



Universiteit
Leiden
The Netherlands

Identification and characterization of the *nodD* gene in *Rhizobium leguminosarum* strain 1001

Squartini, A.; Veen, R.J.M. van; Regensburg Tuink, A.J.G.; Hooykaas, P.J.J.; Nuti, M.P.

Citation

Squartini, A., Veen, R. J. M. van, Regensburg Tuink, A. J. G., Hooykaas, P. J. J., & Nuti, M. P. (1988). Identification and characterization of the *nodD* gene in *Rhizobium leguminosarum* strain 1001. *Molecular Plant-Microbe Interactions*, 1(3), 145-149. doi:10.1094/MPMI-1-145

Version: Publisher's Version

License: [Licensed under Article 25fa Copyright Act/Law \(Amendment Taverne\)](#)

Downloaded from: <https://hdl.handle.net/1887/3763927>

Note: To cite this publication please use the final published version (if applicable).

Identification and Characterization of the *nodD* Gene in *Rhizobium leguminosarum* strain 1001

Andrea Squartini,¹ Ron J. M. van Veen,² Tonny Regensburg-Tuink,² Paul J. J. Hooykaas,² and Marco P. Nuti¹

¹ Dipartimento di Biotecnologie Agrarie, Università di Padova, via Gradenigo 6, 35131 Padova, Italy, and ²Department of Plant Molecular Biology, University of Leiden, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

Received 30 November 1987. Accepted 21 March 1988.

A gene library of the symbiotic 240-kb plasmid of *Rhizobium leguminosarum* strain 1001 was constructed in pUC18. The clones showing homology with a 6.6-kb fragment containing *nodEFDABC* from the *Sym* plasmid pRLIJI were detected by colony hybridization. Additional probes from the symbiotic region of pRLIJI were used to localize the corresponding genes on the

map of pRle1001a. The relative positions of *nod* and *nif* gene clusters are different than those of pRLIJI. A comparison of the amino acid sequence for NodD from pRle1001a with NodD proteins from other *Rhizobium* species showed a high degree of sequence conservation at the amino terminus of the protein.

Additional keywords: Sym plasmids, nodulation genes, NodD protein, *Rhizobium leguminosarum*.

Most of the functions needed for the interaction of fast-growing *Rhizobium* with leguminous plants are encoded by large plasmids (Nuti *et al.* 1977) called *Sym* (symbiotic) plasmids (Hooykaas *et al.* 1981). Such plasmids share various degrees of homology among different species of *Rhizobium*. A set of genes, the *nod* (nodulation) genes, is responsible for the formation of nodules on the roots of the host plant. This process occurs by a highly specific series of recognition events, some of which are controlled by *nod* genes acting as host-specificity determinants.

Some of the *nod* genes, namely *nodDABC*, appear to be present in virtually all *Rhizobium* and were therefore referred to as common *nod* genes. Nevertheless, *nodD* seems to play a role in the host specificity as well (Spaink *et al.* 1987), indicating that, despite the overall conservation of this genetic unit, minor differences may have a significant effect in terms of inducibility by different plant compounds. Flavones and flavanones can in fact turn on some of the *nod* operons, a process that requires the *nodD* product, probably as a positive regulator (Rossen *et al.* 1985).

Probes for the common *nod* and *nif* genes can be used to screen gene libraries from different *Rhizobium* to find the corresponding *nod* and *nif* regions and compare the arrangement of symbiotic regions in different species and strains. Our effort was devoted to *Rhizobium leguminosarum* strain 1001. This strain, originally isolated from *Pisum sativum* nodules, carries a 240-kilobase pair (kbp) symbiotic plasmid, pRle1001a, also called pSym1 (Hooykaas *et al.* 1982). A circular restriction map of the whole plasmid was defined by Prakash *et al.* (1982), including the homology with other *Sym* plasmids, with the *Agrobacterium tumefaciens* Ti plasmids, and with the *nif* structural genes (Prakash *et al.* 1981).

The aims of this work were: to identify and clone the genetic determinants for nodulation of *Vicia* and *Pisum* in *R. leguminosarum* 1001; to compare the organization of genes in the symbiotic region with that found in pRLIJI, the source of the *nod* and *nif* probes; and to further characterize the *nodD* regulatory gene.

MATERIALS AND METHODS

Bacteria and culture conditions. The bacteria used throughout this study were *R. leguminosarum* strains RCR 1001 (Rothamsted Collection, U.K.) and LPR 1105 (1001 rif^r; P. Hooykaas, University of Leyden, The Netherlands), *Escherichia coli* strains 490 (PC 2480 Phabagen Collection, University of Utrecht, The Netherlands), HB 101 (Boyer and Roulland-Dussoix 1969), KA 817 *dam*⁻ *dcm*⁻ (P. van de Putte, University of Leyden), and JM 101 (Messing *et al.* 1981). *Rhizobium* was grown on yeast marmitol broth (Hooykaas *et al.* 1977) or tryptone-yeast extract medium (Beringer 1974), at 28° C. *E. coli* was grown on Luria-Bertani (LB) medium (10 g/L of Bacto-tryptone, 5 g/L of yeast extract, 5 g/L of NaCl, pH 7) at 37° C. Solidified media contained 18 g/L of agar.

