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The *Bradyrhizobium japonicum* Serocluster 123 Hyperreiterated DNA Region, HRS1, Has DNA and Amino Acid Sequence Homology to IS*1380*, an Insertion Sequence from *Acetobacter pasteurianus*

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We have sequenced and analyzed the hyperreiterated DNA region, HRS1, from *Bradyrhizobium japonicum* USDA 424. The 2.1-kb HRS1 fragment is closely linked to the *B. japonicum* common and genotype-specific nodulation genes in serogroup 123 and 127 strains. Southern hybridization analyses indicated that one copy of HRS1 is also located next to the *fixRnifA* locus in *B. japonicum* USDA 424. Nucleotide sequence analysis revealed the presence of a 4-bp target site duplication in HRS1 which is identical to a terminal repeat found in the *B. japonicum* USDA 110 repeated sequence RSα. Computer searches of the PIR (Protein Identification Resource) protein data base revealed a high degree of amino acid sequence homology between a putative 329-amino-acid polypeptide from HRS1 and a large polypeptide from IS*1380*, an insertion sequence from *Acetobacter pasteurianus*. RNA slot blot hybridizations suggest that transcripts showing homology to HRS1 are constitutively produced in strains USDA 424 (serogroup 127) and USDA 438 (serogroup 123).

Bradyrhizobium japonicum serocluster 123 is composed of an important group of indigenous competitors for the nodulation of soybeans in the upper midwestern United States. Biochemical and genetic analyses have indicated that serocluster 123 consists of a genetically diverse group of microorganisms that vary in their nodulation phenotype when inoculated on nodulation-restricting plant introduction (PI) genotypes (16, 19, 22).

We have recently isolated a serocluster 123-specific hyperreiterated DNA region which may contribute to both genetic diversity and genetic instability within the genomes of serocluster 123 strains (13). We have called this region HRS1. DNA probes from HRS1 failed to hybridize (or hybridized poorly) to other *B. japonicum* or *Bradyrhizobium* sp. strains and did not hybridize to DNA from fast-growing strains of *Rhizobium* spp. An interesting feature of this reiterated sequence is that it is closely associated with the common and genotype-specific nodulation genes in the serocluster 123 strain USDA 424 (13). Strain USDA 424 is a broad-host-range serogroup 127 isolate that has the ability to form nodules on the nodulation-restricting PI genotypes (16, 17).

Repeated DNA sequences have also been found in the genomes of several other strains of rhizobia (2, 3, 12, 14, 25–27). The *Rhizobium meliloti* insertion sequence IS*Rm2* has been shown to preferentially transpose into the nitrogen fixation and nodulation genes. In addition, the *R. meliloti* insertion sequence IS*p-Rm2011-2* has been previously shown to promote transcription of adjacent genes (8).

Most of these insertion sequence elements appear to be species specific and do not show significant nucleotide sequence homology to other insertion sequences. Similarity at the amino acid level, however, has been found with the *R. meliloti* insertion sequence IS*Rm-3* (26), and a putative 400-amino-acid polypeptide from IS*Rm-3* shows 52 and 49% similarity to putative transposases from *Staphylococcus aureus* (IS*256*) and *Thiobacillus ferrooxidans* (IS*T2*), respectively (26).

In *B. japonicum* USDA 110, a 1.1-kb repeated sequence, RSα, has been shown to cluster in close proximity to several of the nitrogen fixation genes. Although transposition has not been shown, RSα has properties characteristic of a prokaryotic insertion sequence (7). The RSα nucleotide sequence showed the presence of a 4-bp target site duplication (CTAG) and 5-bp terminal inverted repeats. Spontaneous deletions were generated by homologous recombination between neighboring RSα elements, resulting in the loss of several nitrogen fixation genes (6). It was also demonstrated previously that RSα provides a number of target sites for the integration of cloned DNA into the host genome (1).

