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A MOLECULAR AND GENETICAL ANALYSIS OF SYMBIOTIC GENES IN *LOTUS* RHIZOBIA

By

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A thesis presented in partial
fulfilment of the requirements for the degree
of Doctor of Philosophy in Microbiology at
Massey University,
Palmerston North, New Zealand

October, 1984

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.

ACKNOWLEDGEMENTS

I wish to thank my supervisors Dr B.D.W. Jarvis, Department of Microbiology and Genetics, Massey University, for his constant interest and advice throughout this study and Dr D.B. Scott, Applied Biochemistry Division, Department of Scientific and Industrial Research (D.S.I.R.) for his inspiration and guidance with all aspects of this work and his generosity in sharing his laboratory space and research resources for the past two years.

I also wish to thank Dr C.E. Pankhurst of Applied Biochemistry Division, D.S.I.R., for the advice and assistance with the light and electron microscopy studies and the construction of Rhizobium loti genomic library, as well as for his continued interest in this work.

Special thanks are also due to the following members of D.S.I.R., Palmerston North: Dr J.J. Patel, for his assistance with the bacteriophage typing technique; Dr W.T. Jones, for carrying out the tandem crossed-immunoelectrophoresis experiment; Mr D.H. Hopcroft, for his assistance with the electron microscopy studies and Dr B. Terzaghi for her advice with the transposon mutagenesis experiment.

I would also like to thank the staff members of Rhizobium laboratory, D.S.I.R., for their cooperation and encouragement over the past two years; Mr C.B. Court for his assistance in thesis figures preparation and Mrs G. Shaw for typing the thesis. To all the members of my family, I would like to show my greatest gratitude, for their love and support over the years.

During the period of my Ph.D. programme I have been supported by a study grant from D.S.I.R. which I gratefully acknowledge.

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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^+	*Rhizobium* phenotype characterised by the ability to induce visible nodules on plant roots which are capable of nitrogen fixation.
Nod^+Fix^-	*Rhizobium* 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).