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Hyperreiterated DNA Regions Are Conserved among *Bradyrhizobium japonicum* Serocluster 123 Strains†

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We have identified and cloned two DNA regions which are highly reiterated in *Bradyrhizobium japonicum* serocluster 123 strains. While one of the reiterated DNA regions, pFR2503, is closely linked to the *B. japonicum* common and genotype-specific nodulation genes in strain USDA 424, the other, pMAP9, is located next to a Tn*5* insertion site in a host-range extension mutant of *B. japonicum* USDA 438. The DNA cloned in pFR2503 and pMAP9 are reiterated 18 to 21 times, respectively, in the genomes of *B. japonicum* serocluster 123 strains. Gene probes from the reiterated regions share sequence homology, failed to hybridize (or hybridized poorly) to genomic DNA from other *B. japonicum* and *Bradyrhizobium* spp. strains, and did not hybridize to DNA from *Rhizobium meliloti*, *Rhizobium fredii*, *Rhizobium leguminosarum* biovars trifolii, phaseoli, and viceae, or *Agrobacterium tumefacians*. The restriction fragment length polymorphism hybridization profiles obtained by using these gene probes are useful for discriminating among serologically related *B. japonicum* serocluster 123 strains.

Bacteria belonging to the genera *Bradyrhizobium*, *Rhizobium*, and *Sinorhizobium* establish nitrogen-fixing, root nodule symbioses with members of the family *Leguminoseae*. Many of the genes involved in the earlier stages of nodulation, termed common and host-specific nodulation genes on the basis of their structural and functional conservation (*nod*, *hsn*, and *nol* genes), have been well studied in members of the genus *Rhizobium* (16). Several *nod* and *hsn* loci have also been isolated from the slow-growing root-nodulating bacteria of the genus *Bradyrhizobium*, largely by hybridization homology with *nod* genes from fast-growing species (2, 6, 21). More recently, genotype-specific nodulation (GSN) genes have been isolated from *Rhizobium* and *Bradyrhizobium* strains (9, 19, 28).

The bradyrhizobia have been divided into two groups on the basis of DNA-DNA homology studies and physiological characteristics and consist of strains of *Bradyrhizobium japonicum* and *Bradyrhizobium* spp. (10, 17). The bradyrhizobia have been assigned to distinct serological groups on the basis of immunological reactions of their surface antigens (4, 27). In the northern Midwest United States, the important indigenous competitors for soybean nodulation are strains of *B. japonicum* serocluster 123 (32). Genetic and biochemical analyses indicated that serocluster 123 strains are genetically diverse and that there is an apparent relationship between serogroup, nodulation restriction phenotype on plant introduction (PI) genotypes, and physical organization of the genome (29, 30, 32).

Repetitive DNA sequences have been found in the genomes of a large number of prokaryotic organisms (12, 18, 20, 24, 26, 33). These repeated elements, which include transposons, insertion sequences, and gene duplications (34), are present in bacterial genomes with copy numbers ranging from 2 to 81 (18). Some repeated sequences (RS sequences) have been reported to constitute a major portion of the bacterial genome (33). Repeated elements have been found in the genomes of several *Rhizobium* strains. In *Rhizobium leguminosarum* bv. trifolii and *Rhizobium meliloti*, species-specific RS sequences, namely, RtRs (34), ISRm1 (26, 35), and ISRm2 (5), have also been described. In *R. meliloti*, the 1.4-kbp insertion element ISRm1 is present in about 10 copies per genome. The insertion sequence ISRm2 (5) has been shown to preferentially transpose into *R. meliloti* nitrogen fixation and nodulation genes. Mobile insertion sequences have also been reported in *R. meliloti* (26) and *Bradyrhizobium* spp. (in *Lupinus* spp.) (23).

In *B. japonicum* USDA 110, several repeated elements (RSα, RSβ, RSγ, RSδ, and RSε) have also been identified (8, 11). At least one of these elements, RSα, has properties similar to an insertion element. A unique feature of the *B. japonicum* USDA 110 RS sequences is that they are clustered in close proximity to the nitrogen fixation genes. Moreover, in *B. japonicum* USDA 110, RS sequences have been postulated to play a role in genomic instability (8). Some *B. japonicum* strains have also been shown to contain repeated *nod* gene sequences (30).

