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Genomic Diversity of *Bradyrhizobium* Strains Compatible with Soybean (*Glycine max* L. Merr.) Harboring Various *Rj*–genes.

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Based on the different sequence of putative *Rj–gsn* gene reserved in *Bradyrhizobium* strains, which was concerned with the compatibility between some *Rj*–genotype soybeans and some *Bradyrhizobium* strains, the classification depended on sequence of genomic DNA of *Bradyrhizobium* strains of each nodulation type was thought possible to analyze by the method of restriction fragment length polymorphism (RFLP). Different rare–cutting enzymes *Ase* I, *Dra* I, *Spe* I and *Xba* I were chosen in order to ideally produce fragments greater than 200 kbp.

The dendrogram of *Ase* I was shown that type C strains were found mainly in one of two clusters, and type B strains in the other cluster. The type A strains were found in both clusters at similar frecuency. The dendrogram by *Dra* I was split into three clusters, and the type B and C strains were found exclusively in different cluster. The third cluster had all three types almost uniformly. That of *Sph* I formed small cluster containing type B strains, but it was recognized no tendency that each nodulation type strains formed cluster. That of *Xba* I was split into two clusters, and one cluster was split into two subclusters. However, this dendrogram was not clarified in the difference between two clusters in compared with that of *Dra* I.

The results of cross–agguligation reaction between each nodulation type strains with the antiserum of those typical strains indicate that strong cross–reaction was observed between some strains of type A and B in this experiment. Also, weak cross–reaction was known between some strains of type A and C (Ishizuka *et al.*, 1991).

These experimental results indicated that the type B strains were distantly related to the type C strains in regard to the structure of genomic DNA and that of surface of rhizobial cell, the type A strains were located intermediate between type B and C.

INTRODUCTION

Some soybean cultivars harboring *Rj*–gene(s) can nodulate selectively some specific serotypical strains of *Bradyrhizobium japonicum* and *B. elkanii* (Ishizuka *et al.*, 1991a, 1991b, 1993, Yamakawa *et al.*, 1999). Compatibility between soybean and rhizobium might be determined by an antigenic determinant existing on the surface of the rhizobium. The relationship between the structure of antigenic determinant and the compatibility has not been reported at present, but the structure of the lipopolysaccharide (LPS) containing *O*–antigen was reported from the study using *Rhizobium phaseoli* Tn*5* mutant that might involve successful gram–negative interactions with plant (Carlson *et*

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al., 1987). Thus we though that it was possible to clarify the genomic characteristics concerning to the compatibility of rhizobia with *Rj*–genotypic soybean cultivars by the genomic analysis. Genomic analyses of rhizobia were done in many methods until now, and the classification based on the farmily and species (Young 1992, Young and Downer 1991, Yanagi and yamamoto 1993), the compatibility between rhizobia and host plant species (Bjourson *et al.*, 1992) and the analysis about the hereditary character among some serotype of rhizobia (Judd *et al.*, 1993). However, study dealing with the relationship between the compatibility of soybeans harboring *Rj*–genes with *Bradyrhizobium* strains and the genomic structure of their strains was few (Saeki *et al.*, 2000).

The compatibility of legumes and rhizobia (species specificity) is now going to understand throuth the expressional responses of genes involved in rhizobial strains and species of host legumes (van Rhijin and Vanderleyden 1995). However, it was unclear until now that which gene(s) exiting in *Bradyrhizobium* was concerned to the compatibility (*Rj*–genotype specificity) with *Rj*–genotype of soybean. The gene(s) determining *Rj*–genotype specificity was thought to be different genes with species–specific genes which have been reported until now, namely *nodD1*, *nodD2*, *nodY*, *nodS*, *nodU* and *nodZ* found in *B. japonicum*, *B. elkanii* and *R. fredii* (van Rhijin and Vanderleyden 1995). In other words, although there must be some relationship between the formation of effective nodule and several biochemical processes functioning in rhizobial cell, the genes related to *Rj*–genotype specificity were with doing not become clear. Therefore, the classification of *Bradyrhizobium* strains based on the compatibility with soybeans houboring *Rj*–gene(s) could be impossible to utilize the information of DNA sequences of the known rhizobial genes involved in the nodule formation.

