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Genetic Structure and Symbiotic Characteristics of a *Bradyrhizobium* Population Recovered from a Pasture Soil†

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We examined the genetic structure and symbiotic characteristics of *Bradyrhizobium* isolates recovered from four legume species (*Lupinus albus* [white lupine], *Lupinus angustifolius* [blue lupine], *Ornithopus compressus* [yellow serradella], and *Macroptilium atropurpureum* [siratro]) grown in an Oregon soil. We established that multilocus enzyme electrophoresis (MLEE) can provide insights into the genetic relatedness among *Bradyrhizobium* strains by showing a positive correlation (r^2 = ≥0.90) between the relatedness of *Bradyrhizobium japonicum* strains determined by MLEE at 13 enzyme loci and that determined by other workers using either DNA-DNA hybridization or DNA sequence divergence estimates. MLEE identified 17 electrophoretic types (ETs) among 95 *Bradyrhizobium* isolates recovered from the four hosts. Although the overall genetic diversity among the ETs (H = 0.69) is one of the largest measured to date in a local population of any soilborne bacterial species, there was no evidence of multilocus structure (linkage disequilibrium) within the population. The majority of the isolates (73%) were represented by two closely related ETs (2 and 3) which dominated the root nodules of white lupine, serradella, and siratro. In contrast, ET1 dominated nodules of blue lupine. Although representative isolates from all of the 17 ETs nodulated siratro, white lupine, blue lupine, and big trefoil (*Lotus pedunculatus*), they were either completely ineffective or poorly effective at fixing nitrogen on these hosts. Despite the widespread use of serradella as a surrogate host for lupine-nodulating bradyrhizobia, 7 of the 17 ETs did not nodulate this host, and the remaining 10 ETs were ineffective at fixing nitrogen.

Although the agricultural importance of legumes and nitrogen fixation provides the major impetus for studying *Bradyrhizobium* and *Rhizobium* spp., the two genera offer an opportunity to compare the ecology of inherently slow-growing and potentially fast-growing bacteria in the same soil. Although there is evidence that the ecologies of the two genera are different, the data are inconsistent and consensus has not been reached (2). Early studies by Parker and co-workers in western Australia showed that *Bradyrhizobium* sp. persisted better under the hot and desiccated surface soil conditions of the Australian summer than did *Rhizobium leguminosarum* bv. trifolii (7–9, 32). Other workers, however, have not found that *Bradyrhizobium* sp. possesses a clear advantage over *Rhizobium* sp. in withstanding desiccation (31, 37, 39, 46, 48). For example, Woomer and Bohlool (48) reported that *Bradyrhizobium japonicum* strains did not persist as well as did *Rhizobium* strains in Haplustoll soils exposed to a dry season and receiving only 35 cm of precipitation per year. In contrast, *Bradyrhizobium* sp. survived better than *Rhizobium* sp. in acidic tropohumult soils receiving >100 cm of rainfall per year. Jenkins et al. (22) observed that the surface soil root system of mesquite (*Prosopis glandulosa*) growing in the Sonoran desert of the United States is nodulated primarily with *Rhizobium* sp., while the root system found at the water table several meters below the soil surface was nodulated by *Bradyrhizobium* sp. Jenkins et al. speculated that mesquite-nodulating *Rhizobium* sp. was more tolerant of the extreme and fluctuating environmental conditions at the soil surface, whereas *Bradyrhizobium* sp. preferred the more stable, less extreme environment of the subsurface.

Since we have extensively characterized an indigenous population of *R. leguminosarum* in an Oregon soil (28, 29, 30), a unique opportunity exists to address the comparative ecology of the two genera in the same soil once the *Bradyrhizobium* population is characterized. Although agricultural legumes that are *Bradyrhizobium* hosts are rarely grown in Oregon, we speculated that local soils should harbor lupine-nodulating *Bradyrhizobium* sp. The genus *Lupinus* is widespread throughout the state, occupying niches ranging from seashore dunes to alpine meadows and high deserts (13, 35). To enhance our chances that we recover a full spectrum of the *Bradyrhizobium* genotypes present in a soil population, we chose several host species to be used as trap plants. These were blue lupine (*Lupinus angustifolius*), white lupine (*Lupinus albus*), serradella (*Ornithopus compressus*), i.e., the surrogate host for lupine-nodulating bradyrhizobia (8, 9), siratro (*Macroptilium atropurpureum*), and big trefoil (*Lotus pedunculatus*).