Plasmids. The following plasmids were used: pIJ 1047 (5.5-kbp *Hind*III fragment, *fix* region of pRLIJI [Hombrecher *et al.* 1984], cloned in pBR322), pIJ 1238 (5-kbp *Hind*III fragment, 24-K region of pRLIJI [Hombrecher *et al.* 1984], cloned in pSUP 202), pIJ 1246 (6.6-kbp *Eco*RI fragment, *nod* region of pRLIJI [Hombrecher *et al.* 1984], cloned in pSUP 202), and pSA30 (7-kbp *Eco*RI fragment, *nif*HDK of *Klebsiella pneumoniae* [Cannon *et al.* 1979], cloned in pACYC 184). The large symbiotic plasmid of *Rhizobium* was isolated as described by Prakash *et al.* (1981). Plasmids from *E. coli* were isolated as described by Birnboim and Doly (1979).

DNA manipulation techniques. Restriction endonuclease digestions, DNA cloning, agarose gel electrophoresis, Southern blotting, and nick translation were carried out as described by Maniatis *et al.* (1982). DNA transformation of *E. coli* was performed by the procedure of Dagert and

Address correspondence to Marco P. Nuti.

Nucleotide and/or amino acid sequence data is to be submitted to GenBank as accession number J03671.

Ehrlich (1979). The 240-kbp symbiotic plasmid was digested with *Bam*HI, and the fragments were cloned into a linearized pUC18 (Norrander *et al.* 1983). *E. coli* 490 was transformed as described.

Southern hybridization. Filters were prehybridized in 5× SSPE (20× SSPE stock: 3.6 M NaCl; 0.2 M NaH₂PO₄; 0.02 M Na₂EDTA, pH 6.5), 5× Denhardt solution (0.02% polyvinylpyrrolidone; 0.02% Ficoll; 0.02% bovine serum albumin), 0.5 mg of denatured herring sperm DNA per milliliter, for 5 hr at 72° C; filters were subsequently hybridized, under stringent conditions, in the same solution containing in addition 0.3% sodium dodecyl sulfate (SDS), and the alkali-treated probe.

Filters were washed twice with 5 × SSPE, 0.1% SDS at 72° C for 30 min; twice with 0.5 × SSPE, 0.1% SDS at 72° C for 30 min; and once with 0.5 × SSPE at room temperature. Air-dried filters were wrapped in plastic, and an X-ray film was exposed at -70° C with an intensifying screen.

Colony hybridization. The *E. coli* recombinant clones were plated at a density of 1,000 colony-forming units per plate onto nitrocellulose filters (Schleicher & Schuell), previously laid on well-dried large LB plates supplemented with carbenicillin at 50 µg/ml. Plates were incubated overnight at 37° C. Two replica filters were obtained by touching the master filter, and colonies were allowed to grow for 8 hr on new plates. Colonies were lysed by leaving the filter, colony side up, on Whatman paper 3MM saturated with 0.5 M NaOH, 0.05% Triton X-100 for 10 min, and then on paper with 0.5 M NaOH, 1.5 M NaCl for 10 min. This treatment was followed by a neutralizing step on paper with 1 M Tris, pH 8.3, for 5 min and finally on paper with 0.5 M Tris, pH 8.5, 1.5 M NaCl for 15 min. Filters were air-dried and baked for 24 hr at 80° C in a vacuum oven. The hybridization conditions were the same as for Southern filters.

