Colonization of *Phaseolus vulgaris* nodules by *Agrobacterium*-like strains

**Mhamdi, Ridha; Mrabet, Moncef; Gisèle Laguerre; Tiwari, Ravi; Mohamed Elarbi Aouani**

*Canadian Journal of Microbiology*; Feb 2005; 51, 2; ProQuest pg. 105

---

**Colonization of *Phaseolus vulgaris* nodules by *Agrobacterium*-like strains**

**Ridha Mhamdi, Moncef Mrabet, Gisèle Laguerre, Ravi Tiwari, and Mohamed Elarbi Aouani**

**Abstract:** Non-nodulating *Agrobacterium*-like strains identified among root nodule isolates of common bean were labeled with *gusA*, a reporter gene encoding β-glucuronidase (GUS). Bean plants were then co-inoculated with an infectious *Rhizobium* strain and labeled transconjugants of *Agrobacterium*-like strains. Blue staining of nodules showed that *Agrobacterium*-like strains were able to colonize these symbiotic organs. Isolation and characterization by restriction fragment length polymorphism analysis of PCR-amplified 16S rRNA genes revealed a mixed population of *Rhizobium* and *Agrobacterium*-like strains in all nodules showing GUS activity. PCR amplification of the *nifH* gene and nodulation tests did not show any evidence of acquisition of symbiotic gene by lateral transfer from *Rhizobium* to *Agrobacterium*-like strains. Moreover, these strains were able to invade mature nodules. Based on sequencing of the 16S rRNA gene, one of these *Agrobacterium*-like strains showed 99.4% sequence similarity with *Agrobacterium* bv. I reference strains and 99% similarity with an *Agrobacterium* bv. I strain isolated from *Acacia mollissima* in Senegal. *Agrobacterium tumefaciens* CS8 and the disarmed variant AT123 did not show any ability to colonize nodules. Co-inoculation of bean seeds with *Agrobacterium* and *Rhizobium* strains did not enhance nodulation and plant yield under controlled conditions.

**Key words:** *Agrobacterium*, co-inoculation, *gusA* gene, nodule colonization, *Rhizobium*.

**Résumé:** Des souches non-nodulantes s'apparentant à *Agrobacterium*, identifiées parmi les isolats de nodules de racines du haricot, ont été marquées avec *gusA*, un gène rapporteur codant la β-glucuronidase (GUS). Les plants de haricots ont ensuite été co-inoculés avec une souche infectieuse de *Rhizobium* et marqués avec les souches de type *Agrobacterium* trans-conjugées. La coloration bleue des nodules a révélé que les souches de type *Agrobacterium* sont capables de coloniser cet organe de symbiose. L’isolement et la caractérisation par polymorphisme de restriction des gènes de l’ARNr 16S amplifiés par PCR ont mis en évidence une population mixte de souches de *Rhizobium* et de type *Agrobacterium* dans tous les nodules possédant une activité GUS. L’amplification par PCR du gène *nifH* ainsi que les tests de nodulation n’ont montré aucune évidence d’acquisition de gènes symbiotiques par transfert latéral de *Rhizobium* vers les souches de type *Agrobacterium*. Qui plus est, ces souches sont capables d’envahir des nodules matures. Selon la séquence du gène de l’ARNr 16S, une de ces souches de type *Agrobacterium* possède 99.4% de similarité de séquence avec la souche référencée *Agrobacterium* bv. I et 99% de similarité avec une souche d’*Agrobacterium*, bv. I isolée d’*Acacia mollissima* au Sénégal. La souche d’*Agrobacterium tumefaciens* CS8 et son variant AT123 n’ont révélé aucune capacité de coloniser les nodules. La co-inoculation de grains de haricots avec des souches d’*Agrobacterium* et de *Rhizobium* n’a augmenté ni la nodulation, ni le rendement des plantes en conditions contrôlées.

