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Desta Beyene · Serawit Kassa · Franklin Ampy
Amha Asseffa · Tadesse Gebremedhin
Peter van Berkum

Ethiopian soils harbor natural populations of rhizobia that form symbioses with common bean (*Phaseolus vulgaris* L.)

Abstract The diversity and taxonomic relationships of 83 bean-nodulating rhizobia indigenous to Ethiopian soils were characterized by PCR-RFLP of the internally transcribed spacer (ITS) region between the 16S and 23S rRNA genes, 16S rRNA gene sequence analysis, multilocus enzyme electrophoresis (MLEE), and amplified fragment-length polymorphism. The isolates fell into 13 distinct genotypes according to PCR-RFLP analysis of the ITS region. Based on MLEE, the majority of these genotypes (70%) was genetically related to the type strain of *Rhizobium leguminosarum*. However, from analysis of their 16S rRNA genes, the majority was placed with *Rhizobium etli*. Transfer and recombination of the 16S rRNA gene from presumptively introduced *R. etli* to local *R. leguminosarum* is a possible theory to explain these contrasting results. However, it seems unlikely that bean rhizobia originating from the Americas (or Europe) extensively colonized soils of Ethiopia because *Rhizobium tropici, Rhizobium gallicum*, and *Rhizobium giardinii* were not detected and only a single ineffective isolate of *R. etli* that originated from a remote location was identified. Therefore, Ethiopian *R. leguminosarum* may have acquired the determinants for nodulation of bean from a low number of introduced bean-nodulating rhizobia that either are poor competitors for nodulation of bean or that failed to sur-

D. Beyene · A. Asseffa
Biochemistry Department, Howard University,
Washington DC, USA

S. Kassa · F. Ampy
Biology Department, Howard University,
Washington DC, USA

T. Gebremedhin
Ethiopian Agricultural Research Organization (EARO),
Addis Ababa, Ethiopia

P. van Berkum (✉)
Soybean Genomics and Improvement Laboratory,
USDA, 10300 Baltimore Blvd., ARS, Bldg 010,
BARC-West, HH-4, Beltsville, MD 20705, USA
Tel.: +1 301-5047280, Fax: +1-301-5045728,
e-mail: pberkum@ba.ars.usda.gov

vive in the Ethiopian environment. Furthermore, it may be concluded from the genetic data presented here that the evidence for separating *R. leguminosarum* and *R. etli* into two separate species is inconclusive.

Introduction

Phaseolus vulgaris L. (common bean) is native to the highland regions of Mesoamerica and Andean South America; in both areas beans have been domesticated for more than 7,000 years (Gepts and Bliss 1988; Gepts 1990). Bean is grown in some parts of the Highlands of Ethiopia even though Ethiopia is considered the center of origin for many other leguminous crop plants, including pea, clover, and lentil (Raven and Polhill 1981). Pea was domesticated, as early as the fourth century and since that time has been an integral part of Ethiopian agriculture. As a consequence, presumably Ethiopian soils harbor *Rhizobium leguminosarum*, which forms symbiotic relationships with these native crop legumes.

Initially, all bean rhizobia were classified as *R. leguminosarum* (Jordan 1984). Later, *Rhizobium etli* was proposed as a species separate from *R. leguminosarum* based on results of variation in chromosomal markers determined by multilocus gel electrophoresis (Pinero et al. 1988; Segovia et al. 1993). Variation in the 16S rRNA gene sequences of *R. leguminosarum* and *R. etli* were reported subsequent to the proposal to separate these two species (van Berkum et al. 1996). Similarly, *Rhizobium tropici* as a bean-nodulating species was proposed based on variation in chromosomal markers (Martinez et al. 1991) before Willems and Collins (1993) reported the 16S rRNA gene sequence. The 16S rRNA sequences of *R. leguminosarum* and *R. etli* or *R. tropici* are very similar and vary only by 1 and 2%, respectively (van Berkum et al. 1996). In the case of *R. etli* and *R. leguminosarum,* 16S rRNA gene sequence variation may well be inconclusive evidence

to separate these two species, especially since van Berkum and Eardly (1998) concluded that phylogenetic relationships based on 16S rRNA gene sequence variation are unreliable evidence for resolving species below the genus level.