However, it was thought that some gene(s) concerned with the compatibility between a *Rj*–genotype of soybean and some specific *Bradyrhizobium* strains was made possible the classification into each nodulation type by presuming the different sequence of putative *Rj–genotype specific nodulation* (*Rj–gsn*) gene reserved in the rhizobial strain. So, the phylogeny based on sequence of genomic DNA of *Bradyrhizobium* strains was practiced to analyze by the method of restricted fragment length polymorphism (RFLP). Because this RFLP method shouldn't be necessary special probe and primer for the genomic analysis concerning to the expression of putative *Rj–gsn* gene excluding the digestion with restriction enzyme and electrophoresis of these digested DNA fragments. Genomes of bacterial species vary widely in their base composition. G+C content (Muto and Osawa 1987), as well as frequency of di– and tri–nucleotides (McClelland *et al.*, 1987) account for the relatively infrequent occurrence of particular restriction site. Different rare–cutting enzymes can be chosen in order to ideally produce fragments greater than 200 kbp yielding an electrophoretic pattern whose features reflect an genome constitution of concerned rhizobial strains.

We applied the pulsed field gel electrophoresis (PFGE) of the genome DNA's digests to classify different nodulation types of *Bradyrhizobium* strains and *Ase* I, *Dra* I, *Spe* I and *Xba* I were selected as rare–cutting endonucleases.

MATERIALS AND METHODS

Rhizobia and yeast: Rhizobial strains used in this study are listed in Table 1. They were

Table 1. Nodulation types and sources of *Bradyrhizobium* and *Rhizobium* strains.

Nodulation type		Strain(s)	Source[a]
A	*B.japonicum*	Is–17, Is–55, Is–77, Is–80 -------	1
		USDA110 ----------	3
	B.elkanii	USDA76 ----------	3
B	*B.japonicum*	Is–1, Is–66, Is–74, Is–79,Is–82 --	1
		A1017 ----------	2
		USDA6, USDA122 ----------	3
	B.elkanii	USDA31, USDA33 ----------	3
C	*B.japonicum*	Is–1–0, Is–21, Is–34, Is–61,	
		Is–111 ----------	1
	B.elkanii	USDA61, USDA94 ----------	3
–	*R.sp.*Vigna	MAFF303063 ----------	2
–	*R.loti* (Lupinus)	MAFF303120 ----------	2

[a]Sources:1, Isolates from the nodules of soybean plant grown in the field of the National Institute of Agricurtural Sciences (NIAS), Tsukuba. 2, Culture collection of Ministry of Agriculture, Foresty and Fisheries (MAFF). 3, U.S.Department of Agriculture (USDA), Md. Nodulation types of *B. japonicum* and *B. elkanii* strains were identified by the method of Ishizuka *et al.* (1991a).

maintained on YMA (Vincent, 1970) plates at 4 °C. Yeast (*Saccharomyces cerevisiae* YNN295) used as maker was maintained on YPD (Carle and Olson, 1985) agar plate.

