MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 3, No. 4, pp. 199-206, 1990

The Molecular Genetics of Nodulation Competitiveness in *Rhizobium* and *Bradyrhizobium*

Eric W. Triplett

Department of Agronomy and the Center for the Study of Nitrogen Fixation, University of Wisconsin, Madison 53706-1597 U.S.A.
Received 29 December 1989. Accepted 8 March 1990.

The *Rhizobium* competition problem. Attempts to improve legume productivity in agricultural fields by inoculation with superior strains of root nodule bacteria often fail. This failure is the result of the inability of the superior inoculum strains to occupy nodules in soils with a large population of indigenous rhizobia. This dilemma is referred to as the *Rhizobium* competition problem. Weaver and Frederick (1974) found that inoculation with a strain at a level 1,000 times higher than the number of bradyrhizobia in the soil resulted in one-half of the nodules being occupied by the inoculum strain. Dunigan *et al.* (1984) inoculated soybean plants with a large population of *Bradyrhizobium japonicum* USDA 110 for three consecutive years and found that the inoculum strain became established in the field. This study shows that inoculation of plants with large numbers of a *B. japonicum* strain over a long period can eventually result in the establishment of that strain in the soil. It is not known how widely applicable this strategy is to other conditions.

The need to solve the *Rhizobium* competition problem has increased in recent years because of successful efforts in a number of laboratories to identify genes in *Rhizobium* and *Bradyrhizobium* that increase symbiotic nitrogen fixation rates and/or improve legume yield under controlled conditions. The genes coding for hydrogen oxidation in *B. japonicum* are the best characterized example of this phenomenon. Soybean yield increases approximately 15% when the plants are nodulated by a strain of *B. japonicum* that is capable of recycling the H_2 generated as an obligate product of the nitrogenase reaction compared with plants nodulated with an isogenic strain that fails to oxidize H_2 (Evans *et al.* 1985, 1987; Hanus *et al.* 1981). These experiments were done in soil that lacked an indigenous population of *B. japonicum*.

Other rhizobial genes have been described that improve nitrogenase activity and/or legume productivity (Cannon *et al.* 1988; Spaink *et al.* 1989a). This occurs when the expression of such genes is increased or modified and when the plants are grown under controlled conditions. Such genes include the following: 1) *nifA*, the positive regulator of the nitrogen fixation genes; 2) *dct*, the operon responsible for dicarboxylic acid transport; and 3) *nodD*, a regulatory gene required for the expression of the *nod* genes in *Rhizobium*.

There are also reports in the literature of the use of chemical mutagenesis to generate mutants of *B. japonicum* with increased symbiotic nitrogenase activities compared to the wild-type strains (Maier and Brill 1978; Paau 1989; Scott *et al.* 1979). Williams and Phillips (1983) were able to increase soybean yield in the field by inoculation with one of these chemical mutants. Their experiments were conducted in a soil that lacked indigenous bradyrhizobia. Thus, it is known that the symbiotic properties of *Rhizobium* can be improved by genetic means.

The intent of this review is to describe recent advances in our understanding of competition for nodulation at the molecular level. Space does not permit a discussion of the progress that has been made in our understanding of the ecology of *Rhizobium* in soil including the characterization of indigenous populations and those environmental factors that affect competitiveness. These factors have been reviewed elsewhere (Dowling and Broughton 1986; Schmidt and Robert 1985) as have recent advances in the infection process, nodule development, and symbiotic nitrogen fixation (Gloudemans and Bisseling 1989; Long 1989; Martinez *et al.* 1990).

Two approaches are being used by geneticists to address the *Rhizobium* competition problem. First, researchers in various laboratories are altering the host range of the *Rhizobium* inoculum strains and/or using legume genotypes with restricted *Rhizobium* infectivity. Second, a number of researchers are interested in constructing strains of *Rhizobium* with an increased ability to compete for nodule occupancy. This review will describe both of these approaches but will concentrate on the latter. The discussion of the construction of new strains includes the following: 1) the identification of phenotypes in *Rhizobium* that affect competitiveness and the isolation and characterization of genes responsible for these phenotypes; 2) the isolation and characterization of genes involved in nodulation competitiveness where the mechanism of competitiveness is unknown; 3) methods for the overexpression and transfer of genes involved in nodulation competitiveness to superior N_2-fixing strains of *Rhizobium*; 4) the use of gene probes to identify strains of *Rhizobium* in soil and in nodules; and 5) the problems that need to be addressed during an analysis of the efficacy of recombinant *Rhizobium* strains in field experiments.

Host range as related to competition for nodule occupancy: Host restriction for nodulation. One approach to address the competition problem is to identify a legume host that is not infected by the indigenous rhizobia or

Address correspondence to E. W. Triplett: Department of Agronomy and the Center for the Study of Nitrogen Fixation, University of Wisconsin-Madison, 1575 Linden Dr., Madison, WI 53706-1597 U.S.A.

bradyrhizobia which are present in soil. Once identified, that host is planted along with a strain of root nodule bacteria which is capable of nodulating that host. For example, strains of *B. japonicum* serocluster 123 dominate nodule occupancy in the Upper Midwest even though they are not present in higher concentrations in soil than are other strains of *B. japonicum* (Moawad *et al.* 1984; Schmidt *et al.* 1986). Lines of soybean have been identified that restrict nodulation by strains of *B. japonicum* in serocluster 123 (Cregan *et al.* 1986, 1989a; Sadowsky *et al.* 1987). These genotypes of soybean may permit nodulation by the inoculum strains while preventing nodulation by those strains that normally dominate nodule occupancy. Genetic characterization of both host and microsymbiont determinants involved in these interactions is in progress (Cregan *et al.* 1989b; Rodriguez-Quinones *et al.* 1989). A similar strategy could be pursued in alfalfa and clover since dominant serogroups have also been identified in *R. meliloti* and *R. leguminosarum* bv. *trifolii* in Oregon and Arizona (Demezas and Bottomley 1984; Jenkins and Bottomley 1985; Shishido and Pepper 1990).

