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Classification of Austrian Rhizobia and the Mexican Isolate FL27 Obtained from *Phaseolus vulgaris* L. as *Rhizobium gallicum*

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The phylogenetic positions of four rhizobial strains obtained from nodules of common bean plants (*Phaseolus vulgaris* L.) grown in an Austrian soil and of the Mexican bean isolate FL27 are described. Analysis of the 16S rRNA genes revealed sequences almost identical to that of the *Rhizobium gallicum* type strain, R602sp, with a maximum of two nucleotide substitutions. Comparison of the 16S rRNA gene sequences with those from other bacteria indicated highest similarity to *Rhizobium* sp. strain OK-50, *Rhizobium leguminosarum* IAM 12609, and *Rhizobium etli*. DNA homology determined by DNA-DNA hybridization was high among the Austrian isolates and R602sp^T (45 to 90%) and ranged from 21 to 65% with FL27, but hybridization analysis revealed very low homology to the recognized common bean-nodulating species, *R. leguminosarum* bv. phaseoli, *R. etli*, and *Rhizobium tropici*. Ribosomal gene organization was studied by Southern hybridization with the 16S rRNA gene and temperature gradient gel electrophoresis, indicating identical organizations and the presence of three identical 16S rRNA copies in the genome of this species. The six strains investigated showed different plasmid profiles based on their geographical origins. We propose that the Austrian isolates and the Mexican strain FL27 are members of the species *R. gallicum*.

Bacteria of the genus *Rhizobium* that are able to nodulate common bean plants (*Phaseolus vulgaris* L.) have been traditionally classified as *Rhizobium leguminosarum* bv. phaseoli (13) on the basis of the host plant they infect. Strains belonging to the other subdivisions of this species, *R. leguminosarum* bv. viciae and bv. trifolii, nodulate peas and clovers, respectively, and their symbiotic plasmids carry genes with different host specificities. Nevertheless, rhizobia from common bean plants have been found to be phylogenetically diverse based on different criteria, such as protein profiles (32), multilocus enzyme electrophoresis patterns (6, 28), results of DNA relatedness analysis (16, 37, 44), and differences in their 16S rRNA gene (rDNA) sequences (9, 16, 44). In addition to *R. leguminosarum* bv. phaseoli, two new species, *Rhizobium etli* (38) and *Rhizobium tropici* (20), have been described. Both *R. leguminosarum* bv. phaseoli and *R. etli* carry multiple copies of the nitrogenase reductase gene (*nifH*) on their symbiotic plasmids, but they have different 16S rRNA sequences (17, 30, 38). In contrast, *R. tropici* maintains only a single *nifH* gene copy on its symbiotic plasmid (20). *R. etli* and *R. tropici* show a broad host range, but they nodulate different hosts (9, 20). Several new species among bean-nodulating strains, including *Rhizobium gallicum* and *Rhizobium giardinii*, which comprise the French isolates (1, 16), as well as *Rhizobium* sp. (*Phaseolus*) RCR 3618D of unknown geographical origin, have been proposed (44). The partial 16S rDNA sequence of the *R. gallicum* type strain, R602sp, was found to be identical to that of strain FL27 (16), a Mexican isolate from the common bean which does not fixate N_2 well (28). In general, strains of *Rhizobium* that nodulate bean plants are of American origin, as is their host plant. For a long time it was believed that in Europe, *R. leguminosarum* bv. phaseoli was the only microsymbiont of common bean plants, but recently several species, including *R. etli*, *R. tropici*, *R. gallicum*, and *R. giardinii*, were found in European soils (1, 2, 16, 41).

Rhizobial strains recovered from common bean nodules from an Austrian soil showed high similarity to *R. gallicum* R602sp^T by PCR analysis with repetitive primers and PCR-restriction fragment length polymorphism (RFLP) analysis of the 16S rRNA gene (41). In addition, the *nifH* profiles and the nodulation phenotypes were identical (41). The aim of this study was to confirm that the Austrian isolates are members of the recently described species *R. gallicum*. As the Mexican isolate FL27 exhibited several similarities to *R. gallicum*, it was included in this investigation. Phylogenetic analysis was done by phenotypical characterization as well as sequence analysis of the 16S rRNA gene, analysis of the copy numbers and heterogeneity of ribosomal genes, analysis of plasmid profiles, and DNA-DNA hybridization.