In this study, we present the nucleotide sequence of the USDA 424 hyperreiterated DNA region, HRS1, and show that it has properties similar to those of an insertion sequence element. We also show that HRS1 shares significant amino acid homology with IS*1380*, an insertion sequence identified in *Acetobacter pasteurianus* (24). Moreover, our data suggest that there is a correlation between a restricted nodulation phenotype and the presence of this repeated DNA element.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *B. japonicum* strains were obtained from the Rhizobium Culture Collection of the Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md., and have been previously described (16, 19). All *Bradyrhizobium* strains were grown at 30°C and maintained on AG medium (15). The *Escherichia coli* strains were grown on LB medium (20) at 37°C and supplemented with ampicillin (30 μg/ml) and tetracycline (50 μg/ml) when appropriate.

DNA manipulations. Total bacterial genomic and plasmid DNA was isolated as previously described (19, 20). For hybridizations, DNA was transferred to nylon membranes and hybridized to ^{32}P-labelled probes as previously de-

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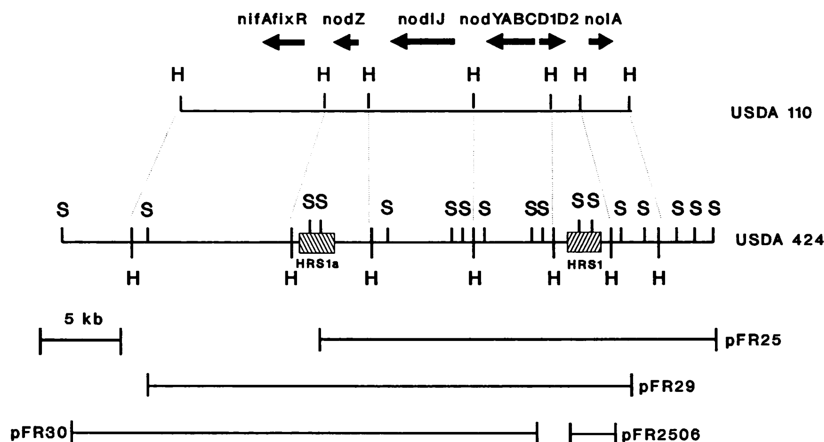

FIG. 1. Comparison of physical and genetic maps of strains USDA 110 and USDA 424 and the physical relationship of two copies of HRS1 and the pFR subclones to the map of USDA 424. The fragments indicated were subcloned into the *Sal*I (S) restriction site of pVK102, except for pFR2506, which was cloned into the *Eco*RI and *Hin*dIII (H) sites in pUC18. Arrows indicate the direction of transcription.

scribed (19). Restriction fragments were separated by horizontal electrophoresis on 0.7% agarose gels and electroeluted onto DEAE nitrocellulose paper as described previously (16). Fragments were ligated (20) into the multiple cloning site of pUC18 (11).

RNA isolation and hybridization. Total RNA from flavonoid-induced and uninduced *B. japonicum* and *E. coli* strains was isolated by the hot-phenol method (10). Cultures were induced for 16 h with 1 μM apigenin as described previously (18). Slot blot RNA-DNA hybridizations were performed as described previously (20). The ^{32}P-labelled DNA probes were prepared by random primer labelling and were hybridized for 18 to 24 h as described previously (19, 20).

DNA sequence analysis. The nucleotide sequence of the hyperreiterated DNA fragment, HRS1, was determined by sequencing double-stranded DNA by the dideoxy chain termination method (21). The sequencing strategy used is shown in Fig. 3A. Each DNA region was independently sequenced at least three times. Primers consisted of the pUC18 universal and reverse primers (United States Biochemical) and commercially generated 15-mer oligonucleotides (Bio-Synthesis, Inc., Lewisville, Tex.) specific for particular regions of HRS1. Computer-aided DNA and protein sequence analyses were performed with the EuGene software package (Department of Microbiology, University of Minnesota). The FASTA and SEARCH computer programs were used to find homologous sequences in the GenBank and PIR (Protein Identification Resource) data bases, respectively.

Nucleotide sequence accession number. The nucleotide sequence presented in this article has been submitted to GenBank and can be found under accession number L09226.