Use of inhibitors of *nod* gene induction to regulate nodule occupancy. Another approach toward solving the competition problem was recently proposed by Cunningham *et al.* (1989). Flavonoids, isoflavones, and chalcones, along with *nodD*, induce expression of the other *nod* genes in *Rhizobium* and *Bradyrhizobium* (Djordjevic *et al.* 1987; Firmin *et al.* 1986; Kosslak *et al.* 1987; Maxwell *et al.* 1989; Peters *et al.* 1986; Peters and Long 1988; Redmond *et al.* 1986; Zaat *et al.* 1987). The amount of flavonoids produced by legumes may be limiting to nodulation and subsequent nitrogen fixation (Kapulnik *et al.* 1987). Inhibitors of *nod* gene induction by flavonoids have also been identified (Cunningham *et al.* 1989; Djordjevic *et al.* 1987; Firmin *et al.* 1986; Kosslak *et al.* 1989). By mutation or recombination, the structure of *nodD* can be altered such that either the expression of the *nod* genes is flavonoid-independent or the *nod* genes are induced by a broader spectrum of inducer molecules (Burn *et al.* 1987, 1989; Johansen *et al.* 1988; Spaink *et al.* 1989a, 1989b). Cunningham *et al.* (1989) showed that inhibitors of *nod* gene induction were also capable of inhibiting nodulation of soybean by *B. japonicum*. Thus, a flavonoid-independent strain should be able to nodulate soybean exclusively in the presence of indigenous bradyrhizobia and the appropriate inhibitor of *nod* gene induction.

However, there are two important practical limitations to this strategy. First, the inhibitors of *nod* gene induction are unstable in soil as suggested by Kosslak *et al.* (1989), although Cunningham *et al.* (1989) suggest that halogenation of these inducers will enhance their stability in soil. Second, there is no single compound that inhibits *nod* gene induction in all strains of *B. japonicum*. Cunningham *et al.* (1989) estimate that effective inhibition of soybean nodulation in the field would require the application of four halogenated inhibitors. Each inhibitor would require registration by the Environmental Protection Agency prior to use in the United States. This is a very expensive process when considering the number of compounds that would need to be registered and the small profit potential of new inoculum strains.

Identification of phenotypes involved in nodulation competitiveness. In an effort to assist in the isolation of genes involved in nodulation competitiveness as well as provide an understanding of mechanisms involved in competition, researchers in several laboratories have identified a number of phenotypes involved in this process. In this review, the term "competitiveness genes" refers to genes that affect competitiveness though this may be an indirect result of their primary function.

Role of motility in nodulation competition. Among the first such reports is that by Ames and Bergman (1981) who found that an ethyl methanesulfonate-induced motility-deficient mutant of *R. meliloti* was less competitive than the wild-type strain for nodulation of alfalfa roots but was otherwise symbiotically competent. Similarly, Mellor *et al.* (1987) isolated nonmotile Tn*5* mutants of *R. l.* bv. *trifolii* TA1 that were less competitive for nodulation than the wild-type parent strain.

Cell surface characteristics and competition. Other workers have identified cell surface characteristics as playing a role in competition. For example, Handelsman *et al.* (1984) found that strains of *R. meliloti* which recognize the alfalfa agglutinin weakly induce earlier nodule formation and are more competitive for nodulation than are high agglutinating strains. Araujo and Handelsman (1989) have isolated nonmucoid Tn*5* mutants of *R. l.* bv. *phaseoli* that are less competitive for nodulation than the wild-type strain. Conversely, Zdor and Pueppke (1989) have selected an EPS^- mutant of *R. fredii* using triphenyltetrazolium chloride and found that this mutant expresses increased competitiveness compared to the wild type. Alterations in EPS^- on the surface of *B. japonicum* also affect competitiveness (Bhagwat *et al.* 1989).

Various mutants of *R. meliloti* have been identified that either lack extracellular polysaccharide (EPS) or overexpress EPS production (Doherty *et al.* 1988; Finan *et al.* 1985; Leigh *et al.* 1985). Mutations in the *exo* genes that decrease the synthesis of calcofluor-binding exopolysaccharide in *R. meliloti* are Inf^-, that is they do not infect alfalfa roots (Doherty *et al.* 1988; Finan *et al.* 1985; Leigh *et al.* 1985). This renders them useless in an analysis of nodulation competitiveness.

Given the proposed role of cell surface characteristics in nodulation competitiveness, a useful tool for the identification of proteins involved in this process may be Tn*phoA*, which is useful for tagging genes that code for membrane-spanning proteins (Manoil and Beckwith 1985). Symbiotically defective mutants of *R. meliloti* were identified by Long *et al.* (1988) using Tn*phoA*. New vectors are available that facilitate mutagenesis by Tn*phoA* (Taylor *et al.* 1989).

Bacteriocin production and competition. The role of bacteriocin production in nodulation competitiveness has been studied in *R. l.* bv. *trifolii*. Hodgson *et al.* (1985) determined that the bacteriocin-producing strain CB782 increased nodule occupancy of clover roots by a bacteriocin-resistant strain when coinoculated with a bacteriocin-sensitive strain. These results were obtained in sterile culture and in nonsterile soil. No isogenic comparisons were made. For example, transposon mutants of CB782 lacking bacteriocin production have not been tested for their ability

to regulate nodule occupancy. Similarly, bacteriocin-resistant mutants of a bacteriocin-sensitive strain have not been assayed for their ability to compete with CB782 relative to the wild type.

An antirhizobial bacteriocin, referred to as trifolitoxin, is produced by *R. l.* bv. *trifolii* T24 (Schwinghamer and Belkengren 1968; Triplett and Barta 1987). Schwinghamer and Belkengren (1968) found that this strain was highly competitive for nodulation when coinoculated with a bacteriocin-sensitive strain of *R. l.* bv. *trifolii*. Unfortunately, T24 is Fix^- (Schwinghamer and Belkengren 1968) and, as a result, is not useful in a solution to the competition problem. Triplett and Barta (1987) found that Tn*5* mutants of T24 lacking trifolitoxin production lost their competitive advantage for nodulation when coinoculated with a trifolitoxin-sensitive strain. The genes for trifolitoxin production and resistance (*tfx*) have been isolated, mapped by Tn*5* mutagenesis, and transferred to other strains of *Rhizobium* (Triplett 1988; Triplett *et al.* 1989). Using marker exchange, the 4.4-kilobase *tfx* region was transferred stably to the genome of a Fix^+, trifolitoxin-sensitive strain, *R. l.* bv. *trifolii* TA1 (Triplett 1990). The resulting recombinant strain stably produced trifolitoxin in the absence of any selection pressure without any decline in its symbiotic properties. This strain also expressed increased nodulation competitiveness when coinoculated with a trifolitoxin-sensitive strain (Triplett 1990). An isogenic recombinant strain that lacks trifolitoxin production did not show increased nodulation competitiveness (Triplett 1990). However, these experiments were done in sterile culture, so it is not known whether the transfer of *tfx* to a *Rhizobium* strain can increase its nodule occupancy in nonsterile soil.

