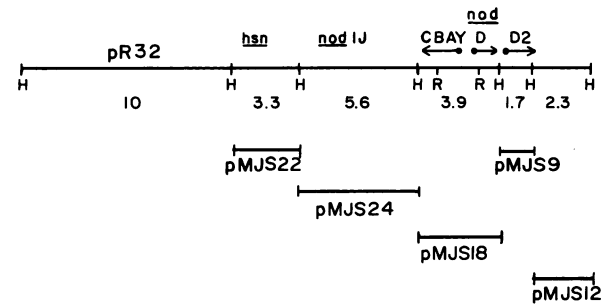


FIG. 1. Physical relationship of pMJS subclones to the nodulation-complementing cosmid pR32. The *Hind*III fragments of pR32 were subcloned into cosmid pVK102. The location of the *hsn* (*Macropitilium atropurpureum*), *nodIJ*, *nodD2*, and *nodDYABC* genes on pR32 are shown. Cosmid pMJS12 contains the previously reported (18) GSN gene region. Numbers below the line are fragment sizes in kilobases.

plement serocluster 123 isolates for the nodulation of the restrictive soybean genotype PI 377578 (18; M. J. Sadowsky, D. Gerhold, G. Stacey, H. H. Keyser, and P. B. Cregan, Abstr. Int. Symp. Mol. Genet. Plant Microbe Interact. 1988, p. IB-9). Total genomic *B. japonicum* DNA was isolated as described previously (19). Restriction enzymes *Eco*RI and *Hind*III were purchased from U.S. Biochemical Corp. (Cleveland, Ohio), and T4 DNA ligase was purchased from New England BioLabs, Inc. (Beverly, Mass.). All enzymes were used according to the specifications of the manufacturers. Restriction fragments were separated by horizontal electrophoresis on 0.7% agarose gels in Tris-EDTA-borate buffer (14). For hybridizations, DNA was transferred to Nytran membranes (Schleicher & Schuell, Inc., Keene, N.H.) as described previously (14). The <sup>32</sup>P-labeled probes were prepared by random primer labeling (Multiprime System; Amersham Corp., Arlington Heights, Ill.) according to the instructions of the manufacturer and were hybridized to filters as described previously (14).

**Nodulation studies.** Plant assays were done in Monmouth fine sandy loam soil limed to a neutral pH with CaMg(CO<sub>3</sub>)<sub>2</sub> and mixed with an equal volume of perlite. This soil contained very low numbers of *B. japonicum* strains (less than one *B. japonicum* cells per gram of soil) as determined by the most-probable-number plant infection method (24). Plants were grown in 17.5-cm (diameter) surface-sterilized, plastic pots containing 2.4 kg of the soil mixture. Pots were planted with four seeds each of *G. max* PI 371607 (a serogroup 123 nodulation-restricting genotype [4-6]) and cv. Williams (a nonrestrictive host). Seeds were surface sterilized (24) before planting and were thinned to two seedlings of each genotype per pot 3 days after emergence. Plants were inoculated with 1.0 ml (about 10<sup>9</sup> cells) of yeast extract-mannitol-grown, stationary-phase *B. japonicum* cultures. After inoculation, seeds were covered with soil and a 1-cm layer of sterile gravel was layered on the soil surface. Plants were grown in the greenhouse as previously described (4). Isolates were inoculated in triplicate, and uninoculated plants served as a negative control. Plants were watered with nitrogen-free nutrient solution (4) as needed and were harvested 35 days after inoculation. Nodule numbers and nodule dry weight were determined (11). Isolates producing a nodule weight and number similar to that made by strain USDA 123 or USDA 127 were categorized as being restricted for nodulation by the PI genotypes, whereas those with nodule numbers and weights not statistically different

