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PLASMIDS IN *Rhizobium Phaseoli*

A THESIS PRESENTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE
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ABSTRACT

Fast growing strains of *Rhizobium* have been divided into four homology groups on the basis of DNA hybridization. Rhizobia from two of these homology groups can form effective nodules with beans (*Phaseolus vulgaris*). Large plasmids associated with nodulation have been demonstrated in *Rhizobium* sp. A study was undertaken to examine the plasmids in rhizobia from the two different homology groups capable of nodulating beans.

The effectiveness of strains of *Rhizobium phaseoli* on bean plants were examined. Spontaneous antibiotic resistant mutants which retained the ability to nodulate beans were selected. Antibiotic resistance marked clones were incubated at elevated temperatures to produce an ineffective mutant strain of *Rhizobium phaseoli* NZP 5492.

Methods of extracting large plasmids from *Rhizobium phaseoli* were developed. Plasmids were visualised by agarose gel electrophoresis and purified by cesium chloride - ethidium bromide density gradient ultracentrifugation.

We demonstrated the presence of plasmids of molecular weight range 66 Md to 316 Md in *Rhizobium phaseoli* strains. Some strains contained a single plasmid while others contained multiple plasmids.

Comparisons were made between whole plasmids and restriction endonuclease digests of the plasmids from the two groups. The fragment pattern obtained from *Eco* RI digests showed differences in fragment numbers and size between plasmids from DNA homology group 1 and DNA homology group 2.

Further studies using gel blotting and hybridization techniques are required to ascertain the degree of homology between the plasmids, both within groups and between groups.

Rhizobium phaseoli NZP 5479 and NZP 5547 a non-nodulating mutant of strain NZP 5479 were examined. Both strains had plasmids of estimated molecular weights 186 Md and 288 Md. There was no detectable difference in the size of the plasmids in the non-nodulating mutant compared to the effective parent strain.

Rhizobium phaseoli NZP 5492 B5/8 (effective) and NZP 5492 B5/1 an ineffective mutant obtained from strain NZP 5492 had plasmids of similar molecular weight. Differences were observed in the *Eco* RI fragment pattern and possible rearrangements to the DNA to account for these differences are presented.

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