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Diversity of Rhizobia Nodulating *Phaseolus vulgaris* L. in Two Kenyan Soils with Contrasting pHs

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Rhizobia were isolated from two Kenyan soils with pHs of 4.5 and 6.8 and characterized on the basis of their host ranges for nodulation and nitrogen fixation, colony morphologies, restriction fragment fingerprints, and hybridization with a *nifH* probe. The populations of rhizobia nodulating *Phaseolus vulgaris* in the two soils were similar in numbers and in effectiveness of N_2 fixation but were markedly different in composition. The population in the Naivasha soil (pH 6.8) was dominated by isolates specific in host range for nodulation to *P. vulgaris*; these all had multiple copies, in most cases four, of the structural nitrogenase gene *nifH*. Only one of the isolates from this soil formed effective nodules on *Leucaena leucocephala*, and this isolate had only a single copy of *nifH*. By contrast, the population in the acid Daka-ini soil (pH 4.5) was composed largely of broad-host-range isolates which had single copies of *nifH*. The isolates from the Daka-ini soil which were specific to *P. vulgaris* generally had three copies of *nifH*, although one isolate had only two copies. These rhizobial isolates are indigenous to Kenyan soils and yet have marked similarities to previously described *Rhizobium* species from other continents.

The common bean (*Phaseolus vulgaris* L.) which nodulates with fast-growing rhizobia is a relatively permissive legume host. The taxonomy of rhizobia which can nodulate *P. vulgaris* has been progressively revised from the initial description of *Rhizobium phaseoli*, which was based solely on the ability to nodulate this host. Recognition that recombination of chromosomal genes between the rhizobial symbionts of beans, clover, and peas could be readily obtained under laboratory conditions (12) and that the host ranges of such strains could be altered simply by replacement of the symbiotic plasmids (2, 13), coupled with earlier observations that strains isolated from beans, clover, and peas could not be reliably distinguished on phenotypic grounds (10), led to the description of a single species, *Rhizobium leguminosarum*, which consists of three biovars (14). Strains which nodulate *P. vulgaris* are biovar phaseoli, those that nodulate peas and vetches (*Vicia* spp.) are biovar viciae, and those which nodulate clovers (*Trifolium* spp.) are named biovar trifolii. *R. leguminosarum* bv. phaseoli strains were found to have reiterations of the genes which encode the enzyme nitrogenase, *nifHDK* (22), a trait not found in strains of the other biovars. Further research on *Phaseolus* rhizobia from soils in Latin America led to the description of two distinct types, one which was specific in host range to *P. vulgaris* and had multiple copies of *nifH* (termed *R. leguminosarum* bv. phaseoli type I) and one which also formed effective nodules on *Leucaena leucocephala* L. and contained only a single copy of *nifH* (termed *R. leguminosarum* bv. phaseoli type II) (20). These two groups were subsequently described as new species, the host-specific type I as *Rhizobium etli* (23) and type II as *Rhizobium tropici* (21). *R. tropici* strains were shown to be more tolerant of acidity on agar plates (20), and this species in fact contains the strains isolated from *P. vulgaris* which had previously been described as being acid tolerant (11, 30). Two further species of *Rhizobium* have been isolated from nodules of *P. vulgaris* grown in French soils (16) and have yet to be fully described, and several additional *Rhizobium* species were found among 146 isolates from American soils (3). It is thus likely that many species await discovery.