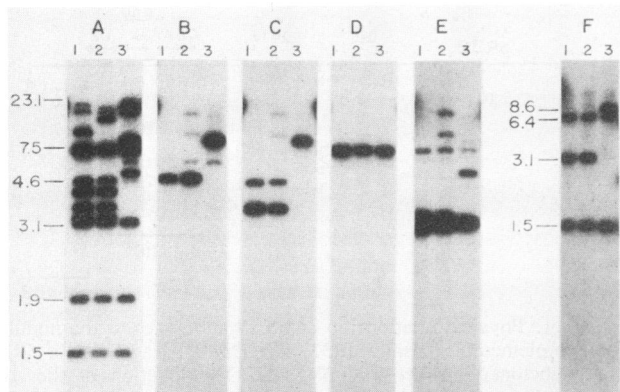


FIG. 2. Hybridization of pR32 and pMJS subclones to *EcoRI*-digested genomic DNA from strains USDA 123 (lanes 1), USDA 127 (lanes 2), and USDA 129 (lanes 3). The probes used were as follows: A, pR32; B, pMJS12; C, pMJS9; D, pMJS24; E, pMJS22; and F, pMJS18. Refer to Fig. 1 for relationship of the probes used to cosmid pR32. Values in margins are in kilobases.

( $P \leq 0.05$ ) than that of strain MN1-1c were designated as not restricted for nodulation. Statistical significance was determined by the analysis of variance and Duncan's New Multiple Range procedures of SAS.

## RESULTS AND DISCUSSION

**Hybridization patterns with nodulation (*nod*) gene probes.** Our initial studies (19) indicated that nodulation-restricted serocluster 123 strains could not be separated into similar groups on the basis of Southern hybridization analyses done with a *nodAB*-specific gene probe. It was therefore of interest to determine if hybridizations done with other *nod* genes (as well as with regions flanking common and host-specific nodulation loci) could be useful in demonstrating relationships among the serocluster 123 isolates. It should be noted that results from previous studies indicated that PI 371607 restricted the nodulation of USDA 123 and USDA 127 but not that of USDA 129 (4–6). When the entire nodulation gene-containing cosmid pR32 was used as a gene probe (Fig. 2A), differential hybridization was found with the three serogroup strains. However, while the hybridization profiles of the two nodulation-restricted strains, USDA 123 and USDA 127 (lanes 1 and 2, respectively), were very similar, that of USDA 129 (lane 3) was markedly different. The two nodulation-restricted strains also had similar, major hybridizing fragments with the *nodDYABC'* (pMJS18) and *nodD2* (pMJS9) gene probes (Fig. 2F and C, respectively), indicating that the genomic arrangements of these sequences are also conserved in these isolates.

When pMJS12 (the 2.3-kb gene region shown to be involved in the genotype-specific nodulation of soybeans [18]) was used as a probe (Fig. 2B), the two restricted strains, USDA 123 and USDA 127, each had a single, major 4.6-kb *EcoRI* hybridizing fragment, while USDA 129 (lane 3) had an 8.3-kb hybridizing fragment. The *nodIJ* gene probe, pMJS24, however, similarly hybridized to a 7.5-kb *EcoRI* fragment in nodulation-restricted and -unrestricted strains (Fig. 2D). While several weakly hybridizing *EcoRI* fragments were detected in strain USDA 127 with the pMJS9, pMJS12, and pMJS22 gene probes (lanes 3 in Fig. 2C, B, and E, respectively), they were not seen in hybridizations done

with other nodulation-restricted serogroup 127 isolates (see below and Fig. 4).

**Fragment length conservation among nodulation-restricted serocluster 123 strains.** Since the pMJS12 gene probe only hybridized to similar sized *EcoRI* fragments (4.6 kb) in the two nodulation-restricted isolates (and produced relatively simple hybridization patterns), we wanted to determine if restriction fragment length was also conserved among a group of previously classified (11, 19) serocluster 123 isolates. When the pMJS12 gene probe was hybridized to *EcoRI*-digested genomic DNAs from 19 serocluster 123 strains (which had previously defined nodulation phenotypes), almost all of the isolates (6 out of 7) that were restricted for nodulation of PI 371607 had a single, major, 4.6-kb hybridizing fragment (Fig. 3A, lanes 13 through 19). All of these isolates belong to *B. japonicum* serogroup 123. The one nodulation-restricted isolate which had a larger 6.1-kb hybridizing fragment, strain KS5-2c (Fig. 3A, lane 17), was previously shown (19) to be different than other nodulation-restricted strains with regards to its *nod* and *nif* gene hybridization profiles, serological affinity, and sodium dodecyl sulfate total protein pattern. All other isolates that were unrestricted (or were partially restricted) for nodulation, with the exception of the broad-host-range (6, 11) isolate, DE3-1a (Fig. 3A, lane 4), had larger (6.1- or 8.7-kb) hybridizing *EcoRI* fragments.