RESULTS

DNA sequence analysis. To determine whether HRS1 contains any of the structural features characteristic of other repeated elements, we sequenced the HRS1-containing DNA region which is located between the *nodD2* and *nolA* genes in *B. japonicum* USDA 424 (Fig. 1). The complete nucleotide sequence of HRS1 is shown in Fig. 2. Comparison of the nucleotide sequence from this region with the nucleotide sequences from *nodD2* (5) and *nolA* (17) shows the presence of a 2,074-bp region of DNA in USDA 424 which is not present in strain USDA 110 (Fig. 1). This region was named HRS1. A computer search of the GenBank DNA sequence data base indicated that the nucleotide sequence of HRS1 shows 53% similarity to the *A. pasteurianus* insertion sequence IS*1380* within a 1-kb overlap of DNA (24). The HRS1 nucleotide sequence does not, however, show any significant nucleotide sequence similarity to previously reported DNA sequences from any *Rhizobium* or *Bradyrhizobium* strains.

Within the nucleotide sequence of HRS1, we found four large open reading frames (ORFs), all beginning with an ATG start codon (Fig. 3B). Each ORF was translated into a peptide sequence and used to search the PIR protein data base. Only ORF1, a putative 329-amino-acid polypeptide, showed significant homology to any previously reported peptide sequences. Results of this data base search are presented in Fig. 4 in the form of an amino acid sequence alignment between ORF1 and a 461-amino-acid polypeptide from IS*1380* (24). These two peptide sequences share 31% identity at the amino acid level. When conservative amino acid replacements are considered, however, the similarity at the amino acid level increases to 78% within an overlap of 278 amino acids.

When translated, ORF1 codes for a 37,500-molecular-weight polypeptide which contains a high number (n = 66) of basic amino acid residues. The high basic amino acid content is characteristic of peptides produced from a variety of insertion sequences (4). Analysis of the DNA region preceding ORF1 showed the presence of a possible promoter region which resembles the *E. coli* consensus Pribnow box and −35 hexamers (9). The putative promoter area begins 170 bp upstream of the putative translational start site of ORF1 (Fig. 2). Also present in the nucleotide sequence is a potential Shine-Dalgarno ribosome-binding site located at the −7 position of ORF1 (Fig. 2) (23).

Analysis of the HRS1 termini. To determine whether HRS1 contains any other structural features characteristic of repeated elements, we examined HRS1 for the presence of inverted or direct repeats. The ends of HRS1 contain a 5-bp direct repeat (GCTAG) which corresponds to a perfect target site duplication (Fig. 2). No inverted repeats, however, were found at the HRS1 termini. Comparison of a portion of this