P. vulgaris is a New World species (15) which was introduced into Africa from Brazil in the 16th century (4) and yet is generally nodulated when grown in African soils which have never been deliberately inoculated. Rhizobia indigenous to African soils have been demonstrated to nodulate and fix nitrogen on *P. vulgaris* (9), but the possibility that rhizobia were also introduced and disseminated in Africa along with the seed remains. In this study we compared the compositions of the populations of rhizobia which nodulate *P. vulgaris* from two Kenyan soils with different pHs. Both soils were from farmers' fields in the same region, were from the same altitude (2,000 m), had been cropped with maize and beans in rotation in the previous years, and had never been inoculated with rhizobia. Our principal objective was to examine whether the stress of soil acidity was associated with a reduction in the number and diversity of rhizobia nodulating *P. vulgaris.*

MATERIALS AND METHODS

Type strains. *R. tropici* CIAT899 was obtained from Judy Kipe-Nolt, *R. etli* CFN42 was obtained from Esperanza Martínez-Romero, *R. etli* Kim5 was obtained from Ian Pepper, and *Rhizobium* sp. strain NUM466 was obtained from the Nairobi Microbiological Resources Center (MIRCEN) collection.

Soils. The two soils were collected from farmers' fields in which beans had been grown frequently but for which there was no history of inoculation with rhizobia. The acid soil (pH 4.5) was an Andosol from Daka-ini near the Gituamba Experimental Station, Murang'a, Kenya. This is an area where tea is commonly grown; the altitude is 2,120 m above sea level, and the annual mean rainfall is 2,003 mm. The near-neutral soil sample (pH 6.8) was an Andosol from Naivasha Marula, Kenya, an area at an altitude of 2,040 m with low-level annual rainfall of 653 mm. Soils were sampled with a spade which was sterilized with alcohol before sampling of each soil. Samples were loosely closed immediately in sterile polyethylene bags and stored fresh at 4°C.

Rhizobial counts and isolation. The numbers of rhizobia present in the soils which could nodulate *P. vulgaris* were estimated for the two soils by the most-probable-number, plant infection technique (31). Seeds of *P. vulgaris* cv. Canadian Wonder were surface sterilized by being rinsed in 95% ethanol and then submerged in 0.2% acidified mercuric chloride for 3 min, and they were then

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germinated on sterile water-agar plates for 2 days. Single seedlings were transplanted into growth pouches (Scientific Products, Evanston, Ill.) containing 100 ml of N-free nutrient solution (7), and the pouches were placed in a growth room at 24°C with 12-h days. Tenfold soil dilutions were inoculated into three replicate pouches 3 days later, and the plants were watered as required with sterile N-free nutrient solution. After four weeks, nodulation was assessed and the number of rhizobia was estimated by using the MPNES program (33). Nodules were collected and surface sterilized, and rhizobia were isolated by standard methods (31), with equal numbers of nodules being sampled from the low and high dilutions. A total of 44 nodules was sampled from plants grown on each soil type; 44 was chosen as the number required to have a 95% probability of sampling all strain types if nine different strains were present, assuming that each has an equal chance of forming a nodule (5). The choice of sample size was thus somewhat arbitrary but based on a compromise between obtaining a representative sample of the rhizobia able to nodulate *P. vulgaris* and the number of isolates which could easily be handled.

Screening for host range of nodulation and N_2 fixation. The abilities of the isolates to nodulate and fix nitrogen on *P. vulgaris*, *L. leucocephala*, and siratro (*Macroptilium atropurpureum*) were tested in separate experiments. Isolates from the acid Daka-ini soil were also tested with *Leucaena diversifolia*. Seed of siratro was obtained from the Wright Stephenson Co. (Silverwater, Australia), *L. leucocephala* seed was collected in Uganda, and *L. diversifolia* seed was collected in Rwanda. In each case three control treatments were included: an uninoculated treatment, an uninoculated treatment watered with NO_3^--N (70 mg liter^{-1}) in the nutrient solution, and treatment with a standard effective *R. tropici* strain, CIAT899. Treatments with each of the 88 isolates and the control treatments were replicated twice, except with siratro, for which three replicates were used.