**Prediction of nodulation restriction among unclassified serocluster 123 field isolates.** Since pMJS12 hybridized to 4.6-kb *EcoRI* fragments in the nodulation-restricted isolates, we investigated its usefulness as a gene probe to predict nodulation restriction among uncharacterized serocluster 123 field isolates. We obtained the *B. japonicum* serocluster 123 strains from soils collected from five midwestern U.S. states and only examined those isolates belonging to serocluster 123, as determined by using antisera prepared against strain USDA 123. Of the 36 isolates examined by Southern hybridization with the pMJS12 gene probe, 15 were found to have a single, major, 4.6-kb hybridizing *EcoRI* fragment (Fig. 3B, C, and D; lanes 20 through 64). The remainder of the isolates had 6.1-, 7.7-, or 8.7-kb hybridizing *EcoRI* fragments. The isolates which had two hybridizing *EcoRI* fragments (Fig. 3C, lanes 51 and 54; Fig. 3D, lane 58) were subsequently shown to contain two colony types and were not examined further. Our hypothesis was that the 15 isolates with the 4.6-kb *EcoRI* fragments would be restricted for nodulation of the PI genotypes. When tested (in soil) in a replicated, double-blind nodulation study, 12 out of 15 isolates (80%) were correctly predicted to be restricted for nodulation of the PI soybean host (Table 1). These isolates produced nodule numbers and weights that were not statistically different ( $P \leq 0.05$ ) from those of the uninoculated PI control and the control restricted strains, USDA 123 and USDA 127. Moreover, the serocluster 123 field isolates did not appear to be impaired in their ability to nodulate soybean, since they produced a similar nodule number and weight on *G. max* cv. Williams as did the unrestricted strains, USDA 129 and MN1-1c.

To determine if there was a relationship between nodulation restriction and serological grouping, we examined the inoculated strains by using cross-adsorbed antisera specific for serogroups 123, 127, and 129. Results of the serological typing (Table 1) indicated that while a majority (9 out of 12) of the restricted strains belonged to serogroup 123 (as did the previously reported nodulation-restricted strains [6, 11, 19]), three of the isolates (OH-5, IN-64, and IA-44) were in serogroup 127. These isolates are therefore phenotypically