MATERIALS AND METHODS

Bacterial strains, phenotypic characterization, and nodulation host range. Four strains, CbS-1, CbS-3, CbS-17, and CbS-18, were isolated from common bean plants grown in soil from fields around the Seibersdorf laboratory in Austria and characterized previously (41). *R. gallicum* R602sp^T, the Mexican isolate FL27, and reference strains were obtained either from G. Laguerre, Dijon, France, or from the culture collection at the Seibersdorf laboratory. All rhizobial strains were maintained on yeast extract-mannitol (YM) medium (46), and FL27, R602sp^T, CbS-1, CbS-3, CbS-17, and CbS-18 were tested for growth on Luria-Bertani medium (21) and on peptone-yeast extract medium (22). In order to test substrate utilization, modified minimal B&D medium (48) was amended with the following carbon sources at a concentration of 1 g/liter: D-glucose, starch, maltose, urea, fructose, D-lactose, D-sorbitol, D-xylose, D-mannose, arabinose, D-ribose, myoinositol, melobiose, raffinose, D-trehalose, methanol, and ethanol. The amino acids L-alanine, L-aspartic acid, L-asparagine, L-arginine, L-cysteine, L-glutamate, L-glutamine, L-histidine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-tryptophan, L-threonine, L-tyrosine, and L-valine were each tested as a sole carbon and nitrogen source at a concentration of 1 g/liter. Resistance to antibiotics was tested by plating the rhizobial strains on YM agar medium containing ampicillin (10 μg/ml), chloramphenicol (30 μg/ml), kanamycin (30 μg/ml), tetracycline (10 μg/ml), nalidixic acid (10 μg/ml), spectinomycin (100 μg/ml), or streptomycin (100 μg/ml).

Growth at different temperatures (20, 25, 30, 35, 37, and 40°C), growth on YM

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medium containing different concentrations of NaCl (1.0, 1.5, and 2% NaCl), and growth at different pH values (pHs 4, 5, 6, 7, 8, 9, and 10) were determined in liquid culture.

The nodulation host range of FL27 was determined as described previously (41) with *Phaseolus vulgaris* cv. Extender, *Vicia faba* cv. Weiselburger, *Pisum sativum* cv. Rheinperle, *Trifolium repens* cv. Reichersberger, *Medicago sativa* cv. Saranac, *Glycine max* cv. Clay, *Vigna unguiculata* cv. Red Caloona, *Leucaena leucocephala* cv. Cunningham, *Gliricidia sepium*, and *Acacia albida*.

Plasmid profiles, DNA isolation, and rDNA hybridization. Plasmid profiles of CbS-1, CbS-3, CbS-17, CbS-18, R602sp^T, FL27, and CIAT 899^T were investigated as described by Hynes et al. (12). Total genomic DNA was prepared as described elsewhere (3) with omission of the CsCl purification step. Genomic DNA was digested with *Hin*dIII, and the resulting fragments were separated by electrophoresis with a 0.7% agarose gel that was blotted onto a Hybond-N membrane (Amersham International, Little Chalfont, Buckinghamshire, United Kingdom). A probe containing the 16S rRNA gene from strain R602sp^T was prepared by PCR with the primers rD1 and fD1 (47) as described previously (41). After isolation of the resulting fragment from a 1% agarose gel with a GeneClean II Kit (Bio 101, Inc., La Jolla, Calif.), it was labeled with [α-^{32}P]dATP by using the Multiprime DNA labeling system (Amersham International) according to the manufacturer's protocol. Hybridization was carried out at 65°C for 2 h in Rapid-Hyb buffer (Amersham International) and washing and autoradiography were done as described elsewhere (39).