P. vulgaris and *L. leucocephala* were grown in 4-in. (10.2-cm)-diameter pots (which had been sterilized by soaking in sodium hypochlorite solution) filled with perlite and watered with sterile N-free nutrient solution (7) containing additional calcium. Siratro was grown in large boiling tubes on plant nutrient agar slopes enclosed with foam bungs in a growth room at 25°C with 12-h days. Siratro seeds were scarified and surface sterilized in concentrated sulfuric acid for 20 min and washed thoroughly in sterile distilled water before being sown. *Phaseolus* and *Leucaena* seeds were sterilized as described for *Phaseolus* seeds above. All experiments were arranged in randomized complete blocks. *P. vulgaris*, *L. leucocephala*, and *L. diversifolia* plants were harvested after 8 weeks, and siratro plants were harvested after 4 weeks. Shoots were separated from the roots, dried at 60°C, ground, and analyzed for N content by Kjeldahl digestion with determination of NH_4-N concentrations in the digests by an automated colorimetric salicylate method. Roots were carefully examined to record nodulation characteristics (distribution, size, color, and number of nodules). Strains were classed as ineffective only when nodules were white and plants accumulated amounts of N less than or similar to those accumulated by the uninoculated controls. When ineffective phenotypes were observed, the effectiveness tests were repeated. There was no evidence of cross-contamination in any of the tests.

DNA analysis of bacterial isolates. DNA was prepared by the genomic DNA miniprep method as described by Wilson (32). Total genomic DNA was digested with restriction endonuclease (*Bam*HI, *Hin*dIII, or *Eco*RI). DNA digests were subjected to electrophoresis on 0.6% agarose gels in Tris-borate-EDTA buffer overnight at 30 V and then blotted onto nylon membranes by using alkaline transfer buffer.

Two different *nifH* fragments were used as probes for DNA hybridization, both internal to the *nifH* coding sequence. A 576-bp *Xho*I fragment was isolated from plasmid pBN370 containing the *nifH* gene from *Bradyrhizobium* sp. (*Parasponia*) strain Rp501 (provided by B. Tracey Nixon), and a 273-bp *Sal*I internal *nifH* fragment was isolated from plasmid pCQ15 containing one of the *nifH* genes from *R. etli* CFN42 (22). DNA fragments were isolated from low-melting-temperature gels and labelled with [^{32}P]dCTP by using Klenow fragment and random priming. The hybridization and washing of filters were done with low stringency as described by Maniatis et al. (19).

RESULTS

Population sizes and isolate characteristics. There was no significant difference between the sizes of the populations of rhizobia capable of nodulating *P. vulgaris* in the two soils, with an estimate of 1.47×10^4 cells per g of soil in both cases. Isolates from the acid Daka-ini soil generally formed dry colonies on yeast-mannitol-agar plates, while the majority of the isolates from the near-neutral Naivasha soil formed wet, gummy colonies (data not presented).

Nodulation and N_2 fixation with *P. vulgaris*. Two of the original 44 isolates from the Daka-ini soil and three isolates from the Naivasha soil failed to nodulate *P. vulgaris*, although their appearance on plates was very similar to that of other isolates and type strains of *Rhizobium* species capable of nodulating *Phaseolus* plants (Table 1). Of the isolates which did form nodules, four isolates from the Daka-ini soil and eight from the Naivasha soil formed only white nodules with *P. vulgaris* which were ineffective (Table 1) as demonstrated by the N accumulation of the plants inoculated with these isolates (Fig. 1). The distribution of the effectiveness of the isolates in N_2 fixation, measured as the amount of N accumulated, among the isolates from each soil was bimodal. One group of isolates from each soil consisted of ineffective and poorly effective strains with N accumulation close to that of the uninoculated control (9.45 mg of N per pot; Fig. 1). The majority of the isolates from both soils had effectiveness similar (25 to 35 mg of N per pot) to that of the *R. tropici* strain CIAT899 (32 mg of N per pot). Strains in the third group of isolates, most of which came from the Daka-ini soil, were more effective than CIAT899, with two isolates leading to plant accumulation of more than 45 mg of N per pot (Fig. 1).

