

Three Replicons of *Rhizobium* sp. Strain NGR234 Harbor Symbiotic Gene Sequences

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***Rhizobium* sp. strain NGR234 contains three replicons: the symbiotic plasmid or pNGR234a, a megaplasmid (pNGR234b), and the chromosome. Symbiotic gene sequences not present in pNGR234a were analyzed by hybridization. DNA sequences homologous to the genes *fixLJKNOPQGHIS* were found on the chromosome, while sequences homologous to *nodPQ* and *exoBDFLK* were found on pNGR234b.**

Bacteria belonging to the genus *Rhizobium* and related genera elicit the formation of nitrogen-fixing nodules on leguminous plants. Usually the genetic information in these soil bacteria is partitioned between the chromosome and large plasmids. In different *Rhizobium* species, most nodulation (*nod*) and nitrogen fixation (*nif* and *fix*) genes are present on one plasmid, known as the symbiotic plasmid (pSym). In contrast, in *Bradyrhizobium*, *Azorhizobium*, and *Mesorhizobium* species, symbiotic information seems to be present on the chromosome.

Rhizobium sp. strain NGR234 (18) is of particular biological interest since it possesses the broadest host range of any strain so far analyzed. It is able to nodulate legumes from more than 110 genera as well as the nonlegume *Parasponia andersonii* (15a). This, together with the complete nucleotide sequence of the 536,165-bp symbiotic plasmid (6), makes this strain an ideal reference in studies aimed at understanding the molecular basis of symbioses between *Rhizobium* and legumes.

There has been controversy as to whether NGR234 contains another plasmid in addition to pSym (4, 12, 14). In the present study we obtained plasmid profiles of *Rhizobium* strains by using a modified Eckhardt procedure (5) as described by Hynes and McGregor (9). As a control we used the well-characterized strain *R. meliloti* 1021 (Fig. 1, lane 1), which has two megaplasmids of 1,400 and 1,700 kb (8). In comparison, NGR234 (Fig. 1, lane 2) possesses the 536-kb pSym (pNGR234a) and a megaplasmid (pNGR234b) which is larger than those of *R. meliloti*. Strain ANU265 (12), a pSym cured derivative of NGR234, also contains pNGR234b (Fig. 1, lane 3).

The original isolate, from which the strain used in the present work, NGR234, was derived, was obtained by M. J. Trinick in Papua New Guinea from the leguminous plant *Lablab purpureus*. We analyzed freeze-dried cultures of the strain dated 1965 and 1971; both cultures gave the same plasmid profile (Fig. 1, lanes 4 and 5, respectively) as that of NGR234.

Most of the known nodulation and nitrogen fixation genes as well as other potential symbiotic loci were located in the nucleotide sequence of pNGR234a (6). Nevertheless, several

genes which play a role in symbiotic nitrogen fixation in other *Rhizobium* species were not found in pNGR234a. These include *fixLJ* (3) and *fixK* (2), whose products participate in the regulation of symbiotic nitrogen fixation; *fixNOQP* (15), which encode a microaerobically induced cytochrome oxidase complex; *fixGHIS*, which probably encode a membrane-bound complex that includes a cation pump involved in nitrogen fixation (10); *nodPQ* (17), which encode enzymes which synthesize the precursors necessary for the sulfation of Nod factors; and several genes that participate in the synthesis of exopolysaccharides.

To localize specific DNA sequences in the genome of NGR234, the different replicons were separated on Eckhardt-type gels and transferred to nylon membranes which were hybridized against various probes. As markers, we used an RNA gene (rDNA) probe for the chromosome and a nitrogenase gene probe for the pSym. Intragenic probes for each gene of *fixLJKNOPQGHIS* and for *nodP* and *nodQ* were obtained as PCR products from *R. meliloti*; a DNA fragment of NGR234 containing the *exoBDFKL* genes was also used as a probe. Examples of the hybridization of different probes are presented in Fig. 1 (lanes 6 to 13). The results indicate that the different *fix* gene sequences analyzed are present in the chromosome while *nodPQ* and *exoBDFKL* gene sequences are present on the megaplasmid. These results were corroborated as follows: DNA from each of the two plasmids, pNGR234a and pNGR234b, was isolated from Eckhardt gel bands; preparations of total DNA and of DNA of each of the plasmids were digested with *Bam*HI; and Southern blots of the three preparations were hybridized against the different probes. As expected, the total DNA hybridized with all the probes; the megaplasmid DNA hybridized only with the *nodP*, *nodQ*, and *exoBDFKL* probes; and the pSym DNA hybridized only with the nitrogenase gene probe (not shown).

Here we show that symbiotic genetic information in NGR234 is partitioned among three replicons. One, which contains ribosomal gene sequences, corresponds to the chromosome; another corresponds to the well-characterized symbiotic plasmid; and the other is a megaplasmid with a very high molecular mass. Megaplasmids (>1,000 kb) have been observed in several *Rhizobium* species, such as *R. meliloti* (1, 8, 16), *R. fredii* (11), *R. galegae* (13, 19), and *R. tropici* (7). Such replicons have been classified as plasmids due to the absence of

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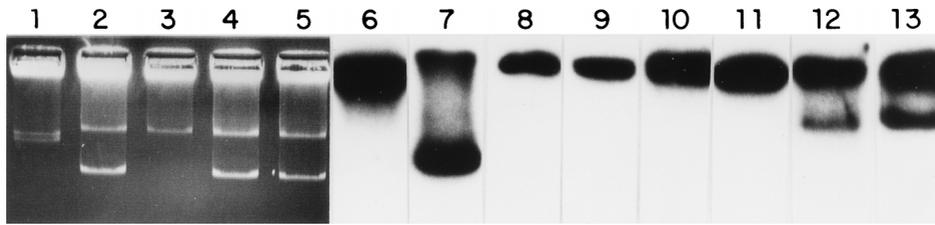


FIG. 1. Plasmid profile of *Rhizobium* strains and localization of specific gene sequences. Lanes 1 to 5, ethidium bromide-stained Eckhardt-type gels containing different strains: *R. meliloti* 1021 (lane 1), *Rhizobium* sp. strain NGR234 (lane 2), ANU265 (lane 3), and cultures of NGR234 originating from 1965 and 1971 (lanes 4 and 5, respectively) (see text). Lanes 6 to 13, autoradiographs of blotted Eckhardt-type gels of *Rhizobium* sp. strain NGR234 hybridized against probes for DNA (lane 6), nitrogenase genes (lane 7), *fixL* (lane 8), *fixK* (lane 9), *fixO* (lane 10), *fixG* (lane 11), *nodP* (lane 12), and *exoBDFKL* (lane 13).

rDNA; however, they might contain genes essential for the survival of the organism.

It is important to note that, according to its electrophoretic mobility, pNGR234b is presumably the largest *Rhizobium* megaplasmid identified so far. This might be the reason why, although Morrison et al. suggested the presence of a second plasmid in NGR234 (12), other work did not evidence such a plasmid (14). We have recently assayed different methods to detect *Rhizobium* plasmids; pNGR234b could be consistently detected only by the one used in this study. Actually, by this method, observation of pNGR234b is reproducible. To date we have analyzed the plasmid profiles of more than 500 individual clones derived from strain NGR234 and of more than 300 derived from the culture from 1965 (not shown). All these colonies presented pNGR234b with the mobility shown in the plasmid profiles of the parental strains. It is interesting that some of the colonies (3 of 800) showed changes in the mobility of pNGR234a compatible with the occurrence of genomic rearrangements involving pSym (13a).

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