DNA sequencing. Fragments of interest were cloned in

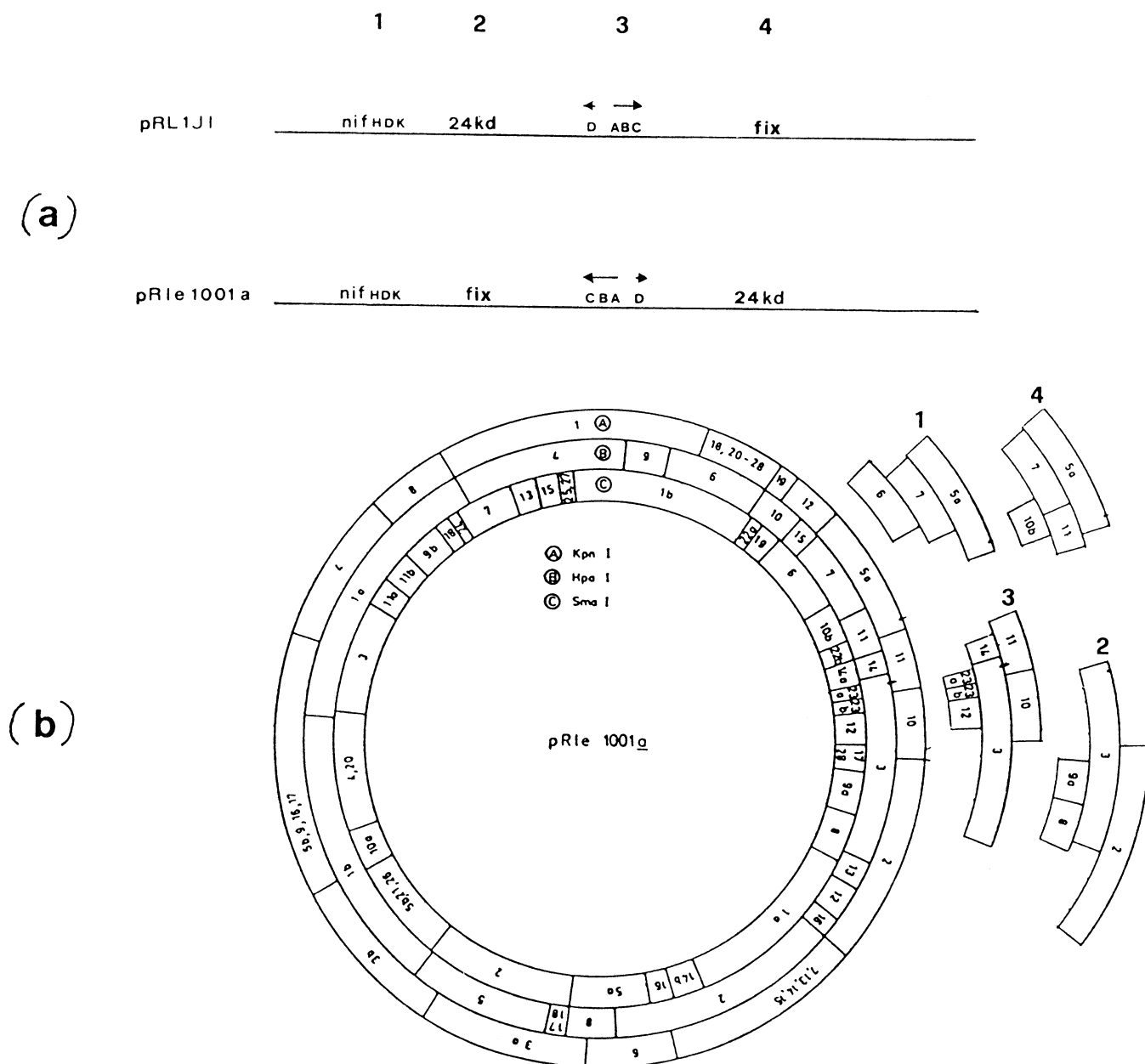


Fig. 1. (a), Location of the homology regions between pRL1J1 and pRle1001a. Relative order of the fragments used as probes in pRL1J1 (24 kDa indicates a gene for a 24-kDa protein), compared to that found on pRle1001a. **(b),** Restriction map of pRle1001a. The fragments hybridizing with the different probes are shown as excised clusters; numbers refer to the probes used.

M13 mp 18/19 (Norlander *et al.* 1983). The transfection of *E. coli* JM 101 and the preparation of template DNA were performed as described by Messing *et al.* (1981). Both strands were sequenced by the method of Sanger *et al.* (1977). Reagents were purchased from Amersham. ³⁵S-ATP was used as label at 8 μ Ci per group of four reactions; Klenow DNA-polymerase was from Boehringer. Samples were boiled for 3 min and run on a 0.4-mm thick polyacrylamide gel, in Tris-borate buffer at 35 mA/1,300 V. The gel was dried with a Bio-Rad gel dryer and Kodak X-0 mat film was exposed overnight at room temperature. Data were analyzed by means of the Beckman Microgenie program on an IBM personal computer.

RESULTS

Regions of pRle1001a are colinear with the *nod* segment from pRLJI. Two different clones were detected by colony hybridization with pIJ 1246 (6.6-kb *nod* fragment from pRLJI) as a probe in the *Bam*HI plasmid bank of pRle1001a (pSym1). These contained adjacent fragments, which partially overlapped the region containing the *Kpn*I fragments 10 and 11 shown on the map of pRle1001a (Fig. 1). A detailed restriction map of the cloned region aligned with that of pRLJI is given in Figure 2. The position of the *nod* genes in pRLJI is also shown. Many restriction sites in the *nod* regions of pRLJI and pSym1 turn out to be conserved, which underscores the close evolutionary relatedness of these regions.

The *nod* and *nif* regions of pRle1001a and pRLJI contain homologous sequences but are arranged in a different order. Four different ³²P-labeled probes from the plasmid pRLJI were hybridized to Southern blots of pRle1001a digested with the enzymes *Bam*HI, *Bgl*II, *Eco*RI, *Hind*III, *Hpa*I, *Kpn*I, *Pst*I, *Sma*I, *Sst*I, and *Xba*I. The following probes were used: pSA30 (*nif*HDK of *K. pneumoniae*); pIJ 1238 containing the gene for a 24-kDa protein close to the *nif* structural genes in pRLJI; pIJ 1246 with *nod*FEDABC of

pRLJI; and pIJ 1047 with the pRLJI *fix* region. The relative positions of these gene clusters on pRLJI (Hombrecher *et al.* 1984) are shown in the upper part of Figure 1. The regions of pRle1001a hybridizing with the probes are also shown in Figure 1. Using the *nif*HDK as a reference point, the locations of the other clusters in pRle1001a are different than those of the corresponding clusters in pRLJI. The *fix* genes map next to the *nif* structural genes on pRle1001a, whereas the 24-kDa protein gene is distal to the *nod* area. The analyses of the cloned *nod* region showed that the order of the *nod* genes is the same, but their orientations are in opposite directions toward the structural *nif* genes.

