Different species and symbiotic genotypes of field rhizobia can nodulate *Phaseolus vulgaris* in Tunisian soils

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Received 18 March 2002; received in revised form 19 April 2002; accepted 19 April 2002

**Abstract**

A collection of 160 isolates of rhizobia nodulating *Phaseolus vulgaris* in three geographical regions in Tunisia was characterized by restriction fragment length polymorphism analysis of polymerase chain reaction (PCR)-amplified 16S rDNA, *nifH* and *nodC* genes. Nine groups of rhizobia were delineated: *Rhizobium gallicum* biovar (bv.) *gallicum*, *Rhizobium leguminosarum* bv. *phaseoli* and bv. *viciae*, *Rhizobium etli* bv. *phaseoli*, *Rhizobium giardinii* bv. *giardinii*, and four groups related to species of the genus *Sinorhizobium*, *Sinorhizobium meliloti*, *Sinorhizobium medicae* and *Sinorhizobium fredii*. The most abundant rhizobial species were *R. gallicum*, *R. etli*, and *R. leguminosarum* encompassing 29–20% of the isolates each. Among the isolates assigned to *R. leguminosarum*, two-thirds were ineffective in nitrogen fixation with *P. vulgaris* and harbored a symbiotic gene typical of the biovar *viciae*. The *S. fredii*-like isolates did not nodulate soybean plants but formed numerous effective nodules on *P. vulgaris*. Comparison of *nodC* gene sequences showed that their symbiotic genotype was not related to that of *S. fredii*, but to that of the *S. fredii*-like reference strain GR-06, which was isolated from a bean plant grown in a Spanish soil. An additional genotype including 16% of isolates was found to be closely related to species of the genus *Agrobacterium*. However, when re-examined, these isolates did not nodulate their original host. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Soil population; *Rhizobium*; *Phaseolus vulgaris*

1. **Introduction**

The rhizobia which are able to form root nodules on common bean (*Phaseolus vulgaris*) are currently classified in five species of the genus *Rhizobium*, *Rhizobium leguminosarum* biovar (bv.) *phaseoli* [1], *Rhizobium tropici* [2], *Rhizobium etli* bv. *phaseoli* [3], *Rhizobium gallicum* bvs. *gallicum* and *phaseoli* [4], and *Rhizobium giardinii* bvs. *giardinii* and *phaseoli* [4]. Other distinct 16S rDNA lineages within the genus *Rhizobium* have been identified from bean isolates [5,6]. Besides the *Rhizobium* species, it was reported that symbiotically effective bean isolates from a Spanish soil had a 16S rDNA allele similar to that of the soybean species, *Sinorhizobium fredii* [7]. However, contrasting with this taxonomical heterogeneity, a recent phylogenetic survey of the nodulation gene *nodC* revealed close relationships among all bean rhizobia but *R. tropici* [6]. In addition to the rhizobia isolated from common bean plants, many other rhizobial strains may induce nodules on *P. vulgaris* when tested as single-strain inoculants [8–12].

The distribution of species that nodulate common beans varied among geographical locations (for a detailed review, see [13]). Originally, *R. etli* and *R. tropici* were isolated from Meso- and South America, respectively [2,3], but they are actually distributed worldwide. *R. etli* was reported to be a prevalent component of bean populations in Europe [7,14], in Central and West Africa [15–17], and in Indonesia [15]. *P. vulgaris* is thought to be native to Mesoamerica and Andean South America [18]. The predominance of *R. etli* strains in common and primitive beans in northwest Argentina suggests that this species may have coevolved with *P. vulgaris* in this region [19].
R. tropici predominated in acid soils from France [20] and Kenya [16], but was scarcely represented in African populations from neutral soils [15,16], and in Mexican and Argentinean populations [19,21]. R. leguminosarum was most prevalent in European soils [20,22–24]. However, this species was also a natural component of South American bean population [19,25]. R. gallicum and R. giardinii were first described in France [4,23,24], but both species also occurred in a Spanish bean population [7]. R. gallicum predominated with R. etli in nodules from bean plants grown in Austrian soils [14].

