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# The *nodD1* Gene from *Rhizobium* Strain NGR234 Is a Key Determinant in the Extension of Host Range to the Nonlegume *Parasponia*

**Gregory L. Bender, Murali Nayudu, Kathryn K. Le Strange, and Barry G. Rolfe**

Plant Molecular Biology, Research School of Biological Sciences, Australian National University, Box 475 P.O., Canberra City, A.C.T. 2601.
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**The narrow host range of *Rhizobium leguminosarum* biovar *trifolii* strain ANU843 can be extended from clovers to the nonlegume *Parasponia* by the addition of the *nodD1* gene from *Rhizobium* strain NGR234. The presence of the *nodD1* gene from NGR234 enabled induction of a *nodA*::Mu*d*II1734 fusion in strain ANU843 by seedling extracts from *Parasponia* and other nonlegumes, including *Trema* (a tree genus closely related to *Parasponia*), *Casuarina* (a genus that forms symbiotic relationships with the actinomycete *Frankia*) and the cereals wheat, rice, and maize. There was also an increase in the range of flavonoids causing induction, suggesting that the *nodD1* gene from the broad host range strain NGR234 is nonspecific in action rather than host specific, as found for the *nodD* genes from narrow host range species. Although the presence of the *nodD1* gene from NGR234 enabled the induction of *nod* genes by a range of nonlegume extracts, no nodules were elicited on any nonlegumes other than *Parasponia*.**

When available nitrogen is limited, some dicotyledonous plants can form a symbiosis with nitrogen-fixing prokaryotes as an alternative means of obtaining nitrogen. Members of the legume family specifically form a symbiosis with *Rhizobium*, *Bradyrhizobium*, or *Azorhizobium* bacteria, whereas nonlegumes interact with actinomycetes of the genus *Frankia*. *Parasponia*, a woody member of the elm family confined to the Malay archipelago, is the only nonlegume known to form a symbiosis with *Rhizobium* or *Bradyrhizobium* strains (Trinick 1973; Akkermans *et al.* 1978).

The infection of *Parasponia* differs in some ways from that of legumes (Lancelle and Torrey 1984; Bender *et al.* 1987a). Root hair curling and the initiation of infection threads within curled hairs, commonly found in legumes, have not been observed in *Parasponia*. The first event in the infection of *Parasponia* appears to be the initiation of cell division within a zone of the root that is highly susceptible to infection (Bender *et al.* 1987a). Cell division produces a break in the epidermis through which bacteria gain entry into the root. Infection threads are then initiated from intercellular colonies within the cortex. Subsequent stages of *Parasponia* nodule development, and final nodule structure, resemble those found in the symbiosis between other nonlegumes and *Frankia* (Lancelle and Torrey 1985). Unlike legume nodules, a *Parasponia* nodule resembles a swollen lateral root with an apical meristem and central vascular bundle.

The activation of *Rhizobium* nodulation (*nod*) genes, and hence the initiation of a legume symbiosis, requires both root exudate and the constitutive expression of the regulatory *nodD* gene (Innes *et al.* 1985; Mulligan and Long 1985; Rossen *et al.* 1985). Hong *et al.* (1987) have shown that the *nodD* gene product binds directly to a conserved promoter sequence called the *nod*-box (Rostas *et al.* 1986) located upstream from *nod* genes. The inducing compounds found in legume exudates or extracts have been identified as flavones, flavanones, or closely related compounds that are thought to interact with NodD to induce expression of *nod* genes (Peters *et al.* 1986; Redmond *et al.* 1986; Firmin *et al.* 1986; Kosslak *et al.* 1987; Rolfe 1988).

*Rhizobium* strain NGR234 has a broad host range among tropical legumes and can form *Parasponia* nodules that do not fix nitrogen (Trinick and Galbraith 1980). Nodulation genes have been localized on a large symbiotic (Sym) plasmid in this strain (Morrison *et al.* 1983; Pankhurst *et al.* 1983). Removal of the Sym plasmid results in the loss of nodulation ability (Morrison *et al.* 1983), whereas transfer of this plasmid to other *Rhizobium* species extends their host range to include plants nodulated by strain NGR234 (Morrison *et al.* 1984; Broughton *et al.* 1984). Lewin *et al.* (1987) have located three host-specific nodulation (Hsn) regions on the Sym plasmid of strain NGR234. HsnI is located next to *nodD*, HsnII is linked to the nitrogenase structural genes *nifHDK*, and HsnIII to *nodC*. All three Hsn regions, when transferred to the appropriate recipients, confer the ability to curl the root hairs of *Macroptilium atropurpureum* (siratro). However, only HsnI enables the formation of nodules on siratro and also specifies the nodulation of *Vigna unguiculata* (cowpea), *Glycine max* (soybean) and *Psophocarpus tetragonolobus*. HsnIII is able to complement a mutation in the *Medicago sativa* (alfalfa)-specific *nodH* gene of *R. meliloti*. Bachem *et al.* (1986) have isolated an 11.4-kb fragment from the Sym plasmid of strain MPIK3030 (a derivative of NGR234) that is specific for siratro nodulation. Genes on this fragment did not complement mutations in either conserved or host-specific nodulation genes in *R. meliloti*.