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1     TTTAGGGACGGGAACGGAACGGGGCCGGCGAGCGACGGAGCAATGCCTTCGCACTGAAGC
61    TAGCCCGTCTTATTCGCGAGAGGGCGCCTGAGAGGCTGGCGAGCGTCGTGTAAAGCGCTG
121   ATGCGGTCGTAGGATTCGGTTGCGAAGCCACCCCATCACCTTCAACCGCGAACGCCACGC
181   CCGCCATGACCGATGATACGATTTCAGACCACTATCGAGAAGCAAGAATTCAACACAAAT
241   AAAACCTCTGCCTCGCGCTCGATCTCTTCATGCTTGATAGTCTCGTTTCGGAGGCTGATC
                                                         -35
301   ACCTACCGCGTCTAATAGGCCGCTCGCGACCCGGTTTCTTTGGACGCGGCACGACGCACG
                -10
361   CTCCACGTTTCGGCACAGAAATGATCGGGAGTCACTCAATCTTTCTTCAACTTAACCCGT
421   CTTATTCGTAAGAGTGCGCCTGAGGGGTAAAGCGCTGATGCGCGTAGGATTCGGTTGCGA
                                   RBS      M  R  V  G  F  G  C  E
481   ACGCAACCCCATCACCTCCAACCGCGAACGCCACGCCCGCCATGACCGACGATACGATTC
       R  N  P  I  T  S  N  R  E  R  H  A  R  H  D  R  R  Y  D  S
541   CGCCCTTCTCGTTTCCAGCCGTTCACGCCAAGAAAGTCACAGCTGCCTTCGATGGTGGGC
       A  L  L  V  S  S  R  S  R  Q  E  S  H  S  C  L  R  W  W  A
601   TCCTAACCTCGAACGGGGGCGTGATGCTTCTGGCGATGGCCGGCGGCGGGACGGTCTCGG
       P  N  L  E  R  G  R  D  A  S  G  D  G  R  R  R  D  G  L  G
661   TTTGGCCAACAATCTGGCCCGGGTGTTCCCGGATCGGCGCGATCCGACGCGGGTCATGCA
       L  A  N  N  L  A  R  E  V  F  P  D  R  R  D  P  T  R  V  M
721   CAGCCTGGTCGATATGTTCCGCGCTCGCATGTTCGCGATCTGCTGCGGCTACGAGGACGC
       H  S  L  V  D  M  F  R  A  R  M  F  A  I  C  C  G  Y  E  D
781   CGACGACCTCGATCATCTGAGGTCCGATCCGGCATTCAAACTGGCTGCGGACGGCGTCCG
       A  D  D  L  D  H  L  R  S  D  P  A  F  K  L  A  A  D  G  V
841   GACACGGGCCGGGATTTGTGTTCCCAGCCGAGCTGTCGCGGCTGGAGAATGCTCGCGCCT
       R  T  R  A  G  I  C  V  P  S  R  A  V  A  A  G  E  C  S  R
901   GCGCGACGTGATCCGGCTGACCTACATTTTGGTCGACGCATGGATGGATAGCTACCCGCG
       L  R  D  V  I  R  L  T  Y  I  L  V  D  A  W  M  D  S  Y  P
961   CGACGCGGCATCCGTCACGCTCGACATCGATGATACCTGCGACGTCGTCCACGGCCATCA
       R  D  A  A  S  V  T  L  D  I  D  D  T  C  D  V  V  H  G  H
1021  GCAGCTCTCGCTGTTCAACGCTCATTATGACGAACGCTGCTTCCTGCCGATCCACGTCTA
       Q  Q  L  S  L  F  N  A  H  Y  D  E  R  C  F  L  P  I  H  V
1081  CGACACGGAGAAGAGCCGGCCCGTGCTGCGTGCTGCGGCCCGGCAAGACGCGCGCGTCGA
       Y  D  T  E  K  S  R  P  V  L  R  A  A  A  R  Q  D  A  R  V
1141  GGTGCGTGCCCATCTGCGCCGCCTGGTACGGCATATCCGGACGCGATGGCACAACACGCA
       E  V  R  A  H  L  R  R  L  V  R  H  R  T  R  W  H  N  T  Q
1201  AATTACGTTCCGTGGCGAGCGCACTATGCCCGCGGAGGCCATGGCGTGGTGCGAGACCAA
       I  T  F  R  G  E  R  T  M  P  A  E  A  M  A  W  C  E  T  N
1261  CGGCATCGACTACATCTTCGGTCTGTCGGCACCAAGCCTCTCGCGCAGAAAAGTCGACGA
       G  I  D  Y  I  F  G  L  S  A  P  S  L  S  R  R  K  V  D  E
1321  GGTCGCCGACGACATCCGCACGCGACGCGCCCATCGAGAACCTGCCGGTTCTGCGTGGCT
       V  A  D  D  I  R  T  R  R  A  H  R  E  P  A  G  S  A  W  L
1381  ATACCGAGACGACGCCACAAGGCAAAGTCCTGGGATCGCGAACGGGCCACTGTCGCCGTA
       Y  R  D  D  A  T  R  Q  S  P  G  I  A  N  G  P  L  S  P  Y
1441  TTGAGGCGACGATGCTCGGCCTCGACATCCGCTTCGTCGTCACCAGCCTCGATGTCGGCT
      ***
1501  CGGACCGAGTGGATCTACGACAGCCTGTATTGCGCGACGAGCCAAGCCGAGAATCTGATC
1561  AAGCTGCATAAGACGCAGCTCGCCTCCGATCGCACCAGCTGCCGTTCGGCGCTCGCCAAC
1621  CAGGTCCGTCTCGTGCTCCATACGGCCGCTTATTGGCTGATGCTGACCGTGCGCGACGCC
1681  ATTCCCAAAGCCCGGGAATTGGCCGCTGCCGAGTTCGCGACGCTGCGTCTTCGGCTCTTG
1741  AAAATCGCCGCCCGTGTCGTCGAGACCACGAGCCGCGCATTCGCCTTGCGTTTGCCGCGG
1801  CATGTCCCGAAGCCGACCTGATCTGCGGCTTGCCCGGCGCGCTGCTGCCGCTCGGTCCTT
1861  GACGGCGCGTCCGCCCCCGTTCGCCCAACCCTTCGCCTTGCGTTTGCCGCGGCATGTCCC
1921  GAAGCCGACCTGATCTGCGGCTTGCCCGGCGCGCTGCTGCCGCTCGGTCCTTGACGGCGC
1981  GTCCGCCCCCGTTCGCCCAACCCATCCCTCAAGCGCGTTGCAAAGTACCGGTCGTCAGGC
2041  GGCGAAAAGCCGAAGGCAATCCTGTGCGCCTCGTCAGACAAGATGTGCGGCCGCATCAAT
2101  CGGGCCCAAAAGCCGCACTCTCACGAATAGACGGCTAGGCGTTTACGTTGCAACGGCCGC
2161  GGCAGGGCGCAGCTCTCTCAAGAAGGTAGGCCAGGCACGGAGCTCTTGCCGTCAACTCCC
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FIG. 2. Nucleotide sequence of the HRS1-containing DNA region from *B. japonicum* USDA 424 and the amino acid sequence of a putative polypeptide encoded by ORF1. The left and right terminal 5-bp direct repeats (GCTAG) mark the ends of HRS1 and are underlined at base pair positions 59 and 2134, respectively. The putative Pribnow box (−10), −35 hexamers (−35), and Shine-Dalgarno ribosome-binding site (RBS) are underlined as shown, and the stop codon (***) is indicated.