Suggestions have been made that bean forms nitrogen-fixing symbiotic relationships with five different rhizobial species (Amarger et al. 1997; Herrera-Cervera et al. 1999; Martinez et al. 1991; Segovia et al. 1993; van Berkum and Eardly 1998; Young 1985). Although bean rhizobia originating from Latin America have been classified into two separate species, *R. etli* and *R. tropici* (Eardly et al. 1995, Martinez et al. 1985, 1991; Pinero et al. 1988; Segovia et al. 1993), some evidence has been reported that *Rhizobium gallicum* and *R. leguminosarum* also may inhabit soils of the Americas (Graham et al. 1999; Sessitsch et al. 1997). Bean rhizobia recovered from African soils were reported to share molecular characteristics reminiscent of *R. etli* and *R. tropici* (Anyango et al. 1995; Diouf et al. 2000; Tjahjoleksono 1993). To explain the high incidence of *R. etli* and *R. tropici* encountered in West African soils, Diouf et al. (2000) proposed that bean rhizobia had been introduced from the Americas together with the seed.

Beans are extensively grown in the Central Highlands of Ethiopia. This crop is not inoculated but does form effective symbioses with native rhizobia (Beyene 1985). Although some agronomic information has been reported for bean rhizobia of Ethiopian origin (Beyene 1985), nothing else is known. Therefore, our objective in this study was to characterize bean rhizobia from different bean-producing areas of Ethiopia for diversity and to determine whether they are related to *R. leguminosarum* or to the Latin-American species *R. etli* and *R. tropici*.

Materials and methods

Bacterial strains and growth conditions

Phaseolus vulgaris cultivar Carioca was grown from seeds in Leonard jars under greenhouse conditions either during May, June, or July for 5 weeks. The seeds were surface-sterilized before sowing (van Berkum et al. 1994) and were inoculated at the time of sowing with soil samples (100 mg/seed) collected from different agro-ecological regions of Ethiopia (Fig. 1). Each jar contained three plants grown under supplemental lighting (14 h/10 h light/dark cycles) and temperatures were maintained at 24 °C/20 °C. Plants were removed from the jars and rhizobia were isolated according to the procedure of Vincent (1970), except for the use of modified arabinose gluconate (MAG) growth media (van Berkum 1990). Single colonies were selected and pure cultures were examined for nodulation of bean in Leonard jars (van Berkum et al. 1994) and were subsequently characterized. Bean-nodulating rhizobia originating from Kenya (Anyango et al. 1995) were not made available to us for analysis in this study.

Determination of nitrogen-fixation capacity

Plant tests were done in triplicate with three plants per jar in a greenhouse as described before (van Berkum et al. 1994). Broth cultures of 13 isolates, each representing different groups identified by analysis of the internally transcribed space (ITS) region, and two culture collection strains were used as inoculum. Culture collection reference strains were *R. etli* (CFN42, USDA 9032) and *R. tropici* (CIAT 899, USDA 9030). The plants were harvested af-
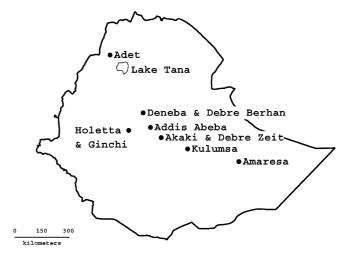