The pattern of nodulation also varied widely between isolates (data not presented). Ineffective isolates formed many small (ca. 1-mm diameter) white nodules (>200 per plant), while most of the effective isolates formed nodules which were

TABLE 1. Nodulation and effectiveness phenotypes of the rhizobial isolates from the two Kenyan soils on *P. vulgaris*, *M. atropurpureum*, *L. leucocephala*, and *L. diversifolia*

Host plant	No. of isolates with the following phenotype[a]:							
	Daka-ini soil[b]				Naivasha soil[c]			
	Nod$^-$	Nod$^+$ Fix$^-$	Nod$^+$ Fix$^+$	Total	Nod$^-$	Nod$^+$ Fix$^-$	Nod$^+$ Fix$^+$	Total
P. vulgaris	2	4	38	44	3	8	33	44
M. atropurpureum	12	25	1	38	40	0	0	40
L. leucocephala	9	2	27	38	39	0	1	40
L. diversifolia	9	2	27	38				

[a] Nod, nodulation; Fix, N_2 fixation.
[b] pH 4.5.
[c] pH 6.8.

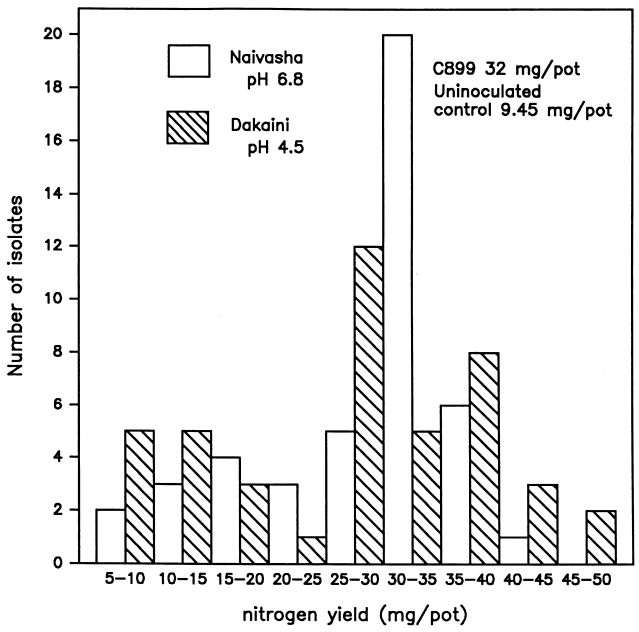

FIG. 1. Distribution of effectiveness in fixing nitrogen with *P. vulgaris* (as indicated by total N accumulation after 8 weeks of growth) of rhizobia isolated from the two Kenyan soils.

TABLE 2. Restriction fragment length polymorphism groups and species similarity of *Rhizobium* isolates composing the populations isolated from the two Kenyan soils with *P. vulgaris* as the trap host

Soil (pH)	RFLP group[a]	No. of isolates	No. of *nifH* bands[b]	*Rhizobium* species similar to
Daka-ini (4.5)	D1	6	1	*R. tropici*
	D2	14	1	*R. tropici*
	D3	4	1	*R. tropici*
	Distinct isolates	11	1 (6)	*R. tropici*
			2 (1), 3 (4)	*Rhizobium* sp. (*Phaseolus*-specific) type I
Naivasha (6.8)	N1	27	4	*Rhizobium* sp. (*Phaseolus*-specific) type I
	N2	3	4	*Rhizobium* sp. (*Phaseolus*-specific) type I
	Distinct isolates	10	4 (1), 3 (2)	*Rhizobium* sp. (*Phaseolus*-specific) type I
			? (6)[c]	
			1 (1)	*R. tropici*

[a] RFLP, restriction fragment length polymorphism.
[b] Numbers in parentheses indicate the numbers of isolates with given numbers of *nifH* bands.
[c] ? indicates that the number of *nifH* bands was not confirmed.

mainly 1 to 3 mm in diameter with 100 to 200 red nodules per plant. Five of the isolates formed few nodules, in extreme cases only three to five nodules per plant, which were dark red and large (3- to 4-mm diameter). There were no obvious differences in nodulation characteristics of isolates between the two soils. Further details of the nodulation characteristics of the isolates have been given by Anyango (1).