**Mots clés :** *Agrobacterium*, co-inoculation, gène *gusA*, colonisation de nodule, *Rhizobium*.

**Introduction**

Leguminous plants can establish a nitrogen-fixing symbiosis with soil bacteria commonly named rhizobia, which belong to several genera of the alpha-proteobacteria including *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Allorhizobium*, *Methyllobacterium*, *Devosia*, and *Blastobacter* (Young and Haukka 1996; van Berkum and Eardly 1998, 2002; Sy et al. 2001; Rivas et al. 2003) as well as to the recently described genera *Burkholderia* and *Ralstonia* of the beta subdivision of proteobacteria (Chen et al. 2001; Moulin et al. 2001). Various other soil microorgan-
isms such as strains of Agrobacterium, Azospirillum, Azotobacter, fluorescent Pseudomonas, or Bacillus have the ability to interact with rhizobia and legumes by stimulating root growth and nodulation (Grimes and Mount 1984; Caetano-Anollés and Bauer 1988; Petersen et al. 1996; Burdman et al. 1997; Camacho et al. 2001; Tokala et al. 2002). Evidence that such beneficial bacteria may colonize root nodules and enhance both nodule growth and bacteroid differentiation was recently given for Streptomyces lydicus WYEC108 (Tokala et al. 2002).

Bacteria related to Agrobacterium bv. 1 were also identified among root nodule isolates of several tropical leguminous plants including Acacia, Prosopis, and Chambeecrista species from Africa (De Lajudie et al. 1999). Nodulation and phytopathogenic tests showed that these isolates were not capable of inducing nodules on a wide variety of leguminous plants or tumor formation on Kalanchee tubiflora and Nicotiana rustica. Similarly, a significant proportion of bacterial isolates from nodules of Phaseolus vulgaris grown in Tunisian soils had 16S rRNA genes similar to those of Agrobacterium tunefaciens (bv. 1) and Agrobacterium rubi reference strains on the basis of RFLP analysis of PCR-amplified 16S rDNA (Mhamdi et al. 2002). No symbiotic genes were detected by PCR amplification of nifH and nodC genes and by hybridization of total DNA with symbiotic gene probes (Mhamdi et al. 1999, 2002). When reexamined for nodulation, these isolates did not nodulate their original host. Collectively, these results led to the hypothesis that the nonnodulating agrobacteria may colonize root nodules by a mixed infection with a rhizobial cell capable of nodule induction, resulting in a mixed population within the nodule. An alternative hypothesis was that the agrobacteria may have acquired symbiotic genes, entered the plant, but subsequently lost their symbiotic genes during symbiosis or after isolation and cultivation or during storage. Lateral transfer of symbiotic genes from rhizobia to agrobacteria has been performed under laboratory conditions (van Brussel et al. 1982; Truchet et al. 1984; Martinez et al. 1987). The recipient Agrobacterium was able to form nitrogen-fixing nodules on P. vulgaris roots (Martinez et al. 1987). There is also evidence that lateral transfer of symbiotic genes did occur during the course of evolution among rhizobia belonging to different species and even different genera, especially among Phaseolus rhizobia (Segovia et al. 1993; Amarger et al. 1997; Laguerre et al. 2001). The fact that certain rhizobial genera and the genus Agrobacterium are phylogenetically closely related and intermingled (Sawada et al. 1993; Willems and Collins 1993) argues in favor of the feasibility of lateral transfer of symbiotic genes under soil conditions. Conversely, conjugal transfer of tumor-inducing plasmid of agrobacteria to soil bacteria related to rhizobia has been reported (Teyssier-Cuvelle et al. 1999).

In the present study, we aimed to (i) further identify the Agrobacterium-like isolates from P. vulgaris nodules, (ii) demonstrate that they are able to colonize the nodules because up to now, there was no direct evidence that the agrobacteria identified among root nodule isolates were indeed present inside the nodules, and (iii) investigate the significance of nodule colonization by these bacteria for nodulation and symbiotic effectiveness of nitrogen fixation.