DNA-DNA hybridization. Dot blot hybridizations were performed as described previously (15) with 25-μl samples containing 400 ng of genomic DNA. In addition, 400 ng of denaturated calf thymus DNA was transferred onto the membrane. The membranes were hybridized with 4 μg of genomic DNA previously digested with *Alu*I and labeled with [α-^{32}P]dATP by using the Multiprime DNA labeling system per dot. Hybridization was carried out under relaxed conditions at 55°C for 2 h in Rapid-Hyb buffer. The filters were washed under stringent conditions at 60°C in a solution containing 0.03 M NaCl, 0.003 M sodium citrate, and 1% sodium dodecyl sulfate. Membranes were cut, and 9- by 9-mm pieces were counted with a liquid scintillation counter (Tri Carb 2200CA; Packard). The amount of radioactivity associated with calf thymus DNA was subtracted, and the percentages of DNA relatedness were determined relative to the signal found in the homologous hybridization.

Analysis of the 16S rRNA genes. The 16S rRNA genes of CbS-1, CbS-3, CbS-17, CbS-18, and R602sp^T were amplified by using the primers FGPS6-63 (with a *Bgl*II site) (16) and P1510Pst (with a *Pst*I site) (23) and a standard protocol (41). The amplified fragments were digested with *Bgl*II and *Pst*I and then cloned into pUC18Not that had been previously cut with *Bam*HI and *Pst*I. As the 16S rRNA gene of strain FL27 showed an internal *Pst*I restriction site, the gene was amplified by using the primers FGPS6-63 (16) and P1510HIII equipped with a *Hin*dIII site (5′-GTG*AAGCTT*GGTTACCTTGTTACGACT-3′), where the *Hin*dIII site is italicized. The resulting fragment was digested with *Bgl*II and *Hin*dIII and then cloned into *Bgl*I- and *Hin*dIII-cut pUC18Not, with *Escherichia coli* DH5α as the recipient. DNA sequence analysis of the cloned 16S rRNA gene fragments was done by using the dideoxy-chain termination method (35) with a model LI-COR 4000L automated sequencer.

For the temperature gradient gel electrophoresis (TGGE), total genomic DNA was used as the template to amplify a fragment of ca. 440 bp comprising the V6 to V8 variable region of the 16S rRNA gene. The primers used, PCR and TGGE, were previously described by Nübel et al. (24).

Sequence alignment and phylogenetic analysis. Gene banks were searched by using the FASTA tool (27), and alignments of selected 16S rDNA sequences were done with the ClustalW multiple-alignment program (10). Short regions of uncertain alignment were excluded from further analyses. Calculation of evolutionary distances was done with the Jukes and Cantor model (14). Phylogenetic trees based on the neighbor-joining method (34) were constructed with 100 bootstrap replicates by using the TREECON for Windows software package (45). A maximum parsimony phylogenetic tree was created with the program PAUP3.1 (43).

Nucleotide sequence accession numbers. The 16S rRNA gene sequences of the strains analyzed have been deposited in GenBank under the accession no. AF008126 (CbS-1), AF008127 (CbS-17), AF008128 (CbS-29), AF008129 (FL27), and AF008130 (R602sp^T).

RESULTS

Phenotypic characterization. The strains CbS-1, CbS-3, CbS-17, CbS-18, R602sp^T, and FL27 showed identical characteristics. They could not utilize the following compounds: starch, urea, methanol, ethanol, L-alanine, L-arginine, L-asparagine, L-cysteine, L-phenylalanine, and L-tryptophan. The strains were not able to grow on Luria-Bertani medium or on YM medium supplemented with high (above 1%) concentrations of NaCl, but they could be cultivated on peptone-yeast extract medium. All strains were able to grow on D-glucose, maltose, fructose, D-lactose, D-sorbitol, D-xylose, D-mannose, arabinose, D-ribose, myoinositol, melibiose, raffinose, D-trehalose, L-aspartic acid, L-glutamate, L-glutamine, L-histidine, L-leucine, L-methionine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine. The strains were able to grow on plates supplemented with ampicillin, chloramphenicol, and nalidixic acid. The optimum pH range was from 6 to 8, while no growth occurred at pHs 4, 9, and 10. All isolates were able to grow at a temperature up to 37°C but did not grow at 40°C. Among the host plants tested, FL27 was able to nodulate common bean plants, cowpea plants, *Leucaena* spp., and *Gliricidia* spp.