Role of effectiveness in competition. There has been a debate in the literature concerning whether the ability to fix nitrogen plays a role in strain nodule occupancy. Some results suggest that the host plant selects for effective *Rhizobium* strains (Labandera and Vincent 1975; Marques Pinto *et al.* 1974; Robinson 1969a, 1969b), while others describe ineffective strains as being more competitive than effective strains (Franco and Vincent 1976; Johnston and Beringer 1976; Nicol and Thornton 1941; Vincent and Waters 1953). Since either spontaneous or Tn*5* Nif^- mutants of *Rhizobium* or *Bradyrhizobium* are unaltered in their competitive ability compared to the wild-type strains (Amarger 1981; Hahn and Studer 1986), effectiveness per se seems to play no role in nodulation competitiveness.

Role of speed of nodulation in competitiveness. A correlation between speed of nodulation and competitiveness has been observed in strains of *R. meliloti*, *R. l.* bv. *trifolii*, and *R. l.* bv. *phaseoli* (Handelsman *et al.* 1984; Stephens and Cooper 1988; Graham and McDermott 1989). Dowling and Broughton (1986) proposed that the speed with which a strain of *Bradyrhizobium* nodulates soybean is strongly correlated with the ability to form nodules to the exclusion of other strains. That is, strains of *Bradyrhizobium* do not compete directly with each other for nodule occupancy but must infect the root prior to the onset of the autoregulatory response. The autoregulatory response is based on the observation that inoculation of one side of a split root one day in advance of inoculation of the opposite side of the root can prevent nodulation on the opposite side (Bhuvaneswari *et al.* 1981; Heron and Pueppke 1987; Pierce and Bauer 1983). Similar observations have been made in *R. l.* bv. *phaseoli* and *R. l.* bv. *trifolii* (Oliveira and Graham 1990; Sargent *et al.* 1987).

Direct evidence for the role of the autoregulatory response in competitiveness was obtained by Sargent *et al.* (1987) who found that a *nodFE*$^-$ mutant of *R. l.* bv. *trifolii*, which infects clover roots normally when inoculated alone, lacks the ability to induce the autoregulatory response. The *nodFE*$^-$ mutant does not prevent nodulation by the opposite side of a split clover root. This mutant also was a poor competitor for nodule occupancy when coinoculated with the parent strain on single roots (Sargent *et al.* 1987).

Further evidence for the role of speed of nodulation in competitiveness comes from the work of Hahn and Hennecke (1988) who examined deletion mutants of *B. japonicum*. Various deletion mutants were constructed that lacked portions of the *nod*-1, *nod*-2, and *nif*-clusterI regions. A deletion in *nod*-2 between RSα12 and RSα3 was found to cause a dramatic delay in nodulation and decline in competitiveness. Other deletions in *nod*-1 and *nif*-clusterI also caused lesser but significant delays in nodulation and decreased competitiveness when compared to the wild-type strain.

Isolation of genes involved in nodulation competitiveness where the mechanism of competitiveness is unknown. In the examples cited above, the investigators chose specific phenotypes that they felt might play a role in nodulation competitiveness. In some of these cases, such as bacteriocin production, this permitted rapid screening of cosmid clones or transposon mutants with altered competitive ability. However, in many cases the mechanism of competitiveness expressed by a *Rhizobium* strain is unknown. In these examples, cosmid clones or transposon mutants with altered competitive ability must be identified by screening directly for the competitiveness phenotype on legume roots. To date, there are two examples of this approach in the literature.

Competitive blocking by *R. l.* bv. *viceae* PF_2. The Afghanistan cultivar of peas is not nodulated by European strains of *R. l.* bv. *viceae* but is nodulated by strain TOM. The European strain PF_2 is capable of blocking nodulation of Afghanistan peas by TOM. Dowling *et al.* (1987) isolated the genes that code for this competitive blocking phenotype (Cnb^+). The Cnb^+ genes are located on a 6.9-kilobase *Hin*dIII fragment on the Sym plasmid of PF_2 (Dowling *et al.* 1987, 1989) that includes much of the *nod* region (Dowling *et al.* 1989). Mutations in *nodC*, *nodD*, and *nodE* abolished the competitive blocking phenotype in PF_2 (Dowling *et al.* 1989). The gene in TOM, *nodX*, necessary for nodulation of Afghanistan peas has been isolated (Davis *et al.* 1988).

The examination of this blocking phenotype could provide very important insights toward a solution to this problem irrespective of the ability of PF_2 to nodulate peas. This may be important in the construction of inoculum strains that can exclusively nodulate legume roots in the presence of indigenous strains.

Competitiveness mutants in *R. fredii*. McLoughlin *et al.* (1987) isolated Tn*5* mutants of *R. fredii* USDA 257 that were significantly less competitive than the wild-type strain. Otherwise, the mutants appeared to be symbiotically identical to the wild-type strain in nodule number, nodule mass, and nitrogenase activity. The isolation of these mutants has not led to the isolation of genes involved in the regulation of nodule occupancy by strains of *R. fredii*.

Methods for the overexpression and transfer of genes involved in nodulation competitiveness to superior N_2-fixing strains of *Rhizobium*: Mechanisms of overexpression of competitiveness genes. In an effort to improve the efficacy of recombinant, competitive *Rhizobium* strains, it may be useful to overexpress the genes involved in competitiveness. One method in developing such strains is to insert the competitiveness genes downstream of a strong promoter such as the *trp* promoter from *Salmonella*. A vector that is useful for this purpose, pTE3, has been described by Egelhoff and Long (1985). The trifolitoxin genes have been placed downstream of the *trp* promoter in pTE3, and trifolitoxin production has been increased as a result (Triplett, unpublished). However, the effects of overexpression of trifolitoxin production on nodulation competitiveness have not been determined. It may also be possible to enhance nodulation competitiveness by transforming rhizobia with multiple copies of genes that enhance competitiveness.

Mechanisms of stable transfer of genes involved in nodulation competitiveness to superior strains of *Rhizobium*. Plasmid transfer of genes involved in competitiveness into *Rhizobium* by conjugation is useful for the initial isolation and analysis of such genes. However, recombinant plasmids are very unstable in *Rhizobium* in the absence of selection pressure (Lambert *et al.* 1987; Long *et al.* 1982; Triplett 1990). Thus, transconjugants with such plasmids are not useful for the field release of recombinant, competitive *Rhizobium* strains since it is not practical for farmers to apply broad spectrum antibiotics to agricultural fields. For that reason, researchers in several laboratories are investigating methods for the integration of foreign genes into the chromosome of *Rhizobium* and *Bradyrhizobium* strains. Williams *et al.* (1988) have constructed a vector, based on the broad host range vector pRK290, that contains an inositol dehydrogenase gene from *R. meliloti* and a unique *Spe*I site within that gene. This vector was used to integrate spectinomycin resistance into the chromosome of several strains of *R. meliloti*. The integration event was encouraged by the introduction of an incompatible P-group plasmid.