Preparation and restriction digestion of intact genomic DNA in agarose plugs: Each strain (Table 1) were grown in 5 mL of PSY medium (Regensburger and Hennecke 1983) for 7 d (28 °C, 160 rpm) and harvested by centrifugation at 8,000 × g for 10 min at 4 °C. The cell pellet was suspended in 10 mL of Pett IV buffer (Smith *et al.*, 1988), sedimented as before, suspended in Pett IV buffer to 2×10^9 cells mL^{-1}, and warmed at 37 °C for 10 min. An equal volume of a 2% (wt/vol) low–melting agarose (In–Cert; FMC Corp., Rockland, Marine) in Pett IV buffer was mixed with prewarmed cells at 40 °C, and 75 μL portions of the mixture were transferred to sample molds (ATTO Co., Tokyo). The molds were placed at 4 °C and allowed to solidify plugs for 30 min. The plugs were removed into lysis solution (Smith *et al.*, 1988) and incubated for 24 h at 37 °C with 100 rpm. The plugs were placed in ESP (0.5 M EDTA of pH 8.0, 1% (wt/vol) Sarkosyl, 1 mg mL^{-1} of proteinase K) solution and incubated at 50 °C for 72 h with 100 rpm. The plugs were stored at 4 °C in fresh ESP solution until restriction endonuclease digestion. Preceding restriction enzyme digestions, the plugs were aseptically placed in sterile tubes containing 600 μL of sterile TE buffer (Ichiyama *et al.*, 1991), furthermore 6 μL of 100 mM phenylmethyl–sulfonyl fluoride (PMSF; ethanol solution) per plug and washed at 37 °C for 2 h with 100 rpm. The plugs were aseptically transferred into TE buffer containing PMSF and incubated overnight at 37 °C. The plugs were washed twice in 1 mL $plug^{-1}$ of sterile 10 mM Tris–HCl (pH 8.0) and 1 mM disodium EDTA at 37 °C for 2 h. For restric-

tion enzyme reactions, the plugs were aseptically placed in a sterile 1.5–mL microcentrifuge tube containing 0.75 mL plug^{-1} of reaction buffer (protocol of restriction enzyme) and equilibrated for 30 min at room temperature. The reaction buffer was removed and 200 μL of freshly prepared restriction enzyme buffer with 100 μg mL^{-1} of nuclease–free bovine serum albumin (Nacalai Tesque Inc., Kyoto) supplied. The restriction enzyme was added 20 units plug^{-1}. The plugs were immersed for 16 h at 4 °C and incubated for 5 h at the optimum temperature for each enzyme. After restriction enzyme digestion, the plugs were washed twice in a TBE buffer solution consisting of 0.1 M Tris, 0.1 M boric acid, and 0.2 mM EDTA, at pH 8.3 for 1 h at room temperature.

PFGE equipment and electrophoresis conditions: Restriction fragments were resolved by the PFGE technique of the biased sinusoidal field gel electrophoresis (BSFGE; Shikata and Kotaka 1991) using a Genofield AE–8900 (ATTO Co., Tokyo) apparatus. These fragments were resolved with TBE buffer containing 10 g L^{-1} of electrophoresis grade agarose (Fast–Lane; FMC Co., Rockland, Marine) gel in 16–cm–long AE6110 (ATTO Co.), at DC 1.2 and AC 7.3 V cm^{-1} with frequency ramped linearly from 0.006 to 0.05 Hz over 24 h and cooled with 10 °C water flowing at approximately 1 to 2 L min^{-1}. The equilibrium temperature in the running buffer was about 15 °C during electrophoresis. Chromosomes of yeast (*Saccharomyces cerevisiae* YNN295) were prepared by the method of Carle and Olson (1985) and used as molecular weight marker. For comparison among different gels, *B. japonicum* USDA110 was included on each gel as an internal standard. Gels were stained with ethidium bromide, washed with distilled water and the image of banding patterns were visualized on a UV transilluminator and photographed.

Cluster analysis: The proportion of restriction fragments (>200 kbp) common to the strains compared (F_{xy}) was calculated by using the formula $F_{xy}=2n_{xy}/(n_x+n_y)$ proposed by Nei and Li (1979) in which n_x and n_y are the total number of restriction fragments observed in the strains x and y, respectively and n_{xy} is the number of restriction fragments shared by them. The distance matrix of each restriction enzyme was made from the genetic distance ($1 - F_{xy}$). A dendrogram was derived from the distance matrix by the unweighted pair–group method algorithm (UPGMA) contained in the computer program Phylip 3.3 (developed by J. Felsenstein, Biology Department, Indiana University, in 1990).