In this article we describe the identification and isolation of two *B. japonicum* gene regions which are highly reiterated in *B. japonicum* serocluster 123 strains. In addition, we show that one of the repeated DNA regions is located within the nodulation gene cluster.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Strains and plasmids used in this study are listed in Table 1. *B. japonicum* USDA 424 (previously designated strain DE3-1a) is a serocluster 123 isolate (serogroup 127) whose nodulation

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TABLE 1. Bacteria used and their sources[a]

Culture	Source
B. japonicum (serocluster 123 strains)	
USDA 123, USDA 127, USDA 129 USDA 162, USDA 171, USDA 185 USDA 422, USDA 423, USDA 424 USDA 426, USDA 427, USDA 429 USDA 430, USDA 431, USDA 436 USDA 438	1
IA5, IA23, IA35, IN56, IN78, IN79, OH6, PA3	2
WI 3058	3
Becker 4N-18	4
B. japonicum (other serogroup strains) USDA 4, USDA 6, USDA 38, USDA 62, USDA 110, USDA 122, USDA 124, USDA 135	1
Bradyrhizobium spp. USDA 31, USDA 46, USDA 61, USDA 76, USDA 94, USDA 130	1
Sinorhizobium fredii	
USDA 191, USDA 205	1
HH 303	5
R. leguminosarum bv. phaseoli TAL 182	5
R. leguminosarum bv. trifolii TAL 1820	5
R. leguminosarum bv. viceae TAL 1399	5
R. meliloti	
TAL 1372	5
8501	6
Rhizobium spp. (Leucaena) TAL 1145	5
Rhizobium spp. (Lens) TAL 634	5
Agrobacterium tumefaciens A136, A138	7

[a] Sources: 1, U.S. Department of Agriculture, Beltsville, Md.; 2, see reference 29; 3, B. Kamicker, University of Wisconsin, Madison; 4, E. L. Schmidt, University of Minnesota, St. Paul, 5, The NifTal Project, Paia, Hawaii; 6, Graham Walker, Massachusetts Institute of Technology, Cambridge; 7, Eugene Nester, University of Washington, Seattle.

is not restricted by any of the serocluster 123-restricting soybean PI genotypes (13, 30). *B. japonicum* USDA 438 (serogroup 123) is restricted for nodulation by several soybean (PI) genotypes and was previously designated SD6-1c (13, 30). All *B. japonicum* strains were from the culture collection of the Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md. All *Bradyrhizobium* strains were grown at 30°C and maintained on AG medium (30), and *Rhizobium* and *Agrobacterium* strains were grown at 30°C in TY medium (3). The *Escherichia coli* strains were grown at 37°C in LB medium (30) supplemented with antibiotics when appropriate.

DNA manipulations. Total bacterial genomic DNA was isolated as described previously (30). A genomic DNA clone bank of strain USDA 424 was prepared as previously described (31). Genomic DNA was partially digested (31) with restriction enzyme *Sal*I, and 20-kb (size-selected) fragments were cloned into the *Sal*I site of cosmid vector pVK102 (15). Cosmids were packaged in vitro by using a Packagene kit (Promega, Madison, Wis.). Plasmid pR32, a nodulation gene-containing cosmid from strain USDA 110 (25), was digested to completion with *Hin*dIII and *Eco*RI, and fragments corresponding to the *nodAB* and the *nolA* gene regions (28) were electroeluted from agarose gels. Fragments flanking the strain USDA 424 *nolA* gene region were cloned into the plasmid pUC18. Gene bank clones were physically mapped by using restriction enzymes *Sal*I, *Bam*HI, *Eco*RI, *Hin*dIII, and *Xho*I. Restriction enzymes were purchased from U.S. Biochemical Corporation, and T4 DNA ligase was from New England Biolabs. Restriction fragments were separated by horizontal electrophoresis on 0.7% agarose gels in Tris-EDTA-borate buffer (31). For hybridizations, DNA was transferred to Nytran membranes (Schleicher & Schuell, Inc., Keene, N.H.) as described previously (31). The ^{32}P-labeled probes were prepared by random primer labeling (Multiprime System, Amersham Corp., Arlington, Ill.) according to the manufacturer's instructions and hybridized to filters as described previously (30). Mutant 671-21 was isolated by random Tn*5* mutagenesis (14) of strain USDA 438 and has the ability to nodulate the serogroup 123-restricting soybean genotypes (26a). Plasmid pMAP1.4 was constructed by cloning the Tn*5*-containing *Eco*RI fragment of mutant 671-21 into *Eco*RI-digested pUC18 DNA. The pMAP1.4 gene region was physically mapped by using restriction enzymes *Eco*RI, *Hin*dIII, *Bam*HI, *Kpn*I, and *Sal*I. Fragments flanking the Tn*5* insertion site in pMAP1.4 were subcloned into the appropriate restriction sites of plasmid pUC18. The plasmid pRJ4108, which contains RSα and RSβ (1), was used as a gene probe to test for sequence homology to the serocluster 123 repeated DNA regions.