Another integration vector, pCU246, has been developed by O'Gara *et al.* (1988). This vector includes a multiple cloning site, the *R. meliloti* adenylate cyclase gene (*cya*), and a gentamycin resistance gene. Acuna *et al.* (1987) have described a vector, pRJ1035, that permits the integration of foreign DNA into *B. japonicum*. This vector is based on the repeated sequences found in *B. japonicum*, RSβ3 and RSα9, and permits marker exchange of foreign DNA into those chromosomal sequences.

A method for the stable integration of genes into the chromosome of gram-negative bacteria that does not require marker exchange is the use of Tn*7* vectors. Such vectors have been described by Barry (1988) and have been used for the integration of foreign genes into *Pseudomonas*. Though these vectors have not been used for the integration of competitiveness genes into *Rhizobium*, they should be useful in that genus since no homologous DNA in the recipient cell is required.

Field release of recombinant, competitive strains of *Rhizobium*. Once genes involved in nodulation competitiveness have been transferred stably to the chromosome of highly effective *Rhizobium* strains, their ability to confer increased competitiveness must be analyzed in the field. This has not yet been done in any system.

Choice of recipient strain for the competitiveness genes. It is wise to place the competitiveness genes into strains well adapted to the soil in which the experiments are to be conducted. That is, the recipient strains must be able to survive the physical and biotic characteristics of the soil such as pH, texture, and possible rhizobial antagonists in the soil. The effectiveness of the genes of interest in regulating nodule occupancy can best be determined by constructing strains that are isogenic except for the expression of the competitiveness genes. Such constructs are described for the trifolitoxin system (Triplett 1990). The strain chosen for the integration of competitiveness genes should be a superior N_2-fixing strain compared to those strains already present in soil. This can be accomplished by choosing a Hup^+ strain or a strain with other symbiotically desirable characteristics.

Regulatory concerns in the field release of recombinant rhizobia. With the appropriate constructs, the field experiments can proceed following approval from the appropriate governmental agencies. In the United States, the Environmental Protection Agency is interested in the spread and survival of the recombinant rhizobia, their influence on the population sizes of indigenous rhizobia, and the effects of the release on total soil microflora. The latter can be determined by measuring the effects of the release on total soil biomass. The ecological concerns of the introduction of recombinant organisms are described very well in a recent review by Tiedje *et al.* (1989a).

New methodology for strain quantification in soil. To determine the ecological effects of the release, the inoculum strain must be distinguished from the indigenous strains in the soil. This can be accomplished by making DNA probes that hybridize to sequences specific to the strain(s) of interest, isolating bacterial DNA from the soil samples, and using the polymerase chain reaction to amplify the amount of the specific sequences in the sample (Trevors and van Elsas 1989). This has been done in the identification of specific *Pseudomonas* gene sequences in soil samples and has permitted the detection of as few as one cell per gram of soil (Steffan and Atlas 1988). This methodology is also being applied to the enumeration of bradyrhizobia in soil (Holben *et al.* 1988; Tiedje *et al.* 1989b).

New methodology for strain identification in nodules. *Rhizobium* strains are commonly identified within nodules by serological techniques, intrinsic or acquired antibiotic resistance, and bacteriophage typing (Somasegaran and Hoben 1985). Recently, gene probes specific for a certain strain of *Rhizobium* have also been used to determine nodule occupancy. Under high stringency conditions,

^{32}P-labeled genomic DNA can be used to distinguish specific strains of *Rhizobium* (Cooper *et al.* 1987; Hodgson and Roberts 1983). Wheatcroft and Watson (1988) have made probes to insertion sequences from *R. meliloti* and used these to distinguish strains of that species. Bjourson and Cooper (1988) have isolated strain-specific DNA by subtraction hybridization to identify closely related strains of *R. loti*. Probes of *fix*, *nif*, and *nod* have been used along with restriction fragment length polymorphism mapping and alloenzyme electrophoresis to distinguish strains and species of *Rhizobium* (Demezas *et al.* 1988; Robert 1989). DNA probes have also been used to differentiate isolates of *B. japonicum* (Sadowsky *et al.* 1989).

Characteristics desirable in a superior inoculum strain of *Rhizobium* or *Bradyrhizobium*. Ideal characteristics of an inoculum strain include the following: 1) the ability to compete for survival in the rhizosphere with other soil microorganisms during the growing season; 2) the ability to compete for nodulation with indigenous rhizobial strains; 3) the ability to provide more efficient nodulation and/or nitrogen fixation rates; and 4) the inability to survive more than one growing season. The fourth characteristic is of interest for three reasons. First, the potential for biological hazards is greatly reduced if the inoculum strains survive only one growing season. Second, the commercial success of an inoculant is enhanced with annual sales. Third, and perhaps most important, it ensures that we will be able to introduce new inoculum strains at any time in the future. Thus, new solutions to the competition problem will not be necessary in the future if better inoculum strains are developed by posterity. To accomplish this end, environmentally triggered promoters could be placed upstream of suicide genes, such as genes coding for broad spectrum antibiotics. Such suicide strategies are being developed for the construction and release of *Pseudomonas* strains for degradation of hazardous organic compounds (Fox 1989; Poulsen *et al.* 1989). These approaches could be readily adapted to *Rhizobium* as well. One set of broad spectrum antibiotic genes in *Escherichia coli*, *gef*, codes for a toxic protein and, based on Southern analysis, has homology with *Rhizobium* DNA (Poulsen *et al.* 1989). Thus, genes useful in the development of suicide strategies may already be present in *Rhizobium* but would require alteration in expression to confer a suicide phenotype under certain environmental conditions.

Summary. The tools of molecular biology are being used to solve the economically important *Rhizobium* competition problem. Genes involved in nodulation competitiveness are being isolated and characterized. Mutational analysis has identified phenotypes that play a role in competition. Such phenotypes include motility, cell surface characteristics, speed of infection, and bacteriocin production. Vectors are available for the stable integration of competitiveness genes into superior N_2-fixing *Rhizobium* and *Bradyrhizobium* strains. Gene probes are being used for the identification of rhizobial strains in nodules. With the advent of sequence amplification by polymerase chain reaction, the sensitivity of detection of specific rhizobial strains in soil is very high.