Materials and methods

Bacterial strains

Rhizobium gallicum bv. gallicum 8a3, Rhizobium leguminosarum bv. phaseoli 31c3, and Agrobacterium-like strains 22c1, 10c2, and 6a6 were isolated from root nodules of P. vulgaris grown in Tunisian soils by standard methods as previously described (Mhamdi et al. 1999). All rhizobial strains were maintained on YEM medium (K2HPO4, 0.5 g/L; MgSO47H2O, 0.2 g/L; NaCl, 0.1 g/L; mannitol, 10 g/L; yeast extract, 0.6 g/L; pH 7) supplemented with 25% glycerol at 70 °C. Escherichia coli strain S17.1 f. PIR harboring a plasmid construct containing the mmitransposon nmr5SSgusA20 (Wilson et al. 1995) was used as a donor in conjugation experiments. This transposon carries the resistance to streptomycin and spectinomycin and expresses gusA constitutively (Wilson et al. 1995).

Labeling of Agrobacterium-like strains by gusA gene

Agrobacterium-like strains 22c1, 10c2, and 6a6 were used as recipients. The Escherichia coli strain containing the relevant β-glucuronidase (GUS) transposon was used as a donor strain. Plate mating was carried on a membrane filter (0.45-μm pore size) in TY (tryptone, 5 g/L; yeast extract, 3 g/L; CaCl2·2H2O, 0.88 g/L; agar, 15 g/L) plates at 28 °C according to Wilson et al. (1994). Since the recipient strains used were naturally resistant to streptomycin, spectinomycin, and kanamycin, the markers carried by the mitransposon nmr5SSgusA20 could not be used to select transconjugants. Thus, the transconjugants were selected on the basis of production of blue colonies on TY medium supplemented with 20 μg kanamycin/mL and 50 μg X-GlcA (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid)/mL, the GUS enzyme substrate. After several cycles of culturing, the transconjugants were then checked for their stability on TY medium supplemented with 50 μg X-GlcA/mL.

Plant tests

Phaseolus vulgaris 'coco' and 'royalnel' were used for nodulation tests, and nitrogen fixation effectiveness was conducted on P. vulgaris 'coco'. These tests were carried out in plastic pots of 0.5 L filled with sterile gravel as previously described (Mhamdi et al. 1999). In infection tests, R. gallicum bv. gallicum 8a3 and R. leguminosarum bv. phaseoli 31c3 were used as infective strains. A mixture of strains 6a6, 10c2, and 22c1 was used as an Agrobacterium-like inoculant. Co-inoculations of rhizobial and agrobacterial strains were conducted in a ratio of 1:1. In the first experiment, bean plants were co-inoculated with a nonlabeled agrobacteria mixture and R. gallicum 8a3. The nodules were then recovered and sterilized. To check the efficiency of the sterilizing method, a sample of a few nodules was incubated in YEM broth for 48 h at 28 °C. Then, the bacterial strains harbored in the nodules were extracted and identified by PCR–RFLP of 16S rDNA. In the second experiment of coinoculation, the Agrobacterium strains were gusA labeled. Both strains, 8a3 and 31c3, were used as rhizobia inoculants. Two ways of inoculant preparation were carried out: (i) Rhizobium and Agrobacterium strains were cultivated separately on YEM broth and (ii) Rhizobium and
*Agrobacterium* strains were cultivated conjointly on solid YEM medium to favor a potential event of symbiotic gene transfer from *Rhizobium* to *Agrobacterium*. The plants were harvested at 10, 15, 20, 25, 35, 45, and 55 DAI (days after inoculation), and the nodules were submitted to staining for GUS activity. In the third experiment, inoculation with agrobacteria mixture was delayed until nodule formation (17 DAI with 8a3). The plants were then recovered at 1, 3, 8, and 10 DAI and submitted to staining for GUS activity. In the effectiveness test, the plants were co-inoculated with a constant dose of *R. gallicum* bv. *gallicum* 8a3 (10^6 CFU per seed) and an increasing ratio of nonlabeled *Agrobacterium* mixture (1:0, 1:10, 1:100, 1:500, and 1:1000). Plants inoculated only with *Agrobacterium* were also included. Ten replicates were performed for each treatment. The plants were harvested at 50 DAI and submitted to nodule numbering and dry matter measures. Statistical analysis of the data was performed by one-way ANOVA and Duncan’s test.