RESULTS AND DISCUSSION

Isolation of a reiterated gene region from strain USDA 424. We have previously shown that the broad-host-range serogroup 123 strain USDA 424 contains a functional GSN gene (*nolA*) region which extends the host range of nodulation-restricted *B. japonicum* serogroup 123 strains (28). To isolate and examine the USDA 424 GSN gene region, we hybridized strain USDA 110-derived *nodAB* and *nolA* gene probes to a strain USDA 424 genomic DNA clone bank. Four overlapping clones (pFR25, pFR26, pFR28, and pFR30), which hybridized to the probes, were isolated from the gene library (Fig. 1). Physical mapping revealed a very high conservation between the USDA 110 and USDA 424 fragments that carry the *nodD1YABC* genes and the GSN gene region (Fig. 1A). However, in strain USDA 424, these regions were separated by a 3.4-kb *Hin*dIII fragment (pFR2503), which is almost twice as long as the corresponding fragment in strain USDA 110. In strain USDA 110, this region has been shown to contain the *nodD2* gene (6). To examine the physical relationship between the USDA 424 *nod* and GSN gene regions in more detail, we digested the 3.4-kb *Hin*dIII fragment cloned in pFR2503 with *Sal*I or *Hin*dIII plus *Eco*RI and the 2.4-kb *Hin*dIII GSN-gene-containing fragment with *Sal*I. The resulting fragments were electroeluted from agarose gels and subcloned in pUC18 (22). These fragments were designated pFR2505 to pFR2510 (Fig. 1B).

When the subcloned fragments from pFR2507 to pFR2510 were eluted from agarose gels and used as hybridization probes against *Hin*dIII-digested genomic DNAs from a large number of *B. japonicum* serocluster 123 strains, *B. japonicum* strains from other serogroups (USDA 6, USDA 38, USDA 110, and USDA 62), and the DNA homology group II *Bradyrhizobium* spp. strains (USDA 31, USDA 46, USDA 61, USDA 76, USDA 94, and USDA 130), strong hybridization homology was found only with the *B. japonicum* strains (Fig. 2 and 3A). All of the probes failed to hybridize to

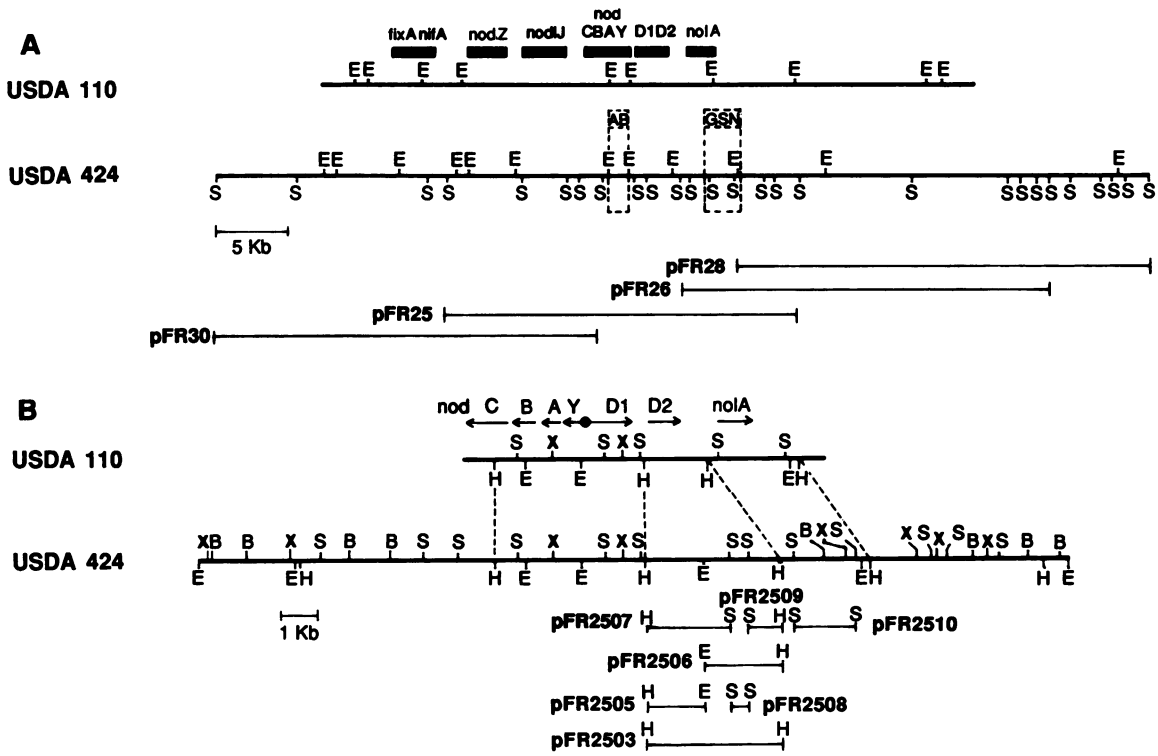

FIG. 1. (A) Comparison of physical and genetic maps of strains USDA 110 and USDA 424 and the physical relationship of pFR cosmid subclones to the map of USDA 424. (B) Physical relationship of USDA 110 and USDA 424 *nod* and *nol* gene regions and the location of corresponding pFR subclones. Symbol: ●, the *nod* box.