The diversity of rhizobial species nodulating P. vulgaris in north Africa in relation to symbiotic effectiveness of rhizobial isolates has been recently investigated [26]. Rhizobia showing genomic characteristics of R. leguminosarum, R. etli and R. gallicum were detected within a sample of 47 bean isolates representative of a larger collection of strains isolated from soils of geographically diverse areas in Tunisia. However, additional genomic groups could not be identified by the DNA probing methods used. Nitrogen fixation with common beans varied from highly effective to ineffective depending on the rhizobial strains, but effectiveness was generally correlated with the classification in genomic groups. In this study, the complete collection of Tunisian rhizobial isolates from bean nodules was characterized by analysis of polymerase chain reaction (PCR)-amplified 16S rDNA and symbiotic genes with the purpose of further investigating the diversity and the geographical distribution of bean rhizobia in Tunisian soils.

2. Materials and methods

2.1. Bacterial strains

The procedure for isolation of the 160 Tunisian bean isolates used in this study has been previously described [26]. They were recovered from various soils in two regions in the north of Tunisia, Cap Bon and Bizerte, where the common bean is traditionally cultivated, and from the south of Tunisia where common beans have probably never been grown. The reference strains included in this study were R. leguminosarum bv. phaseoli H132, R. gallicum bv. gallicum R602spT, R. etli bv. phaseoli CFN42T, R. tropici IIB CIAT899T, R. tropici IIA CFN299, Rhi zobium mongolense USDA1844T, S. fredii MSDJ1537, Sinorhizobium melloti ATCC9930T, Sinorhizobium medicae M1, Sinorhizobium sp. GR-06. The origin and the source of these strains have been indicated in a previous work [6].

2.2. Plant infection tests

Nodulation tests on P. vulgaris and Leucaena leucocephala plants were done as previously described [26]. Seedlings of Glycine max were grown according to the method of Gibson [27] using liquid Jensen’s medium [28]. Medicago sativa plants were grown on slopes of Jensen’s agar medium.

2.3. PCR amplification and restriction digestion

The cells were treated according to Laguerre et al. [29] except that after proteinase K digestion, cells were submitted to three cycles of 1 min in liquid nitrogen and 2 min in boiling water to ensure maximum lysis. Primers fD1 and rD1 described by Weisburg et al. [30] were used for PCR amplification of the 16S rRNA genes. Primers nifHF and nifHI [6] were used to amplify approximately 930 bp of the 890-bp nifH gene. Primers nodCF and nodCI [6] were used to amplify approximately 930 bp of the 1300-bp nodC gene. PCRs and restriction pattern analysis of the PCR products using AluI, CfoI, DdeI, HaeIII, HinfI, MspI, NdeII and RsaI were done as previously described [6,29] except for nifH amplification where the annealing temperature was increased to 63°C.

2.4. Sequencing of nodC DNA

The nucleotide sequence of the rhizobial strain 16b1 obtained in this study was made by Genome Express S.A. (Grenoble, France). A 20-µl aliquot of the crude nodC PCR product obtained by using nodCF/nodCI primers was directly sequenced by using primer nodCI. The less degenerate primer nodCFn [6], which matched the same oligonucleotide sequence as nodCF, was also used as a PCR and sequencing primer. The nodC fragment was extracted from the agarose gel by using a QIAEX II gel extraction kit (Qiagen) followed by ethanol/ammonium acetate precipitation as previously described [6].

Approximately 420–450 nucleotides of each DNA strand were determined and a 819-bp sequence of the nodC fragment was reconstituted. Restriction site analysis of the sequence was performed by using the Bisance software [31], and compared to that experimentally obtained.

2.5. Phylogenetic analysis

The nodC sequence of strain 16b1 has been deposited in the GenBank database under accession number AF481764. The accession numbers of the published nodC sequences used for comparisons were as follows: M13658 (R. leguminosarum bv. viciae 238), AF217271 (R. leguminosarum bv. trifolii USDA2071), AF217263 (R. leguminosarum bv. phaseoli H132), AF217268 (R etli bv. phaseoli CFN42), AF217262 (R etli bv. phaseoli Viking 1), AF217266 (R. gallicum bv. gallicum R602sp), AF217270 (R. gallicum bv. gallicum FL27), AF217265 (R. gallicum bv. phaseoli PhD12), AF217267 (R. giardinii bv. giardinii H152), AF217264 (R. giardinii bv. phaseoli H251), X98514 (R. tropici IIA CFN299), X87578 (Rhizobium galegae HAMB1174), AF217272 (Rhizobium sp. Medicago OR191), M11268 (S. meliloti 1021), M73699 (S. fredii
**3. Results**

The results of the RFLP analysis of the 16S rRNA genes of the 160 bean isolates by using eight restriction endonucleases is shown in Table 1. Nine different taxa could be delineated and identified using the published database of mapped restriction sites in the 16S rRNA gene of rhizobia [29] and by comparison with the reference strains included in this study. One hundred and nineteen isolates were distributed in four species recognized as bean symbionts, *R. leguminosarum*, *R. etli*, *R. gallicum*, and *R. giardinii*. One isolate was closely related to *R. gallicum* and only one difference in four restriction sites for *nodC* gene analysis; ‘*Phaseolus*’ refer to symbiotic genotypes that were closely related to those of reference strains isolated from *P. vulgaris* or *Medicago* spp. plants (see also text).