Serological test: Ten anticera against, *B. japonicum* strains, USDA110, Is–17, –77, and –80 (type A), Is–1 (type B), Is–21, –34, and –61 (type C) produced in previous experiments (Ishizuka *et al.*, 1991b, Yamakawa *et al.*, 1999) were used for this experiment and designated as Anti–110, –17, etc. The antigenic strains were cultured in 3 mL of YMB (Vincent 1970) for 7 d (28 °C, 160 rpm) and harvested by centrifugation at 8,000 × g for 10 min at 4 °C, suspended in 0.85% NaCl plus 0.05% NaN_3 (NaN_3–saline) solution, and heated at 100 °C for 30 min to inactivate flagellar and internal antigens. The heated cells were washed by centrifugation two times under above condition. The cell suspensions were diluted to about 10^9 cells mL^{-1} using NaN_3–saline solution. The cross–agglutination reactions with each antiserum were by the micro–agglutination method using antisera

dilution of 1/400. The reaction was conducted using U–Bottom Microtest Assay Plate (Becton Dickinson and Company, U.S.A), according to the procedure of Somasegaran and Hoben (1985).

RESULTS AND DISCUSSION

A result of electrophoresis of *Dra* I digested fragments was shown in Fig. 1. The image of Fig. 1A was processed by NIH image software and reversed to Fig. 1B. Next the reversed image was corrected the level of background pixel and clarified the band pattern and profiled the pixel concentrations. From the pixel profile a migration distance of each band was measured on the center. The molecular weights (>200 kbp) of the digested genomic DNA of each strain were estimated based on the migration distance of standard DNA (chromosomal DNAs of *Saccharomyces cerevisiae* YNN295). Also about each digested DNA band, the width of their band was estimated from the migration distance of the top and bottom edge of each band. Based on above–mentioned parameters, the diagram of BSFS pattern of each strain was determined as shown Fig. 2.

The number of total and common bands between strains to compare was counted from the diagram of strains to compared and indicated in Table 2. The fractional molecule and denominator of the right diagonal in Table 2 indicate the number of common and total bands between two strains to cross in this table. The pairwise genetic distance is indicated in the left diagonal in Table 2, which was calculated from the number of each bands using the formula of Ni and Li (1979). Based on the value of theses distances, four dendrograms were drawn in Fig. 3A to 3D by the Phylip software.

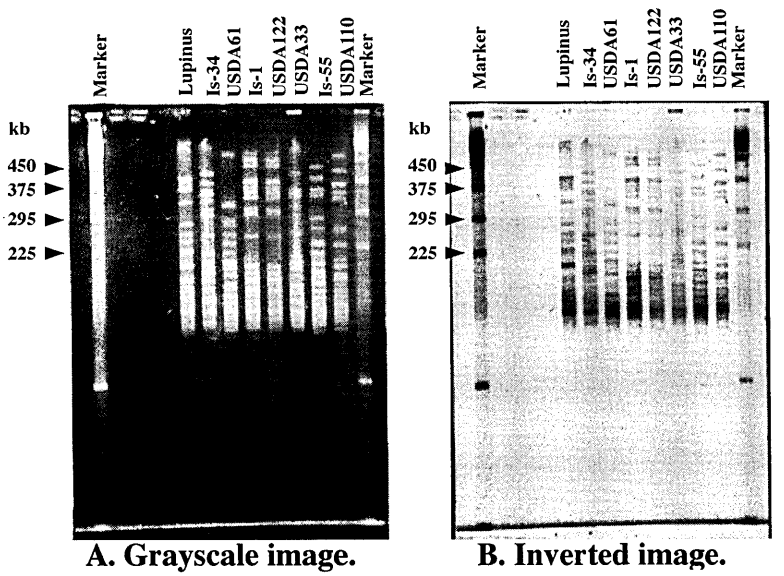

Fig. 1. BSFGE separation pattern of *Dra* I restricted fragments of genomic DNA of *Bradyrhizobium* and *Rhizobium* strains.
Marker: Chromosomes of *Saccharomyces cerevisiae* YNN295.