Leung et al. (28) used multilocus enzyme electrophoresis (MLEE) to obtain information on the genotypic diversity and genetic structure within a local soil population of *R. leguminosarum* bv. trifolii. The suitability of MLEE for that study was confirmed when a positive correlation was found to exist between the overall relatedness of 11 strains established by MLEE, with relatedness previously determined for the same strains by DNA-DNA hybridization (21). Since DNA-DNA hybridization and DNA sequence divergence data were used previously to describe the genetic relatedness among several *B. japonicum* strains from the U.S. Department of Agriculture (USDA) collection (18, 45), we conducted a preliminary experiment with the same strains of *B. japonicum* to determine if MLEE was suitable for examining the relatedness of strains within the genus *Bradyrhizobium*.

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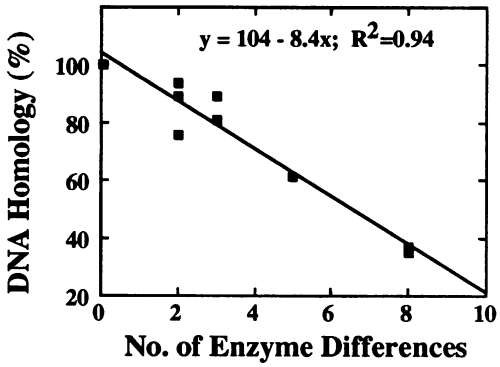

FIG. 1. Comparison of genetic relatedness among strains of *B. japonicum* obtained by MLEE at 13 enzyme loci and by published DNA reassociation experiments (18). Strain USDA 324 (= 61A76) was used as the reference strain (100% value).

MATERIALS AND METHODS

Sources of seed. *L. albus* L. cv. Ultra was obtained from Russ Karrow, Department of Crop and Soil Science, Oregon State University. *L. angustifolius* L. cvs. Yandee and Merrit and *O. compressus* L. cultivar 1209.2 (yellow serradella) were obtained from Lyn Abbott, Department of Soil Science and Plant Nutrition, University of Western Australia, Perth. *Ornithopus sativus* Brot. (French serradella) was obtained as PI 290725 (origin, England), from G. Lovell, Regional Plant Introduction Experiment Station, Griffin, Ga. *M. atropurpureum* (DC.) Urb. was obtained from H. J. Evans, Laboratory for Nitrogen Fixation Research, Oregon State University. *L. pedunculatus* Cav. cv. Marshfield was obtained from Soil Conservation Service, Plant Materials Center, Oregon State University.

TABLE 1. Comparative clustering of USDA strains of *B. japonicum* by DNA reassociation, restriction fragment length polymorphism patterns, and MLEE-derived data

Strain designation[a]	DNA homology group[b]	RFLP group[c]	ET group[d]
USDA 24 (ATCC 11927)	I	ND[e]	1
USDA 38	I	ND	1
USDA 58	I	sT I	1
USDA 115	I	sT I	1
USDA 123	—[f]	ND	?
USDA 123 (ELS)	ND	ND	?
USDA 62	Ia	sT I	1
USDA 110	Ia	sT I	1
USDA 122	—[g]	sT I	1
USDA 127	ND	ND	1
USDA 140	Ia	sT I	1
USDA 29	II	ND	2
USDA 31	II	sT II	2
USDA 46	II	ND	2
USDA 86	II	ND	2
USDA 117	—[h]	ND	1
USDA 130	II	ND	2
USDA 324 (61A76)	II	sT II	2
USDA 74	II	sT II	2

[a] Strain nomenclature from USDA catalog (23).
[b] DNA homology groups are those derived from published DNA reassociation data (18).
[c] Symbiotic groups are defined in Stanley et al. (45).
[d] See cluster analysis in Fig. 2 for MLEE-derived groups.
[e] ND, not analyzed in the original studies (18, 45).
[f] Two strains of USDA 123 with different cultural histories are not closely associated with group I.
[g] DNA homology data are ambiguous, but both restriction fragment length polymorphism and MLEE analyses placed USDA 122 in group 1.
[h] DNA homology data contradict MLEE data for the group assignment of USDA 117.

