ISOLATION AND DNA SEQUENCE ANALYSIS OF A *RHIZOBIUM LOTI* GENE REQUIRED FOR EFFECTIVE NODULATION OF *LOTUS PEDUNCULATUS*

A thesis presented in partial fulfilment of
the requirements for the degree of
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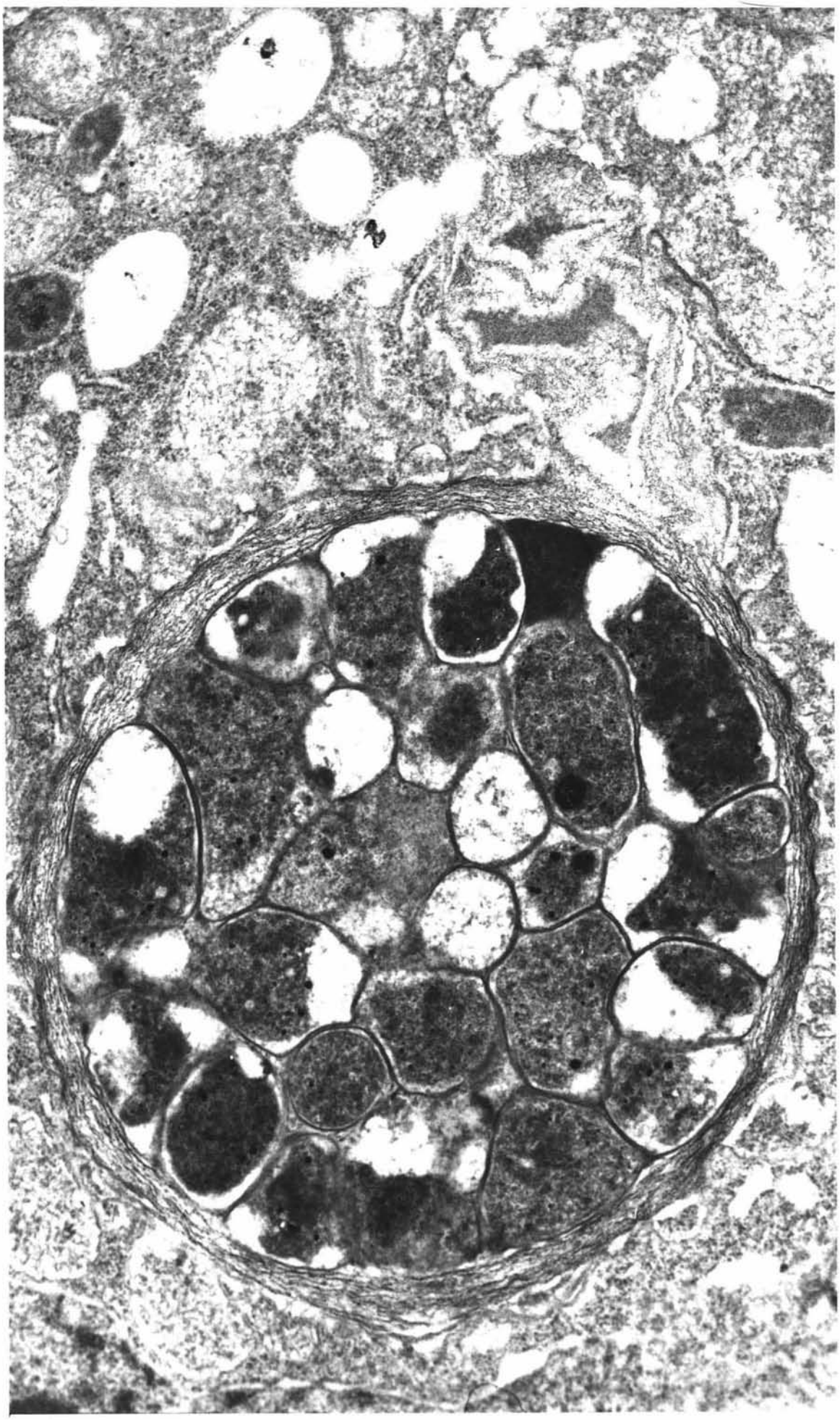
FRONTISPIECE

Transverse section through a swollen infection thread in a *Lotus pedunculatus* nodule. The plant was inoculated with the *Rhizobium loti* mutant strain PN239.