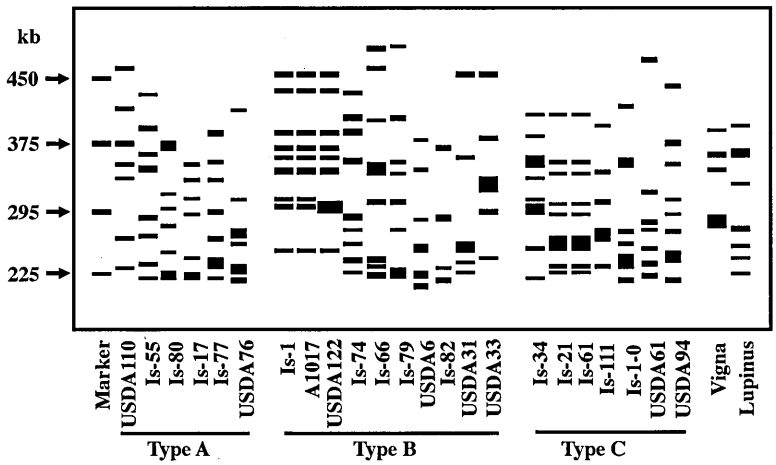

Fig. 2. Diagram of *Dra* I pulsed-field restriction endonuclease digesrtion profies of three nodulation type of *Bradyrhizobium* and two *Rhizobium* strains.
Marker: Chromosomes of *Saccharomyces cerevisiae* YNN295.

Numeric characters shown as percentage for each nodulation type strains in each cluster in Fig. 3 indicate the ratio to disperse around each cluster of each nodulation type strains used for each endonuclease digestion. Fig. 3A indicates the result of *Ase* I digested DNA fragments of each nodulation types of *B. japonicum* strains. The dendrogram was split into clusters I and II at similarity level of 0.45, type C and B strains occupy preferentially in the cluster I and II, respectively, and each type of strains were rarely in each other cluster. The type A strains exited uniformly in the both clusters. The dendrogram of *Dra* I (Fig. 3B) was split into clusters I and II at similarity level of 0.34, and the cluster II was split into two subclusters, IIa and IIb, at similarity level of 0.40. The cluster I contained no type C strain, subcluster IIa also contained no type B strain. Another subcluster IIb contained all nodulation type strains almost uniformly. The dendrogram of *Sph* I formed small cluster containing type B strains, but it was recognized no tendency that each nodulation type strains formed cluster. The dendrogram of *Xba* I (Fig. 3D) was split into clusters I and II at similarity level of 0.45, and the cluster II was split into two subclusters, IIa and IIb, at similarity level of 0.53. However, this dendrogram was not clarified in the difference between two clusters in compared with that of *Dra* I.

The results of cross-agguligation reaction between each nodulation type strains with the antiserum of those typical strains are shown in Table 4. Strong cross-reactions were observed between type A strains USDA110 and Is-17, or Is-77 and -80, but those strains were low similarity each other (Fig. 3A-3D). In type B strains, Anti-1 reacted strongly with antigens USDA-122, A1017 and Is-1, and these three strains showed high similarity above 0.80 and Is-1 and A1017 was similarity 1.00 (Fig. 3A-3D). Furthermore, those three strains cross-reacted with type A strain USDA110. In type C strains, Is-21 and -61 strongly cross-reacted and was similarity 1.00 (Fig. 3A-3D). Also anti-34 reacted positively with antigens Is-34 and -61. These three type C strains formed cluster only in the