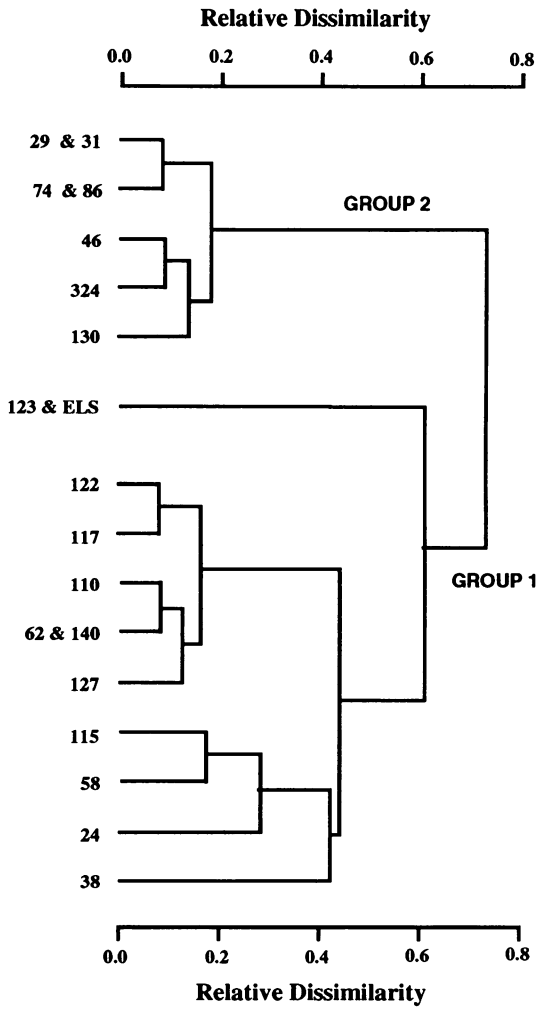

FIG. 2. Relative similarities among the 19 USDA strains of *B. japonicum*. The dendrogram was generated by the average linkage method of clustering based upon the allelic variation at 13 enzyme loci.

Culture collection sources of *B. japonicum* and *Bradyrhizobium* sp. (*Lupinus*) used in this study. Nineteen strains of *B. japonicum* and one of *Bradyrhizobium* sp. (from a *Lupinus* sp.) were obtained from Peter van Berkum, Laboratory for Soybean and Alfalfa Research, USDA-ARS, Beltsville, Md. (see Table 1). Included in this group were USDA 24, (ATCC 11927), which represents one of the American Type Culture Collection (ATCC) strains of *B. japonicum*, and USDA 3051 (ATCC 103190) of *Bradyrhizobium* sp. (*Lupinus*). A derivative of strain USDA 123 of *B. japonicum* (ELS) was obtained from E. L. Schmidt, Department of Soil Science, University of Minnesota, St. Paul. Strain WU425, a commercial inoculant strain of *Bradyrhizobium* sp. (*Lupinus*) was obtained from John Howieson, Western Australian Department of Agriculture, Perth.

Collection and characterization of *Bradyrhizobium* sp. from Abiqua soil. Soil was recovered from a subclover grass pasture

TABLE 2. Allelic profiles of *Bradyrhizobium* ETs recovered from Abiqua soil

ET or isolate	No. of isolates	Allelic variants at enzyme locus[a]:												
		IDH	MDH	6PG	PGM	HBD	G6P	HEX	GDH	PE1	PE2	EST	SKD	ADH
1	11	2	2	3	6	1	1	1	3	4	4	1	6	1
2	43	3	2	4	4	1	0[b]	1	2	5	6	2	3	3
3	22	3	2	4	3	3	2	3	2	5	6	2	2	3
4	4	3	2	5	7	2	3	2	5	4	2	2	7	1
5	1	2	2	4	1	3	3	3	2	5	0	2	0	4
6	2	1	2	4	5	1	3	1	4	4	0	3	5	0
7	1	2	2	4	3	3	1	2	2	5	6	2	2	3
8	1	3	2	2	7	2	3	3	3	0	2	2	7	1
9	1	2	2	4	7	3	2	1	6	4	2	2	6	1
10	1	1	2	4	2	1	2	1	1	4	4	3	3	0
11	1	2	2	4	3	0	2	1	1	5	6	2	0	0
12	1	1	2	1	6	1	2	4	1	3	5	2	1	0
13	2	1	2	5	5	2	2	3	4	3	2	3	7	2
14	1	1	2	4	4	1	2	4	3	3	3	3	2	0
15	1	1	2	4	4	1	2	3	2	4	5	3	3	3
16	1	2	2	2	7	2	3	2	3	0	1	2	7	1
USDA 3051	1	2	2	2	4	1	3	1	2	4	4	3	5	1
No. of alleles		3	1	5	7	3	3	4	6	3	6	3	6	4
Locus diversity (h)		0.69	0.0	0.64	0.88	0.71	0.68	0.74	0.82	0.74	0.88	0.56	0.89	0.78