Although the *nodD1* gene from strain NGR234 is required for the nodulation of all hosts, including *Parasponia* (Bender *et al.* 1987b), there is evidence that this conserved gene also has an important role in determining

Address correspondence to Barry G. Rolfe.

broad host range (Horvath *et al.* 1987; Bassam *et al.* 1988). In this paper we show that *R. leguminosarum* biovar *trifolii* strain ANU843, carrying a 2.1-kb *Pst*I fragment spanning the *nodD1* gene from strain NGR234, can nodulate *Parasponia*, a nonlegume host where the mode of infection differs markedly from that of the homologous host clover. Gene expression studies that use a *nodA*::Mu*d*II1734 fusion suggest that the nodulation of *Parasponia* by strain ANU843 requires the induction of nodulation genes by *Parasponia* seedling extract. In addition, the presence of the NGR234 *nodD1* gene enabled the induction of ANU843 nodulation genes by seedling extracts from a wide range of other nonlegumes, including cereals.

## MATERIALS AND METHODS

**Bacterial strains used.** All bacterial strains and plasmids, their characteristics and the source from which they were obtained, are listed in Table 1.

**Media.** BMM media for *Rhizobium* has been described (Bergersen 1961). GMM minimal media for *Rhizobium* is a derivative of GMY media (Bender and Rolfe 1985) with yeast extract replaced with monosodium glutamate (0.5 g/L). The vitamins biotin (25 μg/L) and thiamine (100 μg/L) were also added for the culture of *R. l.* bv. *trifolii* strains. Herridge plant nutrient solution has been described (Delves *et al.* 1986).

**Bacterial genetics.** Plasmids were constructed as described (Nayudu and Rolfe 1987; Bassam *et al.* 1988) and transferred from *E. coli* strain HB101 to *R. l.* bv. *trifolii* strains via patch mating (Sinclair and Holloway 1982). GMM plates containing tetracycline (4 μg/ml) were used for the selection of *R. l.* bv. *trifolii* transconjugants carrying the plasmid pMN40.

**Seed germination.** *Parasponia andersonii* and *Trema aspera* seeds were germinated as described (Bender and Rolfe 1985). Seeds of *Trifolium repens* (white clover) and *T. subterraneum* (sub clover) were germinated as described by Rolfe *et al.* (1980). *M. atropurpureum* (siratro) seeds were shaken in 98% sulphuric acid for 5 min. The acid was removed by thorough washing in tap water; the seeds were then shaken in 7% sodium hypochlorite for 5 min. After washing in sterile distilled water, the seeds were arranged on a Herridge agar plate and each seed secured by using a single drop of 2% Herridge agar. Plates were then incubated vertically in darkness at 28° C until seedlings reached about 2 cm in length. Seeds of *Casuarina cunninghamiana*, *Helianthus annuus* (sunflower), *Gossypium barbadense* (cotton), *Triticum aestivum* (wheat), *Oryza sativa* (rice), and *Zea mays* (maize) were shaken in a 4% solution of sodium hypochlorite, containing a few drops of Tween-80 detergent, for 5 min. Seeds were then processed as for siratro. After germination, sterile seedlings were used for the collection of seedling extract or for nodulation assays.

**Table 1.** Bacterial strains and plasmids

| | Relevant characteristics | Origin |
|---|---|---|
| *Rhizobium* spp. | | |
| NGR234 | Broad host range strain isolated from *Lablab purpureus* A $Sm^r$ derivative (ANU240) was was used for this study | Morrison *et al.* 1983 |
| *R. l.* bv. *trifolii* | | |
| ANU843 | Wild-type clover-specific strain | Rolfe *et al.* 1980 |
| ANU851 | $Nod^-$ mutant of ANU843 with Tn*5* insert in the *nodD* gene | Djordjevic *et al.* 1983 |
| ANU894 | Strain ANU843 with a translational fusion of *nodA* (pSym) and the *E. coli lac* operon (Mu*d*II1734) | M. Djordjevic (this lab), unpublished |
| *E. coli* | | |
| HB101 | *leu, proA2, thi, hsdS20, recA13,* ($R^-m^+$) | Boyer and Roulland-Doussoix 1969 |
| Plasmids | | |
| pSUP106 | Broad host range *IncQ* vector, stably maintained in *Rhizobium*, $Cm^r$, $Tc^r$ | Simon *et al.* 1983 |
| pMN40 | pSUP106 carrying a 2.1-kb *Pst*I DNA fragment spanning the *nodD1* gene from NGR234 | This work |

**Nodulation assays.** The agar plate plant assays for clover (Rolfe *et al.* 1980), siratro (Cen *et al.* 1982) and *Parasponia* (Bender and Rolfe 1985) have been described. A modified Leonard jar plant-assay system designed by E. Appelbaum of Agrigenetics Inc., Madison, WI was also used for nodulation assays. We have termed this system a "Magenta jar." *Rhizobium* inoculum for Magenta jars was taken from selective plates and suspended in Herridge solution to a density of $10^8$–$10^9$ cells per milliliter. One ml of inoculum was applied to each of five seedlings transplanted to a Magenta jar. The acetylene reduction assay, used to measure the nitrogen-fixation capacity of root nodules, has been described (Bender and Rolfe 1985). Bacteria were isolated from nodules as described by Gresshoff *et al.* (1977).