Nucleotide sequencing of *nodD* from pRle1001a. The sequence of a 1,439-bp stretch containing a 969-bp open reading frame (ORF) is shown in Figure 3. The sequencing strategy is shown in Figure 2. The ORF starts at position 406 and stops at position 1,374. The consensus for a *nod* box is boxed. The nucleotide sequence of the ORF shows a high degree of homology with that of previously published *nodD* genes.

Inverted repeat sequences of 10 bp map immediately downstream from the end of ORF and are separated by 10 bp. A consensus amino acid sequence for DNA binding proteins, as described by Shearman *et al.* (1986), maps at the N terminus of the gene and is shown in brackets in Figure 3. By comparing the nucleotide sequence with a data bank, a homology of 94.7% was found between a 19-bp stretch spanning position 55–73 and the reverse strand of a region flanking the T-DNA of the *A. tumefaciens* octopine Ti plasmid pTi15955 (Barker *et al.* 1983; position 23,932–23,950).

Computer-assisted NodD protein sequence analysis. The amino acid sequence deduced from DNA sequence was compared to that of four other *nodD* genes. The protein alignment is shown in Figure 4. The homology at protein level is 90.7% with NodD from *R. leguminosarum* (pRLJI) (Shearman *et al.* 1986), 74.7% with *R. meliloti* 1021

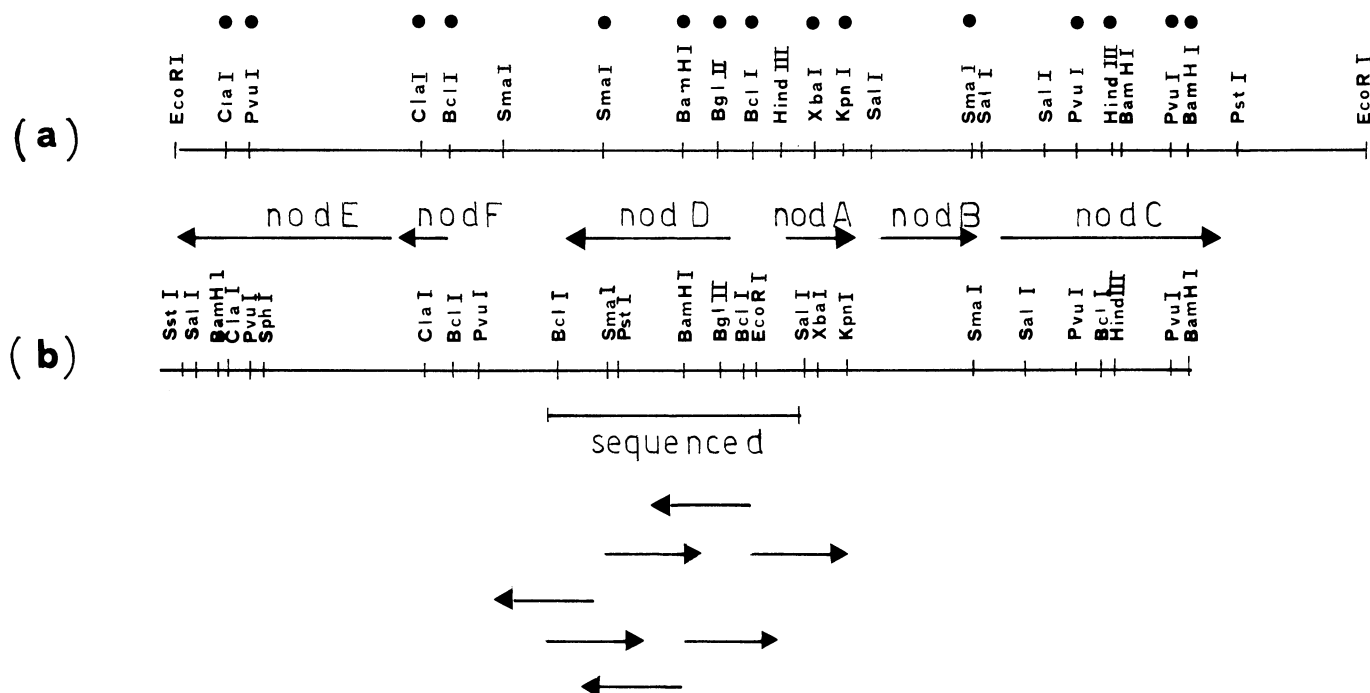


Fig. 2. (a), 6.6-kilobase pair *Eco*RI fragment from the *nod* region of pRLJI used as a probe. The restriction sites conserved in pRle1001a are dotted. (b), Cloned region of pRle1001a. Arrows indicate the sequencing strategy.