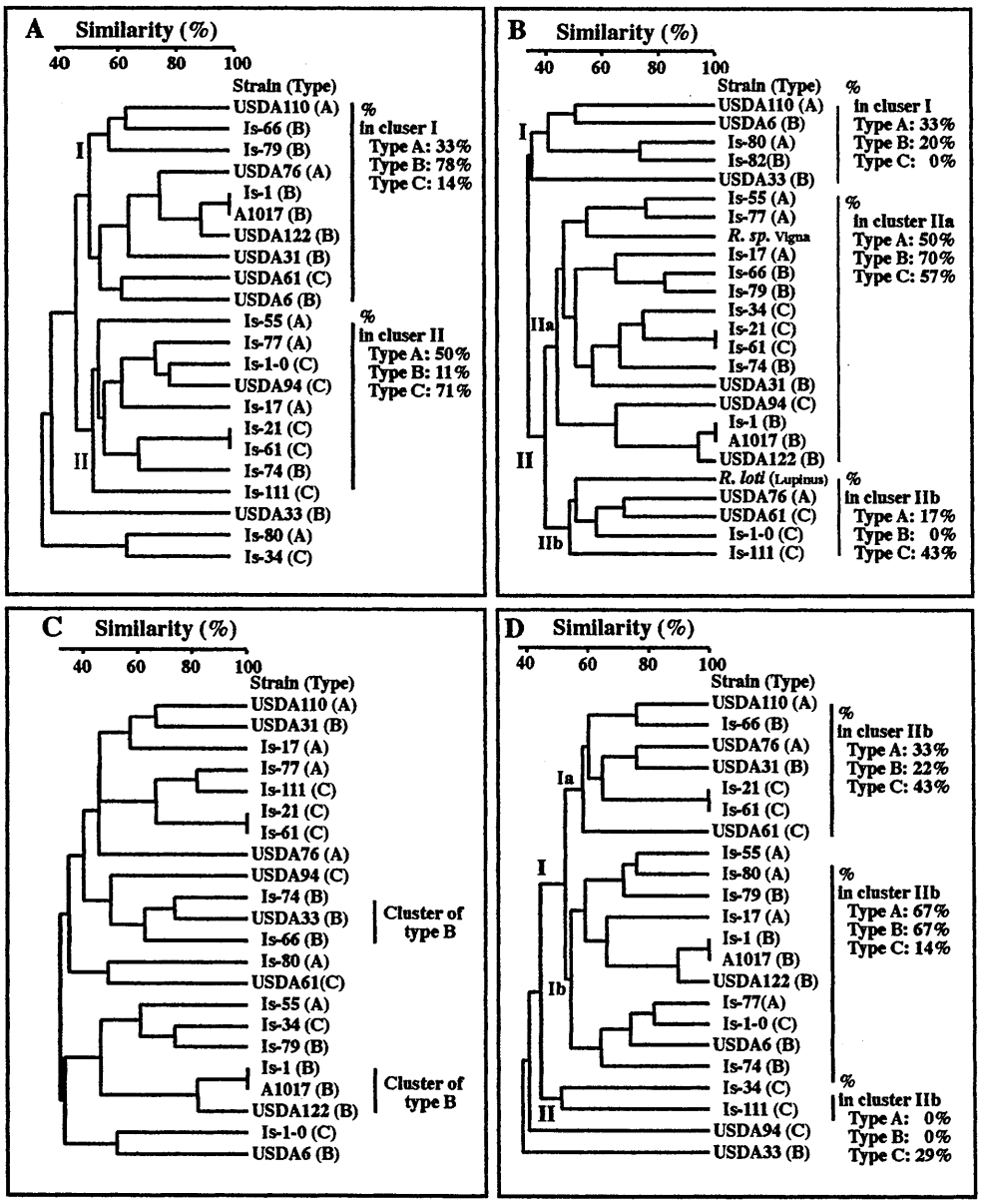

Fig. 3. Dendrogram of similarity of *Ase* I (A), *Dra* I (B), *Spe* I (C) and *Xba* I (D) restriction patterns of the genomic DNA of *Bradyrhizobium* strains.

Table 2. Matrix of pairwise genetic distance between *Bradyrhizobium* and *Rhizobium* strains based on RFLP analysis of *Dra* I digestion fragments of genomic DNA.