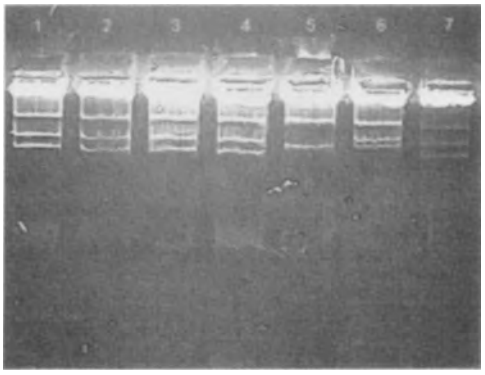

FIG. 1. Plasmid profiles of CbS-1 (lane 1), CbS-3 (lane 2), CbS-17 (lane 3), CbS-18 (lane 4), R602sp^T (lane 5), FL27 (lane 6), and CIAT 899^T (lane 7).

Plasmid profiles. Plasmid analysis showed that the Austrian isolates CbS-1, CbS-3, CbS-17, and CbS-18 carried three plasmids identical in size but that the French strain R602sp^T harbored two plasmids. Strain FL27 and *R. tropici* CIAT 899^T also carried three plasmids but had patterns different from each other and from those of the Austrian isolates. The largest plasmids of the Austrian isolates and of R602sp^T and FL27 appeared to have the same size (Fig. 1).

Ribosomal gene organization. Southern hybridization with the 16S rRNA gene of R602sp^T as a probe showed that isolates CbS-1, CbS-3, CbS-17, CbS-18, R602sp^T, FL27, *R. leguminosarum* bv. phaseoli H131, and *R. etli* CFN 42^T contained at least three copies of the 16S rRNA gene. Both *R. tropici* CIAT 899^T and *R. tropici* CFN 299^T carried one copy of the 16S rRNA gene. The Austrian isolates, R602sp^T and FL27 showed identical patterns with three hybridizing *Hin*dIII fragments of 4.6, 11.0, and 12.9 kb.

TGGE analysis of PCR-amplified segments of 16S rDNAs resulted in single band profiles for each tested strain (data not shown). This result suggested that the sequences in those strains bearing more than one copy of the gene are identical.

DNA-DNA hybridization. Isolates CbS-1, CbS-3, CbS-17, CbS-18, and R602sp^T showed high levels of DNA relatedness ranging from 45 to 90%. Homologies among the European isolates and FL27 ranged from 52 to 65% when FL27 was used as the probe and from 21 to 40% when the same strain was used as the template (Table 1). The average values of the percentages when FL27 was used as the probe and when it was used as the template ranged from 41 to 48% (data not shown). The DNA relatedness values of the Austrian strains, R602sp^T, and FL27 with *R. leguminosarum*, *R. tropici*, and *R. etli* were very low (Table 1).

Analysis of the 16S rRNA gene sequence. The determined 16S rDNA sequences of strains CbS-1 and CbS-3 were identical and differed by only one nucleotide from those of strains CbS-18 and R602sp^T. Two substitutions at different positions were found when these four sequences were compared with the sequences of strains CbS-17 and FL27. FASTA analysis

TABLE 1. Levels of DNA relatedness between CbS-1, CbS-3, CbS-17, CbS-29, R602sp^T, FL27, and other common bean-nodulating *Rhizobium* species