**Staining for GUS activity**

GUS assay buffer was based on standard phosphate buffer containing 50 mmol/L NaPO₄, pH 7.0, 1 mmol/L EDTA, 0.1% Sarkosyl, 0.1% Triton X-100, and 50 µg X-Gluc/µL (Sigma). Plants with the whole root system were incubated for 1–2 days in staining buffer and then extensively washed with deionized water. To isolate bacteria from stained nodules, nodules were sterilized as previously described (Mhamdi et al. 1999), cut in half, and then incubated on solid TY medium supplemented with 50 µg X-Gluc/µL. Bacteria were isolated from colored nodules as previously described (Mhamdi et al. 1999).

**DNA manipulations**

The PCR amplification of the 16S rRNA and *nifH* genes and restriction digestions were performed as previously described (Mhamdi et al. 2002). Three endonucleases, *MspI*, *RsaI*, and *NdeII*, were used to digest 16S rDNA. The nucleotide sequence of the 16S rDNA PCR product from the *Agrobacterium*-like strain 10c2 was determined by using a DTCs-1 kit (Beckman Coulter) and a CEQ 2000 XL sequencer (Beckman Coulter) according to the manufacturer’s instructions. Primers FD1 and RD1 (Weiburg et al. 1991) and 515F (5’TGC CAG CAG CCG CGG AA–3’) were used for sequencing reactions. Restriction site analysis of the sequence was performed using the Bisance software (Dessen et al. 1990). Nucleotide sequence comparisons were performed by using the FASTA program available in the Bisance software.

**Results and discussion**

**Identification of *Agrobacterium*-like isolates by 16S rRNA gene sequencing**

The nucleotide sequence (1147 bp) of the 16S rDNA fragment of the *Agrobacterium*-like strain 10c2 was determined and deposited in the GenBank database under acc. No. AY225504. Sequence comparisons indicated that several strains of *Agrobacterium* bv. 1 (acc. Nos. AJ389893, AJ389892, AJ389897, AJ389904, and D12784) were the closest relatives of strain 10c2, with a similarity value of 99.4% between 16S rDNAs. This result confirms the primary identification based on mapped restriction site analysis of the PCR-amplified fragment (Mhamdi et al. 2002). The 16S rDNA of strain 10c2 was also closely related to that of *Agrobacterium* bv. 1 strain LMG 11915 (99.0% of similarity), which was isolated from a nodule of *Acacia mollissima* in Senegal, but showed only 98.3% of similarity with that of *Agrobacterium* bv. 1 strain LMG 11916 isolated from a nodule of *Prosopis juliflora* (De Lajudie et al. 1991). These results indicate the diversity of agrobacteria able to interact with the *Rhizobium*-legume symbiosis and raise the question of the specificity of the *Agrobacterium*-Rhizobium-legume interactions.