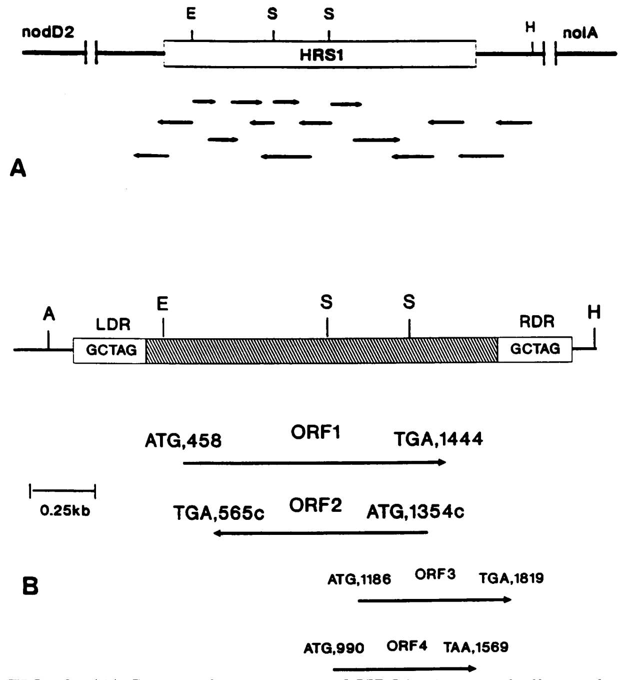

FIG. 3. (A) Sequencing strategy of HRS1. Arrows indicate the direction and extent of sequencing of individual clones. (B) Map of HRS1 showing left and right terminal direct repeats (LDR and RDR) and the locations of potential ORFs. The base pair location of each start and stop codon is indicated for each ORF. E, *Eco*RI; S, *Sal*I; H, *Hin*dIII; A, *Ava*I.

direct repeat (CTAG) with the target site duplications in the *B. japonicum* USDA 110 repeated sequences RSα7, RSα9, and RSα10 (Fig. 5) indicated that there was a high degree of conservation among these direct repeat sequences, suggesting that the integration of these repeated elements may show target site specificity (7).