[a] IDH, isocitrate dehydrogenase; MDH, malate dehydrogenase; 6PG, NAD^+-dependent 6-phosphogluconate dehydrogenase; PGM, phosphoglucomutase; HBD, β-hydroxybutyrate dehydrogenase; G6P, glucose-6-phosphate dehydrogenase; HEX, hexokinase; GDH, glucose dehydrogenase; PE1, leucyl-glycine peptidase; PE2, leucyl-glycyl-glycine peptidase; EST, esterase-naphthyl propionate; SKD, shikimate dehydrogenase; ADH, alcohol dehydrogenase.
[b] A zero indicates that activity of a specific enzyme could not be detected in extracts of these isolates.

located in the Willamette Valley of Oregon. The soil was a silty clay loam of the Abiqua series (Cumulic Ultic Haploxeroll) and has been described in detail elsewhere (1, 3). Soil was sampled with sterile utensils along a transect that ran from the southwest to the northeast corner of an enclosed paddock (100 by 200 m). The soil samples were mixed thoroughly and sieved through a 2-mm-pore-size mesh screen. Soil was corrected for mineral nutrient limitations that would restrict legume growth by supplementing 1-kg samples with 2.1 mg of Na_2MoO_4, 200 mg of KH_2PO_4, and 90 mg of K_2SO_4 to achieve Mo, P, and S concentrations of 1, 55, and 20 mg kg of $soil^{-1}$, respectively. Sterile deionized water was added to achieve a water potential of approximately −0.03 MPa (38 g of water per 100 g of oven-dried soil). Plants were grown in 20-cm-diameter plant pots containing 250-g portions of a 1:1 (vol/vol) vermiculite-perlite mixture (VPM). The VPM was supplemented with 1.25 liters of a plant nutrient solution (30). A portion (1.5 kg) of nutrient-supplemented soil was packed gently on top of the VPM in each pot.

Seedlings of white and blue lupines, siratro, yellow serradella, and big trefoil were germinated from surface-disinfested seeds and sown into three replicate pots of each plant species. After 1 week, the lupine species were thinned to three seedlings per pot, and siratro, serradella, and big trefoil were thinned to 10 seedlings per pot. After 6 weeks of growth under greenhouse conditions, the plants were harvested, and 30 to 50 nodules were recovered randomly from the three replicates of each plant species. Nodules were not detected on the plants of big trefoil. One hundred seventy-five isolates of presumptive bradyrhizobia were isolated into pure culture by standard procedures (47). Isolates from siratro, serradella, blue lupine, and white lupine were coded MA, OC, LG, and LA, respectively. A total of 95 isolates were examined by MLEE. They included about 20 from each of the four plant species and 15 isolates recovered from *L. albus* in an earlier preliminary experiment.

MLEE of the *Bradyrhizobium* isolates. The MLEE procedures and analysis of the data have been described in detail elsewhere (28, 42). A few procedural modifications were found to be essential for work with *Bradyrhizobium* isolates, and these are described below. Each isolate was grown in duplicate 100-ml quantities of yeast extract mannitol broth for 10 days at 27°C. Some enzyme activities were found to be lower than we had experienced with *Rhizobium* extracts, and they were further diminished by repeated thawing and freezing. As a result, certain enzymes were routinely assayed on fresh extracts or on extracts that were thawed once immediately prior to analysis. These enzymes included alcohol dehydrogenase, hex-

TABLE 3. Distribution of ETs among *Bradyrhizobium* isolates recovered from four legume hosts planted in Abiqua soil

Plant species	No. of isolates	% Of isolates of ET:																No. of ETs
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
White lupine	15		28	40										14	7			7	5
White lupine[a]	20	5	60	20	5	5	5												6
Blue lupine	20	50	10	5	5		5	5	5		5					5	5		10
Serradella	20		63	32						5									3
Siratro	20		65	25								5	5						4

[a] These isolates came from a separate collection.