Though the tools for the construction and study of competitive and superior N_2-fixing strains of *Rhizobium* are available, far more genetic analysis of competitiveness is required. Given the number of *Rhizobium*-legume symbioses and the wide variety of environments in which these symbioses are cultured, it is highly unlikely that one solution to the competition problem will be sufficient. No doubt other, as yet undiscovered phenotypes affect competitiveness, and those phenotypes that have been discovered are not highly characterized. In general, basic mechanisms of competitiveness are not well-understood at the molecular level. Such an understanding is necessary for making further advances in solving the *Rhizobium* competition problem.

ACKNOWLEDGMENTS

I am most grateful to Brenda T. Breil for her helpful suggestions and critical comments on this manuscript. The work from this laboratory described in this manuscript has been supported by competitive grants from the United States Department of Agriculture and the University of Wisconsin, Madison, College of Agriculture.

LITERATURE CITED

Acuna, G., Alvarez-Morales, A., Hahn, M., and Hennecke, H. 1987. A vector for the site-directed, genomic integration of foreign DNA into soybean root-nodule bacteria. Plant Mol. Biol. 9:41-50.

Amarger, N. 1981. Competition for nodule formation between effective and ineffective strains of *Rhizobium meliloti*. Soil Biol. Biochem. 13:475-480.

Ames, P., and Bergman, K. 1981. Competitive advantage provided by bacterial motility in the formation of nodules by *Rhizobium meliloti*. J. Bacteriol. 148:728-729.

Araujo, R. S., and Handelsman, J. 1989. Nodulation competitiveness of *Rhizobium leguminosarum* bv. *phaseoli* mutants with altered cell surface characteristics. (Abstr.) Page 72 in: Proc. North Am. Symbiotic Nitrogen Fixation Conf., 12th; 1989 July 3–Aug. 3; Dep. of Genetics, Iowa State Univ. of Sci. and Technol., Ames. Available from Iowa State Univ.

Barry, G. F. 1988. A broad-host-range shuttle system for gene insertion into the chromosomes of Gram-negative bacteria. Gene 71:75-84.

Bhagwat, A. A., Sloger, C., and Keister, D. L. 1989. Isolation of extracellular polysaccharide defective mutants of *B. japonicum* 61A89 and USDA 438. (Abstr.) Page 97 in: Proc. North Am. Symbiotic Nitrogen Fixation Conf., 12th; 1989 July 3–Aug. 3; Dep. of Genetics, Iowa State Univ. of Sci. and Technol., Ames. Available from Iowa State Univ.

Bhuvaneswari, T. V., Bhagwat, A. A., and Bauer, W. D. 1981. Transient susceptibility of root cells in four common legumes to nodulation by rhizobia. Plant Physiol. 68:1144-1149.

Bjourson, A. J., and Cooper, J. E. 1988. Isolation of *Rhizobium loti* strain-specific sequences by subtraction hybridization. Appl. Environ. Microbiol. 54:2852-2855.

Burn, J. E., Hamilton, W. D., Wootton, J. C., and Johnston, A. W. B. 1989. Single and multiple mutations affecting properties of the regulatory gene *nodD* of *Rhizobium*. Mol. Microbiol. 3:1567-1577.

Burn, J., Rossen, L., and Johnston, A. W. B. 1987. Four classes of mutations in the *nodD* gene of *Rhizobium leguminosarum* biovar *viciae* that affect its ability to autoregulate and/or activate other *nod* genes in the presence of flavonoid inducers. Genes Dev. 1:456-464.

Cannon, F. C., Beynon, J., Hankinson, T., Kwiatkowski, R., Legocki, R. P., Ratcliffe, H., Ronson, C., Szeto, W., and Williams, M. 1988. Increasing biological nitrogen fixation by genetic manipulation. Pages 735-740 in: Nitrogen Fixation: Hundred Years After. H. Bothe, F. J. de Bruijn, and W. E. Newton, eds. Gustav Fischer, Stuttgart.

Cooper, J. E., Bjourson, A. J., and Thompson, J. K. 1987. Identification of *Lotus* rhizobia by direct DNA hybridization of crushed root nodules. Appl. Environ. Microbiol. 53:1705-1707.

Cregan, P. B., and Keyser, H. H. 1986. Host restriction of nodulation by *Bradyrhizobium japonicum* strain USDA 123 in soybean. Crop Sci. 26:911-916.

Cregan, P. B., Keyser, H. H., and Sadowsky, M. J. 1989a. Soybean genotype restricting nodulation of a previously unrestricted serocluster 123 bradyrhizobia. Crop Sci. 29:307-312.

Cregan, P. B., Keyser, H. H., and Sadowsky, M. J. 1989b. Host genetic control of restricted nodulation of specific serocluster 123 *Bradyrhizobium japonicum*. (Abstr.) Page 12 in: Proc. North Am. Symbiotic Nitrogen Fixation Conf., 12th; 1989 July 3–Aug. 3; Dep. of Genetics, Iowa State Univ. of Sci. and Technol., Ames. Available from Iowa State Univ.

Cunningham, S. D., Kollmeyer, W. D., and Stacey, G. 1989. The selection and use of exogenously applied chemicals to control competition for nodule formation between *Bradyrhizobium japonicum* strains. (Abstr.) Page 26 in: Proc. North Am. Symbiotic Nitrogen Fixation Conf., 12th; 1989 July 3–Aug. 3; Dep. of Genetics, Iowa State Univ. of Sci. and Technol., Ames. Available from Iowa State Univ.

Davis, E. O., Evans, I. J., and Johnston, A. W. B. 1988. Identification of *nodX*, a gene that allows *Rhizobium leguminosarum* biovar *viciae* strain TOM to nodulate Afghanistan peas. Mol. Gen. Genet. 212:531-535.

Demezas, D. H., and Bottomley, P. J. 1984. Identification of two dominant serotypes of *Rhizobium trifolii* in root nodules of uninoculated field-grown subclover. Soil Sci. Soc. Am. J. 48:1067-1071.

Demezas, D. H., Watson, J. M., Reardon, T. B., and Gibson, A. H. 1988. A molecular approach to *Rhizobium* ecology. Pages 196-197 in: Molecular Genetics of Plant-Microbe Interactions, 1988. R. Palacios and D. P. S. Verma, eds. American Phytopathological Society, St. Paul, MN.

Djordjevic, M. A., Innes, R. W., Wijffelman, C. A., Schofield, P. R., and Rolfe, B. G. 1987. Clovers secrete specific phenolic compounds which either stimulate or repress *nod* gene expression in *Rhizobium trifolii*. EMBO J. 6:1173-1179.

Doherty, D., Leigh, J. A., Glazebrook, J., and Walker, G. C. 1988. *Rhizobium meliloti* mutants that overproduce the *R. meliloti* acidic calcofluor-binding exopolysaccharide. J. Bacteriol. 170:4249-4256.