Fig. 1 Map of Ethiopia that indicates the location of the different sites where soil samples were collected for isolation of bean rhizobia
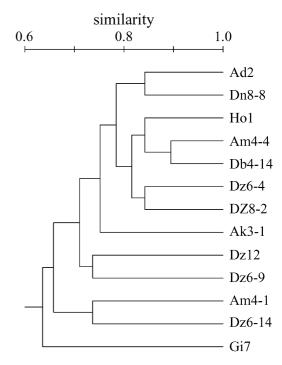

Fig. 2 Similarity among the 13 representative Ethiopian bean isolates based on fingerprint patterns of the internally transcribed space (ITS) region obtained after digesting PCR products with *Msp*I

ter 5 weeks to determine nitrogenase activity by acetylene reduction assay of whole roots (van Berkum and Sloger 1979) and to measure nodule and shoot dry matter. The data were analyzed for significant variation by Duncan's New Multiple Range test using the software package Unistat version 4.0 (Unistat, UK).

ITS-RFLP and amplified fragment-length polymorphism analysis

Genomic DNA was purified from each isolate grown in MAG broth for 2 days using a QIAamp tissue kit (Qiagen, Valencia, Calif., USA) and the procedure specified by the manufacturer.

The ITS regions between the 16S and 23S rRNA genes of each isolate were amplified by PCR with final reaction volumes of 40 µl and using the protocols described by van Berkum and Fuhrmann

(2000). The presence of a single PCR product in each reaction mixture generated from each of the templates was verified in 10-μl subsamples by horizontal gel electrophoresis (Sambrook et al. 1989). The remainder of each sample was then used in a *Msp*I restriction digestion and the products were incubated overnight at 37 °C. Subsequently, the restriction fragments were precipitated with two volumes of 100% (v/v) ethanol for 10 min and collected using a microcentrifuge at full speed for 10 min. Each sample was washed with 70% (v/v) ethanol and dried in a Speed Vac Concentrator (Savin Instruments, Hicksville, N.Y., USA). The precipitates were dissolved in 4 μl of a 5:1 (v/v) mixture of TBE buffer and tracking dye (dye II, Sambrook et al. 1989) and the molecular sizes of the restriction fragments in 2 μl of each sample were determined by horizontal gel electrophoresis using a gel mixture of 1% (w/v) agarose and 1% (w/v) GelTwin (Baker, Phillipsburgh, N.J., USA) with 0.5 μg ethidium bromide/ml. The gels were examined on a trans-illuminator and were photographed after electrophoresis for 2 h at 120 V. The presence or absence of restriction fragments of each molecular size was scored for each lane across the gel to produce a rectangular data matrix using the software DNA Proscore (DNA Proscan, Nashville, Tenn., USA). The matrix was used to generate simple matching coefficients, which were clustered using SAHN to generate a phenogram using NTSYSpc version 1.6 (Rohlf 1988). Amplified fragment-length polymorphism (AFLP) analysis with each isolate was done and the data were analyzed according to the methods described by van Berkum and Fuhrmann (2000).

Table 1 PCR-RFLP profiles of the Ethiopian isolates using analysis of the internally transcribed space (ITS) region between the 16S and 23S rRNA genes