**Do *Agrobacterium*-like isolates effectively colonize nodules?**

Since the *Agrobacterium* bv. 1 isolates from *P. vulgaris* nodules were not capable of inducing any nodule formation when inoculated to their original host (Mhamdi et al. 2002), we decided to examine the possibility of recovering these strains from nodule extracts by co-inoculation with an infective rhizobial strain. In the first experiment, bean plants were co-inoculated with nonlabeled *Agrobacterium* bv. 1 strains and *R. gallicum* strain 8a3. Isolates showing morphological and growth characteristics similar to the *Agrobacterium*
Fig. 2. Staining for β-glucuronidase activity in nodules cut in half. The nodules were recovered from 50-DAI-old bean plants co-inoculated by the infective R. gallicum strain 8a3 and Agrobacterium bv. 1 isolates labeled with the gusA gene. The nodules were sterilized, cut in half, and then incubated for 48 h on the agar surface of TY medium supplemented with X-GlC A. The staining was observed at different levels inside the nodules. (a) Restricted staining in the cortical zone; (b) uniform staining in the cortical zone; and (c) generalized staining touching the cortical and infection zones.

Table 1. Colonization of mature nodules by Agrobacterium-like isolates.

<table>
<thead>
<tr>
<th>Days after inoculation (DAI)</th>
<th>Total no. of analyzed nodules</th>
<th>Nodules expressing GUS activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>196</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>156</td>
<td>32</td>
</tr>
<tr>
<td>10</td>
<td>273</td>
<td>59</td>
</tr>
</tbody>
</table>

*Note:* Bean plants were inoculated with R. gallicum strain 8a3; at 17 DAI they were re-inoculated with agrobacteria. The plants were then harvested at different intervals and submitted to GUS activity staining.

Fig. 3. Colonization of mature nodules induced by R. gallicum 8a3 (17 DAI old) by Agrobacterium-like strains 1 DAI with agrobacteria mixture. The nodules were harvested and submitted to GUS activity staining. Stained spots indicate the beginning of agrobacteria invasion.

incubant and different from the co-inoculated R. gallicum strain (i.e., the bigger size and red coloration of the Agrobacterium colonies on YEM agar medium supplemented with red Congo and the ability to grow on Luria-Bertani agar medium) were isolated from approximately 20% of nodules formed on the 2 cultivars of bean used. The identity of these strains was then confirmed by PCR-RFLP of the 16S rRNA gene. Nevertheless, further evidence is still required to show that the agrobacteria strains were effectively recovered from the inside of nodules. In fact, external contamination could not be undeniably excluded.

In the second experiment, co-inoculation was performed with an infective rhizobial strain, R. gallicum bv. gallicum 8a3 or R. leguminosarum bv. phaseoli 31c3, and a mixture of gusA-labeled Agrobacterium strains. The plants inoculated only with Agrobacterium strains did not show any nodule structure on their roots. Nodules expressing GUS activity were detected on the root system of the co-inoculated plants after 15 DAI (Fig. 1). The development of blue color was observed with both types of inoculants ((i) and (ii), Plant tests section), both bean varieties cultivated, and both rhizobial strains used (R. gallicum 8a3 and R. leguminosarum 31c3). No staining was observed in nodules of control plants inoculated only with nonlabeled rhizobial strains 8a3 and 31c3. The number of colored nodules increased with time and could exceed 50% of the nodules per plant at 45 DAI (data not shown). No correlation was found between color development and size or shape of nodules. Nevertheless, some nodules were wholly colored, while others were dotted.

To determine if stained nodules harbor only Agrobacterium strains or a mixed population of agrobacteria and rhizobia, we attempted to isolate bacteria by crushing stained nodules on YEM agar medium. Unfortunately, no bacterial cells were able to grow, possibly because the staining buffer killed the bacteria. To overcome this problem, nodules were sterilized, cut in half, and then incubated on agar medium supplemented with X-GlC A. This method of staining permitted better localization of the blue color inside the nodules and different levels of staining could be thus distinguished (Fig. 2). Some nodules showed a central staining, whereas in others, the staining was essentially localized in the cortex. Extraction of bacteria from blue-colored zones revealed 2 types of colonies on the basis of morphology and size. Incubation on TY medium supplemented with X-GlC A showed that large colonies all expressed a GUS activity, while the
small colonies did not. The large and small colonies were assumed to be agrobacteria and rhizobia, respectively. The 16S restriction patterns of the big colonies were identical to those of the Agrobacterium gusA labeled inoculants, while those obtained from the small colonies conformed to those of the rhizobial co-inoculants. These results clearly demonstrated that agrobacteria and rhizobia coexisted as a mixed population in the stained nodules.