Isolation of a second copy of HRS1 in USDA 424. In order to determine whether any additional copies of HRS1 are linked to the *nod* gene region in strain USDA 424, we examined three overlapping cosmid clones which show hybridization homology to *nodYABC* (Fig. 1). Using *Hin*dIII, *Eco*RI, and *Sal*I restriction endonuclease digestions and sequential hybridizations to ^{32}P-labelled HRS1, *nodZ*, and *nifAfixR* probes, we identified the location of a second copy of HRS1, called HRS1a, which is located between the *nodZ* and *nifAfixR* genes in strain USDA 424. The repeated sequence HRS1a is inserted in the opposite orientation relative to HRS1 (Fig. 1).

Transcription analysis. To determine whether any RNA transcripts originate within HRS1, total RNA was extracted from flavonoid-induced *B. japonicum* USDA 110 and USDA 424 and was hybridized to the 2.2-kb HRS1-containing probe, pFR2506. This probe, which was used to sequence HRS1, completely spans all four of the potential ORFs (Fig. 1). Hybridization homology to pFR2506 was seen only with RNA from USDA 424 (Fig. 6, row A) and was not seen with RNA from USDA 110.

Flavonoid-induced and uninduced RNA was also isolated from USDA 438 and hybridized to pFR2506. There was no discernible difference in hybridization intensity between the induced and uninduced RNAs from USDA 438, suggesting that a transcript(s) originating within the cloned DNA region

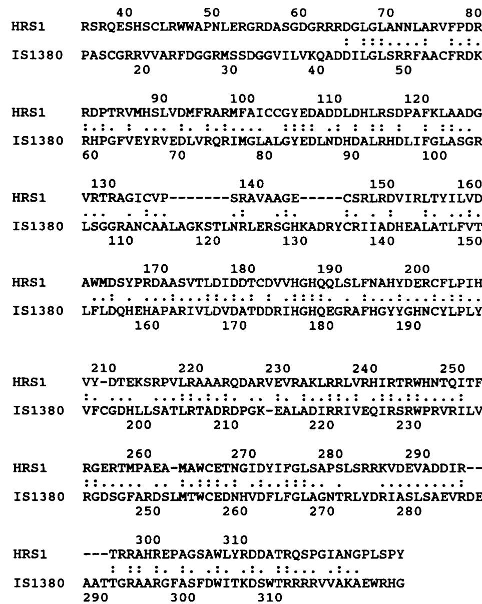

FIG. 4. Comparison of amino acid sequences from HRS1 (ORF1) and IS*1380*. Gaps have been introduced for maximum alignment. Identical (:) and conserved (.) residues are indicated.

may be constitutively produced in this serogroup 123 strain (data not shown). Note, however, that this experiment was done under only one type of inducing condition.

To determine whether the production of RNA transcripts homologous to pFR2506 is present in HRS1-containing recombinant *E. coli* strains, total RNA was extracted from apigenin-induced and uninduced cultures of *E. coli* containing cosmid clone pFR25 and hybridized to the probe, pFR2506. Cosmid pFR25 is an HRS1-containing, pVK102 clone which was originally used to isolate HRS1 (13). The induced and uninduced RNAs from *E. coli* (pFR25) did not hybridize to pFR2506, indicating that transcripts homologous to HRS1 are not produced in this recombinant strain (data not shown).