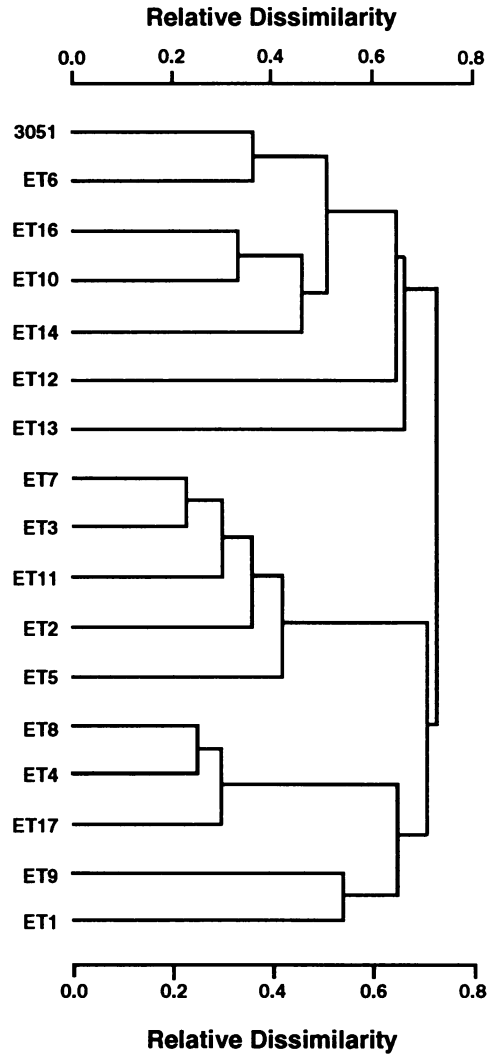

FIG. 3. Relative similarities among the 17 ETs of *Bradyrhizobium* sp. recovered from the Abiqua soil. The dendrogram was generated by the average linkage method of clustering based upon the allelic variation at 13 enzyme loci.

TABLE 4. Symbiotic characteristics of isolates representing each of the 17 ETs of *Bradyrhizobium* recovered from Abiqua soil and of strains USDA 3051 and WU425

Isolate representing ET:	Effectiveness status[a] on:				
	Blue lupine (g/plant)	Siratro (mg/plant)	Big trefoil (mg/plant)	Serradella	
				Nodulation	Effectiveness
ETs					
1	0.64(e)	66(e)	(i)	+	(i)
2	0.52(e)	61(e)	35.5(e)	+	(i)
3	0.53(e)	71(e)	(i)	+	(i)
4	0.63(e)	41(i)	(i)	+	(i)
5	0.85(e)	41(i)	(i)	+	(i)
6	0.68(e)	40(i)	(i)	−	
7	0.42(i)	45(i)	(i)	−	
8	0.58(e)	42(i)	(i)	+	(i)
9	0.36(i)	69(e)	24(e)	+	(i)
10	0.38(i)	62(e)	(i)	+	(i)
11	0.60(e)	57(e)	(i)	−	
12	0.45(i)	52(e)	(i)	−	
13	0.66(e)	34(i)	(i)	+	(i)
14	0.50(i)	43(i)	(i)	−	
15	0.47(i)	45(i)	(i)	+	(i)
16	0.47(i)	45(i)	(i)	−	
17	0.33(i)	52(e)	(i)	−	
USDA 3051	1.7(E)	27(i)	(i)	+	(i)
WU425	1.7(E)	52(e)	21(e)	+	73(e)
Uninoculated	0.4	36.5(i)	2	−	
Nitrate fed	2.8	107	77	−	100

[a] (i), the plants grew no better than uninoculated controls; (e), shoot dry weight was significantly greater than that of uninoculated controls but less than the shoot yield of nitrate-fed plants ($P < 0.05$); (E), in the case of blue lupine, both USDA 3051 and WU425 produced shoot dry weights that were significantly greater than those produced by strains in the (e) category of effectiveness (the yields were still significantly less than those of nitrate-fed plants [$P < 0.05$]).

okinase, NAD-dependent 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, and β-hydroxybutyrate dehydrogenase. Starch gels were sliced into two halves of approximately 4-mm thickness, rather than into three or four slices of 2- or 3-mm thickness. Fifteen enzymes were found to be active and focused into discrete bands after electrophoresis in one or another of the three electrode-gel buffer combinations described elsewhere (28). These were as follows: (i) an electrode buffer of Tris-citrate at pH 8.0 (isocitrate dehydrogenase, malate dehydrogenase, leucyl-glycine peptidase, leucyl-glycyl-glycine peptidase, phosphoglucomutase, and glucose-6-phosphate dehydrogenase); (ii) an electrode buffer of Tris-citrate at pH 6.3 (NAD^+-dependent 6-phosphogluconate dehydrogenase, β-hydroxybutyrate dehydrogenase); and (iii) an electrode buffer of borate at pH 8.2 (shikimate dehydrogenase, hexokinase, alcohol dehydrogenase, glucose dehydrogenase, nucleoside phosphorylase, esterase-naphthyl propionate, and indophenol oxidase). While activities of some enzymes were undetectable or nonreproducible in *B. japonicum* extracts (glucose-6-phosphate dehydrogenase and alcohol dehydrogenase), others were undetectable in *Bradyrhizobium* extracts (indophenol oxidase and nucleoside phosphorylase). As a consequence, a different combination of 13 enzymes was assayed for *Bradyrhizobium* sp. and *B. japonicum*. Each isolate was characterized by its own combination of electromorphic variants of the 13 enzymes assayed. A distinct electromorphic profile was referred to as an electrophoretic type (ET). Genetic diversity (h) at an enzyme locus was calculated as $h = [1 - \Sigma x_i^2]\,[n/(n-1)]$, where x_i is the frequency of the ith allele at the locus and n is the number of ETs in the population of interest. The relative similarities among ETs were revealed with a cluster analysis program designed by T. S. Whittam (Pennsylvania State University) and were displayed in the form of a dendrogram. To determine the extent to which the populations exhibited linkage disequilibrium (nonrandom combinations of alleles between loci), the observed allelic mismatch frequency distribution was obtained by comparing each ET with every other ET once (for a total of $n(n-1)/2$ comparisons, where n is the number of ETs), and for each paired comparison, the number of dissimilar alleles (mismatches) was recorded. An equation for computing the variance of this distribution has been derived (5, 6), and the process is described in detail elsewhere (28).