Dowling, D. N., and Broughton, W. J. 1986. Competition for nodulation of legumes. Annu. Rev. Microbiol. 40:131-157.

Dowling, D. N., Samrey, U., Stanley, J., and Broughton, W. J. 1987. Cloning of *Rhizobium leguminosarum* genes for competitive nodulation blocking on peas. J. Bacteriol. 169:1345-1348.

Dowling, D. N., Stanley, J., and Broughton, W. J. 1989. Competitive nodulation blocking of Afghanistan pea is determined by *nodDABC* and *nodFE* alleles in *Rhizobium leguminosarum*. Mol. Gen. Genet. 216:170-174.

Dunigan, E. P., Bollich, P. K., Hutchinson, R. L., Hicks, P. M., Zaunbrecher, F. C., Scott, S. G., and Mowers, R. P. 1984. Introduction and survival of an inoculant strain of *Rhizobium japonicum* in soil. Agron. J. 76:463-466.

Egelhoff, T. T., and Long, S. R. 1985. *Rhizobium meliloti* nodulation genes: Identification of *nodDABC* gene products, purification of *nodA* protein, and expression of *nodA* in *Rhizobium meliloti*. J. Bacteriol. 164:591-599.

Evans, H. J., Harker, A. R., Papen, H., Russell, S. A., Hanus, F. J., and Zuber, M. 1987. Physiology, biochemistry, and genetics of the uptake hydrogenase in rhizobia. Annu. Rev. Microbiol. 41:335-361.

Evans, H. J., Hanus, F. J., Haugland, R. A., Cantrell, M. A., Xu, L.-S., Russell, S. A., Lambert, G. R., and Harker, A. R. 1985. Hydrogen recycling in nodules affects nitrogen fixation and growth of soybeans. Pages 935-942 in: Proc. World Soybean Res. Conf., 3rd; 1984; Iowa State Univ. of Sci. and Technol., Ames. Westview Press, Boulder, CO.

Finan, T. M., Hirsch, A. M., Leigh, J. A., Johansen, E., Kuldau, G. A., Deegan, S., Walker, G. C., and Signer, E. R. 1985. Symbiotic mutants of *Rhizobium meliloti* that uncouple plant from bacterial differentiation. Cell 40:869-877.

Firmin, J. L., Wilson, K. E., Rossen, L., and Johnston, A. W. B. 1986. Flavonoid activation of nodulation genes in *Rhizobium* reversed by other compounds present in plants. Nature 324:90-92.

Fox, J. L. 1989. Contemplating suicide genes in the environment. ASM News 55:259-261.

Franco, A. A., and Vincent, J. M. 1976. Competition amongst rhizobial strains for the colonization and nodulation of two tropical legumes. Plant Soil 45:27-48.

Gloudemans, T., and Bisseling, T. 1989. Plant gene expression in early stages of *Rhizobium*-legume symbiosis. Plant Sci. 65:1-14.

Graham, P. H., and McDermott, T. R. 1989. *Bradyrhizobium*, root and rhizosphere interactions to improve soybean productivity. Pages 1–18 in: The Physiology, Biochemistry, Nutrition and Bioengineering of Soybeans: Implications for Future Management. R. D. Munson, ed. Potash and Phosphate Institute, Atlanta, GA.

Hahn, M., and Hennecke, H. 1988. Cloning and mapping of a novel nodulation region from *Bradyrhizobium japonicum* by genetic complementation of a deletion mutant. Appl. Environ. Microbiol. 54:55-61.

Hahn, M., and Studer, D. 1986. Competitiveness of a *nif*$^-$ *Bradyrhizobium japonicum* mutant against the wild-type strain. FEMS Microbiol. Lett. 33:143-148.

Handelsman, J., Ugalde, R. A., and Brill, W. J. 1984. *Rhizobium meliloti* competitiveness and the alfalfa agglutinin. J. Bacteriol. 157:703-707.

Hanus, F. J., Albrecht, S. L., Zablotowicz, R. M., Emerich, D. W., and Russell, S. A. 1981. Yield and N content of soybean seed as influenced by *Rhizobium japonicum* inoculants possessing the hydrogenase characteristic. Agron. J. 73:368-372.

Heron, D. S., and Pueppke, S. G. 1987. Regulation of nodulation in the soybean-*Rhizobium* symbiosis. Strain and cultivar variability. Plant Physiol. 84:1391-1396.

Hodgson, A. L. M., and Roberts, W. P. 1983. DNA colony hybridization to identify *Rhizobium* strains. J. Gen. Microbiol. 129:207-212.

Hodgson, A. L. M., Roberts, W. P., and Waid, J. S. 1985. Regulated nodulation of *Trifolium subterraneum* inoculated with bacteriocin-producing strains of *Rhizobium trifolii*. Soil Biol. Biochem. 17:475-478.

Holben, W. E., Jansson, J. K., Chelm, B. K., and Tiedje, J. M. 1988. DNA probe method for the detection of specific microorganisms in the soil bacterial community. Appl. Environ. Microbiol. 54:703-711.

Jenkins, M. B., and Bottomley, P. J. 1985. Evidence for a strain of *Rhizobium meliloti* dominating the nodules of alfalfa. Soil Sci. Soc. Am. J. 49:326-328.

Johansen, E., Stencel, L., and Appelbaum, E. 1988. Isolation of *Bradyrhizobium japonicum* mutants with hyperinducible common *nod* genes. Page 464 in: Nitrogen Fixation: Hundred Years After. H. Bothe, F. J. de Bruijn, and W. E. Newton, eds. Gustav Fischer, Stuttgart.

Johnston, A. W. B., and Beringer, J. E. 1976. Mixed inoculations with effective and ineffective strains of *Rhizobium leguminosarum*. J. Appl. Bacteriol. 40:375-380.

Kapulnik, Y., Joseph, C. M., and Phillips, D. A. 1987. Flavone limitations to root nodulation and symbiotic nitrogen fixation in alfalfa. Plant Physiol. 84:1193-1196.

Kosslak, R. M., Bookland, R., Barkei, J., Paaren, H. E., and Appelbaum, E. R. 1987. Induction of *Bradyrhizobium japonicum* common *nod* genes by isoflavones isolated from *Glycine max*. Proc. Natl. Acad. Sci. USA 84:7428-7432.

Kosslak, R. M., Schroth, R., Paaren, H. E., and Appelbaum, E. R. 1989. Differential expression of *nodABC* genes in *Bradyrhizobium japonicum* by flavonoid compounds. (Abstr.) Page 13 in: Proc. North Am. Symbiotic Nitrogen Fixation Conf., 12th; 1989 July 3–Aug. 3; Dep. of Genetics, Iowa State Univ. of Sci. and Technol., Ames. Available from Iowa State Univ.