Association of HRS1 with genetic diversity and Nod$^-$ phenotypes. In order to ascertain whether all serogroup 127 strains contain a copy of HRS1 inserted between the *nodD2* and *nolA* genes, we hybridized pMJS9 (a 1.7-kb, *Hin*dIII fragment from USDA 110 which contains *nodD2* [15, 17]) to *Eco*RI-digested DNA from nine serogroup 127 strains (IA44, IN64, MN9, IA67, USDA 430, USDA 185, USDA 424, USDA 171, and USDA 127). Results in Fig. 7 show that

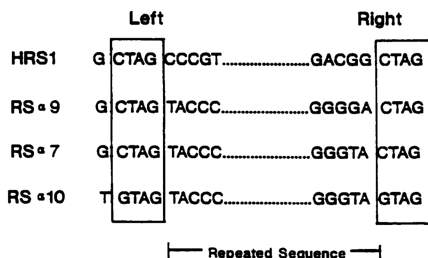

FIG. 5. Nucleotide sequence comparison of the left and right terminal direct repeats of HRS1 and the direct repeats found in three different copies of RSα.

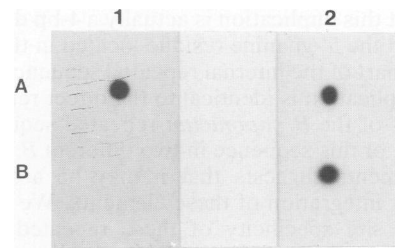

FIG. 6. RNA slot blot hybridization analysis of probes HRS1 (pFR2506) (lane 1) and *nodYABC* (pMJS18) (positive control) (lane 2) hybridized to total RNA extracted from flavonoid-induced *B. japonicum* USDA 424 (row A) and USDA 110 (row B).

strains USDA 185, USDA 171, and USDA 430 each had only one hybridizing *Eco*RI fragment approximately equal in size to the corresponding, non-HRS1-containing fragment in USDA 110. All other serogroup 127 strains (and serogroup 123 strains) showed two hybridizing fragments equal in size to those seen in USDA 424. This indicates that HRS1 is present in the same *Hin*dIII fragment in these strains, since the presence of HRS1 introduces an *Eco*RI site into the *nodD2*-containing *Hin*dIII fragment and, after digestion with *Eco*RI and hybridization with pMJS9, yields two distinct hybridizing bands. An analogous result was seen when genomic DNA from these eight serogroup 127 strains was digested with *Hin*dIII and hybridized to pMJS9.

In order to determine whether HRS1 influences the nodulation phenotype of these serogroup 127 strains, we compared their observed nodulation responses with the soybean PI genotype 371607 (16) with the presence or absence of HRS1 between the *nodD2* and *nolA* genes. All of the serogroup 127 strains which have HRS1 inserted between the *nodD2* and *nolA* genes, with the exception of USDA 424, are restricted for nodulation by PI 371607 (16). Those serogroup 127 strains (and USDA 110) which do not have HRS1 inserted between the *nodD2* and *nolA* genes are not restricted for nodulation by PI 371607. Taken together, our results suggest that there is a correlation between nodulation restriction by PI 371607 and the presence of HRS1 between the *nodD2* and *nolA* loci.

DISCUSSION

Using DNA sequence analysis, we show that HRS1 has several of the characteristics of known insertion sequences. The most significant feature is the presence of a 5-bp target site duplication (GCTAG) at the ends of HRS1. It is possible,

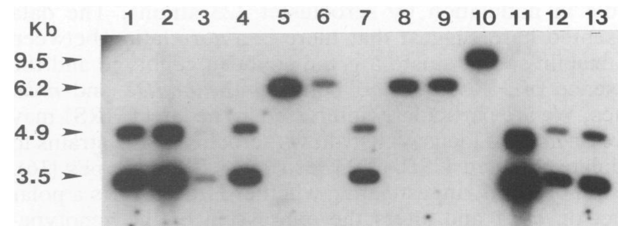

FIG. 7. Southern hybridization of probe *nodD2* (pMJS9) to *Eco*RI-digested genomic DNA from *B. japonicum* serocluster 123 strains. Lanes: 1, IA44; 2, IN64; 3, MN9; 4, IA67; 5, USDA 430; 6, USDA 185; 7, USDA 424; 8, USDA 171; 9, USDA 110; 10, USDA 129; 11, USDA 123; 12, USDA 438; 13, USDA 127. Molecular sizes (in kilobases) are on the left.