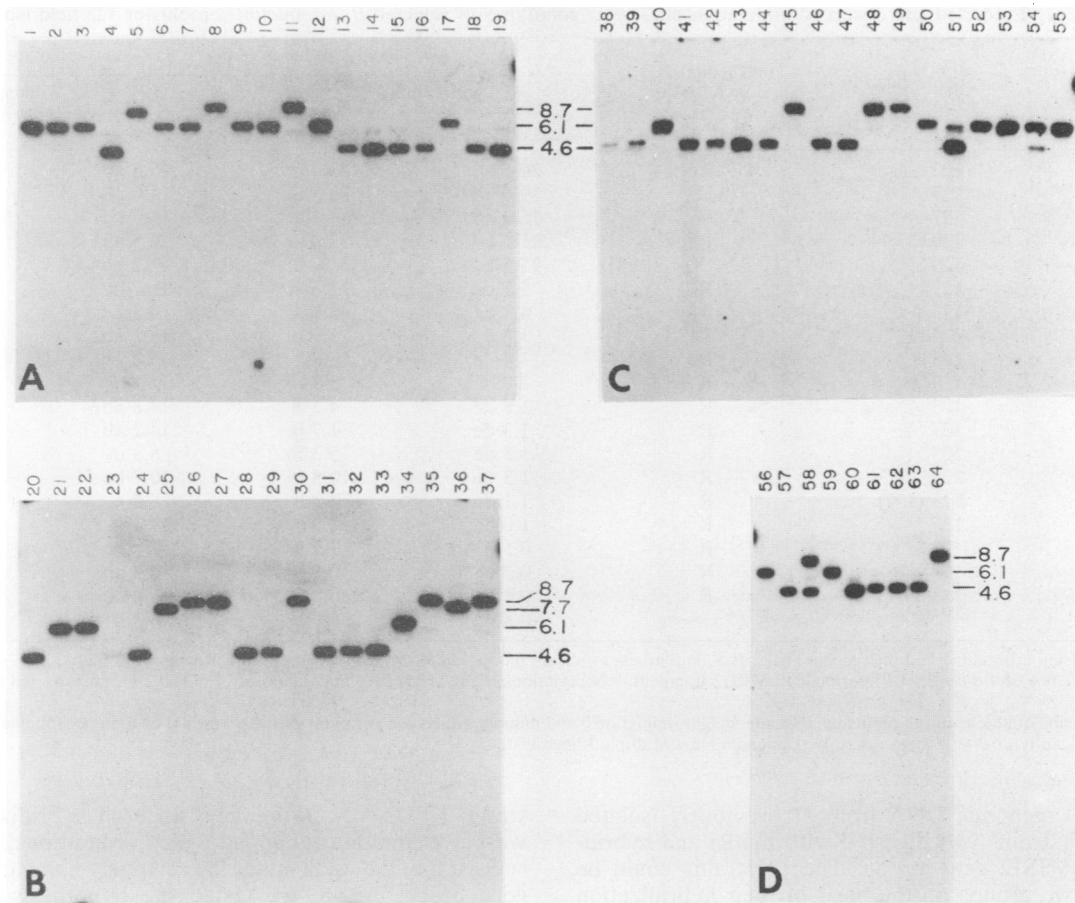


FIG. 3. Southern hybridization of pMJS12 to *Eco*RI-digested genomic DNAs from *B. japonicum* serocluster 123 field isolates. Lanes 13 through 19 in panel A contain DNAs from those strains which were previously classified as being restricted for nodulation of PI 371607. The DNAs in panel A, lanes 1 through 12, are from strains known to be unrestricted or partially restricted for nodulation of the PI genotype. Panels B, C, and D contain DNAs from additional serocluster 123 isolates whose nodulation phenotypes on PI 371607 were unknown. Lanes: 19, 28, 47, and 62, DNA from the serotype strain USDA 123; 29, 46, and 63, DNA from USDA 127; 30, 45, and 64, DNA from USDA 129. Lanes: 1, MN1-1c; 2, USDA 185; 3, USDA 228; 4, DE3-1a; 5, AR1-3a; 6, NC3-1a; 7, IA3H2-7; 8, NJ2-1a; 9, IA3H2-8; 10, KS6-3b; 11, NJ14-c; 12, AK9-3a; 13, MN5-4a; 14, USDA 162; 15, SC2-3c; 16, SD6-1c; 17, KS5-2c; 18, MN6-1b; 19, USDA 123; 20, IA-35; 21, IA-12; 22, IA-37; 23, MN-9; 24, OH-13; 25, IN-77; 26, MN-11; 27, MN-12; 28, USDA 123; 29, USDA 127; 30, USDA 129; 31, OH-9; 32, OH-6; 33, IA-67; 34, IL-68; 35, MN-8; 36, IL-25; 37, MN-3; 38, IN-78; 39, IN-56; 40, IA-25; 41, IA-5; 42, IN-34; 43, IA-23; 44, IN-79; 45, USDA 129; 46, USDA 127; 47, USDA 123; 48, IN-2; 49, IN-9; 50, OH-3; 51, IA-51; 52, IN-55; 53, IA-39; 54, IA-4; 55, OH-2; 56, IA-49; 57, IA-44; 58, IA-56; 59, OH-1; 60, IN-64; 61, OH-5; 62, USDA 123; 63, USDA 127; 64, USDA 129. Those strains with a prefix that is a two-letter postal code abbreviation were isolated in soils obtained from those states. All isolates reacted with fluorescent antibodies prepared against strain USDA 123. Molecular-size values are in kilobases.

similar to strain USDA 127, which is also restricted for nodulation of the PI genotypes. Strains IA-67 and IN-56, which were two of the three field isolates with incorrectly predicted nodulation responses, however, were also in serogroup 127 but nodulated the PI genotype to an extent equal to that of the unrestricted strain, USDA 129. In addition, since isolate IN-78 had a 4.6-kb hybridizing fragment and still nodulated the PI genotype, the serological and plant nodulation results (Table 1) indicated that serogroup 129 strains are also more diverse than was originally thought (19).