Host range of the isolates. Only isolates which nodulated beans were tested on other hosts, and some isolates were lost, reducing the total number tested on other hosts. None of the 40 isolates from the Naivasha soil nodulated siratro, and only one nodulated *L. leucocephala* effectively (Table 1). By contrast, 26 of the 38 isolates from the Daka-ini soil formed nodules on siratro, of which only one formed effective nodules. Twenty-nine of the isolates formed nodules with *L. leucocephala*, and only two of these formed only nodules ineffective in N_2 fixation. *L. diversifolia* showed a response identical to that of *L. leucocephala* in nodulation and effectiveness of N_2 fixation with the isolates from the Daka-ini soil.

"Strain" groupings based on restriction fragment fingerprints. The isolates were placed into groups on the basis of restriction fragment fingerprints generated by digestion of total DNA with the enzyme *Bam*HI. On this basis 27 of the isolates from the Naivasha soil were placed into one group (N1), 3 isolates formed another group (N2), and the remaining isolates had distinct restriction fragment fingerprints (Table 2). Three groups in the Daka-ini soil which contained 6 (group D1), 14 (group D2), and 4 (group D3) isolates were identified, and the remaining 11 isolates which were characterized had distinct restriction fragment fingerprints. The restriction fragment fingerprint of the *R. tropici* type strain CIAT899 had a pronounced similarity to the restriction fragment fingerprint of group D1.

All but two of the isolates in the three groups from the Daka-ini soil, groups D1, D2, and D3, had a broad host range, nodulating all three hosts (though all were ineffective in N_2 fixation with siratro). The two isolates in group D1 which failed to nodulate siratro (isolates 3 and 29) were effective in N_2 fixation on the two *Leucaena* species. Isolate 40 actually formed effective nodules on siratro but ineffective nodules on the *Leucaena* species, and it was placed in group D3. Isolate 32, which had a distinct restriction fragment fingerprint, nodulated all three hosts ineffectively. Of the other isolates with distinct restriction fragment fingerprints, five were specific in nodulation to *P. vulgaris*, four with three *nifH*-hybridizing (*Bam*HI) fragments and one with two *nifH*-hybridizing (*Bam*HI) fragments.

***nifH* hybridization.** Of the 23 isolates tested from the Naivasha soil, only one (isolate 45) had a single *nifH*-hybridizing (*Bam*HI) fragment of 20 kb (Fig. 2A), and this was also the isolate which effectively nodulated *L. leucocephala*. All of the other isolates from the Naivasha soil had four *nifH*-hybridizing *Bam*HI fragments except for two isolates (isolates 66 and 71) which had three such fragments (Fig. 2B and C). Hybridization with the *nifH* probe in *Bam*HI digests of 35 of the isolates from the Daka-ini soil was examined. The majority of these isolates had only a single *Bam*HI-*nifH* band (Fig. 2D), three isolates had three *Bam*HI-*nifH* bands (Fig. 2A, B, and D), and one isolate had only two *nifH* bands (data not shown).

The majority of the Naivasha isolates had an 11-kb *Bam*HI fragment which hybridized with the *nifH* probe in common with the *R. etli* strains Kim5 and CFN42. A further fragment of 6 kb was similar in size to a hybridizing fragment in CFN42 but not Kim5, while the fragments of 18 and 5 kb were similar in size to hybridizing bands in Kim5 and CFN42 had a smaller hybridizing fragment (Fig. 2B and C). The host-specific isolates from the Daka-ini soil also shared multiple *nifH*-hybridizing *Bam*HI fragments which were similar in size to those from the Naivasha soil except that the large hybridizing band at 18 kb was lacking (Fig. 2D, isolate 33). All of the broad-host-range isolates had a single hybridizing fragment of 2.5 kb (Fig. 2D), which was the same size as the hybridizing fragment of the *R. tropici* strain CIAT899.