Strain	Nod-type	USDA 110	Is-55	Is-80	Is-17	Is-77	USDA 76	Is-1	USDA A1017	USDA 122	Is-74	Is-66	Is-79	USDA 6	Is-82	USDA 31	USDA 33	Is-34	Is-21	Is-61	Is-111	Is-1-0	USDA 61	USDA 94	Vigna	Lupinus
USDA110	A		6/15	4/14	6/14	8/15	6/15	4/16	4/16	4/15	6/16	8/16	6/15	8/16	4/11	6/13	6/13	4/17	8/16	8/16	6/13	8/15	2/14	6/16	2/12	0/14
Is-55	A	0.60		4/15	8/15	12/16	6/16	8/17	8/17	8/16	10/17	6/17	6/16	6/17	8/12	6/14	6/14	4/18	10/17	10/17	8/14	10/16	6/15	10/17	8/13	6/15
Is-80	A	0.71	0.73		6/14	4/15	4/15	8/16	8/16	6/15	4/16	3/16	8/15	8/16	8/11	4/13	6/13	6/17	8/16	8/16	6/13	4/15	8/14	4/16	2/12	6/14
Is-17	A	0.57	0.47	0.57		10/15	6/15	6/16	6/16	4/15	8/16	10/16	10/15	4/16	6/11	6/13	6/13	10/17	6/16	6/16	2/13	6/15	6/14	10/16	2/12	6/14
Is-77	A	0.47	0.25	0.73	0.33		6/16	6/17	6/17	4/16	10/17	8/17	8/16	2/17	4/12	8/14	8/14	10/18	10/17	10/17	6/14	8/16	4/15	8/17	6/13	8/15
USDA76	A	0.60	0.63	0.73	0.60	0.63		2/17	2/17	2/16	8/17	6/17	8/16	6/17	4/12	4/14	0/14	8/18	12/17	12/17	8/14	10/16	10/15	6/17	2/13	6/15
Is-1	B	0.75	0.53	0.50	0.63	0.65	0.88		18/18	16/17	8/18	6/18	6/17	4/18	2/13	6/15	6/15	12/19	10/18	10/18	6/15	6/17	2/16	12/18	6/14	2/16
A1017	B	0.75	0.53	0.50	0.63	0.65	0.88	0.00		16/17	8/18	6/18	6/17	4/18	2/13	6/15	6/15	12/19	10/18	10/18	6/15	6/17	2/16	12/18	6/14	2/16
USDA122	B	0.73	0.50	0.60	0.73	0.75	0.88	0.06	0.06		8/17	6/17	6/16	4/17	2/12	6/14	4/14	8/18	8/17	8/17	6/14	6/16	2/15	10/17	6/13	2/15
Is-74	B	0.63	0.41	0.75	0.50	0.41	0.53	0.56	0.56	0.53		8/18	8/17	8/18	4/13	8/15	6/15	12/19	12/18	12/18	8/15	10/17	10/16	8/18	6/14	10/16
Is-66	B	0.50	0.65	0.63	0.38	0.53	0.65	0.67	0.67	0.65	0.56		14/17	6/18	4/13	4/15	6/15	8/19	12/18	12/18	8/15	10/17	4/16	6/18	2/14	6/16
Is-79	B	0.60	0.63	0.47	0.33	0.50	0.50	0.65	0.65	0.63	0.53	0.18		6/17	4/12	4/14	2/14	10/18	12/17	12/17	8/14	8/16	6/15	6/17	6/13	4/15
USDA6	B	0.50	0.65	0.50	0.75	0.88	0.65	0.78	0.78	0.76	0.56	0.67	0.65		6/13	6/15	4/15	8/19	6/18	6/18	6/15	6/17	8/16	6/18	4/14	6/16
Is-82	B	0.64	0.33	0.27	0.45	0.67	0.67	0.85	0.85	0.83	0.69	0.69	0.67	0.54		2/10	2/10	2/14	4/13	4/13	2/10	4/12	4/11	2/13	2/9	4/11
USDA31	B	0.54	0.57	0.69	0.54	0.43	0.71	0.60	0.60	0.57	0.47	0.73	0.71	0.60	0.80		4/12	6/16	10/15	10/15	4/12	8/14	6/13	6/15	2/11	6/13
USDA33	B	0.54	0.57	0.54	0.54	0.43	1.00	0.60	0.60	0.71	0.60	0.60	0.86	0.73	0.80	0.67		6/16	4/15	4/15	2/12	2/14	4/13	6/15	2/11	4/13
Is-34	C	0.76	0.78	0.65	0.41	0.44	0.56	0.37	0.37	0.56	0.37	0.58	0.44	0.58	0.86	0.63	0.63		14/19	14/19	4/16	8/18	4/17	10/19	8/15	4/17
Is-21	C	0.50	0.41	0.50	0.63	0.41	0.29	0.44	0.44	0.53	0.33	0.33	0.29	0.67	0.69	0.33	0.73	0.26		18/18	8/15	6/17	6/16	6/18	6/14	4/16
Is-61	C	0.50	0.41	0.50	0.63	0.41	0.29	0.44	0.44	0.53	0.33	0.33	0.29	0.67	0.69	0.33	0.73	0.26	0.00		8/15	6/17	6/16	6/18	6/14	4/16
Is-111	C	0.54	0.43	0.54	0.85	0.57	0.43	0.60	0.60	0.57	0.47	0.47	0.43	0.60	0.80	0.67	0.83	0.75	0.47	0.47		8/14	4/13	6/15	4/11	6/13
Is-1-0	C	0.47	0.38	0.73	0.60	0.50	0.38	0.65	0.65	0.63	0.41	0.41	0.50	0.65	0.67	0.43	0.86	0.56	0.65	0.65	0.43		8/15	10/17	6/13	8/15
USDA61	C	0.86	0.60	0.43	0.57	0.73	0.33	0.88	0.88	0.87	0.38	0.75	0.60	0.50	0.64	0.54	0.69	0.76	0.63	0.63	0.69	0.47		8/16	4/12	8/14
USDA94	C	0.63	0.41	0.75	0.38	0.53	0.65	0.33	0.33	0.41	0.56	0.67	0.65	0.67	0.85	0.60	0.60	0.47	0.67	0.67	0.60	0.41	0.50		6/14	10/16
Vigna	—	0.83	0.38	0.83	0.83	0.54	0.85	0.57	0.57	0.54	0.57	0.86	0.54	0.71	0.78	0.82	0.82	0.47	0.57	0.57	0.64	0.54	0.67	0.57		6/12
Lupinus	—	1.00	0.60	0.57	0.57	0.47	0.60	0.88	0.88	0.87	0.38	0.63	0.73	0.63	0.64	0.54	0.69	0.76	0.75	0.75	0.54	0.47	0.43	0.38	0.50	