**Genetic diversity among *B. japonicum* serogroup 127 strains.** Since results from this and our previously reported (6, 11, 19) studies indicated that serogroup 127 strains have varied nodulation responses on the restrictive soybean genotypes, they suggested that this serogroup may be composed of a group of genetically diverse organisms. To examine this

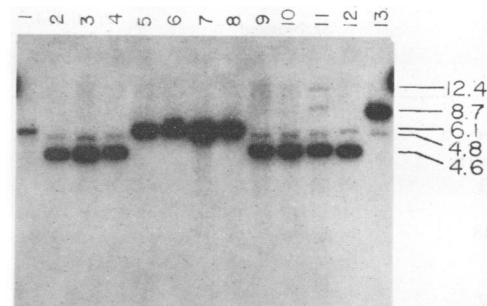


FIG. 4. Southern hybridization of pMJS12 to *Eco*RI-digested genomic DNAs from *B. japonicum* serogroup 127 strains. Lanes: 1, WI 3105; 2, WI 3058; 3, Webster 48; 4, PA 3; 5, Becker 4 N18; 6, USDA 171; 7, USDA 185; 8, MN1-1c; 9, DE3-1a; 10, SC2-3c; 11, USDA 127; 12, USDA 123; 13, USDA 129. Values in margin are in kilobases.

TABLE 1. Predicted and observed nodulation of *G. max* genotypes of selected *B. japonicum* serocluster 123 field isolates

Isolate	Serological group <sup>a</sup>	Predicted nodulation response <sup>b</sup>	Observed nodulation response on <i>G. max</i> genotypes <sup>c</sup>			
			PI 371607		Williams	
			No.	Wt	No.	Wt
IA-67	127	R	20.5 a	38.2 a	15.5 ab	52.8 abcd
MN1-1c	127	U	19.6 ab	35.8 a	14.0 ab	47.7 abcd
IN-56	127	R	16.5 ab	41.7 a	15.2 ab	43.0 bcd
IN-78	129	R	11.5 c	35.8 a	14.5 ab	42.7 cd
USDA 129	129	U	15.5 bc	32.6 a	12.2 bc	41.7 d
OH-5	127	R	5.5 de	12.6 b	12.7 bc	64.3 abc
IA-35	123	R	4.7 de	46.3 a	10.8 bc	50.8 abcd
IA-23	123 (127)	R	3.8 de	5.9 b	14.2 ab	51.7 abcd
OH-13	123	R	3.4 de	11.8 b	15.2 ab	68.7 a
USDA 123	123	R	2.9 de	8.7 b	17.5 a	58.7 abcd
IN-79	123	R	1.8 de	4.1 b	14.8 ab	56.7 abcd
USDA 127	127	R	1.3 de	1.7 b	14.2 ab	62.0 abcd
OH-9	123	R	1.3 de	15.3 b	8.3 c	76.0 a
IN-34	123	R	1.3 de	10.8 b	10.7 bc	42.8 bcd
MN-9	123 (127)	R	1.1 de	3.8 b	8.5 c	64.7 ab
IN-64	127	R	1.0 de	2.9 b	21.1 a	85.3 a
IA-5	123	R	0.5 e	1.3 b	12.5 bc	50.7 abcd
IA-44	127	R	0.3 e	0.5 b	15.3 ab	57.3 abcd
OH-6	123	R	0.1 e	0.2 b	15.0 ab	45.8 bcd
Uninoculated			0.8 de	3.0 b	4.3 d	11.6 e

<sup>a</sup> Determined using cross-adsorbed fluorescent antibodies. Parentheses indicate partial reaction with antisera specific for strain USDA 127.

