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A MOLECULAR AND GENETICAL ANALYSIS
OF SYMBIOTIC GENES IN LOTUS RHIZOBIA

By

KAW-YAN CHUA

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ABSTRACT

Relative DNA homologies were determined among 24 strains of fast-growing rhizobia and 26 strains of slow-growing rhizobia with particular reference to those which nodulate Lotus species. Two major DNA homology groups were identified which shared less than 10% relative DNA homology. Fast-growing Lotus rhizobia were grouped with related fast-growing strains in DNA homology group I, and these strains are designated as Rhizobium loti. Slow-growing strains from Lotus, (Bradyrhizobium spp. (Lotus)), Glycine max (Bradyrhizobium japonicum), Ornithopus and Lupinus formed DNA homology group II which was further divided into 4 DNA homology subgroups. Bradyrhizobium spp. (Lotus) were shown to be genetically distinct from B. japonicum.

Plasmid profiles were determined for strains of R. loti and Bradyrhizobium spp. (Lotus). All R. loti strains contained a single large indigenous plasmid, whereas strains from Bradyrhizobium spp. (Lotus) carried multiple plasmids of molecular weights ranging from 130-280 MDal. A plasmid-cured derivative of R. loti NZP2213 was isolated and found to still form effective nodules on Lotus tenuis, suggesting that nodulation and nitrogen fixation genes are not plasmid-borne in this strain. The functions of the indigenous plasmids carried in Lotus rhizobia are unknown.

Symbiotic mutants of R. loti strain NZP2037 were isolated by random Tn5-mutagenesis. Mutants included strains blocked in root hair curling (Hac), nodule initiation (Noi), bacterial release (Bar) and nitrogen fixation (Cof) on Lotus pedunculatus.

The nodulation (nod) gene region from R. loti strain NZP2037 was isolated from a pLAFR1-NZP2037 gene library using the cloned Tn5 containing EcoRI fragment from the Nod⁻ mutant as a probe. Two cosmids were isolated and were found to complement the NZP2037 Nod⁻ mutant. Hybridisation and complementation experiments confirmed that a 7.1 kb EcoRI fragment present in both nod cosmids carried gene sequences involved in nodulation. An EcoRI and Hind III restriction enzyme map of the nod gene region in R. loti NZP2037 was constructed using nod cosmids pPN305 and pPN306.

Using the cloned R. loti NZP2037 nod gene region (7.1 kb EcoRI fragment from pPN305) as a hybridisation probe, highly conserved DNA sequences from other strains of R. loti and Bradyrhizobium spp. (Lotus) were identified. The nod gene region from Bradyrhizobium spp. (Lotus) strain NZP2309 was isolated by direct 'in planta' complementation of the R. loti Nod⁻ mutant using a NZP2309 pLAFR1 gene library.

Comparative physical and genetical studies showed that the R. loti NZP2037 nod gene region isolated shared functional similarities with previously isolated nod gene regions from R. trifolii and R. meliloti despite the fact that only weak DNA homology was observed between the corresponding regions. This suggested that at least some of the nod gene sequences carried on the cloned R. loti nod gene region belong to the highly conserved 'common' nod gene sequence category.

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ABBREVIATIONS

Nm	neomycin
Km	kanamycin
Cb	carbenicillin
Tc	tetracycline
Ap	ampicillin
Sp	spectinomycin
Cm	chloramphenicol
Str	streptomycin
Kb	kilobases
MES	2-(N-morpholino)ethanesulfonic acid
SDS	sodium dodecyl sulphate
Nod ⁺ Fix ⁺	<u>Rhizobium</u> phenotype characterised by the ability to induce visible nodules on plant roots which are capable of nitrogen fixation.
Nod ⁺ Fix ⁻	<u>Rhizobium</u> phenotype characterised by the ability to induce visible nodules on plant roots which are not capable of nitrogen fixation.

Abbreviations not defined in this list are "accepted" abbreviations (Biochemical Journal (1983) 209, 1-27).