genomic DNAs from wild-type strains of *R. leguminosarum* bvs. viceae and trifolii and *Agrobacterium tumefaciens*. While the GSN gene region probe (pFR2510) was highly conserved in all the strains tested (Fig. 3B), the 3.4-kb *Hin*dIII fragment (present in pFR2503) was extremely reiterated (up to 21 times) in all of the *B. japonicum* serocluster 123 strains. With *Hin*dIII-digested genomic DNAs, there was little or no relationship between hybridization patterns and serogroup. Since the hyperreiteration (i.e., extremely reiterated hybridization pattern) was found with several of the subclones (pFR2507, pFR2508, and pFR2509) of the 3.4-kb *Hin*dIII fragment, our results suggested that a repeated element was either distributed in several places in the DNA fragment or overlaps these three cloned fragments. A 500-bp *Sal*I fragment (cloned in pFR2508) was the smallest fragment tested showing a hyperreiterated hybridization pattern in the serocluster 123 strains (Fig. 2B). Similar results were obtained when all of the gene probes were hybridized to *Eco*RI-digested genomic DNAs from the same *B. japonicum* strains (data not shown).

When the same gene probes (inserts from pFR2507, pFR2508, pFR2509, and pFR2510) were hybridized to *Hin*dIII-digested genomic DNAs from strains in other *B. japonicum* serogroups (USDA 4, USDA 6, USDA 38, USDA 110, and USDA 62, which represent members of *B. japonicum* DNA homology groups I and IA [10]), hyperreiteration was not found (Fig. 3C to F). However, in some of the strains, up to four hybridizing fragments could be seen with the pFR2507 and pFR2509 gene probes. With the pFR2508 and pFR2510 (GSN) probes, however, the strains had only one strongly hybridizing fragment (Fig. 3D and F). Weak or no hybridization homology (with any of the tested gene probes) was found with DNAs from several *B. japonicum* sp. strains. While *Bradyrhizobium* sp. strain USDA 94 failed to hybridize to probes pFR2507, pFR2508, and pFR2509, strains USDA 31, USDA 46, USDA 61, USDA 76, and USDA 130 had a limited number (two to four) of weakly hybridizing *Hin*dIII fragments (data not presented). All of these strains are in DNA homology group II (10) (or fatty acid group II [17]) and have been shown to have genetically diverged from the homology group I, serocluster 123 strains. It should be noted, however, that all hybridizations were done under high-stringency conditions and that more hybridizing fragments may be exhibited by using less-stringent reaction conditions. Nevertheless, these results indicated that the hyperreiteration profile seen with the tested gene probes is a unique property of *B. japonicum* serocluster 123 strains.

The presence of the reiterated DNA regions located between the USDA 424 *nodAB* and GSN genes is of particular interest to us. We are currently investigating whether these reiterated DNA regions alter the genomic stability of strain USDA 424. We have previously shown that the GSN gene *nolA* from strain USDA 110 is fairly unstable when transferred to serocluster 123 strains (28). It is possible that reiterated sequences, flanking both *nod* and GSN genes, affect the genomic stability of this region and in some manner influences the genotype-specific nodulation of soybeans. Given the high degree of reiteration seen within and outside of the nodulation gene region, genetic recombination between reiterated sequences seems probable.

Isolation of repeated DNA sequences from strain USDA 438. *B. japonicum* USDA 438 is a serogroup 123 isolate which is restricted for nodulation by several USDA 123-restricting soybean PI genotypes (13, 28). We have previously reported