Numbers above the diagonal represent the proportion of shared fragments (F_{xy}) estimated as the ratio of the number of comigrating bands to the total number of bands between pair of *Bradyrhizobium* and *Rhizobium* strains (x and y). Numbers below the diagonal represent genetic distances estimated from the F_{xy} values by the method proposed by Nei and Li (1979).

Table 3. Serological reactions of *Bradyrhizobium* strains of each nodulation type against various antiserum.

Nodulation type	*Brady-rhizobium* strain	Antiserum							
		USDA110	Is-17	Is-77	Is-80	Is-1	Is21	Is-34	Is-61
		Type A				Type B	Type C		
A	USDA110	+	+						
	Is-17	+	+						
	Is-55								
	Is-77			+	+				
	Is-80			+	+				
	USDA76								
B	USDA6			+	+				
	USDA122	+				+			
	A1017	+				+			
	Is-1	+				+			
	Is-66								
	Is-74			+	+				
	Is-79								
	Is-82								
	USDA31								
	USDA33								
C	Is-1-0								
	Is-21						+		+
	Is-34							+	
	Is-61						+	+	+
	Is-111								
	USDA61								
	USDA94								

dendrogram by *Dra* I. From Strong cross-reaction was observed between some type A and type B strains in this experiment, or weak cross-reaction was reported between some type A and type C strains (Ishizuka *et al.*, 1991b). From these cross-reaction of each nodulation type strains, it was thought that there were type B and type C strains in the distant relation in the structure of antigenic determinant, and type A strains was middle position between those strains. The dendrogram that reflect most suitably the results of agglutination reaction was estimated to be that constructed by *Dra* I. Also the dendrogram using only longer fragment than 200 kbp in this experiment was useful for classifying the nodulation type strains.

The results of RFLP analysis and cross-agguligation reaction indicated that the type B strains were distantly related to the type C strains in regard to the structure of genomic DNA and that of surface of rhizobial cell, the type A strains were located intermediate between type B and C.

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