Strain[a]	% DNA relatedness with[b]:									
	CbS-1	CbS-3	CbS-17	CbS-18	R602sp^T	FL27	H131	CIAT 899^T	CFN 299^T	CFN 42^T
CbS-1	100	76	75	69	90	60	7	7	19	11
CbS-3	83	100	80	87	83	65	7	5	13	12
CbS-17	90	83	100	88	82	62	6	5	9	14
CbS-18	45	68	86	100	87	52	2	1	7	7
Rhizobium sp. strain R602sp^T	55	82	79	74	100	61	5	3	8	14
Rhizobium sp. strain FL27	26	21	40	30	35	100	5	14	6	12
R. leguminosarum bv. phaseoli H131	6	4	3	4	11	5	100	6	12	22
R. tropici CIAT 899^T	8	5	5	5	8	5	4	100	33	7
R. tropici CFN 299^T	7	5	5	4	5	5	3	14	100	11
R. etli CFN 42^T	7	12	5	5	9	6	18	4	7	100

[a] DNA used as a template.
[b] DNA used as a probe. DNA relatedness values of strains CbS-1, CbS-3, CbS-17, CbS-18, R602sp^T, and FL27 are mean values obtained from the results of two independent experiments.

indicated several members of the alpha subclass of *Proteobacteria* as having the most related 16S rDNA sequences. All further comparisons were based on 1,431 nucleotides comprising more than 93% of the 16S rRNA gene. *Rhizobium* sp. strain OK-50 (26), *R. leguminosarum* IAM 12609 (26), and *R. etli* had the highest homology values, namely, 97.9, 97.9, and 97.6%, respectively. Phylogenetic dendrograms constructed by both the distance and the parsimony method showed essentially the same topology and similar evolutionary distances; the latter method yielded only one most parsimonious tree. The neighbor-joining phylogenetic tree (Fig. 2) showed a well-defined and compact cluster comprising *R. gallicum* R602sp^T; the Austrian isolates CbS-1, CbS-17, and CbS-18; and the Mexican isolate FL27. This cluster was clearly separated from other well-recognized rhizobial lineages and supported by high bootstrap values.

DISCUSSION

High relatedness among common bean-nodulating strains isolated in Austria (CbS-1, CbS-3, CbS-17, and CbS-18) and the French *Rhizobium* strain R602sp^T was demonstrated by using several approaches targeting symbiotic and chromosomal regions of the genome as well as the nodulation phenotype (41). In a recent study, strain R602sp^T as well as other isolates obtained from French soils was classified as a new species, *R. gallicum*, with R602sp as the type strain (1). The Mexican isolate FL27 and strain R602sp^T showed identical partial 246-bp 16S rRNA gene sequences as well as identical PCR-RFLP profiles of their 16S rRNA genes (16). In the present study, sequence analysis of 93% of the 16S rRNA genes revealed high homology among the four Austrian isolates, R602sp^T, and FL27, with a maximum of two nucleotide substitutions. One nucleotide difference in FL27 resulted in an internal *Pst*I restriction site that was absent in all European isolates. Comparison of the 16S rDNA sequences with those of other bacteria confirmed the findings of Amarger et al. (1), and the 16S rDNA sequences showed highest similarity to *Rhizobium* sp. strain OK-50 isolated from *Pterocarpus klemmei* in Japan (26), to *R. leguminosarum* IAM 12609 (26), and to *R. etli*. This finding is in agreement with Eardly et al. (5), who reported high similarity between FL27 and *R. etli* by sequence analysis of a 260-bp segment of their 16S rRNA genes. Phylogenetic dendrograms always positioned the Austrian isolates and strain FL27 in the *R. gallicum* cluster, and *R. gallicum* was found to be a member of a lineage different from those of described *Rhizobium* species.