**β-galactosidase assays.** Freshly germinated seedlings were submerged in a minimum volume of 50% ethanol in a screw-topped vial. Nitrogen gas was bubbled through the solution for 5 min, and the vial was sealed and placed in darkness for 2 hr. The solution was then passed through filter paper to remove suspended matter. One-ml aliquots of the filtrate were immediately dried overnight under vacuum. The dried extract was then resuspended in 1.2 ml of water by vigorous shaking. Log-phase *Rhizobium* cells (0.4 ml of a BMM liquid culture at an absorbance of 0.20–0.25, wavelength 600 nm) were added without shaking and left at 28° C for 3 hr. β-galactosidase activity was then assayed as in Miller (1972), with 4 hr allowed for color development.

**Microscopy.** Plant tissue for light and electron microscopy was prepared and examined as described (Bender *et al.* 1987b).

## RESULTS

Bassam *et al.* (1986) reported that a 6.7-kb *Hin*dIII fragment from strain NGR234 carries the *nodD1* gene and two loci involved in the host-specific nodulation of siratro but not the other hosts of NGR234. Mutational analysis of the 6.7-kb fragment showed that only the two Hsn loci and not the *nodD* gene were involved in extension of the host range of *R. l.* bv. *trifolii* strain ANU843 to siratro. Subsequent analysis of a 17-kb *Xho*I fragment (Bassam *et al.* 1988), which includes the 6.7-kb fragment, has shown that a mapping error was made and that the *nodD1* gene is a key element in determining the broad host range of NGR234. This is consistent with Horvath *et al.* (1987), who have shown that mutations in the *nodD1* gene of strain MPIK3030 (a derivative of NGR234) prevent host range extension. In addition, the use of the Magenta jar plant

assay (Bassam *et al.* 1988), rather than an agar plate assay (Bassam *et al.* 1986), has shown that the *nodD1* gene can extend the host range of strain ANU843 from clovers to not only siratro but other legume hosts of strain NGR234.

**Cloning of the *nodD1* gene from *Rhizobium* strain NGR234.** To test the observation further by Bassam *et al.* (1988) that the *nodD* gene is an essential determinant for the broad host range of NGR234, we cloned the smallest possible DNA fragment containing the NGR234 *nodD1* gene into the broad host range vector pSUP106 (Fig. 1). A 2.1-kb *Pst*I fragment was cloned from the NGR234 Tn*5* mutant number 7 (Bassam *et al.* 1986) into the plasmid pSUP106 by replacing the small *Pst*I fragment carrying the *cos* site on pSUP106. This plasmid construct (pMN40) contains a 0.8-kb segment of IS*50* which is part of a Tn*5* arm and about 0.4 kb of an intergenic region (Bassam *et al.* 1986) which separates Tn*5* from the *nodD1* gene. DNA sequence analysis of the equivalent 0.4-kb region upstream from the *nodD1* gene in strain MPIK3030 (Horvath *et al.* 1987) did not reveal an open reading frame, suggesting that the *nodD1* gene is the only *nod* gene present on pMN40. The 2.1-kb fragment contains a functional copy of the NGR234 *nodD1* gene, as shown by the ability of a *nodD1* mutant of NGR234 (ANU1255), carrying plasmid pMN40, to nodulate siratro and *Parasponia*.

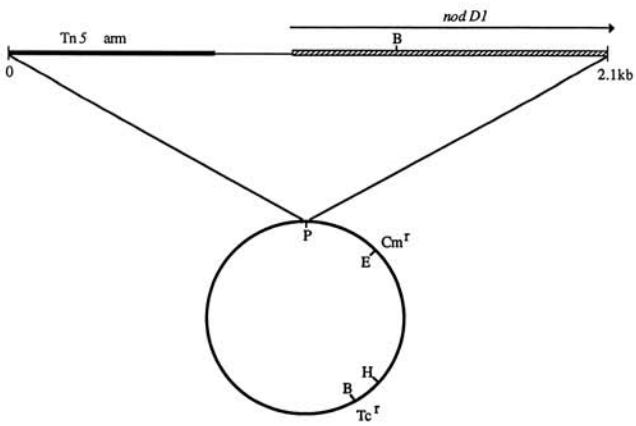


**Fig. 1.** Map of plasmid pMN40 showing the location of a 2.1-kb *Pst*I fragment carrying the *nodD1* gene from *Rhizobium* strain NGR234 cloned into the broad host range vector pSUP106. The 2.1-kb *Pst*I fragment was cloned from the Tn*5* mutant number 7 (Bassam *et al.* 1986) into pSUP106 by replacing the small *Pst*I fragment carrying the *cos* site on pSUP106. The direction of transcription, indicated, is from data published by Horvath *et al.* (1987) for the *nodD1* gene of strain MPIK3030. This plasmid contains a segment of IS*50* that is part of the Tn*5* arm. It also contains an intergenic region of about 0.4 kb (Bassam *et al.* 1986) that contains no open reading frames (Horvath *et al.* 1987). The location of the antibiotic resistance genes and restriction enzyme sites are indicated. $Cm^r$, gene encoding resistance to chloramphenicol; $Tc^r$, gene encoding resistance to tetracycline. Restriction enzymes: E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; P, *Pst*I.