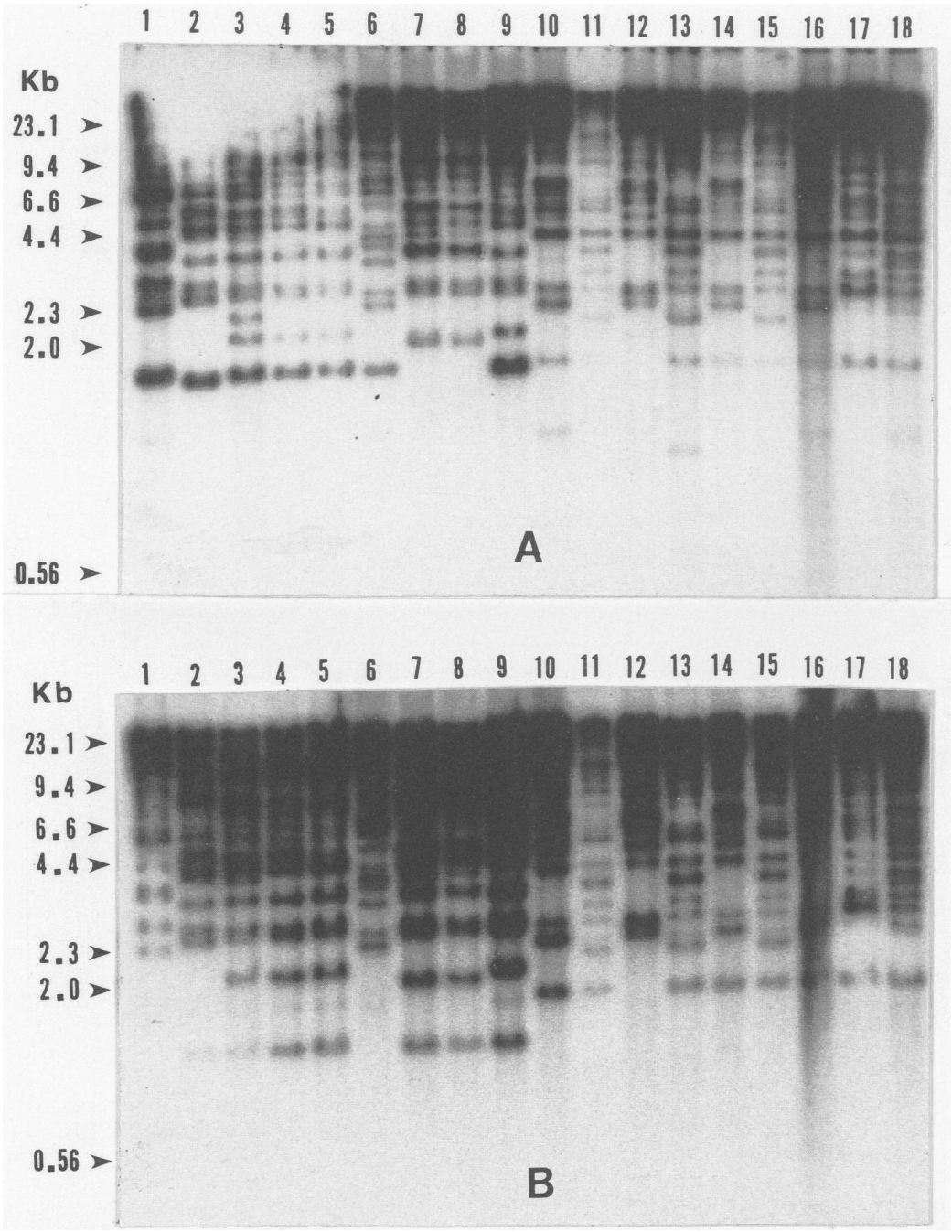

FIG. 2. Hybridization of probes pFR2507 (A) and pFR2508 (B) to *Hin*dIII-digested genomic DNA from *B. japonicum* serocluster 123 strains. Lanes: 1, USDA 430; 2, USDA 422; 3, USDA 423; 4, USDA 427; 5, USDA 426; 6, USDA 428; 7, USDA 435; 8, USDA 129; 9, USDA 429; 10, USDA 431; 11, USDA 425; 12, USDA 424; 13, USDA 127; 14, USDA 432; 15, USDA 437; 16, USDA 438; 17, USDA 162; and 18, USDA 123.

the isolation of a USDA 438:Tn*5* mutant (671-21) which has an extended host range for nodulation of the USDA 123-restricting soybean PI genotypes (26a). The DNA regions containing (and flanking) the Tn*5* insertion were cloned in pUC18, and we have named this plasmid pMAP1.4 (Fig. 4). Together, the flanking regions contain about 12 kb of *B. japonicum* genomic DNA. Hybridization analyses done by using plasmid pR32 (25) indicated that the Tn*5* insertion in mutant 671-21 was not in the *B. japonicum* common, host-specific, or GSN genes (*nodD1YABC*, *nodSUIJ*, *nodZ*, *nodD2*, or *nolA*) (data not shown).

When the entire cloned DNA region (pMAP1.4) was used as a hybridization probe against genomic DNAs from *B. japonicum* strains in several serogroups, extensive hybridization homology was found only with the serocluster 123 strains, with one exception (Fig. 5). In the serocluster 123