Although ribosomal gene sequences play an important role in the description of new species, in many cases there is only a limited correlation between DNA relatedness and 16S rDNA homology. This finding was reported for *Rhizobium* (25) but also for other bacterial genera, such as *Aeromonas* and *Plesiomonas* (18). Recently, van Berkum et al. (44) suggested that although the level of 16S rDNA sequence similarity among bean rhizobia is high, the DNA relatedness data indicate different species. Stackebrandt and Goebel (42) demonstrated that the correlation between 16S rDNA homology and DNA-DNA reassociation is not necessarily linear, indicating that distinct species can show high 16S rDNA sequence similarities. Assessment of DNA relatedness was proposed as an important criterion for the description of new species of root- and stem-nodulating bacteria (8). The strains tested in the present study had very low DNA homology with the recognized common bean-nodulating species, i.e., *R. leguminosarum*, *R. tropici*, and *R. etli*. This result is in agreement with the work of Martínez-Romero (19), who found low DNA relatedness between *R. etli* and FL27. High DNA homology was found among the European isolates. The Mexican isolate FL27, however, had lower DNA relatedness to the European strains. It is not clear why generally higher values were obtained when a particular strain was used as a probe than when the same strain was used as a template. However, the average values of the percentages when FL27 was used as a probe and when it was used as a template in combination with the 16S rDNA similarities are within the possible range suggested by Stackebrandt and Goebel (42). One explanation for the lower DNA relatedness of FL27 to the other *R. gallicum* strains may be the presence of plasmids in FL27 showing little homology with those of the European isolates. These plasmids may carry up to 25% of the genetic information in *Rhizobium* (29), and plasmids are prone to losses or alterations. High-frequency plasmid-borne rearrangements, including sequence amplification, deletion, cointegration, and loss, have been particularly observed in *R. etli* CFN 42^T plasmids (4, 33). In addition, exchange of plasmids among *Rhizobium* populations has been reported (31, 36). Interestingly, the Mexican, French, and Austrian strains showed plasmid profiles that reflected their geographical origins.

Data obtained by Martínez-Romero et al. (20) suggested that the RFLPs of rRNA in *Rhizobium* operons are species