**Extension of *Rhizobium* host range to *Parasponia*.** Plasmid pMN40 was transferred to *R. l.* bv. *trifolii* strain ANU843 selecting for the $Tc^r$ marker of plasmid pSUP106. Strain ANU843(pMN40) could still nodulate clover, but its host range was now extended to include *Parasponia* and the tropical legume siratro (Table 2). All *Parasponia* plants grown in Magenta jars were nodulated with an average of three nodules per plant for strain ANU843(pMN40) and five per plant for strain NGR234. In contrast, when using the agar plate assay, strain ANU843(pMN40) did not nodulate *Parasponia*, and NGR234 nodulated only 10% of plants. *Parasponia* nodules formed by strain ANU843(pMN40) were similar in external appearance to the nodules formed by strain NGR234 (Fig. 2). Sections of ANU843(pMN40) nodules revealed a smaller infected zone with bacteria encased in thin-walled peribacteroid-like membranes called threads (Trinick 1979), as opposed to infection threads, which have thicker walls. The poor growth of *Parasponia* plants nodulated by either strain ANU843(pMN40) or strain NGR234 and the absence of acetylene reduction by nodules indicated that no nitrogen was fixed by resident bacteria.

All siratro plants were nodulated by either strain ANU843(pMN40) or NGR234 when using agar plate assays. Siratro nodules induced by ANU843(pMN40) were similar in external appearance to those formed by strain NGR234. However, there were no infection threads or plant cells occupied by bacteroids. It is interesting to note that strain ANU851 (a *nodD* mutant of strain ANU843) carrying pMN40 formed nodules that were infected with bacteroids (Fig 3). In comparison with nodules formed by NGR234, nodule development for ANU851(pMN40) was incomplete with a smaller infection zone, and the nodules produced did not fix nitrogen, unlike those of the parent strain NGR234. No difference was observed in the internal structure of *Parasponia* nodules formed by strains ANU843(pMN40) and ANU851(pMN40). Bacteria isolated from *Parasponia*, siratro, and clover nodules retained the appropriate antibiotic-resistance markers, and pMN40 DNA could be visualized in strains ANU843(pMN40) and ANU851(pMN40) by using a modified Echardt method

**Table 2.** Plant response to *R.l.* bv. *trifolii* strain ANU843 carrying the *nodD1* gene from strain NGR234[a]

| Plant spp. | NGR234 | ANU843 | ANU843(pMN40) | ANU851 | ANU851(pMN40) |
|---|---|---|---|---|---|
| *Trifolium repens* (white clover) | $Nod^-$ | $Nod^+Fix^+$ | $Nod^+Fix^+$ | $Nod^-$ | $Nod^+Fix^+$ |
| *T. subterraneum* (sub clover) | $Nod^-$ | $Nod^+Fix^+$ | $Nod^+Fix^+$ | $Nod^-$ | $Nod^+Fix^+$ |
| *Macroptilium atropurpureum* (siratro) | $Nod^+Fix^+$ | $Nod^-$ | $Nod^+Fix^-$ | $Nod^-$ | $Nod^+Fix^-$ |
| *Parasponia* | | | | | |
| Agar plates | $Nod^+Fix^-$ | $Nod^-$ | $Nod^-$ | $Nod^-$ | $Nod^-$ |
| Magenta jars | $Nod^+Fix^-$ | $Nod^-$ | $Nod^+Fix^-$ | $Nod^-$ | $Nod^+Fix^-$ |

[a]Strain NGR234 nodulated only 10% of *Parasponia* plants when using an agar plate assay, in contrast to 100% of plants when using Magenta jars. $Nod^+$, nodules formed on all inoculated plants; $Nod^-$, no plant response observed; $Fix^+$, the presence of nitrogen fixation as evidenced by an obvious benefit to plant growth, compared with controls and acetylene reduction by root nodules; $Fix^-$, poor plant growth, with pale green to yellow leaves and no detectable acetylene reduction. Two experimental repeats were used each, with 10 plants per strain. Agar plate assays only were used for clover and siratro.