**Table 1**

<table>
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<th>nodC type</th>
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<th>No. of isolates from</th>
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<tr>
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</table>

**Total**

78 74 8 160

**Notes**: 1. The 16S rDNA, *nifH*, and *nodC* types represent the combination of the restriction patterns obtained with the following restriction enzymes: *AluI*, *CfoI*, *DdeI*, *HaeIII*, *HinIII*, *MspI*, *NdeII*, and *RsaI* for 16S rDNA analysis, and *AluI*, *CfoI*, *HaeIII*, *HinIII*, *MspI*, and *NdeII* for *nifH* and *nodC* fragment analysis. 2. Biovar identification was done on the basis of the 16S rDNA analysis. 3. Species identification was done on the basis of *nifH* and *nodC* gene analysis; ‘*Phaseolus*’ and ‘*Medicago*’ refer to symbiotic genotypes that were closely related to those of reference strains isolated from *P. vulgaris* or *Medicago* spp. plants (see also text). 4. Nd, not determined; no, unsuccessful PCR amplification.

**USDA257**, X73362 (*Sinorhizobium* sp. NGR234), AF217269 (*Sinorhizobium* sp. Phaseolus GR-06), X52958 (*Mesorhizobium loti* NZP2037), AF217261 (*Mesorhizobium amorphae* ACCCC196665), U53327 (*Mesorhizobium sp. Oxytropis* N33), AJ249393 (*Mesorhizobium sp. Astragalus 7653R*), AF32201282 (Bradyrhizobium japonicum USDA110), AF105431 (Bradyrhizobium sp. SNU001), AF222753 (Bradyrhizobium sp. Lupinus WM9), AF284858 (Bradyrhizobium sp. Aeschynomene ORS285), L18897 (Azorhizobium caulinodans ORS571T), AJ306730 (Burkholderia sp. Aspalatus STM678).

Molecular sequence analyses were performed by using programs available in the Bisance software. Nucleotide sequences were aligned with Clustal W [32]. Phylogenetic trees of *nodC* gene were inferred using the Phylogenetic Inference Package (PHYLIP, [33]) with neighbor-joining analyses from Kimura’s [34] two-parameter nucleotide distances, and the maximum likelihood method. Confidence in the neighbor-joining tree was assessed by bootstrap analysis with the SEQBOOT and CONSENSE programs of PHYLIP.
3.2. Host specificity

When re-examined for nodulation, the Agrobacterium-like isolates did not nodulate their original host. The S. medicae and S. melloti isolates nodulated M. sativa in addition to P. vulgaris. However, the nodules made on common bean plants were small and white indicating a non-effective nitrogen-fixing symbiosis. By contrast, the nodules induced by S. fredii-like isolates on common beans were typical of an effective nitrogen-fixing symbiosis. These isolates were not able to form nodules on soybean plants.

3.3. RFLP analysis of nifH and nodC gene fragments

The nifH gene fragment of the expected size was amplified for the 152 isolates tested, except for the R. giardinii and the Agrobacterium-like isolates. Amplification of the nodC fragment was also achieved for all these isolates, except again for the Agrobacterium-like isolates. For the R. giardinii and some of the S. melloti-like isolates, two bands were obtained. A small piece of agarose containing the nodC fragment of the expected size was used as a template in a new PCR as previously described [6].

The results of the restriction analysis using six restriction endonucleases are given in Table 1. Eleven composite nif types and composite nod types were identified, and the combined data of the nod and nif gene analysis revealed 15 symbiotic (nod–nif) genotypes among the collection of isolates. The restriction patterns were analyzed by comparison with those of the reference strains included in this study and in the available database [6].

Two nif types, A and B, were recorded among the R. gallicum isolates. Type A was harbored by 62% of the isolates and was identical to that of the type strain of the species, R. gallicum bv. gallicum R602sp\(^{\text{T}}\). Type B shared all its restriction patterns with strain R602sp or R. gallicum bv. gallicum FL27 except for the CfoI restriction patterns, but the differences could be explained by the gain or loss of only one restriction site. The isolate that showed a 16S rDNA type closely related to that of R. gallicum also had nif type A. Consequently, all these isolates could be classified into bv. gallicum.