**How do Agrobacterium-like isolates invade nodules?**

PCR amplification of the nifH gene was performed on Agrobacterium and Rhizobium strains recovered from stained nodules. No fragment of the expected size could be amplified from the Agrobacterium strains. The 2 rhizobial strains used as co-inoculants gave the expected fragment. The nodulation tests confirmed this result, since no nodule could be observed at 50 DAI when the Agrobacterium strains reisolated from stained nodules were inoculated to P. vulgaris.

Our results suggest that the agrobacteria did not acquire symbiotic genes by horizontal transfer, although we could not definitely exclude the possibility of a transitory acquisition of a symbiotic plasmid, unstable in nodules or after isolation in our culture and storage conditions. The symbiotic plasmid specific to bv. phaseoli has been previously shown to be unstable during cultivation of the rhizobial strains in laboratory conditions (Soberon-Chavez et al. 1986; Flores et al. 1988; Romero et al. 1991).

It seems that Agrobacterium-like isolates invade nodules through a mechanism different from symbiotic infection. This hypothesis is supported by the nonnodulating ability of the Agrobacterium-like strains and by the fact that all stained nodules were co-infected by rhizobia and agrobacteria. On the other hand, the infection of the roots by the agrobacteria seems not to be restricted to the Rhizobium infection thread, since different-colored bands, simulating infection foci, were observed in many cases on the same nodule. With time, the staining appeared to be more uniform, which suggested that the agrobacteria could colonize the nodules even after their formation.

To test this hypothesis, a third co-inoculation experiment, where inoculation with agrobacteria was delayed until nodule formation (17 DAI), was conducted. Interestingly, stained nodules were observed from 1 d after Agrobacterium inoculation on (Table 1). The coloration begins with restricted dots, 1 or 2 on the same nodule, and then the infected zone proliferates with time (Fig. 3). At 8 DAI, 20% of nodules were stained, and in some cases, the nodules were totally stained.

This finding indicates that nodule colonization by Agrobacterium-like isolates could occur after nodule formation. It seems likely that mixed infection with a rhizobial cell or the possibility of symbiotic gene acquisition by Agrobacterium-like isolates does not play a role in this mechanism.

**Do other agrobacteria share this ability?**

The Agrobacterium strains isolated from root nodules of P. vulgaris in this work and those isolated by de Lajudie et al. (1991) from A. mellissima and P. juliflora were assigned to bv. 1. It would be interesting to investigate if the capacity of colonizing nodules is restricted to these strains of agrobacteria or is enlarged to other reference strains. In our work, we were not able to confirm the colonization of nodules either by the wild strain of A. tumefaciens C58 or with the disarmed variant AT123 in co-inoculation trials with strain 8a3. It seems that tumor-inducing genes do not play a role in this colonization. De Lajudie et al. (1999) showed that the Agrobacterium isolated from nodules were avirulent. However, the possibility of loss of the Ti plasmid should not be excluded, since plants usually favor the selection of nonpathogenic forms inside the tumors (Nautiyai and Dion 1990; Canfield and Moore 1991; Belanger et al. 1995).

We are currently investigating other reference strains of agrobacteria. Laguerre et al. (1993) previously identified nonsymbiotic rhizobia from soil. It would be interesting to determine if these strains have the ability to colonize nodules as did these agrobacteria.

These results are viewed in the context of the recent discovery of several unsuspected bacterial species and genera involved in natural symbiosis with legume plants. Some of them were also described as natural endophytes of cereals (rice, corn, and wheat), suggesting that mechanisms for interaction with plants and new physiological functions other than the classical legume nodulation process are yet to be understood.