As the strains of *Rhizobium* specific to *P. vulgaris* previously examined had only three *nifH* bands, we further examined the possibility that the observation of four bands was due to the restriction enzyme *Bam*HI cutting at a site within the region hybridizing with the 273-bp fragment of *nifH* in one copy of *nifH*. We therefore repeated the *nifH* hybridization after digestion of the DNA with two other restriction enzymes, *Hin*dIII and *Eco*RI, with three of the isolates from the Naivasha soil and the standard strain of *R. etli*, CFN42 (Fig. 3). The three Kenyan isolates produced four bands with *Hin*dIII, of which the smallest (2.4 kb) gave a less strong hybridizing signal, and five bands with *Eco*RI, four of which were close together between 4 and 5 kb. For *Hin*dIII and *Eco*RI three clear hybridizing bands were found with the *nifH* probe in DNA digests of strain CFN42.

DISCUSSION

Species of rhizobia and effectiveness in N_2 fixation. The populations of rhizobia nodulating *P. vulgaris* in the two soils were similar in numbers and in effectiveness of N_2 fixation but were markedly different in composition. The population in the Naivasha soil was dominated by isolates specific in host range for nodulation to *Phaseolus* species which all had multiple copies of the structural nitrogenase gene *nifH* (Table 2). Only one of the isolates from this soil formed effective nodules on *L. leucocephala*, and this isolate had only a single copy of *nifH*. By contrast the population in the acid Daka-ini soil was composed largely of broad-host-range isolates which had single copies of *nifH* (Table 2). The isolates from the Daka-ini soil which were specific to *Phaseolus* species generally had three copies of *nifH*, although one isolate had only two copies. These rhizobial isolates are indigenous to Kenyan soils and yet have marked similarities to previously described *Rhizobium* species from