(Egelhoff *et al.* 1985), 72.3% with NodD from *R. trifolii* ANU843 (Schofield and Watson 1986), and 64.8% with NodD from *Bradyrhizobium parasponiae* (Scott 1986). A computer prediction of the secondary structure by the algorithm of Garnier *et al.* (1978) for our NodD is given in Figure 4.

DISCUSSION

Homology between pRle1001a and pRLJI. As expected, these two *R. leguminosarum* plasmids share sufficient homology to allow the identification and cloning of corresponding regions. However, there are some noteworthy differences. Although the nucleotide sequence

of the *nodD* genes is greater than 90% conserved, there are significant differences nevertheless in the restriction map of the regions examined (see Fig. 2). Furthermore, the order of the gene clusters is different, as shown in Figure 1. This suggests that a large fragment encoding the *fix*, the *nod* genes, and the gene for the 24-kDa protein has undergone an inversion event.

Nucleotide sequence. The putative start site of a second ORF (strongly homologous to *nodA*) on the complementary strand is shown in Figure 3. This start site for *nodA* was chosen by comparing the *nodA* sequence from two *R. leguminosarum* strains with that from an *R. meliloti* strain. The inverted complementary sequence found downstream of *nodD* is a potential transcriptional terminator. A 19-bp sequence at the N terminus of *nodA* is also found on the pTi15955 from *A. tumefaciens*. Its location is 49 bp to the right of the right border of the T_R-DNA. The significance of this homology, if any, is unknown.

NodD protein sequence analysis. The higher degree of amino acid sequence conservation is at the amino terminus of the gene. A sequence comparison of five genes indicates a strong consensus at the first 40 amino acid residues (Fig. 4).

CCAGCTTCGC	CCAGTCTCGA	ACGGCTGGC	ATTGAAAGCG	TGCGCGTCCG	GCCCATAGGG	59
TCCTTCAAAA	AAACCTCAGC	GAGTTCCGCG	TGGTCTGAGG	CTTCCAGCTC	ATTTTCCGAG	
CATATTTTCC	ATCCGACTTG	AGAAGACATG	CAAAAGCTCC	AACCTGTTTC	CTTTCCAACG	179
CTGCCACGTT	CAAAAGTGGC	CSTGATTGTT	TGGCATCTAA	TGGATTGCCC	CTTTGACAAT	
TCGCGCGGTT	AAGAGAATTA	AGAATTCAAA	TTGGGCGCTT	GGCAATTTC	CTTTTTCGTT	299
CCGCGCGCTT	GATATTGATC	ATGTTCCGCG	TTTCTCTATA	GCGATCCGAA	AGATTTGCTAA	
AATGATTGTT	TGGAATGGCA	ATCATCTATG	GAATGGATAT	AAACTT ATG	CGT TTT	414
				Met	Arg Phe	
AAA GGC CTA GAT CTT AAT CTT CTT GTA GCG CTC GAC CGT CTA ATG						
Lys Gly Leu Asp Leu Asn Leu Leu Val Ala Leu Asp Arg Leu Met						
ACC GAG CGC AAG CTG ACA GCA GCG GCA CGA GCC ATC AAC CTC AGT						504
Thr Glu Arg Lys Leu Thr Ala Ala Ala Arg Ala Ile Asn Leu Ser						
CAA CCG GCG ATG AGC GCT GCC ATC TCT AGG TGG CGC GAC TAT TTC						
Gln Pro Ala Met Ser Ala Ala Ile Ser Arg Trp Arg Asp Tyr Phe						
CGC GAC GAC CTT TTT ATC ATC CAG AGA GCG GAG CTA AAT CCG ACC						594
Arg Asp Asp Leu Phe Ile Ile Gln Arg Arg Glu Leu Asn Pro Thr						
CCG GCT GCA GAG CCA CTT GCC CCC GTC GTG CGC GAG GGC CTG CTG						
Pro Ala Ala Glu Pro Leu Ala Pro Val Val Arg Glu Ala Leu Leu						
CAT ATT CAG CTT TTC GTC ATC GCA TGG GAT CCA ATA AAC CTT GCG						684
His Ile Gln Leu Ser Val Ile Ala Trp Asp Pro Ile Asn Pro Ala						
GAG TTC GAC CGC CGA TTC AGA ATT ATC CTT TGA GAT TTC ATG GCC						
Glu Phe Asp Arg Arg Phe Arg Ile Ile Leu Ser Asp Phe Met Ala						
TTG CTC TTC TTC GAA ABA ATC ATA GTG CGC TTA GCT CGG GAG GCG						774
Leu Val Phe Phe Glu Lys Ile Val Arg Leu Ala Arg Glu Ala						
CCA GGG GTC AGC TTC AAG TTG CTG CCA CTT GAC GAC GAT CCC GAG						
Pro Gly Val Ser Phe Lys Leu Leu Pro Leu Asp Asp Asp Pro Glu						
GAG CTT CTC CGC CGT GGG GAT GTT GAT TTT CTG ATC CTA CCC GAT						864
Glu Leu Leu Arg Arg Gly Asp Val Asp Leu Ile Leu Pro Asp						
CTA TTC ATG TCT GGC GCC CAT CGC AAG GCA AGG CTT TTC GAA GAG						
Leu Phe Met Ser Gly Ala His Arg Lys Ala Arg Leu Phe Glu Glu						
AGA CTG GTG TGC GTC GGC TGC TCC ACC AAG GAG CAG TTG CAA GGG						954
Arg Leu Val Cys Val Gly Cys Ser Thr Asn Glu Gln Leu Gly						
AAG CTC TTC CTG GAG CAA TAT ATG TCC ATG GGA CAT GTT GCG GCT						
Lys Leu Phe Leu Glu Gln Tyr Met Ser Met Gly His Val Ala Ala						
AAG TTC GGA CGT GGT CTT AAG CCT TCC GGT GAG CAA TGG TTA TTG						1044
Leu Phe Gly Arg Gly Leu Lys Pro Ser Val Glu Gln Trp Leu Leu						
CTG CAG CAA GGT CTT AAG AGG GGT ATT GAA CTC GTC GTC CCG GGG						
Leu Gln Gln Gly Leu Lys Arg Arg Ile Glu Leu Val Val Pro Gly						
TTT AAG TTG ATC CCG CTG CTG TCA GGC ACT AAT CCA ATA GCA						1134
Phe Asn Leu Ile Pro Pro Leu Ser Gly Thr Asn Arg Ile Ala						
ACC ATC CCC CTG GCG CTG GTC AAA CAT TAC GAA CAA ACT ATC CCC						
Thr Ile Pro Leu Arg Leu Val Lys His Tyr Glu Gln Thr Ile Pro						
CTG CCG ATT ATT GAG CAT CCA CTT CTT TCG TTC ACT GAG						1224
Leu Arg Ile Ile Glu His Pro Leu Leu Ser Phe Thr Glu						
GCT GTC CAA TGG CCG GCT CTT CAC AAG TCT GAT CCT GGA AAC ATA						
Ala Val Gln Trp Pro Ala Leu His Asn Ser Asp Pro Gly Asn Ile						
TGG ATG CCG GAG ATT ATG ATC CAA GAG GCT TCG CCG CAT TGG AAT						1314
Trp Met Arg Glu Ile Met Ile Gln Glu Ala Ser Arg His Trp Asn						
CCG AGG CCG AAA CTT GTA CGT CTT AAG CGA CCG CGG TCA TTT CAC						
Pro Arg Pro Lys Val Val Arg Leu Lys Arg Pro Arg Ser Phe His						
AGC CCG AGT AGT TAG ACGGGCGGTGATCATTCGACCTATGCGCGTCCAACGA						1415
Ser Arg Ser END						