<sup>b</sup> Based on presence of 4.6-kb *EcoRI* hybridizing pMJS12 fragment. Abbreviations: R, restricted for nodulation of PI 371607; U, unrestricted for nodulation of PI 371607.

<sup>c</sup> Values are means of at least three replicates. Nodule weight (milligrams) and number values are per plant. Values within a column not followed by the same letter differ significantly at  $P = 0.05$  as tested by Duncan's New Multiple Range test.

in more detail, genomic DNA from 11 previously isolated serogroup 127 strains was digested with *EcoRI* and hybridized to the pMJS12 gene probe. The 11 strains could be divided into two groups on the basis of their hybridization profiles (Fig. 4). The first group contained isolates with 4.6-kb *EcoRI* hybridizing fragments (WI 3058, Webster 48, PA 3, DE3-1a, SC2-3c, and USDA 127), while the second group had 6.1-kb *EcoRI* hybridizing fragments (WI 3105, Becker4-N18, USDA 171, USDA 185, and MN1-1c). Interestingly, while all of the group 1 isolates had a single, weakly hybridizing *EcoRI* fragment (about 4.8 kb in size), none of the serogroup 127 isolates (or the nodulation-restricted serogroup 123 strains in Fig. 3) had the three weakly hybridizing *EcoRI* fragments (4.8, 8.7, and 12.4 kb) detected in the type

strain, USDA 127. Moreover, since all hybridizations and washes were done at high-stringency conditions, our results suggest that the weak bands are relatively homologous to the gene probe. Again, we tested the hypothesis that those isolates with the 4.6-kb *EcoRI* fragments would be restricted for nodulation of the PI genotypes, whereas those with the larger hybridizing fragments (6.1 kb) would not be restricted. When nine serogroup 127 strains were tested in a replicated nodulation test, all of the isolates with the 4.6-kb fragments (group 1) were correctly predicted to be restrictive for nodulation of the PI soybean host (Table 2). While there was some statistical overlap in the nodule number data, the group 1 isolates nevertheless produced two to five times fewer nodules than did the nonrestricted strains and are considered

TABLE 2. Predicted and observed nodulation of *G. max* genotypes by *B. japonicum* serogroup 127 strains

Isolate	Serological group <sup>a</sup>	Predicted nodulation response <sup>b</sup>	Observed nodulation response on <i>G. max</i> genotypes <sup>c</sup>			
			PI 371607		Williams	
			No.	Wt	No.	Wt
MN1-1c	127	U	55.5 a	236.7 a	51.2 a	279.2 bc
USDA 185	127	U	45.0 ab	128.0 bc	72.3 a	239.7 c
Becker4-N18	127	U	38.3 abc	232.7 a	79.5 a	273.7 bc
USDA 171	127 (123)	U	36.3 abc	217.5 a	60.0 a	340.7 ab
WI 3105	127	U	33.5 abc	141.5 b	84.5 a	208.5 c
Webster 48	127	R	17.0 bc	89.0 bcd	58.7 a	326.5 ab
WI 3058	127	R	9.8 c	59.0 cd	84.5 a	208.5 c
PA 3	127	R	9.8 c	64.5 cd	67.0 a	332.3 ab
USDA 127	127	R	9.8 c	61.8 cd	63.0 a	387.8 a
Uninoculated			5.3 c	18.8 d	8.0 b	115.5 d

<sup>a</sup> Determined by using cross-adsorbed fluorescent antibodies. Parentheses indicate partial reaction with antisera specific for strain USDA 123.

<sup>b</sup> Based on presence of 4.6-kb *EcoRI* hybridizing pMJS12 fragment. Abbreviations: R, restricted for nodulation of PI 371607; U, unrestricted for nodulation of PI 371607.

<sup>c</sup> Values are means of at least three replicates. Nodule weight (milligrams) and number values are per plant. Values within a column not followed by the same letter differ significantly at  $P = 0.05$  as tested by Duncan's New Multiple Range test.

restricted for nodulation of the PI genotypes. The group 1 isolates, however, did produce nodule weights on PI 371607 that were not significantly different ( $P \leq 0.05$ ) from the uninoculated PI control (except Webster 48) and the negative control restricted strains, USDA 127. As before, group 1 and group 2 strains were not affected for nodulation of the commercial soybean cultivar, Williams.