however, that this duplication is actually a 4-bp direct repeat (CTAG), with the 5′ guanine residue located in the right end repeat being part of the internal repeated sequence. The 4-bp target site duplication is identical to the direct repeats found in two copies of the *B. japonicum* repeated sequence RSα. The presence of this sequence in two different *B. japonicum* repeated elements suggests that it may be a site-specific target site for integration of these elements. We do not find the potential site specificity of these repeated sequences surprising, since many prokaryotic insertion sequence-like elements show different degrees of preference for particular nucleotide hot spots (4). We find it particularly interesting, however, that HRS1 and RSα have the same (or similar) target site duplications, since they otherwise lack significant nucleotide sequence homology.

If HRS1 is indeed an insertion sequence, the absence of terminal inverted repeats is not without precedent. The *Pseudomonas atlantica* insertion sequence IS*492* and the *Salmonella typhimurium* insertion sequence IS*200* do not contain terminal inverted repeats (4). However, since we have not shown that HRS1 can transpose, we have not ruled out the possibility that HRS1 is actually the remnant of a larger transposable element which has undergone a nucleotide rearrangement resulting in the loss of all or part of its terminal inverted repeat sequences.

The presence of a putative polypeptide (ORF1) which shows significant sequence homology to a polypeptide from IS*1380* strengthens the hypothesis that HRS1 is an insertion sequence. While neither polypeptide shows significant amino acid sequence homology to any previously reported insertion sequence proteins, both contain high numbers of basic amino acids, a characteristic of many proteins required for transposition of insertion sequence elements.

RNA-DNA slot blot hybridizations clearly show that transcripts originating within HRS1 are produced by strains USDA 424 and USDA 438. Although we have not determined experimentally that ORF1 is transcribed, analysis of the nucleotide sequences preceding each ORF indicates that ORF1 is the only ORF which has the features necessary for transcription.

Another interesting feature of HRS1 is the overlap of ORF1 and ORF2 and their locations on opposite strands of DNA. The overlapping arrangement of ORFs has been seen previously in a number of other insertion sequences, including IS*10*, IS*50*, and IS*903* (4). These insertion sequences all contain a large ORF required for transposition and one or more smaller ORFs found on the opposite strand which are of sufficient length that they cannot be discounted on the basis of possible random occurrences of ORFs in any given stretch of DNA (4).

Of great interest to our laboratory is whether HRS1 plays a role in nodulation by serocluster 123 strains. The data presented here suggest that there is a correlation between the inability to nodulate a given soybean genotype and the presence of HRS1 located between the *nodD2* and *nolA* genes. We are particularly interested in the effect HRS1 may have on *nolA*, a locus which allows serocluster 123 strains to nodulate selected USDA 123-restricting PI genotypes (16). We are presently investigating whether HRS1 exerts a polar effect on *nolA* and alters the expression of the genotype-specific nodulation region in nodulation-restricted strains and whether the integration of HRS1 into the genome may have disrupted the upstream regulatory domains of *nolA*, also preventing expression of this gene in restricted strains. We are also examining the effect that HRS1a may have on the *nifAfixR* genes in strain USDA 424. Although the nucleotide sequence has not been determined, restriction digests indicate that HRS1a is inserted in the opposite orientation relative to HRS1. It is interesting to note that in both HRS1 and HRS1a, ORF1 is in the same orientation as the surrounding *nod* and *nif* genes. Moreover, since ORF1 may be transcriptionally active, it could be influencing the transcription of downstream DNA regions.

In summary, due to the presence of target site duplications and its homology to known insertion sequence elements, our results indicate that HRS1 possesses the characteristics of a prokaryotic insertion sequence. While a direct relationship between HRS1 and nodulation restriction has not been fully established, our results suggest that there is a correlation between the ability to nodulate a given PI genotype and the presence of HRS1 between *nodD2* and *nolA*. In addition, the possibility of transposition by HRS1 and its related elements, coupled with their high copy number, further supports our contention that these elements contribute to both genetic diversity and genetic instability within serocluster 123 strains.

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