Labandera, C. A., and Vincent, J. M. 1975. Competition between an introduced strain and native Uruguayan strains of *Rhizobium trifolii*. Plant Soil 42:327-347.

Lambert, G. R., Harker, A. R., Cantrell, M. A., Hanus, F. J., Russell, S. A., Haugland, R. A., and Evans, H. J. 1987. Symbiotic expression of cosmid-borne *Bradyrhizobium japonicum* hydrogenase genes. Appl. Environ. Microbiol. 53:422-428.

Leigh, J. A., Signer, E. R., and Walker, G. C. 1985. Exopolysaccharide deficient mutants of *Rhizobium meliloti* that form ineffective nodules lacking nitrogenase. Proc. Natl. Acad. Sci. USA 82:6231-6235.

Long, S., McCune, S., and Walker, G. C. 1988. Symbiotic loci of *Rhizobium meliloti* identified by random Tn*phoA* mutagenesis. J. Bacteriol. 170:4257-4265.

Long, S. R. 1989. *Rhizobium*-legume nodulation: Life together in the underground. Cell 56:203-214.

Long, S. R., Buikema, W. J., and Ausubel, F. M. 1982. Cloning of *Rhizobium meliloti* nodulation genes by direct complementation of Nod$^-$ mutants. Nature 298:485-488.

Maier, R., and Brill, W. J. 1978. Mutant strains of *Rhizobium japonicum* with increased ability to fix nitrogen for soybean. Science 201:448-450.

Manoil, C., and Beckwith, J. 1985. Tn*phoA* transposon probe for protein export signals. Proc. Natl. Acad. Sci. USA 82:8129-8133.

Marques Pinto, C., Yao, P. Y., and Vincent, J. M. 1974. Nodulating competitiveness amongst strains of *Rhizobium meliloti* and *R. trifolii*.