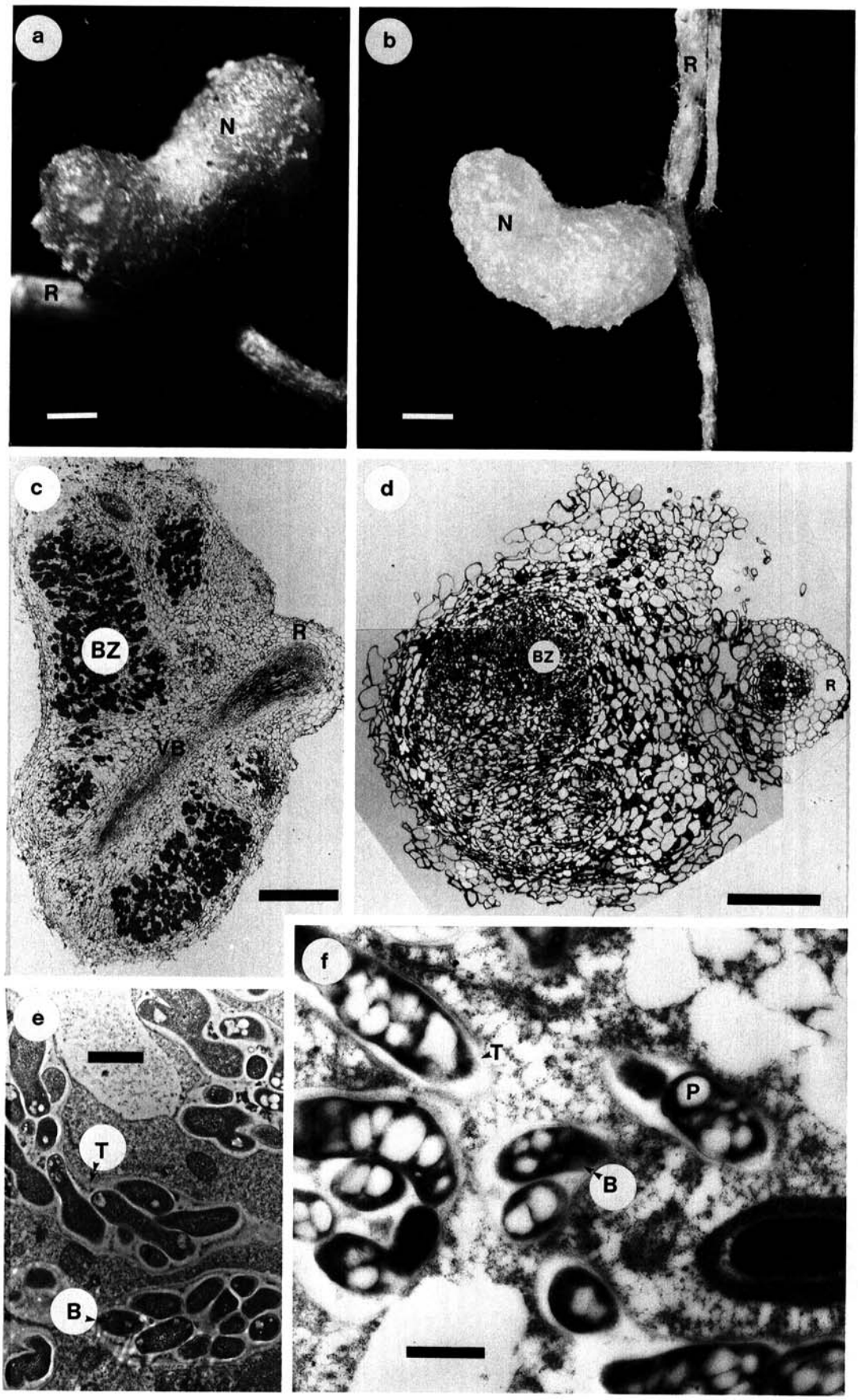


**Fig. 2.** Response by *Parasponia* to *R. l.* bv. *trifolii* strain ANU843 carrying the *nodD1* gene from strain NGR234. Nodules (N) formed by strains NGR234 (**a**) and ANU843(pMN40) (**b**) on *Parasponia* roots (R). Sections of *Parasponia* roots and attached nodules formed by strains NGR234 (**c**) and ANU843(pMN40) (**d**) showing zones of bacterial infection (BZ); VB, nodule vascular bundle; bars = 1 mm. TEM sections taken through the infection zones showing strain NGR234 (**e**; bar = 1 $\mu$m) and ANU843(pMN40) (**f**; bar = 0.5 $\mu$m) bacteria in the bacteroid (B) form surrounded by a thread (T) membrane; P, $\beta$-hydroxybutyrate granules.

