

Semi- Annual progress Report September 1, 1986 to January 31, 1987

Project No. 6.233 "Genetic Engineering of rhizobia for nodulation efficiency"

Grant No. DPE-5542-G-SS-6039-00

Background.

Bradyrhizobium japonicum strain 61A76 is naturally composed of two distinct subpopulations of cells; one forms translucent and friable colonies on yeast extract mannitol gluconate medium (YEMG) while other forms slimy compact colonies on this medium. The former type breeds true on subculturing, while the latter produces both friable and slimy populations on subculturing. Regardless of the culture age, approximately 70% of the population in broth cultures of the slimy type consists of the friable true breeding variant.

Scope of the project and research done between September 1, 1986 and December 31, 87.

One of the objectives of this proposal was to understand the genetic basis of the mixed colony morphology in the slimy variant. In order to understand this phenomenon, we have constructed a gene bank of the strain 61A76 in the reconstructed cosmid vector pLAFR1. The original vector pLAFR1 has a tetracyclin resistance gene as the marker. Our preliminary results indicated that strain 61A76 possessed high intrinsic resistance to tetracyclin. Therefore we replaced the tetracyclin marker gene in the vector with the Neomycin phosphotransferase gene (NPT) cloned from Tn5. The reconstructed vector possessed kanamycin resistance as the marker.

During this period we have also obtained variants of 61A76 that possess only the slimy compact phenotype. To our surprise two such variants tested failed to nodulate soybean cv. Williams. They also failed to deform root hairs. Experiments are underway to mate the gene bank into these variants and select the recombinants for mixed colony and Nod⁺ phenotype. These experiments will identify the clones that possess the genes present in the parent that are responsible for its mixed colony phenotype.