<table>
<thead>
<tr>
<th>Co-inoculation ratio</th>
<th>Nodule value per plant</th>
<th>Nodule number</th>
<th>Nodule dry mass (g)</th>
<th>Root dry mass (g)</th>
<th>Shoot dry mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/1</td>
<td>406a</td>
<td>0.42a</td>
<td>0.70a</td>
<td>2.85a</td>
<td></td>
</tr>
<tr>
<td>10/1</td>
<td>344a</td>
<td>0.38a</td>
<td>0.72a</td>
<td>2.75a</td>
<td></td>
</tr>
<tr>
<td>100/1</td>
<td>411a</td>
<td>0.42a</td>
<td>0.69a</td>
<td>2.70a</td>
<td></td>
</tr>
<tr>
<td>500/1</td>
<td>337a</td>
<td>0.37a</td>
<td>0.70a</td>
<td>2.81a</td>
<td></td>
</tr>
<tr>
<td>1000/1</td>
<td>345a</td>
<td>0.33a</td>
<td>0.65a</td>
<td>2.54a</td>
<td></td>
</tr>
<tr>
<td>1/0</td>
<td>0b</td>
<td>0b</td>
<td>0.30b</td>
<td>0.32b</td>
<td></td>
</tr>
<tr>
<td>Noninoculated</td>
<td>0b</td>
<td>0b</td>
<td>0.26b</td>
<td>0.31b</td>
<td></td>
</tr>
</tbody>
</table>

*Values of the mean ± 10 replicates. Means with the same letter are not significantly different for each independent variable (P < 0.05).
discovered in these bacteria (Yanni et al. 1997; Biswas et al. 2000; Chaintreuil et al. 2000; Dazzo et al. 2003).

Impact on symbiotic interaction
No significant effect of Agrobacterium inoculation was observed on the number of nodules or the root, nodule, and shoot biomass (Table 2). The characteristics of the plants inoculated with Agrobacterium strains alone were similar to those of the noninoculated control plants. This result indicated that the Agrobacterium strains had no effect on nodulation and effectiveness of strain 8a3 under controlled conditions on sterile gravel. Plant growth promoting rhizobacteria have been shown to improve nodulation and in some instances nitrogen fixation of bean plants based on co-inoculation trials under nonsoil conditions (Grimes and Mount 1984; Burdman et al. 1997; Camacho et al. 2001). The positive effect of a Bacillus sp. strain was confirmed in soil conditions (Camacho et al. 2001). However, the positive effect of co-inoculation was also shown to be dependant on the concentration of both rhizobial and plant growth promoting rhizobacteria rhizobacterial inoculants (Burdman et al. 1997). Positive effects may be reduced or annulled by high concentrations of inoculants. It was suggested that such synergistic effects could be due to the excretion of substances as phytohormones (Hirsch et al. 1989; Yahalom et al. 1990; Camacho et al. 2001), improvement of phosphorus uptake (Grimes and Mount 1984), and (or) suppression of pathogenic agents (Kloeper 1993). However, to our knowledge, there is no evidence that plant growth promoting rhizobacteria able to enhance the Rhizobium-legume symbiosis act through nodule colonization, except as recently described for a S. lydicus strain (Tokala et al. 2002).

The identification in different laboratories of nodule isolates related to Agrobacterium bv. 1 recovered from various legumes and the direct evidence resulting from the present work that the agrobacteria are able to cocolonize nodules deserve further investigation to discover the biological significance of this new bacteria-plant association. Assuming that nutrient status, density of indigenous rhizobial populations, and other environmental factors should influence this association, we are currently investigating the response of the plant to inoculation with agrobacteria under soil conditions including nodulation by indigenous populations of rhizobia.

Acknowledgments
This work was supported by grants from the Ministere de l’Enseignement Superieur, de la Recherche Scientifique et de la Technologie, Projet Interactions Legumineuses-Microorganismes.

References

© 2005 NRC Canada