Examination of host range and symbiotic effectiveness of an isolate representing each ET from the *Bradyrhizobium* population. Leonard jar assemblies (47) were used for growing the large-seeded lupine species. The composition of the N-free mineral nutrient solution is described elsewhere (29). For the

TABLE 5. Symbiotic effectiveness of isolates of ET1 and ET2 on white and blue lupines

ET	Isolate	Shoot dry wt (g/plant) on[a]:	
		White lupine	Blue lupine
1	LGS1-7	1.5(e)	0.5(i)
	LGS2-14	1.1(e)	0.5(i)
	LGS3-19	0.8(e)	0.6(e)
2	LAS1-11	1.3(e)	0.9(e)
	LAS2-22	0.5(i)	0.5(i)
	LAS3-31	0.9(e)	0.8(e)
None	WU425	3.2(E)	1.6(E)
	Nitrate fed	5.3	3.9
	Uninoculated	0.3	0.4

[a] See Table 4, footnote *a*, for definitions of (E), (e), and (i).

nitrate-supplemented controls, the concentration of K_2SO_4 was reduced to 0.18 g/liter, and KNO_3 was added at 0.8 g/liter. Four replicate assemblies each containing two blue lupine seedlings were inoculated with 5-ml portions of a 10-day-old culture of an isolate representing each of the 17 ETs identified in the soil population of *Bradyrhizobium* sp. In addition, other seedlings were either inoculated with strains USDA 3051 or WU425 or left uninoculated and provided with a nutrient solution supplemented with or without nitrate. Plants were transferred to the greenhouse and arranged in a randomized complete block design. After 46 days of growth, the plants were harvested and the root systems were analyzed for nodulation. The symbiotic effectiveness of the isolates was determined by comparing the shoot dry weights of plants inoculated with the different ETs with those of plants that were either nitrate-fed, inoculated with WU425, or left uninoculated. The small-seeded species (serradella, siratro, and big trefoil) were treated almost identically to blue lupine with the exception that 5-g quantities of VPM were added to cotton-stoppered test tubes (3.0-cm diameter) and soaked with 25-ml volumes of the plant nutrient solution. Seedlings were transferred to the tubes, inoculated, and incubated under the same growth conditions as described above.

Comparative symbiotic effectiveness of multiple isolates from ET1 and ET2 on white and blue lupine. Growth conditions were the same as those described above for blue lupine. Four replicate Leonard jar assemblies containing either white or blue lupine seedlings were inoculated with 5-ml portions of one of three isolates of ET2 (LAS1-11, LAS2-22, or LAS 3-31) or with one of three isolates of ET1 (LGS 1-7, LGS 2-14, or LGS 3-19). After 50 days of growth, plants were harvested, roots were examined for nodulation, and shoot dry weights were compared with those of plants that were either nitrate fed, inoculated with WU425, or left uninoculated.