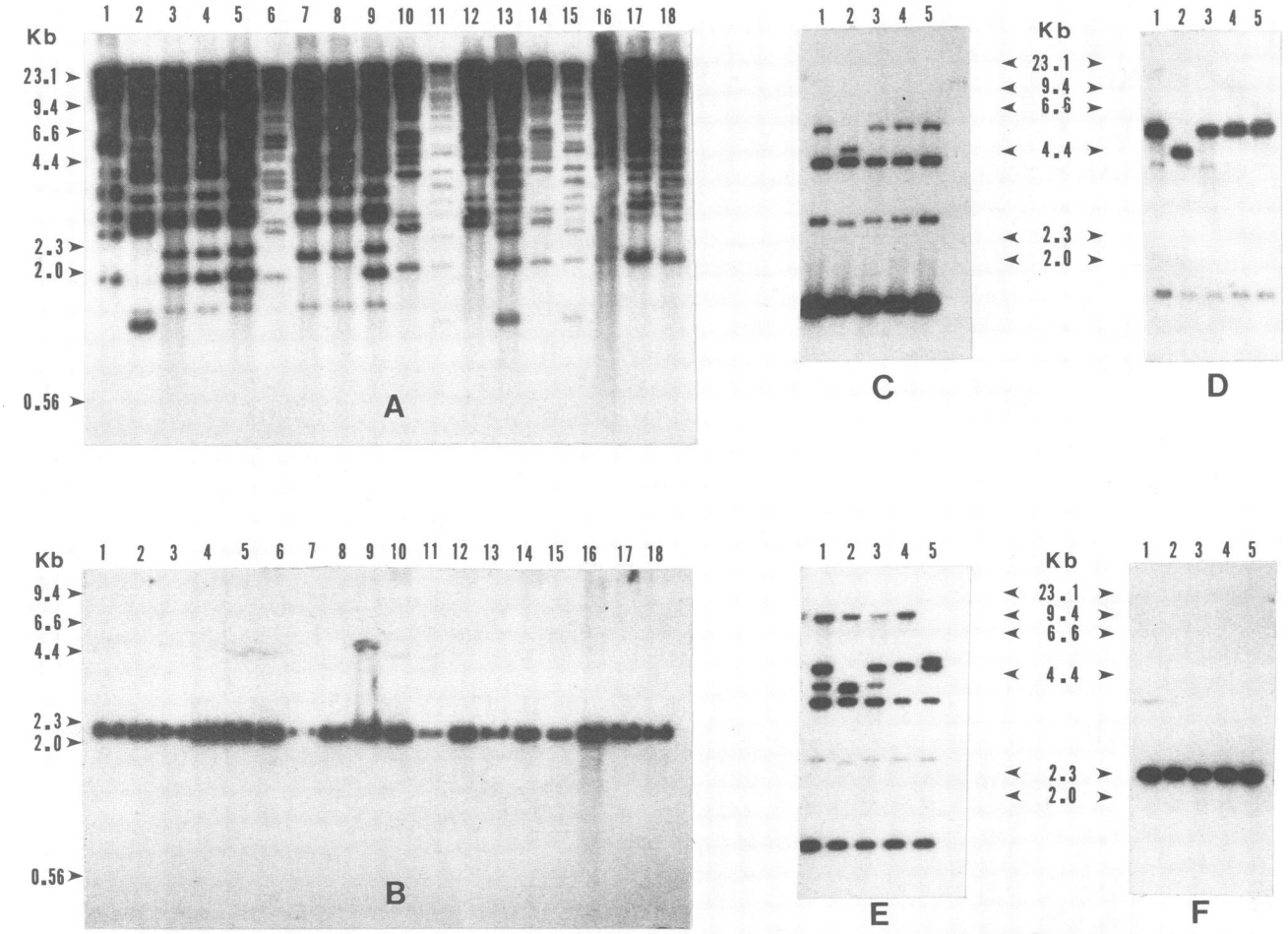

FIG. 3. Southern hybridization of probes pFR2509 (A) and pFR2510 (B) to *Hin*dIII-digested genomic DNA from *B. japonicum* serocluster 123 strains. Lanes in panels A and B: 1, USDA 430; 2, USDA 422; 3, USDA 423; 4, USDA 427; 5, USDA 426; 6, USDA 428; 7, USDA 435; 8, USDA 129; 9, USDA 429; 10, USDA 431; 11, USDA 425; 12, USDA 424; 13, USDA 127; 14, USDA 432; 15, USDA 437; 16, USDA 438; 17, USDA 162; 18, USDA 123. DNA from strains in panels A and B were the same. Hybridization of probes pFR2507 (C), pFR2508 (D), pFR2509 (E) and pFR2510 (F) to *Hin*dIII-digested DNA from *B. japonicum* strains. Lanes in panels C to F: 1, USDA 4; 2, USDA 6; 3, USDA 38; 4, USDA 110; and 5, USDA 62. DNA from strains in panels C to F were the same.

strains (USDA 123, USDA 127, USDA 129, USDA 438, and mutant 672-21), the pMAP1.4 gene probe produced an extremely reiterated hybridization pattern (Fig. 5, lanes 12, 14, 15, 18, and 19, respectively). In some instances, up to 21 hybridizing fragments could be seen by using the pMAP1.4 probe. This suggested that a DNA sequence(s) within pMAP1.4 was hyperreiterated in the genome of *B. japonicum* serocluster 123 strains. Interestingly, the extra-slow-growing, alkaline-tolerant strain USDA 135 (7) also showed a hyperreiterated hybridization pattern with the pMAP1.4 probe.

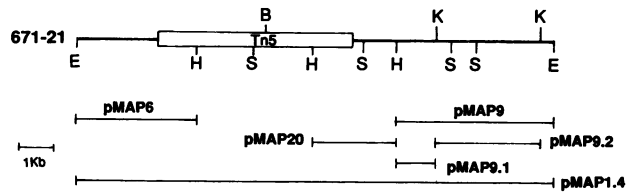

FIG. 4. Physical relationship of subclones of the pMAP1.4 gene region to the Tn*5* insertion site in the USDA 438::Tn*5* mutant 671-21. The fragments indicated were subcloned into the corresponding restriction sites of pUC18.

The other *Bradyrhizobium* strains examined either failed to hybridize (USDA 31, USDA 46, and USDA 61) or hybridized very weakly (USDA 4, USDA 6, USDA 38, USDA 62, USDA 76, USDA 110, USDA 122, and USDA 124) with the pMAP1.4 probe (Fig. 5). With the exception of strain USDA 122, all of the remaining strains had three to four weakly hybridizing *Eco*RI fragments.

