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ANALYSIS OF GENOMIC REARRANGEMENT AND PLASMID CONJUGATION OF AN  
INOCULANT STRAIN OF *RHIZOBIUM LEGUMINOSARUM* BV *TRIFOLII*.

A Thesis Presented in Partial Fulfillment of the Degree of  
Doctor of Philosophy.

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## ABSTRACT

Variation of plasmid profile is the hallmark of Rhizobium leguminosarum bv trifolii strains isolated from the nodules of pasture plants. Previous attempts to demonstrate that these variant types were derived from the original inoculant were inconclusive. Subsequent laboratory based simulations revealed that the broad host range plasmid RP4 was capable of generating stable alterations in the plasmid and total genomic DNA profile. This variation involved an apparent loss of pSym, with concurrent loss of the nod and nif genes, but these strains produced nodules on white clover plants. The strains recovered from the nodules, while not identical, were clearly derived from the pSym<sup>-</sup> strain. A plasmid closely corresponding in size to pSym was detected in the nodule re-isolates and repeated trials of this experiment involving antibiotically marked (apparently) sym<sup>-</sup> strains confirmed this observation. This left the conclusion that the DNA was still there but in a form which was difficult to detect by conventional DNA hybridization procedures, a result which is not totally without precedent (Downs and Roth, 1987).

The second portion of this project involved an investigation of the transmissability of plasmids from the inoculant strain 2668. The strain was marked with Tn5 with the expectation that some of the movable DNA pieces would carry a Tn5 insert and could thus be selected for. A transmissible symbiotic plasmid was detected, as had been previously observed in other rhizobia by Johnston et al (1978). The plasmid was shown to be transferable, in an altered form, to soil microorganisms of unidentified genera, to a sym<sup>-</sup> strain of R. leguminosarum bv trifolii, to its original parent 2668 and to E. coli. In all strains, with the exception of E. coli the nodulation genes were functional, producing normal looking nodules (on the outside) and in many strains nitrogen was also fixed, though not generally as well as by the parent 2668. The significance of a self-transmissible broad host range symbiotic plasmid is discussed in the context of the microbial ecology of rhizobia.