Four nif types, C, D, E and F, were delineated among the isolates identified as R. etli. Type C and F were typical of bv. phaseoli since they were also found in reference strains of this biovar within R. etli, R. leguminosarum, or R. gallicum species [6]. Differences between types D and E, and types C and F are explainable by gain or loss of only one or two restriction sites so that we concluded that they were also characteristic of bv. phaseoli. Thirty-two percent of the isolates identified as R. leguminosarum also had nif types D and F and were thus assigned to bv. phaseoli. A third nif type, G, was identified for the remaining R. leguminosarum isolates. This genotype was identical to that of the R. leguminosarum bv. viciae reference strain 8401.

The isolates related to the species S. melloti were distributed into two nif types, H and J. Type H was identical to that of the type strain of the species, ATCC9930. Type J shared no restriction patterns with type H and only 26% of its restriction fragments. However, AflI restriction pattern of type J was identical to that of the reference strain of S. medicae, M1. HinfI, MspI and NdeII restriction patterns were identical to those of the R. mongolense type strain USDA1844, which was isolated from Medicago ruthenica [35]. Type J shared 81% of restriction fragments with the nif type of R. mongolense USDA1844 and 47% with that of S. medicae M1. The unique S. medicae-like isolate showed the same nif type as the reference strain of S. medicae, M1. Thus, the nifH analysis corroborates for at least the isolates with nif types H and I.

By contrast, the five isolates assigned to S. fredii showed a nif type (K), which differed to that of the S. fredii reference strain MSDJ1537 for all restriction patterns, and to those of the other reference strains of the database. However, nif type K shared 80% of its restriction fragments with the nif type of the S. fredii-like strain GR-06 isolated from a bean plant grown in a Spanish soil [7], and only 41% of them with that of S. fredii MSDJ1537.

The results of the RFLP analysis of the nodC gene fragments corroborate the conclusions drawn from the nifH gene analysis. The isolates classified in R. gallicum bv. gallicum had the same nod type as the type strain R602sp. The isolates classified in R. etli bv. phaseoli or in R. leguminosarum bv. phaseoli had nod types similar to those of bv. phaseoli reference strains. Two nod types, E and F, were identified for the R. leguminosarum isolates with the bv. viciae nif type G. The nod type E corresponded to that of R. leguminosarum bv. viciae strain 8401. Type F differed from all the previously identified nod types among R. leguminosarum bv. viciae strains and other rhizobia. However, it shared 50% of its restriction fragments with type E, which is a high value when considering the high degree of restriction fragment polymorphism among the rhizobial nodC fragments [6]. The isolates with the nif type H typical of S. melloti had a nod type that differed from the nod type of strain ATCC9930\(^{\text{T}}\) by only one additional MspI restriction site. The nod type of the S. melloti-like isolates with the nif type J shared 48 and 56% of its restriction fragments with those of the reference strains ATCC9930 and 2011 of S. melloti, and 38% with that of R. mongolense. The S. medicae-like isolate had the same nod type as the S. medicae reference strain M1. The S. fredii-like isolates had a specific type, but its closest relative was again the nod type of the S. fredii-like strain GR-06 with 44% of shared restriction fragments. By contrast, it shared less than 30% of fragments with the nod type of S. fredii strain MSDJ1537.

The isolates assigned to the R. giardinii species had the same nod type as the type strain of the species, H152, which belongs to the bv. giardinii. Strains in this biovar
probably lack \textit{nifKDH} genes [4, 6], which agrees with the lack of amplification of the \textit{nifH} fragment from these isolates.

3.4. Sequencing of \textit{nodC} gene

The nucleotide sequence of the \textit{nodC} gene fragment of a representative of the \textit{S. fredii}-like isolates, strain 16b1, was determined and a phylogenetic analysis was performed in order to clarify its relationships with other rhizobia. The \textit{nodC} gene of strain 16b1 was found to be closely related to that of the \textit{S. fredii}-like strain GR-06 (Fig. 1), with a similarity value of 93.3%. The two strains were clustered with all the other \textit{Phaseolus} rhizobia except \textit{R. tropici} as previously reported for the \textit{nodC} gene of strain GR-06 [6]. The similarity values between the \textit{nodC} gene fragments of strain 16b1 and strains of \textit{bv. phaseoli} or \textit{bv. gallicum} varied from 88.1 to 89.5%. The similarity between the \textit{nodC} gene fragments of strain 16b1 and the soybean symbiont \textit{S. fredii} USDA191 was only 80%.