Determining the size of the *Bradyrhizobium* soil population with a plant infection-soil dilution procedure. Pairs of siratro or serradella seeds were transplanted into cotton-stoppered test tubes (20 by 2.5 cm) containing sterile VPM supplemented with 25 ml of plant nutrient solution. Pairs of white lupine seedlings were planted into Erlenmeyer flasks (250 ml) containing VPM instead of into test tubes. A fivefold dilution series of Abiqua soil was prepared in distilled water, and 1-ml portions of each dilution were used to inoculate four replicates of each plant species. Seedlings were placed in a growth chamber, and roots were examined for nodulation after 6 weeks. Because nodules were not detected on plants grown under the above conditions, the possibility that the soil population existed below the detection limit of the method (<10/g) was considered. As a consequence, the following procedure was adopted. Portions of Abiqua soil (100 g) and sterile VPM (20 g) were mixed with 100-ml volumes of plant nutrient solution and placed in sterile Erlenmeyer flasks (500 ml). Two seedlings of white lupine or 10 seedlings of serradella or siratro were transplanted into six replicate flasks of each plant species. Seedlings inoculated with strain WU425 as well as uninoculated and nitrate-fed seedlings were included. Aerial contamination was excluded from the flasks by using cotton stoppers, and water was added under a laminar-flow hood. After 6 weeks of growth, the plants were examined and nodules were found on each species, indicating that *Bradyrhizobium* sp. exists in Abiqua soil at low densities (<10/g of soil).

RESULTS

Evaluating MLEE for determining the relatedness among *Bradyrhizobium* isolates. The basic assumption underlying the ability of MLEE to describe the genetic relatedness among a bacterial population is that the loci encoding the enzymes analyzed should be representative of the total genome. If this assumption is true, one would expect to find a positive relationship between genetic relatedness obtained by MLEE with that determined by either DNA-DNA hybridization of total genomic DNA or by restriction fragment length polymorphism analyses carried out with chromosomal gene probes. Two studies have been reported in the literature in which the relatedness among several strains of *B. japonicum* was determined by either DNA-DNA hybridization (18) or by percent sequence divergence estimated from hybridization patterns to chromosomal gene probes (45).

We conducted an MLEE analysis with 19 of the same strains of *B. japonicum* which were used in one or the other of the two studies cited above (Table 1). From an analysis of 13 enzymes, positive correlations ($r^2 > 0.9$) were obtained between strain similarities predicted by MLEE and the similarities predicted by DNA-DNA hybridization (Fig. 1) and by percent sequence divergence (data not shown). Fifteen electrophoretic types (ETs) were identified among the *B. japonicum* strains, and they clustered into two major groups (I and II) (Fig. 2). All of the strains which fell into groups sT I and sT II of Stanley et al. (45) were found in groups 1 and II, respectively. With the exception of USDA 117, all of the strains in groups I and II corresponded with DNA homology groups 1 (or 1a) and II, respectively, of Hollis et al. (18) (Table 1).

MLEE analysis of *Bradyrhizobium* isolates recovered from Abiqua soil. A total of 95 bradyrhizobial isolates chosen from the two white lupine collections and from blue lupine, serradella, and siratro were analyzed by MLEE. Seventeen ETs were identified from an analysis of 13 enzyme loci (Table 2). With the exception of malate dehydrogenase, the enzyme loci were polymorphic, ranging from three to seven alleles per locus. The *h* values per enzyme locus ranged between 0.56 and 0.89, with an overall mean genetic diversity per locus (*H*) of 0.69. Despite the large genetic diversity within this population, the observed and expected variances of ET mismatches were not significantly different at the 95% confidence level, indicating that the population was not composed of genetically distinct lineages. The relative similarities among the ETs and the ATCC *Bradyrhizobium* sp. (*Lupinus*) strain, USDA 3051, are shown in a dendrogram (Fig. 3). The majority (73.2%) of the isolates were represented by two closely related ETs (2 and 3) and were found in one cluster of five ETs. Isolates represented by ET1 were the only other substantial population in

the collection and, with the exception of one isolate, were recovered only from blue lupine, where they occupied 50% of the nodules. Along with the dominance of ET1 on blue lupine, a greater number of ETs was recovered from blue lupine than from any other host (Table 3).

Host range of the different ETs. Each of 17 isolates chosen from each of the 17 ETs exhibited a broad host range (Table 4). Siratro, big trefoil, blue, and white lupines were nodulated by all of these isolates. Nevertheless, the symbiotic effectiveness potential of all isolates was poor when the plant yield was compared with the plant yield of those either inoculated with an inoculant-quality strain, WU425, or supplemented with nitrate. In the case of blue lupine and siratro, about 50% of the isolates showed a mediocre ability to fix nitrogen, and the rest were completely ineffective. Only two ETs (2 and 9) fixed nitrogen on big trefoil, and none did so on serradella. Furthermore, seven ETs did not nodulate the latter host. Although isolates of ET1 were more dominant nodule occupants than ET2 on soil-grown blue lupine, isolates of both ETs were equally mediocre in effectiveness on the two hosts (Table 5). Indeed, ET1 isolates were somewhat better fixers of nitrogen on white lupine than on blue lupine.