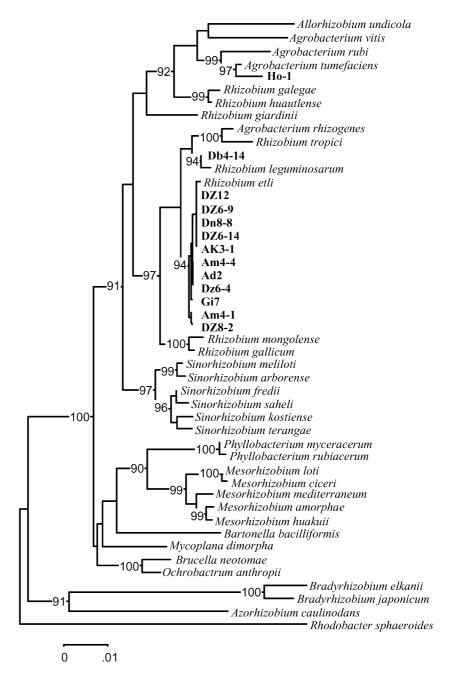| Profile	Representative isolates	Number of isolates	Isolates included in each profile
1	Ad2	1	Ad2
2	Ak3-1	3	Ak3-1, Ak3-2, Ak3-3
3	Am4-1	2	Am4-1, Am4-2
4	Am4-4	2	Am4-3, Am4-4
5	DZ6-4	2	DZ6-2, DZ6-4
6	DZ6-9	3	DZ6-9, DZ6-10, DZ6-13
7	DZ6-14	2	DZ6-14, DZ6-15
8	DZ8-2	2	DZ8-1, DZ8-2
9	DZ12	4	DZ6, DZ6-1, DZ7, DZ12
10	Gi7	26	Ak1, Ak2, Ak3, Ak4, Ak5, Ak7, Ak9, Ak11, Ak12, Ak14, Am1, Am2, Am5, Am6, Am7, Gi2, Gi3, Gi4, Gi5, Gi6, Gi7, Gi8, Ku2, Ku3, Ku4, Ku5
11	Ho1	1	Ho1
12	Db4-14	25	Db4-1, Db4-2, Db4-4, Db4-6, Db4-7, Db4-8, Db4-9, Db4-12, Db4-13, Db4-14, Db4-15, Db4-16, Db4-17, Db4-18, Db4-20, Db4-21, Ku11-1, Ku11-3, Ku11-4, Ku11-6, Ku11-7, Ku11-8, Ku11-9, Ku6, Ku7
13	Dn8-8	10	Dn8-1, Dn8-2, Dn8-3, Dn8-4, Dn8-5, Dn8-7, Dn8-8, Dn8-9, Dn8-10, Dn8-11

PCR amplification and sequencing analysis of 16S rRNA genes

PCR amplification and sequencing analysis of the 16S rRNA gene were as described by van Berkum et al. (1996). PILEUP of the GCG Wisconsin software package was used to align sequences to derive Jukes-Cantor distances for the construction of a neighbor-joining tree using the software package MEGA (Kumar et al. 1993). The Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999) was used to determine whether another hypothetical tree, constrained for monophyly of *R. leguminosarum* with isolates Ad2, Ak3-1, Am4-1, Am4-4, Dn8-8, DZ12, DZ6-4, DZ6-9, DZ6-14, and DZ8-2, would be less likely than the unconstrained distance tree. The 16S rRNA gene sequences of the representative isolates Ad2, Ak3-1, Am4-1, Am4-4, Db4-14, Dn8-8, DZ12, DZ6-14, DZ6-4, DZ6-9, DZ8-2, Gi7, and Ho-1 have been deposited in GenBank under accession numbers AY210704 to AY210716.

Multilocus enzyme electrophoresis

One isolate that represented each of 13 different PCR-RFLP ITS types was included, the reference type strains *R. etli* (CFN 42, USDA 9032), *R. tropici* (CIAT 899, USDA 9030), and *R. leguminosarum* (USDA 2370), as well as the West African bean strains ISRA 350, ISRA 362, and ISRA 27 originating from Senegal and Gambia (Diouf et al. 2000). ISRA-77 was not included in our analysis because, according to Diouf et al. (2000), it has the same electrophoretic type (ET) as ISRA-362. Cultures were grown in 50 ml

Table 2 The effect of rhizobial inoculation on nitrogenase activity, nodule dry mass and shoot dry matter of *Phaseolus vulgaris* grown in Leonard jars in a greenhouse. Numbers in the same column flanked by one or more same letters are not significantly different at the 5% level of probability as determined by Ducan's new multiple range test