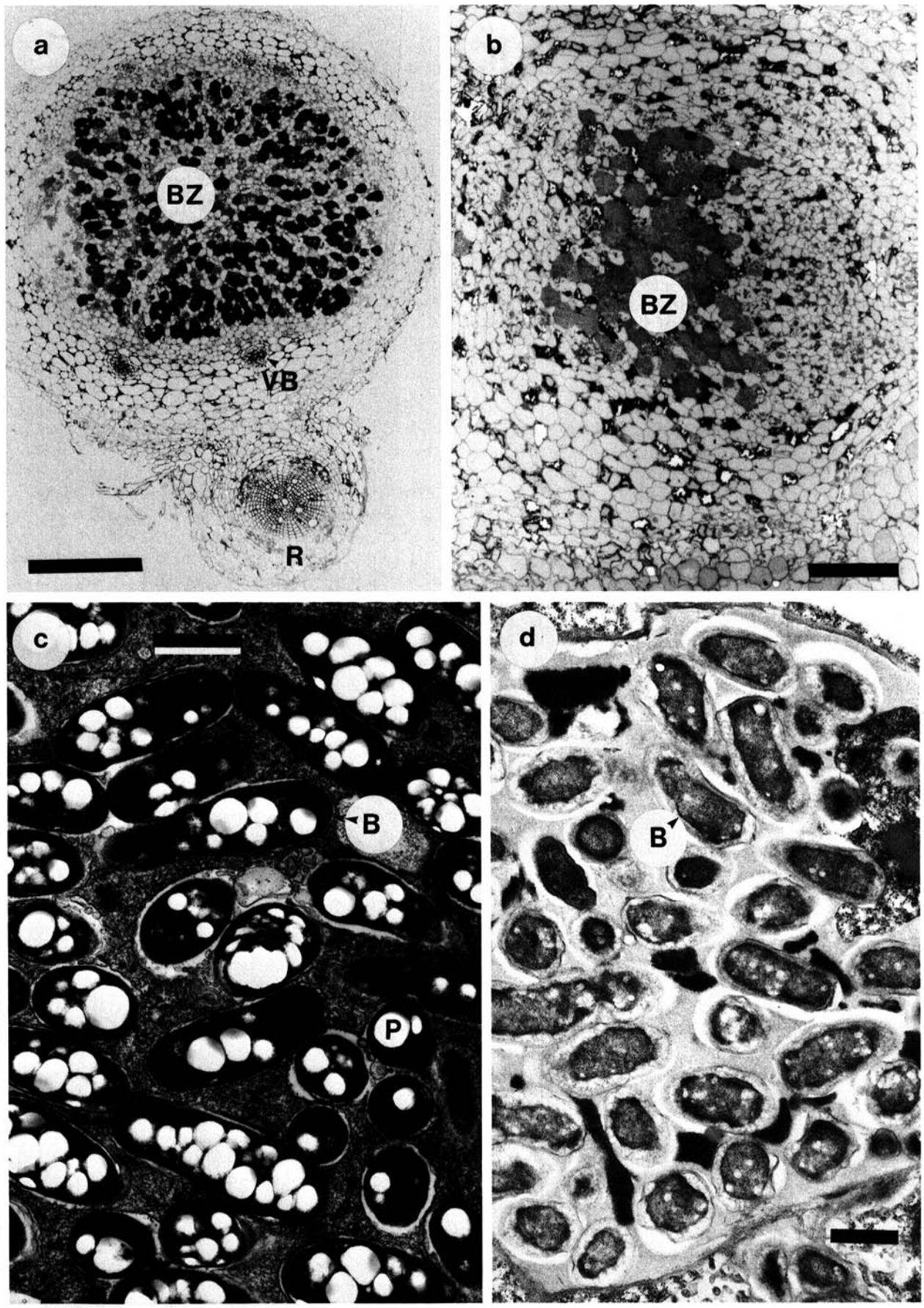


**Fig. 3.** Response by *Macroptilium atropurpureum* to *R. l.* bv. *trifolii* strain ANU851 carrying the *nodD1* gene from strain NGR234. Nodules formed by strains NGR234 (**a**; bar = 1 mm) and ANU851(pMN40) (**b**; bar = 0.5mm) showing zones of bacterial infection (BZ); VB, nodule vascular bundle; R, root. TEM micrographs of the infection zones showing bacteroids of strains NGR234 (**c**) and ANU851(pMN40) (**d**); B, bacteroid; P, β-hydroxybutyrate granule; bars = 0.5 μm.

(Plazinski *et al.* 1985a). When retested these bacteria could still nodulate the same hosts.

**Induction of *R. l.* bv. *trifolii* strain ANU843 nodulation genes by nonlegume extract.** Plasmid pMN40 was transferred into strain ANU894, which contains a translational fusion of the *E. coli lac* genes (Mu*d*II1734) with the *nodA* gene of the *nodABC* operon on the resident Sym plasmid of strain ANU843. This construction was used to assay the induction of the *nodABC* operon by purified plant signal compounds and extracts from seedlings of *Parasponia* and various other plants (Table 3). The addition of the NGR234 *nodD1* gene increased the range of extracts that could induce *nod* gene expression in strain ANU894. Extract from *Parasponia* induced activity, as did extract from siratro. This was expected because both of these plants are nodulated by strains NGR234 and ANU843(pMN40), but not by strain ANU843. A surprising result was that stimulatory substances were also found in extracts from plants nodulated by neither strain. Extract from seedlings of *Trema aspera*, a species found in the same family as *Parasponia* and known to contain plant hemoglobin genes (Landsmann *et al.* 1986; Bogusz *et al.* 1988), essential for a *Rhizobium* symbiosis, induced *nod* gene activity. Extracts from *C. cunninghamiana*, a nonlegume that forms a nitrogen-fixing root nodule symbiosis with the actinomycete *Frankia*, induced activity, as did extracts from sunflower and cotton and the cereals wheat, rice, and maize (Table 3). The presence of the *nodD1* gene from NGR234 also increased the range of purified flavonoid compounds that induced expression of the *nodABC* operon (Table 3). These compounds included umbelliferone, which is normally inhibitory to the induction of *nod* genes in strain ANU843 (Djordjevic *et al.* 1987). The expression of the *nodABC* operon was increased up to 40-fold by the presence of the NGR234 *nodD1* gene with the additional compounds tested. It appears that the NGR234 *nodD1* gene enables *nod* gene induction by plant signals in a nonspecific manner.

