APPLICATION OF DNA HYBRIDISATION TO THE TAXONOMY OF *RHIZOBIUM TRIFOLII* AND RELATED SPECIES

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ABSTRACT

Rhizobia were grown in Yeast Mannitol broth in the presence and absence of K_2HPO_4. Increasing the concentration of yeast extract in the medium resulted in an increase in growth up to a certain concentration of yeast extract. Growth of rhizobia was inhibited by further increases in yeast extract concentration, especially in the presence of K_2HPO_4. The inhibition is due to glycine present in the yeast extract and is increased by the presence of monovalent cations.

The molecular weight of DNA extracted from rhizobia was determined by centrifugation in alkaline sucrose gradients. It was found that ^{32}P-labelled DNA was more fragile under shear than unlabelled DNA. Unlabelled DNA required 75 seconds and ^{32}P-labelled DNA required 56 seconds sonication to reduce the fragment size to 230,000 Daltons.

Labelled DNA prepared by a phenol-chloroform method was contaminated with polysaccharide and only low homologous hybridisation could be obtained. A hydroxyapatite-urea method of purifying DNA was developed which produced polysaccharide-free DNA. When labelled DNA was prepared by this method homologous hybridisation averaged 72%. Polysaccharide contamination of unlabelled DNA preparations did not effect the % relative reassociation.

Complete reassociation of heterologous DNA required a longer incubation time than did homologous DNA. The homologous reaction was complete after 32 hours (Cot 200) whereas heterologous DNA required an incubation of 40 hours (Cot 250) to achieve maximum reass ociation.

Deoxyribonucleic acid homologies were determined among 27 strains of *Rhizobium trifolii*, 4 strains of *R. leguminosarum* and 4 of *R. phaseoli*. Results from

related strains indicate that DNA homologies correlate with serological relationships and ability to form nodules on legume roots can be lost without detectable change in homology with an independent reference strain. All rhizobia which nodulated effectively on T. repens, T. subterraneum , T. ambiguum, and Vicia hirsuta formed one population with an average relatedness of 70% (range 49-94%) and ΔTm(e) of 0.2-7.5°C with respect to reference strains capable of nodulating the first two clover species. Two strains from African Trifolium species and one from a Japanese species were less closely related. The average relatedness of strains from Phaseolus vulgaris with clover rhizobia was 46% (range 37-50%) and Δ Tm(e) 6.5-10.0°C. Taxonomic revisions consistent with these observations are discussed. It is proposed that R. trifolii and R. leguminosarum should be combined and called Rhizobium leguminosarum Frank. Within this species various biotypes should be designated according to their plant specificity R. phaseoli should be retained as separate species and examined in more detail. The results are discussed in relation to proposed genetic basis for plant specificity.

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