Strain/isolate	Shoot dry matter (mg/plant)	Acetylene reduction (μmol C_2H_4/plant/h)	Nodule dry matter (mg/plant)
Rhizobium etli, CFN 42	1210 defgh[*]	16.7 bcd	237 cdef
Rhizobium tropici, CIAT 899	2100 ab	17.7 bcd	313 bcde
Ad2	740 hi	17.0 bcd	119 fg
Ak3-1	1306 cdefg	13.7 cde	326 bcde
Am4-1	1676 bcdef	16.0 bcd	353 bcd
Am4-4	1410 bcdefg	22.7 bc	236 cdef
DZ6-4	976 fgh	13.3 cde	230 cdef
DZ6-9	2060 ab	30.7 a	418 ab
DZ6-14	1950 abcd	31.0 a	338 bcd
DZ8-2	1816 abcde	23.0 b	373 abc
DZ12	470 i	6.3 ef	180 ef
Gi7	1966 abc	30.7 a	505 a
Ho1	1540 bcdefg	19.0 bc	326 bcde
Db4-14	1410 bcdefg	16.7 bcd	260 bcdef
Dn8-8	1066fgh	10.7 de	247 cdef
Control	210i	0.0 f	0 g

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Fig. 3 Phylogenetic relationships of 13 Ethiopian PCR-RFLP reference isolates reconstructed from aligned 16S rRNA gene sequences
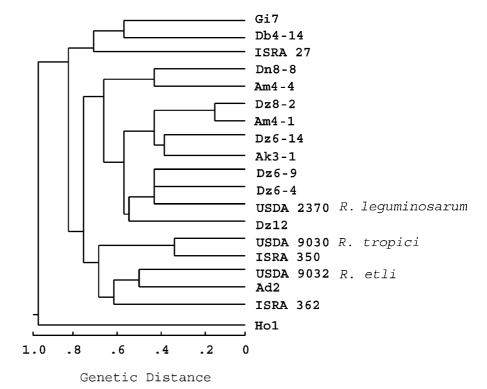

MAG broth for 24 h at room temperature; cell collection by centrifugation and subsequent protein extraction were according to the methods described by van Berkum et al. (1998). The protein extracts were divided into aliquots and stored at –20 °C. The aliquots from each extract were thawed and applied to cellulose-acetate membranes; proteins were separated by electrophoresis (Herbert and Beaton 1993) and subsequently stained according to the methods described by Selander et al. (1986). The following eight enzymes were included in the analysis: isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme, malate dehydrogenase, phosphoglucomutase, phosphoglucose isomerase, and xanthine dehydrogenase. Distinctive mobility variants (electromorphs) of each enzyme, numbered in order of decreasing anodal mobility, were equated with alleles at the corresponding structural gene locus. Allele profiles or ETs were equated with multilocus genotypes. The genetic distance between pairs of ETs was estimated as the proportion of loci at which dissimilar alleles (mismatches) occurred. Clustering of ETs from a matrix

of pairwise genetic distances was derived by the unweighted pair group method (Sneath and Sokal 1973). Genetic diversity (h) at an enzyme locus was calculated as $h=[1-Sx_i 2] [n/(n-1)]$, where x_i is the frequency of the ith allele at the locus and n is the number of ETs in the population. Computer programs for the analysis were written by T.S. Whittam (Pennsylvania State University).

Results

A total of 83 rhizobial cultures were isolated from nodules of *P. vulgaris* plants inoculated with soil samples collected from different sites in Ethiopia (Fig. 1). The number of isolates from each region were 1, 13, 9, 16, 13, 10, 7, 1, and 13 from Adet (Ad), Akaki (Ak), Amaresa (Am), Debre Berhan (Db) Debre Zeit (DZ), Deneba (Dn), Ginchi (Gi),

Fig. 4 Genetic distance among the 13 Ethiopian ITS PCR-RFLP genotypes, *Rhizobium leguminosarum* (USDA 2370), *Rhizobium etli* (USDA 9032), *Rhizobium tropici* (USDA 9030), ISRA 27, ISRA 350 and ISRA 362 derived by multilocus enzyme electrophoresis from allele variation at eight enzyme loci

Holetta (Ho) and Kulumsa (Ku), respectively. Each isolate was identified by the acronym of the region from which it originated followed by the number of the nodule from which the isolation was made. Each of the 83 isolates was verified for nodulation of bean; preliminary effectiveness scores were estimated from plant color and height.