The induction of the *nodA*::Mu*d*II1734 fusion by *Parasponia* extract is consistent with the observation that the *nodD1* gene from strain NGR234 is also required for *Parasponia* nodulation by strain ANU843. Although the *nod* genes of ANU894 can be induced by signal compounds from nonlegumes other than *Parasponia*, the inoculation of *Trema*, *Casuarina*, sunflower, cotton, wheat, rice, and maize with strains NGR234, ANU843(pMN40), and ANU851(pMN40) did not produce nodules on these hosts.

**Table 3.** Response by *R. l.* bv. *trifolii* strain ANU894, carrying the *nodD1* gene from strain NGR234, to flavonoids and seedling extracts[a]

| | | β-galactosidase activity | |
|---|---|---|---|
| | **Inducer** | **ANU894** | **ANU894 (pMN40)** |
| None | | 22 | 24 |
| Compounds | | | |
| Flavones | 7,4′-dihydroxyflavone | 509 | 636 |
| | Luteolin | 123 | 470 |
| | Kaempferol | 14 | 490 |
| Flavanones | 7,4′-dihydroxyflavanone | 310 | 400 |
| | Naringenin | 122 | 95 |
| Isoflavones | Genistein | 10 | 440 |
| | Daidzein | 9 | 450 |
| Coumestan | Coumestrol | 9 | 450 |
| Coumarin | Umbelliferone | 10 | 140 |
| Dicotyledons | | | |
| | Siratro | 17 | 474 |
| | *Parasponia* | 25 | 410 |
| | *Trema* | 10 | 440 |
| | *Casuarina* | 26 | 140 |
| | Sunflower | 26 | 261 |
| | Cotton | 15 | 310 |
| Monocotyledons | | | |
| | Wheat | 5 | 371 |
| | Maize | 11 | 237 |
| | Rice | 34 | 134 |

[a]Values are in units of β-galactosidase activity (Miller 1972) following induction of a *nodA*::Mu*d*II1734 fusion in the symbiotic plasmid of *R. l.* bv. *trifolii* strain ANU843 (strain ANU894) carrying pMN40. A concentration of $1 \times 10^{-6}$ M was used for each compound, except for umbelliferone, which was used at $1 \times 10^{-5}$ M. Values shown are an average of two readings with four experimental repeats. Standard deviation for all values was less than 30%.

## DISCUSSION

It has been reported previously that transfer of the *nodD1* gene from *Rhizobium* strain NGR234 to *R. meliloti* (Horvath *et al.* 1987) and *R. l.* bv. *trifolii* (Bassam *et al.* 1988) extended the host range of the recipients to new legume hosts such as siratro. This paper extends these observations to a nonlegume. Specifically, the NGR234 *nodD1* gene enables *R. l.* bv. *trifolii* strain ANU843 to nodulate *Parasponia*, which has a rather different pathway of infection and nodule development to that of legumes (Lancelle and Torrey 1984, 1985; Bender *et al.* 1987a). This suggests that the different pathways of infection are largely due to a difference in plant response between legumes and *Parasponia* and not to major differences in the bacterial genome. It is possible that the induction of conserved nodulation genes, present in most *Rhizobium* species, is sufficient for the nodulation of this host. The NGR234 *nodD1* gene also enables strain ANU843 to respond to a broadened spectrum of purified flavonoid inducers and extracts from other nonlegumes. The induction by monocotyledons was unexpected because these plants are not only outside the known host range of *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium*, but are also outside the host range of the pathogenic member of the Rhizobiaceae, *Agrobacterium*. It is interesting to note that extracts from seedlings of wheat and oats can induce *vir* genes in *A. tumefaciens* (Usami *et al.* 1988).

The induction of *nod* genes in strain ANU894 by *Parasponia* extract was correlated with the ability of ANU843(pMN40) to nodulate *Parasponia*. However, the induction of *nod* genes by extracts from other nonlegumes was not correlated with nodule formation. Similarly, the induction of *vir* genes by wheat and oat extracts does not lead to crown gall formation (Usami *et al.* 1988). Previous work in our lab has shown that strain ANU845 (A Sym$^-$ derivative of ANU843), carrying *nodD*, *nodF*, and *nodABC* genes from ANU843 on a multicopy vector, was able to curl the root hairs of rice and maize plants (Plazinski *et al.* 1985b). In light of the present work, this is unexpected, because the *nodD* gene from ANU843 does not enable the induction of nodulation genes in the presence of extract from these plants. The response observed may be due to the high copy number of *nod* genes, because no effect was observed when a low copy number vector was used, or perhaps it is due to a cloning artifact. The resolution of this question is the focus of a series of experiments currently in progress.