DISCUSSION

Our findings have bearing upon a number of issues that pertain to *Bradyrhizobium* sp. specifically and to genetic structure of local populations of soilborne bacterial species in general. Although an analysis of *B. japonicum* was not the major goal of this work, the MLEE data are clearly supportive of this species being composed of at least two distinct groups of strains (16, 18, 19, 24, 25, 45). In addition, since statistical analysis of the MLEE data revealed that large linkage disequilibrium exists between groups 1 and II (data not shown), we confirm that the two groups are genetically distinct lineages. These data provide additional support for the proposition of others (25) that the soybean-nodulating *Bradyrhizobium* sp. should be thought of as two different "species" (group 1 being *B. japonicum* and group II being *Bradyrhizobium elkani*).

Although MLEE has been used widely to explore aspects of genetic diversity and population genetic structure among *Rhizobium* species (10, 11, 17, 28, 38, 40, 41, 44, 49, 50), we believe this work to be the first of its kind to address a local soil population of the genus *Bradyrhizobium*. Despite that our data provide direct evidence for a high degree of genetic diversity ($H = 0.69$) within this soil population of *Bradyrhizobium* sp., they provide no evidence of genetic structure (linkage disequilibrium or nonrandom association of alleles) existing within the population. As a consequence, it is worth emphasizing that the data presented in Fig. 4 (in contrast to Fig. 2) have no phylogenetic significance. The ETs should not be considered genetically isolated genotypes despite there being large dissimilarities between them in many cases. This finding is somewhat surprising since Pinero et al. (38) obtained an equally high H value which was accompanied by strong linkage disequilibrium when *Phaseolus*-nodulating *Rhizobium* strains of diverse origins were examined with MLEE. Indeed, the latter findings subsequently led to the subdivision of *Phaseolus*-nodulating *Rhizobium* sp. into three genetically distinct species, i.e., *R. leguminosarum*, *Rhizobium tropici*, and *Rhizobium etli* (33, 41).

In agreement with our findings, however, other workers have shown recently that linkage disequilibrium can be minimal within local soil populations of *R. etli* (44) and *Bacillus subtilis* (20) and within global populations of the pathogen *Neisseria gonorrhoeae* (34, 36). Furthermore, Leung et al. (28) showed that linkage disequilibrium in a local soil population of *R. leguminosarum* bv. trifolii was due entirely to the presence of one genetically distinct lineage which contributed only 8 of the 53 ETs identified in the population. When these eight genotypes were excluded from the analysis, the remainder of the genotypes in the population were found to be in linkage equilibrium. These data imply that genetic exchange occurs sufficiently often among members of the same local soil population to hinder the development of linkage disequilibrium through periodic selection and immigration. The low density (<10/g) of *Bradyrhizobium* sp. in Abiqua soil is probably due to the prolonged absence of an appropriate legume host. Under these conditions, it is unlikely that there have been many opportunities for selective clonal expansion of specific genotypes and concomitant development of genetic structure in the population. However, neither is it easy to envisage how this low-density bacterial population could be organized in a manner conducive for extensive recombination to occur. A more comprehensive examination of the microscale spatial distribution of this *Bradyrhizobium* population is needed.

Many studies have described host plant X strain symbiotic interactions between different lupine species and *Bradyrhizobium* sp. (12 [and references cited therein], 14, 26). Our work extends those findings by providing the first direct evidence for two different agriculturally important lupine species recovering distinctly different chromosomal types from the same soil population of *Bradyrhizobium* sp. At this time, however, it remains unclear whether uncultivated lupine species are the native hosts of this local soil population or whether the population is the legacy of some other legume species that previously occupied the site. Indeed, the poor effectiveness of all of the ETs on all of the test hosts is reminiscent of a situation in which *Bradyrhizobium* isolates recovered from native Australian woody legumes were found to be generally ineffective on herbaceous test hosts (27). Our observation that yellow serradella (*O. compressus*) possesses more stringent nodulating requirements for *Bradyrhizobium* sp. than lupine species is entirely consistent with other findings (15). In that earlier study (15), a high proportion of "cowpea miscellany" rhizobia recovered from native Australian legumes would not nodulate french serradella even though they nodulated *Lupinus digitatus* (now *Lupinus cosentinii* Guss.). Researchers need to be aware of the limitations of serradella for determining the soil population density of lupine-nodulating *Bradyrhizobium* sp. by the plant infection-soil dilution procedure (4, 8, 9, 43).

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