Not all of the isolates, however, fit neatly into the predicted nodulation groups. For example, while isolate SC2-3c (Fig. 4, lane 10) was previously shown to be only partially restricted for the nodulation of the PI genotypes (11), it nevertheless had *nif* (19) and pMJS12 hybridization profiles similar to those of other isolates, which are restricted for nodulation. In addition, the serogroup 127 isolates DE3-1a, IA-67, and IN-56 (Fig. 3, lanes 4, 33, and 39, respectively) remain particularly enigmatic in that, while they appear serologically and phenotypically similar to strain MN1-1c, they have 4.6-kb hybridizing *Eco*RI fragments and are unrestricted for the nodulation of PI 371607. We are currently examining these strains in more detail to learn more about the relationship between restricted nodulation and the presence of the 4.6-kb *Eco*RI fragment.

One of the major goals of our research is to identify and use plant genotypes which would eliminate nodulation by indigenous strains in serocluster 123. Results of this and previous studies (5, 6, 11) indicate, however, that serocluster 123 comprises strains having a high degree of genetic diversity, which ultimately affects their ability to nodulate specific soybean hosts. Thus, the genetic factors from several plant genotypes may need to be combined together into one host in order to eliminate nodulation by most, if not all, serocluster members. We are currently investigating whether the nodulation restriction of PI 371607 and PI 417566 can be combined into one genotype. We will, however, also need to examine additional soybean genotypes for their ability to restrict nodulation by the heterogeneous serogroup 127 members.

In summary, results of this study indicate that the pMJS12 gene probe is useful for predicting nodulation restriction among uncharacterized *B. japonicum* serocluster 123 field isolates. Although DNA hybridization probes have been used to identify *Rhizobium meliloti* (25), *Rhizobium loti* (3), and *Rhizobium leguminosarum* bv. *trifolii* (22) strains from cultures and in nodules, to our knowledge, there have been no reports of a nonreiterated *B. japonicum* nodulation gene probe that can be used to differentiate (and phenotypically group) serologically related organisms. While a *nodAB* gene probe was not useful in grouping serocluster 123 into nodulation groups (19), results from the current study suggest that hybridization patterns obtained with other nodulation gene probes (pMJS9, pMJS24, or pMJS18) can be used to differentiate among phenotypically divergent, yet serologically related, strains. The nodulation groups made by using the pMJS12 nodulation gene probe may, in fact, be more justified than the groupings we previously made with *nif* gene hybridization patterns (19).

While the exact function of gene(s) on pMJS12 is unknown, they have been shown to be involved in the genotype-specific nodulation of soybean (18; Sadowsky et al. Abstr. Int. Symp. Mol. Genet. Plant Microbe Interact. 1988). Moreover, although it is not known if the arrangement of sequences homologous to pMJS12 in the serocluster 123 strains affects their expression, the observed fragment-length polymorphisms obtained with the probe are, nevertheless, well correlated with nodulation restriction. Thus, the pMJS12 gene probe may be useful in determining the

suitability of field sites for the placement of soybean germplasm which contain alleles that restrict the nodulation of specific *Bradyrhizobium* populations. In addition, we are currently investigating whether pMJS12-derived gene probes can be used to directly detect and quantify (10, 16, 23) nodulation-restricted serocluster isolates in bulk soil and rhizosphere samples.

Our results also suggest that serogroup 127 comprises genetically diverse strains that can be divided into at least two groups on the basis of hybridization profiles obtained by using the pMJS12 gene probe. These two groups consist of organisms that have three nodulation phenotypes on the USDA 123 restrictive soybean genotypes. Lastly, while it appears that there is some relationship between the ability of an organism to nodulate the restrictive soybean genotypes and its serological constitution, the few exceptions to groupings made on the basis of this correlation and the diversity within serogroup 127 itself indicate that the system is most likely more complex than was previously thought and warrants further study.

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