GGCATCACTTACAGGACGGCC

Fig. 3. Nucleotide sequence of the *nodD* gene of pRle1001a. The conserved nucleotides between this plasmid and pRLJI are underlined; those conserved in the *Rhizobium meliloti* 1021 megaplasmid are overlined. Asterisks represent the conserved amino acids between pRle1001a and pRLJI. The boxes indicate the consensus for the *nod* box. The dot (.) indicates the presumed start of *nodA* on the complementary strand. The broken line from position 55 to 73 overlies the region conserved in pTi15955. A consensus amino acid sequence for DNA-binding proteins is shown in brackets.

pRle1001a	MRFKGLDLNL	LVALDRLMTE	RKLTAAARAI	NLSQPMASAA	40
pRLI1	MRFKGLDLNL	LVALDALMTE	RKLTAAARSI	NLSQPMASAA	
f.ANU 843	MRFKGLDLNL	LVALDALMTE	RKLTAAARSI	NLSQPMASAA	
.1021	MRFKGLDLNL	LVALDALMTE	RKLTAAARSI	NLSQPMASAA	
asp.ANU 289	MRFKDLNL	LVALDALMTE	RNLTAARKI	NLSQPMASAA	
ensus	mrf--ldlnl	lvald-lmte	r-ltaaar-i	nlsqpmasaa	

ISRWRDYFRD	DLFIIQRREL	NPTPAAEPLA	PVVRALLHI	QLSVIADWPI	90
ISRLRDYFRD	DLFIMQRREL	VPTPRAEALA	PAVREALLHI	QLSVIADWPI	
IGRLRAYFND	ELFLMQRRRL	VPTPRAEALA	PAVREALLHI	QLSVIADWPI	
IARLRTYFGD	ELFSMQGREL	IPTPRAEALA	PAVRDALLHI	QLSVIADWPI	
IARLRSYFRD	ELFTMRGREL	VLTPGAEALA	GPVREALLHI	QLSIISRDAF	
i-r-r-yf-d	-lf-----r-l	--tp-ae-la	--vr-allhi	qls-i----	

NPAEFDRRFR	IILSDFMALV	FFEKIIIVRLA	REAPGVSKFL	LPLDDDPPEL	140
NPAESDRRFR	IILSDFMALV	FFDKIILRLA	REAPGVSKFL	LPLDDDPPEL	
VPAESDRRFR	IVLSDFMTLV	FFEKIVIKRVA	REAPGVSKFL	LHVNDPDER	
NPAQSDRRFR	IILSDFMILV	FFARIIVERVA	REAPGVSKFL	LPLDDDPHEL	
DPAQSSRRFR	VILSDFMTIV	FFRRIVDRIA	QEAPAVRFEL	LPFSDEPSEL	
-pa---rrfr	--lsdfm--v	ff-----r-a	-eap-v-f-l	l---d-p-e-	