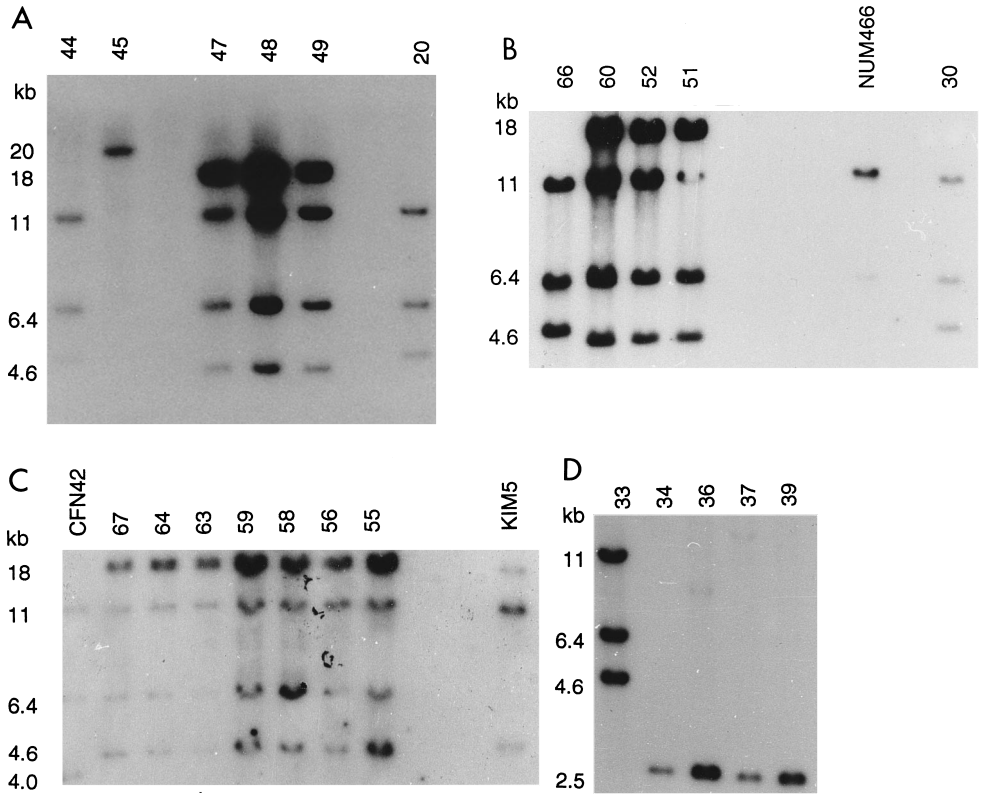

FIG. 2. Hybridization of a 273-bp *nifH* probe with restriction enzyme (*Bam*HI) digests of total DNA from *Rhizobium* isolates from the Daka-ini soil (isolate numbers below 45) and the Naivasha soil (isolate numbers 45 and above) and standard strains CFN42, KIM5, and NUM466.

other continents. The isolates specific to *P. vulgaris* are similar to the species *R. etli* and *R. leguminosarum* bv. phaseoli, while the broad-host-range isolates have marked similarities to *R. tropici.*

Without further genetic analysis we cannot be sure as to the species identity of these Kenyan isolates. The relative distribution of the host-specific and broad-host-range types does, however, fit with what might be expected from the known ecological preferences of *R. leguminosarum* and *R. etli* when compared with those of *R. tropici*. Acid-tolerant strains of rhizobia isolated from *Phaseolus* nodules are invariably *R. tropici*, which is in fact more tolerant of acidity than other fast-growing or slowly growing rhizobia (11). It has been demonstrated that *R. tropici* is able to form a greater proportion of the nodules on *Phaseolus* beans in competition with *R. etli* or *R. leguminosarum* bv. phaseoli strains under acid conditions (1, 6). The predominance of *R. tropici*-like strains isolated from the acid soil in this study was not due to the experimental conditions favoring competition for nodulation over the specific *Phaseolus* rhizobia, as isolations were made from nodules formed on *Phaseolus* plants grown in growth pouches inoculated with dilute soil suspensions.

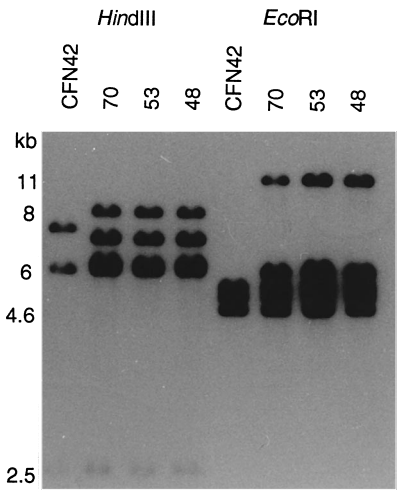

FIG. 3. Hybridization of a 273-bp *nifH* probe with total DNA from three *Rhizobium* isolates from the Naivasha soil and a standard strain of *R. etli*, CFN42, digested with *Hin*dIII and *Eco*RI.

Number of *nifH* copies. All previous reports of reiteration of the nitrogenase genes in host-specific *Phaseolus* rhizobia have demonstrated the presence of three fragments hybridizing with a *nifH* probe in DNA digested with *Bam*HI (20, 22, 25). We observed three such bands also for most of the host-specific isolates from the acid Daka-ini soil. However, an additional *nifH*-hybridizing band was observed in *Bam*HI-digested DNA of the majority of the isolates from the Naivasha soil. Additional hybridizing bands were also observed on digestion with *Hin*dIII and *Eco*RI (Fig. 3). Given that four (or more) hybridizing bands were observed in genomic digests using three different six-base-cutter restriction enzymes and that the probe used was a 273-bp fragment internal to the structural gene, the likelihood that there are actually four copies of sequences homologous to *nifH* is high.

All of the broad-host-range isolates from the Daka-ini soil had only a single fragment hybridizing with the *nifH* probe

(Fig. 2; Table 2), and this fragment was similar in size to the *nifH* band of *R. tropici* CIAT899. By contrast, the single broad-host-range isolate from the Naivasha soil had a much larger DNA fragment which hybridized with the *nifH* probe (Fig. 2A).