PCR-RFLP of the 16S-23S ITS region

From the analysis of the ITS regions, 13 distinct types among the 83 Ethiopian bean isolates were identified (Fig. 2). The majority of the isolates (74%) belonged to types 10, 12, and 13 (Table 1). With the exception of Ad2 and DZ12, the representative isolates formed effective nitrogen-fixing symbioses with bean, since plant dry matter scores were significantly increased over non-inoculated controls (Table 2). Dry matter accumulation with each of the effective isolates compared well with that obtained with the type strains for *R. etli* (CFN 42) and *R. tropici* (CIAT 899). Nitrogenase activity by acetylene reduction was observed, with the ineffective isolates possibly indicating late onset of the symbiosis.

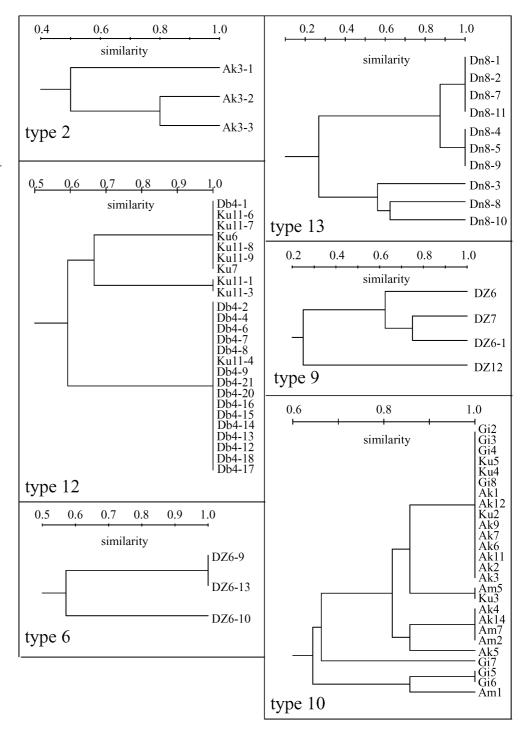Phylogeny based on 16S rRNA sequences

Except for Db4-14 and Ho-1, all the Ethiopian isolates representing the 13 ITS PCR-RFLP types were placed with *R. etli* (Fig. 3). Only Db4-14 was placed together with the type strain for *R. leguminosarum*. None of the isolates grouped with *Rhizobium mongolense, R. gallicum, Rhizobium giardinii*, or *R. tropici*.

Multilocus enzyme electrophoresis

The genetic relatedness of the 13 isolates representing the Ethiopian bean rhizobia was compared with three reference strains of species that nodulate bean (*R. etli, R. tropici*, and *R. leguminosarum*) and with three representatives (ISRA 27, ISRA 350, and ISRA 362) from a collection of bean rhizobia originating from Senegal and Gambia (Diouf et al. 2000). The type strains for *R. gallicum* and *R. giardinii* were not included in this analysis because none of the isolates from Ethiopia were related to these two species according to 16S rRNA gene sequences. A total of 19 distinctive ETs were identified based on the allelic profile of the eight enzymes (Fig. 4). The mean number of alleles was 5.5, ranging from 4 to 7 electromorphs, and the mean genetic diversity (*H*) was 0.732. From clustering analysis of the data it was confirmed that two strains originating from West Africa (ISRA 362 and ISRA 350) were closely related to *R. etli* and *R. tropici* (Fig. 4). The third West African reference strain (ISRA 27) was more divergent. The majority (nine isolates) of the Ethiopian reference cultures clustered with the type strain for *R. leguminosarum*, while one (Ad2) clustered with ISRA 362 and the *R. etli* type strain, USDA 9032 (Fig. 4). The remaining two isolates (Gi7 and Db 4-14) were more closely related to ISRA 27; all three were more divergent from the three type strains used as reference. None of the Ethiopian reference isolates were placed with *R. tropici* type strain USDA 9030 and West African reference strain ISRA 350.