LRRGDVDFLI	LPDLFMSGAH	RKARLFEERL	VVVGCSSTNEQ	LQKGLFLEQY	190
LRRGDVDFLI	LPDLFMSGAH	PRARLFEERL	VCVGPTNEQ	LPQGLSLEQY	
LRSGDLDFLI	LPDQFMGATH	PSAKLFEDKL	VCVGCPNSQK	LRGKLSKFRF	
LRRGDVDFLI	FPDVFMSGAH	PKAKLFDEAL	VCVGCPNTKK	LGNIISFETY	
LRRGEVDFLI	LPDLFMSGAH	PKATLFDTEL	VCVGCPNTKK	LSRPLTFEY	
lr-g--dfli	-p--fms--h	--a-lf--l	v-gc--n--	l-----	

MSMGHVAAKF	GRGLKPSVEQ	WLLMQGLKLR	RIELVVPGFN	LIPPLLSGIN	240
MSMGHVAAKF	GRGLKPSVEQ	WLLMQHGLKLR	RIELVVPGFN	LIPPLLSGTN	
MSMGHVAAMF	GRTLKPSIEQ	WLLLEHGFKR	REIVVVPGFN	SIPMLLQGTN	
MSMGHVAAGF	GREMKPSVEQ	WLLLEHGFNR	RIELVVPGFN	LIPPLLSGTN	
NSMGHVTAGF	GRALRPNLEE	WFLLEHGLKLR	RIEIVVVGFS	LIPPLLGTS	
-smghv-a-f	gr---p-e-	w-l--g--r	r-e-vv-gf-	-ip-l-l-g--	

RIATIPRLV	KHYEQTIPLR	IIHPLPLLS	FTEAVQWPAL	HNSDPGNIWM	290
RIATIPRLV	KHYERTIPLR	IIHPLPLVS	FTEAVQWPAL	HNTDPGNIWM	
RIATLPLLV	RHFETIPLQ	IVHPLPLLS	FTEALQWPLL	HNSDPGNIWM	
RIATLPLRV	KYEQTIPLR	IVTSPPLPLF	FTEAIQWPAI	HNTDPGNIWM	
RIGTMPLRLA	RHFETKMPQL	IVEPPLPLPT	FTEAVQWPAF	HNTQPASIWM	
ri-t-pl-l-	---e---pl-	---plp---	f-tea-qwp--	hn--p--lw-	

REIMIQEASR	HWNPRPKVVR	LKRPRSFHSR	SS		322
REIMIQEALR	RIGI				304
RNIILEEASR	IETSSERCSQ	EPRATQSW			318
REILLQEASR	IDPQSDTCTL	PRALTCMLIK	NSSLFSGAA		329
RRIILEEASN	MASEHREPT	PORALDSRPR	RCKNIIINHS	TAWPASSF	338

Fig. 4. Protein alignment of different NodDs. The computer prediction for the secondary structure is indicated above the sequence. Solid line (—) represents α -helix structures, broken line (---) represents β -sheet, and dotted line (....) indicates the reverse turn, as described by Garnier *et al.* (1978).

According to the computer prediction, the secondary structure of the 1-40 amino acid region is an α -helix.

The predicted secondary structures of five NodD proteins are shown in Figure 4. These show that the unconserved regions are in most cases characterized as reverse turn.

The border regions between an α -helix and a β -sheet domain (e.g., from residue 75 to 89, 120 to 128, and 170 to 175) are generally better conserved than other parts of the sequence. The highest degree of NodD amino acid conservation is between *R. leguminosarum* and *R. trifolii* proteins. The *B. parasponiae* sequence is the most divergent and is more closely related to the *R. meliloti* protein than that of the two *R. leguminosarum* strains. The region from residue 157 to 170 is predicted as an α -helix and is quite variable in all the species.

The amino acid sequence between residues 271 and 290 is relatively well conserved and precedes the only part of the protein that does not show any consensus. From residue 290 up to the carboxy terminus end, the various NodD are completely unrelated and vary in length. Even the two Nod proteins from *R. leguminosarum* differ in length by 18 residues. The predominant secondary structure predicted for this end is reverse turn.

It has now been shown (Spaink *et al.* 1987) that the *nodD* gene plays an important role in host specificity. The predicted secondary structures of five *nodD* proteins that we have described could provide a useful guide for structural and functional analyses of these proteins.

ACKNOWLEDGMENTS

We thank John Allan Downie for providing the probes and Frank Cannon for critically reading the manuscript. Mark Van Haaren, Leo Melchers, Amke den Dulk-Ras, and Norbert Sedee are gratefully acknowledged for discussion and cooperation. We thank Lidia Zaltron for typing the manuscript.

This research was supported by CNR (Italy), Special Grant I.P.R.A., paper 1,612.

An account of this work was presented at the third International Symposium on Plant-Microbe Interactions, Montreal, Quebec, July-August 1986.