Although the broad host range function of the *nodD1* gene is clearly evident in strain NGR234, the *nodD* genes from narrow host range strains are much more specific in action. Spaink *et al.* (1987) have shown that all three *nodD* genes from *R. meliloti*, *R. leguminosarum*, and *R. l.* bv. *trifolii* can interact with *Trifolium repens* (white clover) root exudate, but only the *R. l.* bv. *trifolii nodD* gene can respond to exudate from *T. pratense* (red clover). Transfer of the *R. l.* bv. *trifolii nodD* gene to *R. meliloti* and *R. leguminosarum* also enabled the induction of *nod*::*lacZ* fusions in these strains by red clover root exudate. Thus, the *nodD* gene from *R. l.* bv. *trifolii* determines red clover host specificity. Honma and Ausubel (1987) and Gyorgypal *et al.* (1988) have shown that broad host range in *R. meliloti* is determined by three copies of the *nodD* gene. The three *nodD* genes are allelic forms with divergent flavonoid specificity and activate nodulation genes in response to exudates from different hosts (Gyorgypal *et al.* 1988).

It is interesting to speculate that *Rhizobium* have evolved different strategies to nodulate a broader range of hosts. *R. meliloti* has acquired additional *nodD* alleles with divergent C-terminal regions (Gyorgypal *et al.* 1988) to interact with signals from different hosts, whereas strain NGR234 has retained a single functional *nodD* gene with alterations in the C-terminal region (Horvath *et al.* 1987) that enable nonspecific interaction. These considerations and our own observations support the conclusion by Spaink *et al.* (1987) and Horvath *et al.* (1987) that the *nodD* gene should no longer be recognized as a common *nod* gene but as a gene that plays a key role in determining host range. The NGR234 *nodD1* gene is unusual in that it does not determine host specificity but rather nonspecificity for host interaction.

*Bradyrhizobium* strains ANU289 and Rp501 have a broad host range similar to that of NGR234, and both can form nitrogen-fixing nodules on *Parasponia*. Mutation of the *nodD* gene adjacent to the *nodABC* operon in strain ANU289 (Scott *et al.* 1987) does not affect nodulation on either *Parasponia* or siratro, which suggests that this strain has another copy or copies of the *nodD* gene capable of interacting with legume and/or *Parasponia* plant signals. This assumption may be correct, as additional copies of the *nodD* gene have recently been located in strain ANU289 (K. Scott, personal communication). A *nodD* gene that has no apparent function in siratro or *Parasponia* nodulation is also found next to the *nodABC* operon in *Bradyrhizobium* strain Rp501 (Marvel *et al.* 1987).

The *nodD1* gene from strain NGR234, in the presence of extracts from a range of nonlegumes, did not enable nodulation of any nonlegume other than *Parasponia*. Similarly, interaction with alfalfa signal by the *nodD1* gene from strain NGR234 was not sufficient for alfalfa nodulation (Horvath *et al.* 1987). Only the transfer of *R. meliloti* host-specific nodulation genes could accomplish this (Horvath *et al.* 1986; Putnoky and Kondorosi 1986). Hence, the presence of a *nodD* gene capable of interacting with a signal from a particular legume host does not guarantee nodulation of that host. Nodulation may also require the presence of particular host-specific genes. The existence of *Parasponia*-specific DNA regions has been shown for strain NGR234 (Bender *et al.* 1987b) and *Bradyrhizobium* strains ANU289 (Scott *et al.* 1987) and Rp501 (Marvel *et al.* 1987). The NGR234 *nodD1* gene, when transferred to *R. l.* bv. *trifolii* strains ANU843 and ANU851, enabled nodules to form with a less well-developed structure than those of NGR234. This suggests that ANU843 lacks genes needed for *Parasponia* nodule development. Transfer of an 18-kb DNA fragment, containing a nonfunctional *nodD* gene, the *nodABC* operon, and a presumptive *Parasponia*-specific region from strain ANU289 to strain ANU843, extended the host range from clover to *Parasponia* but not siratro (Scott *et al.* 1987). Similarly, a Tn*5* mutation in a presumptive *Parasponia*-specific region adjacent to *nodC* in *Bradyrhizobium* strain Rp501 abolished *Parasponia* but not siratro nodulation (Marvel *et al.* 1987).

Strains NGR234, ANU843(pMN40), and ANU851 (pMN40) form *Parasponia* nodules that do not fix nitrogen. However, a mutant of strain NGR234 (ANU2895), which carries a specific chromosomal mutation affecting the regulation of acidic polysaccharide synthesis, can form nitrogen-fixing nodules on *Parasponia* plants (Chen 1987). These observations, and the ability of strain ANU843 (pMN40) to nodulate *Parasponia* in Magenta jars and not on agar plates, suggest that prolific nodulation of *Parasponia*, *Parasponia* nodule structure, and the ability to form nitrogen-fixing nodules on this host are dependent on genetic background. Genes other than a *nodD* gene that interacts with *Parasponia* plant signals, such as host-specific nodulation genes (Bender *et al.* 1987b), along with the appropriate exopolysaccharide genes and nodule development genes, are essential for the development of nodules that can supply *Parasponia* with fixed nitrogen.

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