Diversity of isolates within the populations. Our initial hypothesis had been that the diversity of *Phaseolus* rhizobia would be reduced under stressed conditions such as those associated with soil acidity. In fact, the converse was true. The distribution of restriction fingerprint types in the Naivasha (pH 6.8) soil was very uneven, with one fingerprint group of specific *Rhizobium* isolates comprising almost 70% of the population (27 isolates). The remainder comprised one small group of three isolates, and the remaining nine isolates had distinct restriction fragment fingerprints (Table 2). In the Daka-ini (pH 4.5) soil, the broad-host-range rhizobia, similar to *R. tropici*, predominated and there were three restriction fragment fingerprint groups (comprising 40, 17, and 11% of the isolates) and 11 distinct isolates. Thus, rather than there being reduced diversity in the presence of the acid soil stress, there was apparently a similar degree of diversity. However, the key difference was that the predominant types in the populations in the two soils appeared to belong to two different species of *Rhizobium* phenotypically similar to *R. etli* (Naivasha) and *R. tropici* (Daka-ini). The predominance of the *R. tropici*-type isolates in the Daka-ini soil is presumably due to their better adaptation to acidity (11), although stresses in acid soils not only are the direct effect of low pH but include toxicities of aluminium which can be severe in many acid tropical soils.

Origin of *Phaseolus* rhizobia in African soils. Lie et al. (17) suggested that the centers of diversity of legume hosts were also likely to be the centers of diversity of their compatible rhizobia. We have demonstrated that rhizobia indigenous to African soils with no history of deliberate inoculation can nodulate and fix nitrogen with *P. vulgaris*. For legumes such as *P. vulgaris*, which is now well documented to be a permissive host which can nodulate with a large number of *Rhizobium* species (16), we may find a wide diversity of compatible rhizobia even in soils into which the legume has been comparatively recently introduced. In other work we collected soils from montane forests in Uganda at altitudes above those at which any agriculture (including shifting cultivation) is practiced and found that the soils contained rhizobia which were able to form effective symbioses with *P. vulgaris* (9). However, the possibility that rhizobia were also introduced into Africa along with the crop still remains (9). A large number of *Trifolium* species and some *Vicia* spp. are native to Kenya (18), and it is possible that the host-specific *Phaseolus* rhizobia are *R. leguminosarum* strains which have acquired the ability to nodulate *Phaseolus* plants. The broad-host-range rhizobia may have evolved to nodulate *Acacia* spp. or other native legumes, although we have not tested their ability to nodulate and fix nitrogen with legumes other than *Phaseolus*, *Leucaena*, and *Macroptilium* spp. Trinick (28, 29) demonstrated similarities between fast-growing rhizobia isolated from *L. leucocephalus* (a New World species) and *Acacia farnesiana* plants in Papua New Guinea, but these strains could not nodulate *P. vulgaris*. The *R. tropici* strain CIAT899 can form effective nodules on a wide range of tropical legumes, including *Acacia nilotica* (22a).

The frequent occurrence of *Rhizobium* isolates which were more effective in N_2 fixation than CIAT899, which is commonly recommended as an inoculant strain for *Phaseolus* spp., is indicative of the benefits which could be achieved by rigorous screening of large numbers of isolates from natural populations. The frequent lack of response in yield of *P. vulgaris* to inoculation with *Rhizobium* spp. in East and southern Africa (26) is often due to the presence of large populations of indigenous, compatible rhizobia which are effective in N_2 fixation as shown in this study. Poor nodulation and N_2 fixation of *P. vulgaris* in Africa are most commonly due to a lack of available phosphorus in the soil or other limiting environmental factors (8, 24, 27).

It remains to be seen whether the predominance of broad-host-range *R. tropici*-type rhizobia in acid soils and the predominance of host-specific rhizobia in near-neutral soils are general phenomena in bean-growing areas of East Africa.

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