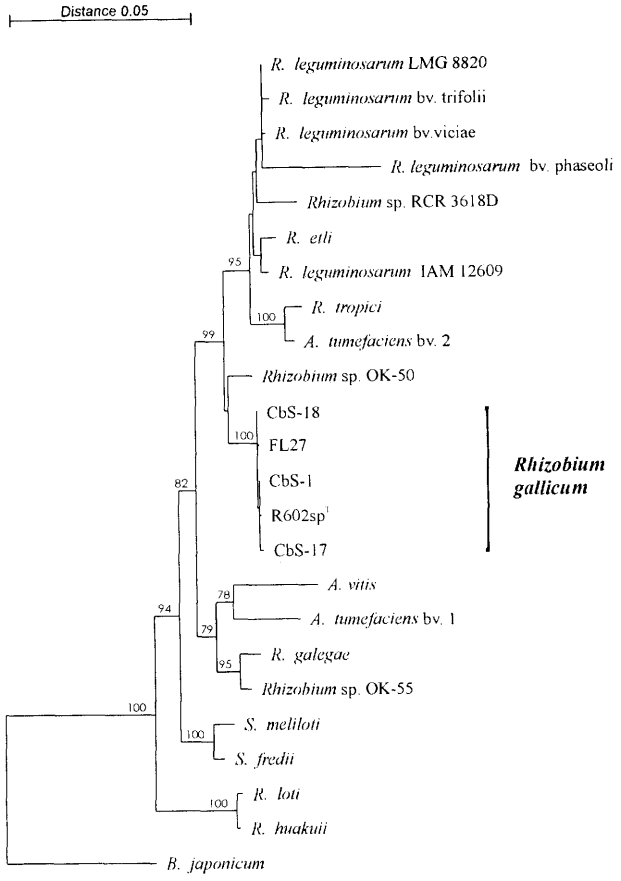

FIG. 2. Neighbor-joining phylogenetic tree based on 1,431 nucleotides of the 16S rDNAs of different rhizobia and related genera. Percentages of 100 bootstrap replicates are shown when they are above 75%. The *R. gallicum* cluster including R602sp^T, FL27, and the Austrian isolates is clearly separated from other rhizobial groups and supported by a 100% bootstrap value. Accession no. of the 16S rDNA sequences used are X67227 (*R. leguminosarum* LMG 8820), U31074 (*R. leguminosarum* bv. trifolii T24), U29386 (*R. leguminosarum* bv. viciae ATCC 10004), U29388 (*R. leguminosarum* bv. phaseoli RCR 3644), U29387 (*Rhizobium* sp. strain RCR 3618D), U28916 (*R. etli* CFN 42^T), D14513 (*R. leguminosarum* IAM 12609^T), U38469 (*R. tropici* type IIB CIAT 166), D01257 (*Agrobacterium tumefaciens* bv. 2 IFO 13257), D01271 (*Rhizobium* sp. strain OK-50), AF008126 (strain CbS-1), AF008127 (strain CbS-17), AF008128 (strain CbS-18), AF008129 (strain FL27), AF008130 (*R. gallicum* R602sp^T), D012528 (*A. vitis* NCPPB 3554), D01256 (*A. tumefaciens* bv. 1 NCPPB 2437), D11343 (*R. galegae* ATCC 43677), D01266 (*Rhizobium* sp. strain OK-55), D14509 (*Sinorhizobium meliloti* IAM 12611^T), D14516 (*S. fredii* ATCC 35423^T), D12791 (*Rhizobium loti* IAM 3588), D13431 (*Rhizobium huakuii* IFO 15243), and D13429 (*Bradyrhizobium japonicum* USDA 94), which was used as an outgroup.

specific but that riboprobing based on pulsed-field gel electrophoresis fingerprints resulted in strain-specific patterns among *Rhizobium galegae* strains (11). The identical 16S rRNA organizations in FL27, R602sp^T, and the Austrian isolates is an additional factor indicating the close relationship of these strains. Our study as well as results obtained by Huber and Selenska-Pobell (11) suggests that the presence of three rRNA operons is common among rhizobia. Previously, Southern analysis of R602sp^T and the Austria isolates with a *nifH* gene fragment resulted in hybridization with a fragment of the same size, indicating the presence of one copy of the *nifH* gene and related *nif* gene organization (41). Based on differences in the symbiotic plasmid and nodulation host range, *R. gallicum* was divided in two biovars, phaseoli and gallicum, containing either three copies or a single copy of the *nifH* gene (1). No differences in the results of phenotypic analysis were found among the Austrian strains, R602sp^T, and FL27, and the results correlated well with those reported by Amarger et al. (1). In a former study the Austrian strains and R602sp^T showed the same nodulation host range for nodulating not only bean plants but also cowpea plants, *Leuceana*, and *Gliricidia* (41). Although isolated from a very different geographical region, FL27 exhibited the same nodulation host range, indicating the presence of the same or a related symbiotic plasmid among the European strains and FL27.

From our analyses it is evident that the Austrian isolates are members of *R. gallicum* bv. gallicum, and we also propose that FL27 belongs to this species. As Mesoamerica, where FL27 was isolated, is one center of origin of the common bean plant (7), it is possible that *R. gallicum* has been imported to Europe as a seed contaminant. Bean rhizobia belonging to species originating in the Americas, such as *R. tropici* and *R. etli*, have been found in Europe (2, 41). In addition, Martínez-Romero (19) suggested that *R. leguminosarum* bv. phaseoli strains are a result of plasmid transfer in historic times because of the high similarity of their symbiotic plasmids with that from *R. etli*. The origin of *R. giardinii*, a recently described species that nodulates bean plants and that was obtained from France, is unknown (1). However, two biovars, *R. giardinii* bv. giardinii and bv. phaseoli, have been described and it was suggested that *R. giardinii* bv. phaseoli as well as *R. gallicum* bv. phaseoli received their symbiotic plasmids from *R. leguminosarum* (1). On the other hand, FL27 was isolated from bean plants grown in a *Leucaena* field and was demonstrated to be a poor N_2 fixer in common bean nodules (28), and therefore it is uncertain whether the common bean plant is the true host of *R. gallicum* bv. gallicum. Nevertheless, the Austrian isolates possess high competitive ability in nodulating bean plants in a soil where they adapted well (40, 41). Further studies will be needed to understand the origin of bean plant-nodulating rhizobia in Europe as well as the role and frequency of plasmid transfer in their evolution.

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