Magnification approximately 3500 times
Photograph courtesy of Clive Pankhurst



ERRATA

p4	line 17	'Spanik' (twice) should be 'Spaink'
p11	line 7	'is indicated' should be 'is indicated by X'
p17	line 19	'Sodium chloride, 0.5' should be 'Sodium chloride, 5.0'
p18	line 14	'*lac*I^9' should be '*lac*I^q'
p20	line 17	'$MnCl_2.4Cl_2O$' should be '$MnCl_2.4H_2O$'
p22	line 21	'Tn5' should be 'Tris'
p27	line 12	'0.5 µg/ml' should be '0.5 mg/ml'
p33	line 14	'BC1G' should be 'BCIG'
p36	line 1	'Whitfield' should be 'Whitfeld'
p51	line 4	'*bar*$^-$' should be '*bar*'
p54	line 6	'(2 µl/ml)' should be '(2 µg/ml)'
p54	line 21	Insert '(Fig. 14)' after '*Xho*I'
p66	Fig. 18	Add 'Vertical lines indicate the location of Tn5 insertions which result in a Fix^+ (longer vertical line) and Fix^- (shorter vertical line) phenotype on *Lotus pedunculatus*'
p76	Fig. 22	'(arrowed)' should be '(two examples of which are arrowed)'
p77	Fig. 23	'the sequence read from them (B)' should be 'part of the sequence read from them (B)' Add 'The sequences given in B (left to right) read from bottom to top in the segments of gels from the + strand and from top to bottom in the segments of gels from the - strand. Due to poor reproduction not all bands are readily visible.'
p81	line 7	'cosmids' should be 'plasmids'
p81	line 33	'base repeat' should be 'base direct repeat'
p83	Fig. 26	'sequence of two Tn5 - *Rhizobium* junctions.' should be 'sequences of Tn5 - *Rhizobium* DNA junctions from two different mutants.'

ABSTRACT

A *Rhizobium loti* gene required for effective nodulation of the host *Lotus pedunculatus* has been identified by transposon Tn5 mutagenesis. Cosmids from a *R. loti* gene library which complemented a previously isolated mutant strain, PN239, (Chua *et al* 1985; J. Bacteriol. 162; 335-343) at this locus were identified by *in planta* complementation. A physical map of these cosmids was constructed and the site of insertion of the Tn5 was mapped to a 7.5 kb *Eco*RI fragment common to all cosmids which complemented the mutation. This 7.5 kb *Eco*RI fragment was subcloned into pBR328 and pLAFR1 and a more detailed physical map constructed. The 7.5 kb *Eco*RI fragment in pLAFR1 was able to complement the Tn5 mutation when introduced into strain PN239.

Further Tn5 mutagenesis of the 7.5 kb *Eco*RI fragment was carried out in *E. coli* and the mutations were homogenotised into *R. loti* NZP2037. Three additional mutations were isolated which caused a Fix^- phenotype on *Lotus pedunculatus*. The Tn5 inserts which caused a Fix^- phenotype were mapped to positions adjacent to the position of the original mutation in strain PN239. All other Tn5 insertions isolated in the 7.5 kb *Eco*RI fragment gave a Fix^+ phenotype on *Lotus pedunculatus*.

A region was sequenced which was involved in effective nodulation of *Lotus pedunculatus* as indicated by the position of the Tn5 insertions. Analysis of the consensus sequence of 2307 bases identified a potential open reading frame (ORF) of 576 base pairs, coding for a putative protein of 21.2 kD. The positions of the Tn5 insertions causing a Fix^- phenotype and the adjacent Tn5 insertions which did not affect fixation were determined in the sequence. The position and orientation of the ORF identified was consistent with the sequenced positions of these Tn5 insertions.

A fragment containing most of the ORF identified from the sequence was used as a hybridization probe to various strains of rhizobia. Homology was only demonstrated with DNA from other *R. loti* strains. *R. loti* strains containing Tn5 insertions which were Fix$^-$ on *Lotus pedunculatus* were found to be fully effective on *Lotus corniculatus*. These observations suggest that the gene characterised in this investigation may be involved in the host specificity of *R. loti* for *Lotus pedunculatus.*

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