To ascertain whether the hyperreiterated hybridization pattern was characteristic of other members of the serocluster, we hybridized pMAP1.4 to *Eco*RI-digested genomic DNAs from 17 additional *B. japonicum* serocluster 123 strains. This included one, eight, and nine strains in serogroups 129, 127, and 123, respectively. The results in Fig. 6 show that extensive, reiterated hybridization homology was found with all of the serocluster 123 strains, with the exception of strain USDA 185. While some of the strains had

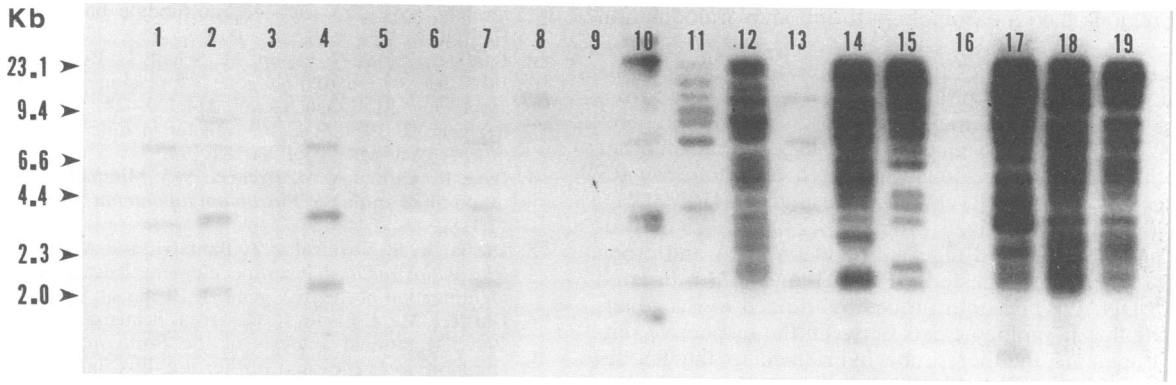

FIG. 5. Southern hybridization of the pMAP1.4 probe to *Eco*RI-digested genomic DNA from *B. japonicum* and *Bradyrhizobium* spp. strains. Lanes: 1, USDA 4; 2, USDA 6; 3, USDA 31; 4, USDA 38; 5, USDA 46; 6, USDA 61; 7, USDA 62; 8, USDA 76; 9, USDA 94; 10, USDA 110; 11, USDA 122; 12, USDA 123; 13, USDA 124; 14, USDA 127; 15, USDA 129; 16, USDA 130; 17, USDA 135; 18, USDA 438; and 19, mutant 671-21.

up to 21 *Eco*RI fragments which hybridized to the pMAP1.4 probe, strain USDA 185 only had five hybridizing fragments. All of the strains had at least one hybridizing *Eco*RI fragment that was more intense than the rest, suggesting that the probe differentially hybridized to the genomic DNA fragments. This could be due to either the genomic accumulation of reiterated sequences of a specific size class or to the fact that the different hybridizing fragments have slightly different sequence homology with the various portions of the pMAP1.4 gene probe. The results shown in Fig. 6 also indicate that, with the pMAP1.4 probe, there was no relation between a serogroup designation of a strain and its hybridization pattern. Plasmid pMAP1.4 did not hybridize to genomic DNAs from wild-type strains of *R. leguminosarum* bvs. viceae, trifolii, and phaseoli, *Rhizobium fredii* (strains USDA 191, USDA 205, and HH303), *R. meliloti* (strain 8501), *Rhizobium* spp. strains for *Lens* and *Leucaena* spp., and *A. tumefaciens*.

To establish which region(s) of pMAP1.4 was responsible for the hyperreiterated hybridization profile, we subcloned the *Hin*dIII, *Eco*RI-plus-*Hin*dIII, and *Kpn*I fragments of pMAP1.4 into pUC18 (Fig. 4). The subclones, designated pMAP6, pMAP20, pMAP9, pMAP9.1, and pMAP9.2 were individually hybridized to *Eco*RI-digested genomic DNAs from serocluster 123 strains. Subclones pMAP9, pMAP9.1, and pMAP9.2 showed hybridization patterns identical to that seen with pMAP1.4 (data not shown). This suggested that each of the subclones contained a similarly structured genetic element. In addition, while the pMAP6 and pMAP20 probes did not show the reiterated hybridization pattern, their hybridization profiles did provide a means to differentiate among serocluster 123 isolates.