LITERATURE CITATIONS

- Barker, R. F., Idler, K. B., Thompson, D. V., and Kemp, J. D. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi 15955. *Plant Mol. Biol.* 2:335-350.
- Beringer, J. E. 1974. R-factor transfer in *Rhizobium leguminosarum*. *J. Gen. Microbiol.* 84:188-198.
- Birnboim, H. C., and Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res.* 7:1513-1523.
- Boyer, H. W., and Roulland-Dussoix, D. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* 41:459-472.
- Cannon, F. C., Riedel, G. E., and Ausubel, F. M. 1979. Overlapping sequences of *Klebsiella pneumoniae nif* DNA cloned and characterized. *Mol. Gen. Genet.* 174:59-66.
- Dagert, M., and Ehrlich, S. D. 1979. Prolonged incubation in calcium chloride improves the competence of *E. coli* cells. *Gene* 6:23-28.
- Egelhoff, T. T., Fisher, R. F., Jacobs, T. W., Mulligan, T. T., and Long, R. S. 1985. Nucleotide sequence of *Rhizobium meliloti* 1021 *nod*-genes; *nodD* is read divergently from *nodABC*. *DNA* 4:241-248.
- Garnier, J., Osguthorpe, D. J., and Robson, B. 1978. Analysis of the accuracy and implications of single methods for predicting the secondary structure of globular proteins. *J. Mol. Biol.* 120:97-120.
- Hombrecher, G., Gotz, R., Dibb, N. J., Downie, J. A., Johnston, A. W. B., and Brewin, N. J. 1984. Cloning and mutagenesis of nodulation genes from *Rhizobium leguminosarum* TOM, a strain with an extended host range. *Mol. Gen. Genet.* 194:293-298.
- Hooykaas, P. J. J., Klapwijk, P. M., Nuti, M. P., Schilperoort, R. A., and Rorsch, A. 1977. Transfer of the *Agrobacterium tumefaciens* Ti plasmid to avirulent agrobacteria and to *Rhizobium explanta*. *J. Gen. Microbiol.* 98:477-484.
- Hooykaas, P. J. J., Van Brussel, A. A. N., den Dulk-Ras, A., Van Slogteren, G. M. S., and Schilperoort, R. A. 1981. *Sym* plasmids of *Rhizobium trifolii* expressed in different rhizobial species and in *Agrobacterium tumefaciens*. *Nature* 291:351-352.
- Hooykaas, P. J. J., Snijdwint, F. G. M., and Schilperoort, R. A. 1982. Identification of the *Sym* plasmid of *Rhizobium leguminosarum* strain 1001 and its transfer to and expression in other rhizobia and *Agrobacterium tumefaciens*. *Plasmid* 8:73-82.
- Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Messing, J., Crea, R., and Seeburg, P. M. 1981. A system for shotgun DNA sequencing. *Nucleic Acid Res.* 9:309-321.
- Norlander, J., Kempe, T., and Messing, J. 1983. Improved M13 vectors using oligonucleotide-directed mutagenesis. *Gene* 26:101-106.
- Nuti, M. P., Ledebøer, A. M., Lepidi, A. A., and Schilperoort, R. A. 1977. Large plasmid in different *Rhizobium* species. *J. Gen. Microbiol.* 100:241-249.
- Prakash, R. K., Schilperoort, R. A., and Nuti, M. P. 1981. The large plasmids of fast-growing rhizobia: Homology studies and location of structural nitrogen fixation (*nif*) genes. *J. Bacteriol.* 145:1129-1136.
- Prakash, R. K., Van Veen, R. J. M., and Schilperoort, R. A. 1982. Restriction endonuclease mapping of a *Rhizobium leguminosarum sym* plasmid. *Plasmid* 7:271-280.
- Rossen, L., Shearman, C. A., Johnston, A. W. B., and Downie, J. A. 1985. The *nodD* gene of *Rhizobium leguminosarum* is autoregulatory and in the presence of plant exudate induces the *nodA,B,C* genes. *EMBO J.* 4:3369-3373.
- Sanger, F. C., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463-5467.
- Schofield, P. R., and Watson, J. M. 1986. DNA sequence of *Rhizobium trifolii* nodulation genes reveals a reiterated and potentially regulatory sequence preceding *nodABC* and *nodFE*. *Nucleic Acid Res.* 14:2891-2903.
- Scott, K. 1986. Conserved nodulation genes from the non-legume symbiont *Bradyrhizobium* sp. (*Parasponia*). *Nucleic Acid Res.* 14:2905-2919.
- Shearman, C. C., Rossen, L., Johnston, A. W. B., and Downie, J. A. 1986. The *Rhizobium leguminosarum* nodulation gene *nodF* encodes a polypeptide similar to acyl-carrier protein and is regulated by *nodD* plus a factor in pea root exudate. *EMBO J.* 5:647-652.
- Spaink, M. P., Wijffelman, C. A., Pees, E., Okker, R. J. M., and Lugtenberg, B. J. J. 1987. *Rhizobium* nodulation gene *nodD* as a determinant of host specificity. *Nature* 328:337-339.