Aust. J. Agri. Res. 25:317-329.
Martinez, E., Romero, D., and Palacios, R. 1990. The *Rhizobium* genome. CRC Crit. Rev. Plant Sci. 9:59-93.
Maxwell, C. A., Hartwig, U. A., Joseph, C. M., and Phillips, D. A. 1989. A chalcone and two related flavonoids released from alfalfa roots induce *nod* genes of *Rhizobium meliloti*. Plant Physiol. 91:842-847.
McLoughlin, T. J., Merlo, A. O., Satola, S. W., and Johansen, E. 1987. Isolation of competition-defective mutants of *Rhizobium fredii*. J. Bacteriol. 169:410-413.
Mellor, H. Y., Glenn, A. R., Arwas, R., and Dilworth, M. J. 1987. Symbiotic and competitive properties of motility mutants of *Rhizobium trifolii* TA1. Arch. Microbiol. 148:34-39.
Moawad, H. A., Ellis, W. R., and Schmidt, E. L. 1984. Rhizosphere response as a factor in competition among three serogroups of indigenous *Rhizobium japonicum* for nodulation of field grown soybeans. Appl. Environ. Microbiol. 47:607-612.
Nicol, H., and Thornton, H. G. 1941. Competition between related strains of nodule bacteria and its influence on infection of the legume host. Proc. R. Soc. London, B 130:32-59.
O'Gara, F., Boesten, B., and Fanning, S. 1988. The development and exploitation of "marker genes" suitable for risk evaluation studies on the release of genetically engineered microorganisms in soil. Pages 50-64 in: Risk Assessment for Deliberate Release. W. Klingmuller, ed. Springer-Verlag, Berlin.
Oliveira, L. A., and Graham, P. H. 1990. Speed of nodulation and competitive ability among strains of *Rhizobium leguminosarum* bv. *phaseoli*. Arch. Microbiol. 153:311-315.
Paau, A. S. 1989. Improvement of *Rhizobium* inoculants. Appl. Environ. Microbiol. 55:862-865.
Peters, N. K., Frost, J. W., and Long, S. R. 1986. A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. Science 233:977-980.
Peters, N. K., and Long, S. R. 1988. *Rhizobium meliloti* nodulation gene inducers and inhibitors. Plant Physiol. 88:396-400.
Pierce, M., and Bauer, W. D. 1983. A rapid regulatory response governing nodulation in soybean. Plant Physiol. 73:286-290.
Poulsen, L. K., Larsen, N. W., Molin, S., and Andersson, P. 1989. A family of genes encoding a cell-killing function may be conserved in all Gram-negative bacteria. Mol. Microbiol. 3:1463-1472.
Redmond, J. W., Batley, M., Djordjevic, M. A., Innes, R. W., Kuempel, P. L., and Rolfe, B. G. 1986. Flavones induce expression of nodulation genes in *Rhizobium*. Nature 323:632-634.
Robert, F. M. 1989. *Rhizobium* interspecies and interstrain differentiation using biotinylated DNA probes. (Abstr.) Page 86 in: Proc. North Am. Symbiotic Nitrogen Fixation Conf., 12th; 1989 July 3–Aug. 3; Dep. of Genetics, Iowa State Univ. of Sci. and Technol., Ames. Available from Iowa State Univ.
Robinson, A. C. 1969a. Competition between effective and ineffective strains of *Rhizobium trifolii* in the nodulation of *Trifolium subterraneum*. Aust. J. Agri. Res. 20:827-841.
Robinson, A. C. 1969b. Host selection for effective *Rhizobium trifolii* by red clover and subterraneum clover in the field. Aust. J. Agri. Res. 20:1053-1060.
Rodriguez-Quinones, F., Sadowsky, M. J., Cregan, P. B., and Keyser, H. H. 1989. Cloning and characterization of the GSN region from *Bradyrhizobium japonicum* strain DE3-1a. (Abstr.) Page 15 in: Proc. North Am. Symbiotic Nitrogen Fixation Conf., 12th; 1989 July 3–Aug. 3; Dep. of Genetics, Iowa State Univ. of Sci. and Technol., Ames. Available from Iowa State Univ.
Sadowsky, M. J., Cregan, P. B., and Keyser, H. H. 1989. A DNA probe for use in determining restricted nodulation among *Bradyrhizobium japonicum* serocluster 123 field isolates. (Abstr.) Page 55 in: Proc. North Am. Symbiotic Nitrogen Fixation Conf., 12th; 1989 July 3–Aug. 3; Dep. of Genetics, Iowa State Univ. of Sci. and Technol., Ames. Available from Iowa State Univ.
Sadowsky, M. J., Tully, R. E., Cregan, P. B., and Keyser, H. H. 1987. Genetic diversity in *Bradyrhizobium japonicum* serogroup 123 and its relation to genotype-specific nodulation of soybean. Appl. Environ. Microbiol. 53:2624-2630.
Sargent, L., Huang, S. Z., Rolfe, B. G., and Djordjevic, M. A. 1987. Split-root assays using *Trifolium subterraneum* show that *Rhizobium* infection induces a systemic response that can inhibit nodulation of another invasive *Rhizobium* strain. Appl. Environ. Microbiol. 53:1611-1619.
Schmidt, E. L., and Robert, F. M. 1985. Recent advances in the ecology of *Rhizobium*. Pages 379-385 in: Nitrogen Fixation Research Progress. H. J. Evans, P. J. Bottomley, and W. E. Newton, eds. Martinus Nijhoff, Boston.
Schmidt, E. L., Zidwick, M. J., and Abede, H. H. 1986. *Bradyrhizobium japonicum* serocluster 123 and diversity among member isolates. Appl. Environ. Microbiol. 51:1212-1215.
Schwinghamer, E. A., and Belkengren, R. P. 1968. Inhibition of rhizobia by a strain of *Rhizobium trifolii*: Some properties of the antibiotic and of the strain. Arch. Mikrobiol. 64:130-145.
Scott, D. B., Hennecke, H., and Lim, S. T. 1979. The biosynthesis of nitrogenase MoFe protein polypeptides in free-living cultures of *Rhizobium japonicum*. Biochim. Biophys. Acta 565:365-378.
Shishido, M., and Pepper, I. L. 1990. Identification of dominant indigenous *Rhizobium meliloti* by plasmid profiles and intrinsic antibiotic resistance. Soil Biol. Biochem. 22:11-16.
Somasegaran, P., and Hoben, H. J. 1985. Methods in legume-*Rhizobium* technology. Page 367 in: NifTAL Project, Dep. of Agron. and Soil Sci., Univ. of Hawaii, Honolulu.
Spaink, H. P., Okker, R. J. H., Wijffelman, C. A., Tak, T., Roo, L. G., Pees, E., van Brussel, A. A. N., and Lugtenberg, B. J. J. 1989a. Symbiotic properties of rhizobia containing a flavonoid-independent hybrid *nodD* product. J. Bacteriol. 171:4045-4053.
Spaink, H. P., Wijffelman, C. A., Okker, R. J. H., and Lugtenberg, B. J. J. 1989b. Localization of functional regions of the *Rhizobium nodD* product using hybrid *nodD* genes. Plant Mol. Biol. 12:59-73.
Steffan, R. J., and Atlas, R. M. 1988. DNA amplification to enhance detection of genetically engineered bacteria in environmental samples. Appl. Environ. Microbiol. 54:2185-2191.
Stephens, P. M., and Cooper, J. E. 1988. Variation in speed of infection of "no root hair zone" of white clover and nodulating competitiveness among strains of *Rhizobium trifolii*. Soil Biol. Biochem. 20:465-470.
Taylor, R. K., Manoil, C., and Mekalanos, J. J. 1989. Broad-host-range vectors for delivery of Tn*phoA*: Use in genetic analysis of secreted virulence determinants of *Vibrio cholerae*. J. Bacteriol. 171:1870-1878.
Tiedje, J. M., Colwell, R. K., Grossman, Y. L., Hodson, R. E., Lenski, R. E., Mack, R. N., and Regal, P. J. 1989a. The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. Ecology 70:298-315.
Tiedje, J. M., Holben, W. E., and Jansson, J. K. 1989b. Fate of genes and genetically engineered microorganisms in soil and rhizosphere. Page 23 in: The Rhizosphere and Plant Growth. Beltsville Symp. Agri. Res., 14th. Allanheld, Osmun & Co., Totowa, NJ.
Trevors, J. T., and van Elsas, J. D. 1989. A review of selected methods in environmental microbial genetics. Can. J. Microbiol. 35:895-902.
Triplett, E. W. 1988. Isolation of genes involved in nodulation competitiveness from *Rhizobium leguminosarum* bv. *trifolii* T24. Proc. Natl. Acad. Sci. USA 85:3810-3814.
Triplett, E. W. 1990. Construction of a symbiotically effective strain of *Rhizobium leguminosarum* bv. *trifolii* with increased nodulation competitiveness. Appl. Environ. Microbiol. 56:98-103.
Triplett, E. W., and Barta, T. M. 1987. Trifolitoxin production and nodulation are necessary for the expression of superior nodulation competitiveness by *Rhizobium leguminosarum* bv. *trifolii* strain T24 on clover. Plant Physiol. 85:335-342.
Triplett, E. W., Schink, M. J., and Noeldner, K. L. 1989. Mapping and subcloning of the trifolitoxin production and resistance genes from *Rhizobium leguminosarum* bv. *trifolii* T24. Mol. Plant-Microbe Interact. 2:202-208.
Vincent, J. M., and Waters, L. M. 1953. The influence of the host on competition amongst clover root-nodule bacteria. J. Gen. Microbiol. 9:357-370.
Weaver, R. W., and Frederick, L. R. 1974. Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. I. Greenhouse studies. Agron. J. 66:229-232.
Wheatcroft, R., and Watson, R. J. 1988. A positive strain identification method for *Rhizobium meliloti*. Appl. Environ. Microbiol. 54:574-576.
Williams, L. E., and Phillips, D. A. 1983. Increased soybean productivity with a *Rhizobium japonicum* mutant. Crop Sci. 23:246-250.
Williams, M. K., Cannon, F., McLean, P., and Beynon, J. 1988. Vector for the integration of genes into a defined site in the *Rhizobium meliloti* genome. Pages 198-199 in: Molecular Genetics of Plant-Microbe Interactions, 1988. R. Palacios and D. P. S. Verma, eds. American Phytopathological Society, St. Paul, MN.

Zaat, S. A. J., Wijffelman, C. A., Spaink, H. P., van Brussel, A. A. N., Okker, R. J. H., and Lugtenberg, B. J. J. 1987. Induction of the *nodA* promoter of *Rhizobium leguminosarum* Sym plasmid pRL1JI by plant flavanones and flavones. J. Bacteriol. 169:198-204.

Zdor, R. E., and Pueppke, S. G. 1989. Nodulation competitiveness of Tn*5*-induced mutants of *Rhizobium fredii* 208 that are altered in motility and extracellular polysaccharide production. (Abstr.) Page 33 in: Proc. North Am. Symbiotic Nitrogen Fixation Conf., 12th; 1989 July 3–Aug. 3; Dep. of Genetics, Iowa State Univ. of Sci. and Technol., Ames. Available from Iowa State Univ.