To ascertain whether genomic DNA fragments cloned in pMAP9.1 and pMAP9.2 contained any sequences homologous to several of the known *B. japonicum* nodulation genes, we probed *Hin*dIII digestions of cosmid pR32 (containing the strain USDA 110 *nodD1YABC*, *nodSU*, *nodIJ*, *nodD2*, and *nolA* genes [25, 28]) with pMAP9.1 and pMAP9.2. Both plasmids failed to hybridize to pR32, indicating that the

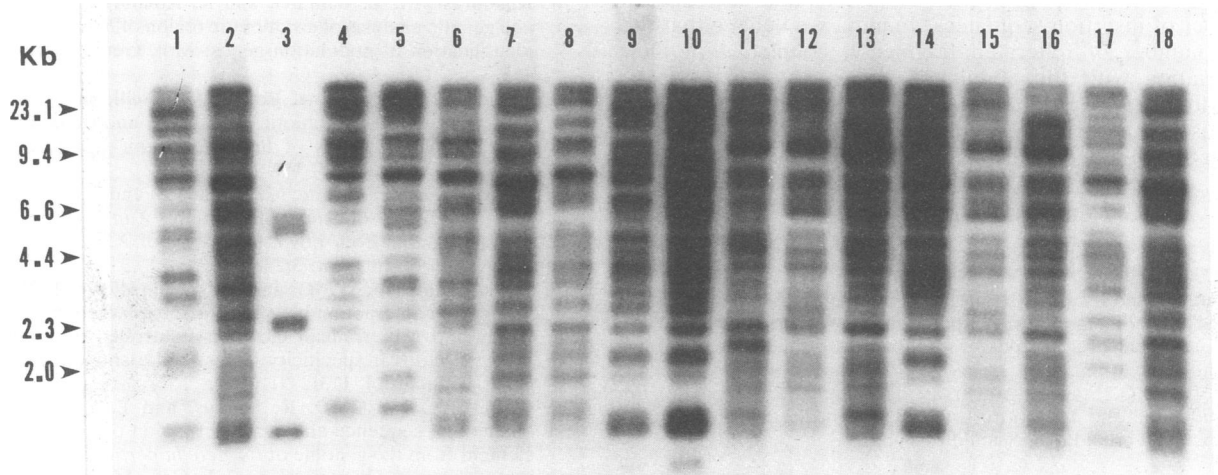

FIG. 6. Southern hybridization of pMAP1.4 probe to *Eco*RI-digested genomic DNA from *B. japonicum* serocluster 123 strains. Lanes: 1, USDA 430; 2, USDA 424; 3, USDA 185; 4, USDA 171; 5, Becker 4-N18; 6, IN56; 7, IN78; 8, IN34; 9, WI 3058; 10, PA3; 11, USDA 432; 12, IA5; 13, IA23; 14, IA35; 15, OH6; 16, USDA 436; 17, USDA 162; and 18, USDA 123.

cloned regions have no homology to the known nodulation genes.

We also tested whether the two reiterated DNA regions (cloned in pFR2503 and pMAP9), which were isolated from different serocluster 123 members (serogroup 123 and serogroup 127), shared any sequence homology with each other and with two of the previously reported *B. japonicum* RS sequences (RSα and RSβ) cloned in plasmid pRJ4108 (1, 11). To do this, we hybridized probe pFR2503 to *Hin*dIII-digested pMAP9 and *Xho*I-digested pRJ4108 DNA and probe pMAP9 to *Hin*dIII-digested pFR2503 and *Xho*I-digested pRJ4108 DNA. While both probes hybridized to the other, indicating that homology exists between the respective reiterated fragments, neither probe hybridized to the RS sequences cloned in plasmid pRJ4108. Thus, the newly isolated reiterated DNA regions appear collectively unique and do not share any sequence homology with the RS sequences and RS repeated elements. As was previously demonstrated by Acuña et al. (1), the USDA 110 RS repeated elements provide a number of target sites for the integration of cloned DNA into the host genome. The utility of this, however, is dependent on the stability of the reiterated fragments and their potential for excision. We are currently investigating whether the newly isolated reiterated regions can be used, in an analogous manner, to integrate foreign DNA into the genomes of serocluster 123 strains.

In summary, our results indicate that pFR2503 and pMAP9 contain similar, reiterated DNA sequences which are associated with both the known nodulation genes and with other portions of the *B. japonicum* serocluster 123 genome. The sequence located on a 3.4-kb *Hin*dIII fragment in strain USDA 424 is specific to *B. japonicum* and allows for the easy identification of serocluster 123 strains. The fact that the USDA 424 reiterated sequence is so closely clustered to the common *nod* operon suggests that it may play a role in nodulation by this strain. We are currently investigating whether the 3.4-kb DNA fragment affects expression of the GSN region and thereby alters the symbiotic behavior of serocluster 123 strains. However, since another similar reiterated gene region, from strain USDA 438, was not found near or within the *B. japonicum nod* gene region, our results indicate that all of these repeated elements do not preferentially accumulate in the nodulation genes. The location of the repeated elements in the genome may be simply due to random DNA jumping events, similar to that seen with a number of insertion sequences. Finally, we believe that the high number of reiterated fragments, coupled with their association with the nodulation regulatory regions, may contribute to both genetic instability and genetic diversity because of either DNA jumping events or homologous recombination within the serocluster 123 genome.

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