4. Discussion

\textit{P. vulgaris} grown in Tunisian soils was nodulated by rhizobia distributed in seven species of the genera \textit{Rhizobium} and \textit{Sinorhizobium} as identified from 16S rDNA analysis. An additional genotype including 16% of isolates was found to be closely related to species of the genus \textit{Agrobacterium}. The most abundant rhizobial species were \textit{R. gallicum}, \textit{R. etli}, \textit{R. leguminosarum} encompassing 29–20% of the isolates each.

Apart from the \textit{Agrobacterium}-like isolates, a high proportion (19%) of rhizobial isolates did not appear to be specific symbionts of common beans as revealed by symbiotic gene analysis, and confirmed by plant tests. Thirteen percent of isolates were assigned to \textit{R. leguminosarum} \textit{bv. viciae} and 6% to \textit{Medicago} specific sinorhizobial species. Two subgroups with distinct symbiotic genotypes and also distinct 16S rDNA hybridization patterns [26] were recorded among the isolates assigned to \textit{S. meliloti}. One subgroup was genetically similar to the type strain of the
species, while the second subgroup showed a \( nifH \) type that was more closely related to the *Medicago* species *R. mongolense*. The *R. leguminosarum* bv. *viciae* and *Medicago* sinorhizobial isolates were ineffective in symbiosis with common beans ([26], the present study). Previous studies have mentioned promiscuous nodulation of beans by *R. leguminosarum* bv. *viciae* indigenous rhizobia [36], and by strains of *S. meliloti* [8,10,37]. The Tunisian isolates identified as *Medicago* sinorhizobia were indeed able to induce nodules on alfalfa, a plant usually cultivated in the south Tunisia soils from which most of them were isolated. These soils had not been cultivated with common beans, and the plants were poorly nodulated suggesting that effective bean rhizobia were absent. One *S. meliloti* isolate was identified among isolates from the northern region, but it also originated from a soil where the plants were poorly nodulated. The high proportion of *R. leguminosarum* bv. *viciae* recovered from Bizerte soils is more puzzling. Some of them were isolated from poorly nodulated plants, but some others from well-nodulated plants. The nodules produced on common beans by the non-specific rhizobia were small and white, and when isolating strains from highly nodulated plants, we usually selected the biggest nodules which may have biased the sampling of indigenous rhizobia. The extent of the promiscuous nodulation of common beans and its impact on the symbiosis effectiveness need to be further evaluated.

The *Agrobacterium*-like isolates represented 16% of the population. The failure of amplification of \( nifH \) and *nodC* fragments among these isolates corroborates the 16\( S \) rDNA-based identification, as well as the results of a previous characterization indicating that no hybridization signal was obtained when probing their plasmid and genomic DNAs with symbiotic gene probes [26]. Also, plant tests showed that these isolates were unable to nodulate their original host. Their presence in nodules could be explained either by a mixed infection with a rhizobial cell, or by the acquisition of a symbiotic plasmid by the *Agrobacterium* which might be highly unstable and lost during the isolation and preservation processes. A similar case was reported by De Lajudie et al. [38] who isolated *Agrobacterium* strains from tropical legumes. It would be interesting to evaluate the impact of these *Agrobacterium*-like isolates on the nodulation and the effectiveness of common beans in co-inoculation trials.

The identification of *R. gallicum* bean isolates by RFLP analysis of 16\( S \) rDNA confirmed the previous study of Mhamdi et al. [26] reporting that this species occurs in Tunisian soils. Biovars *gallicum* and *phaseoli* differ in their host range, strains of *bv. gallicum* being able to form nitrogen-fixing nodules with legumes other than *P. vulgaris* [4]. All the *R. gallicum* isolates analyzed, which corresponded to isolates able to nodulate *L. leucocephala* [26], had a symbiotic genotype typical of *bv. gallicum*. So far, *R. gallicum* bean isolates were only detected in European soils [4,7,14,23]. *R. gallicum* was widespread in Cap Bon and Bizerte soils where it represented 36 and 26% of the isolates, respectively. The 16\( S \) rDNA type 2 detected in one isolate and closely related to but distinct from that of the *R. gallicum* type strain, was similar to one genotype identified in Spain where it was exclusively associated to *bv. phaseoli* [7]. The Spanish isolates were only distantly related to the *R. gallicum* type strain based on multilocus enzyme electrophoresis (MLEE) cluster analysis. Likewise, the Tunisian isolate did not cluster with the typical *R. gallicum* strains based on RFLP analysis of 16\( S-23S \) rDNA intergenic spacer and on phenotypic characters [39]. This result strengthens the hypothesis formulated by Herrera-Cervera et al. [7] that the isolates carrying the type 2 16\( S \) rDNA allele could belong to a species distinct from *R. gallicum* and that exchanges of 16\( S \) rDNA alleles may have occurred between the two species.