The nine isolates, Ad2, Am4-4, Ak3-1, Am4-1, Dn8-8, DZ6-14, DZ6-4, DZ6-9 and DZ12 were placed with *R. leguminosarum* by multilocus enzyme electrophoresis

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Fig. 5 Genotype heterogeneity within each of six ITS PCR-RFLP types based on results of amplified fragment-length polymorphism analysis. The isolates of PCR-RFLP patterns type 1, type 3, type 4, type 5, type 7, type 8, and type 11 were not included in this analysis since there were only one or two isolates within each type. These types included one isolate each from Adet and Holetta; two isolates each of the two types from Amaressa, and two isolates each of the three types from Debre Zeit

(MLEE). This contrasted their placement with *R. etli* by sequence analysis of the 16S rRNA genes. Therefore, the likelihood for a hypothetical phylogenetic tree having these isolates constrained as a monophyletic group together with *R. leguminosarum* and Db4–14 instead of *R. etli* was tested. This hypothetical tree was compared to the tree generated with the unconstrained data set using the Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999). The probability value obtained was $p = 0.023$, which is lower than 0.05 and, therefore, constraining the data set to force

monophyly of the 9 isolates with *R. leguminosarum* and Db4–14 produced a change in tree topology, which was significantly less likely than the original tree.

AFLP analysis

The potential for genetic diversity within each ITS PCR-RFLP type was examined by AFLP analysis and MLEE. AFLP analysis was done with six types (90% of the iso-

lates) that included groups with three or more isolates. MLEE was done only with the three largest groups (ITS PCR-RFLP types 10, 12, and 13), representing 74% of the isolates. Within types 2, 6, 9, 10, 12, and 13, respectively, 3, 2, 4, 7, 3, and 5 distinct genotypes were observed by AFLP profile polymorphisms (Fig. 5). There were 4, 1, and 2 ETs within groups 10, 12, and 13, respectively, each with a mean genetic diversity (*H*) of 0.31, 0, and 0.17, respectively.

Discussion

To our knowledge, there is only the communication of Diouf et al. (2000) in which a molecular systematic approach was used to characterize bean-nodulating rhizobia originating from African soils. Diouf et al. (2000) concluded from MLEE data that West African soils harbored only *R. etli* and *R. tropici*. The MLEE results from our study and those of Diouf et al. (2000) significantly differ because *R. leguminosarum* was predominantly detected in Ethiopian soils. Soils of Kenya (neighboring Ethiopia) have been reported to harbor bean-nodulating rhizobia similar to *R. leguminosarum*, *R. etli*, and *R. tropici* (Anyango et al. 1995), but the authors indicated that their characterization was presumptive since little molecular systematic evidence had been gathered. Variation in rhizobial populations between Ethiopia (our study) and the West African nations of Senegal and Gambia (Diouf et al. 2000) can be expected because of dissimilarity in native legume species and differences in geographic location and topography.

Another difference between bean-nodulating rhizobia in Ethiopian and those in West African soils is the level of genetic diversity. We detected 13 ITS PCR-RFLP types among 83 isolates, while Diouf et al. (2000) reported only two among 58 isolates, and one of the two was subdivided into three. Differences in the level of genetic diversity also were apparent from the MLEE data. We detected that each PCR-RFLP representative strain had a different ET and that up to four ETs or seven AFLP polymorphisms could be detected within each PCR-RFLP group. In contrast, Diouf et al. (2000) reported only four ETs among 54 isolates with a mean genetic diversity of 0.44 when they excluded the four isolates with characteristics of *R. tropici*. The mean genetic diversity we observed was 0.73 among the isolates representing the 13 PCR-RFLP types, none of which grouped with the *R. tropici* type strain CIAT 899.