*R. etli* isolates harbored typical *bv. phaseoli* symbiotic genotypes. As *R. gallicum*, this species was abundant in Cap Bon soils (44% of isolates), but was scarcely represented in Bizerte soils (5% of isolates). *R. leguminosarum* bv. *phaseoli* isolates were only found in Bizerte soils where it represented 13% of the isolates. Three isolates from Cap Bon were identified as *R. giardinii* bv. *giardinii*. The identification of this biovar was confirmed by plant tests indicating that these isolates were ineffective in symbiosis with *P. vulgaris* and able to nodulate *L. leucocephala* as previously reported [4]. So far, *R. giardinii* bv. *giardinii* isolates have been only detected in French soils, although this species associated to the *bv. phaseoli* was also detected in Spain [7].

Five isolates from Cap Bon and Bizerte soils had a 16\( S \) rDNA type similar to that of *S. fredii*. It has been reported that some soybean *S. fredii* strains have the capacity to induce nodules on beans [9]. However, we found that the Tunisian *S. fredii*-like isolates were not able to nodulate soybean. By contrast, they formed numerous effective nodules on *P. vulgaris* [26]. Also, their symbiotic genotype was not related to that of *S. fredii* reference strains. A similar case was reported within the Spanish bean rhizobial population analyzed by Herrera-Cervera et al. [7]. Both RFLP analysis of \( nifH \) gene and comparison of the *nodC* sequences indicated that the Spanish and the Tunisian bean *S. fredii*-like rhizobia were closely related. The *nodC* genes of these rhizobia were clustered with those of the other bean symbionts except *R. tropici*, as previously reported for the Spanish *S. fredii*-like strain GR-06 [6].

In Cap Bon and Bizerte regions, the soils are neutral and bean had been extensively cultivated. However, differences in the distribution of the bean nodulating rhizobial species between the two regions were observed, mainly with regard to the relative abundance of two species, *R. etli* and *R. leguminosarum*. These differences might be related to environmental conditions, but *R. gallicum* and *S. fredii*-like isolates were recovered from both regions. By contrast, in the south of Tunisia where bean cropping had not been practiced, no bean-adapted rhizobia could be
recovered. These results suggest that rhizobia adapted to bean have been introduced, probably with the seeds. There is evidence that rhizobia may remain viable on the seed surface for years [40]. Additional data supporting this hypothesis were the conservation of symbiotic genotypes among local isolates and reference strains originating from different countries. The biovar phaseoli seems to be specific for the P. vulgaris symbiosis. The symbiotic genes which are characteristic for this biovar are located on a plasmid and were probably spread to different species by horizontal gene transfer from R. etli which is supposed to be the original donor species and to originate from the Americas [3,7,19]. The origin of biovars gallicum and girardii have a wider host range although they had been so far almost exclusively isolated from bean nodules in Europe. However, rhizobial strains classified in R. gallicum bv. gallicum have also been recovered from Onobrychis and Oxytropis spp. [29,41], and R. gallicum bv. gallicum rhizobia isolated from P. vulgaris are able to nodulate Onobrychis vicifolia [4]. Our study provides further evidence that P. vulgaris can be nodulated by diverse rhizobia including broad host range bacteria and non-specific rhizobia that cannot be considered as symbionts since they are ineffective in nitrogen fixation.

The promiscuous nodulation of common beans with non-specific rhizobia may result from low abundance of natural populations of bean-compatible rhizobia in Tunisian soils which needs to be investigated further. If so, inoculation of common beans with efficient rhizobial strains may be the means to improve biological nitrogen fixation and common bean productivity. This study provides an ecological framework that can be used in selecting efficient rhizobial strains that are adapted to local environmental factors.

Acknowledgements

This work was supported by MRST (PRC, nitrogen fixation), AUPELF-UREF (LAF 310) and by Franco-Tunisian (RFR) grants. We thank F. Revoy and M. Bours for technical assistance.

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FEMSEC 1366 13-6-02