Based on results of MLEE, we placed nine of the 13 PCR-RFLP representative isolates with *R. leguminosarum*, one with *R. etli*, two with the West African isolate ISRA 27, and none with *R. tropici*. Diouf et al. (2000) indicated that ISRA 27 belongs to *R. etli*. However, we wish to point out that Diouf et al. (2000) reported a wider genetic diversity by MLEE among the *R. etli* reference strains used in their study than between the type strains for *R. etli* (CFN 42) and *R. tropici* (CIAT 899). Similarly, we observed from the MLEE data that ISRA 27 was genetically more different than the reference strains of *R. etli*, *R. tropici*, and *R. leguminosarum* (USDA 2370) were to each other.

The most logical conclusion would be that the *R. etli* reference strains F8 and F16 that were used by Diouf et al. (2000), should not have been assigned to *R. etli*. Consequently, subgroup II.3 (ISRA 27) bean-nodulating isolates from Senegal and our isolates Gi7 and Db4–14 also probably should not be assigned to *R. etli*.

Although nine Ethiopian isolates grouped with *R. leguminosarum* by MLEE analysis, we placed them with *R. etli* by sequencing analysis of the 16S rRNA gene. We attempted to constrain the phylogenetic tree by changing the placement of these nine isolates into a group with *R. leguminosarum*. However, this produced a tree topology that was significantly less likely by the Shimodaira and Hasegawa test (Shimodaira and Hasegawa 1999). Therefore, we concluded that these nine isolates with ETs characteristic of *R. leguminosarum* had divergent 16S rRNA alleles that were reminiscent of the sequences reported for *R. etli* (van Berkum et al. 1996). Our observation is similar to that reported by Eardly et al. (1995) for bean-nodulating rhizobia of Colombian origin, in which 17 ETs characteristic of *R. etli* had 16S rRNA gene PCR-RFLP patterns reminiscent of *R. leguminosarum*. Our results and those of Eardly et al. (1995) differ since the Colombian *R. etli* had 16S rRNA alleles of *R. leguminosarum* while our Ethiopian *R. leguminosarum* had 16S rRNA alleles of *R. etli*. Eardly et al. (1995) concluded that the most plausible explanation would be transfer and recombination of the 16S rRNA gene either in part or as a whole. Certainly, the possibility for transfer and recombination between divergent 16S rRNA alleles in rhizobia has been demonstrated (van Berkum et al. 2001, 2003). The placement of Ho-1 with *Agrobacterium* may be explained by recombination since this isolate had an ITS-PCR RFLP pattern similar to Am4-4, Db4-14, Dz6-4, and Dz8-2, which were placed with *R. etli* and *R. leguminosarum* by 16S rRNA gene sequence.

It seems unlikely that bean rhizobia originating from the Americas (or Europe) extensively colonized soils of Ethiopia because we failed to detect *R. tropici, R. gallicum* and *R. giardinii* and identified only a single isolate of *R. etli* that was ineffective for nitrogen fixation and originated from a remote location. Therefore, it is not completely clear how *R. leguminosarum* in Ethiopian soils acquired the genetic information for nodulation of bean, nor is it apparent how Ethiopian rhizobia gained the 16S rRNA gene sequence characteristic of *R. etli* by recombination. Ethiopian *R. leguminosarum* may have acquired the determinants for nodulation of bean from a low number of introduced bean-nodulating rhizobia that either are poor competitors for nodulation of bean or that failed to survive in the Ethiopian environment. Furthermore, it may be concluded from the genetic data presented that the evidence for separating *R. leguminosarum* and *R. etli* into two separate species is inconclusive.

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