DETERMINANTS OF NODULATION COMPETITIVENESS IN *RHIZOBIUM ETLI*

Final Report

For Period September 30, 1996 - September 29, 1999

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January 2000

Prepared for DOE Field Office, Chicago

THE U.S. DEPARTMENT OF ENERGY
AWARD NO. DE-FG02-96ER20248

DISCLAIMER

Nitrogen is a major limiting nutrient in crop production. Chemical fertilizers, which are used extensively to meet crop nitrogen requirements, contribute to the high energy inputs of modern agriculture and cause human health and environmental problems through run-off and groundwater contamination. Legumes and their bacterial associates have long been used in crop rotations to replenish soil nitrogen, but effective and reliable biological nitrogen fixation for some legumes, such as beans, is prevented by the lack of nodulation competitiveness of many *Rhizobium* strains used as inoculants. The result is that the inoculant strains will not occupy the host's nodules and no benefit will be derived from inoculation. Many indigenous soil strains of *Rhizobium etli* bv. *phaseoli*, the symbiont of bean, nodulate but fix little or no nitrogen and therefore the nodulation competitiveness problem is significant for achieving maximum nitrogen benefit from bean crops. Nodulation competitiveness is poorly understood and has been the focus of few mechanistic studies even though it is the single greatest barrier to extracting benefit for agriculture from biological nitrogen fixation. This project was directed toward developing an understanding of the basis of nodulation competitiveness.

RosR is a determinant of nodulation competitiveness and cell surface characteristics of *Rhizobium etli* and has sequence similarity to a family of transcriptional repressors. To understand how RosR affects these phenotypes, we mutagenized a *rosR*$^-$ derivative of *R. etli* strain CE3 with a mini-Tn*5* that contains a promoterless *gusA* at one end, which acts as a transcriptional reporter. Using a mass-mating technique, we introduced *rosR* into each mutant *in trans*, and screened for mutants that expressed different levels of GUS activity in the presence and absence of *rosR*. A screen of 18,000 mutants identified 52 insertions in genes negatively regulated and one in a gene positively regulated by RosR. Nucleotide sequence analysis of the regions flanking the insertions suggests that RosR regulates genes of diverse function including those involved in polysaccharide production, carbohydrate metabolism, and those in a region containing sequence similarity to *virC1* and *virD3* from *Agrobacterium tumefaciens*. Two of the mutants produced colonies with altered morphology and were more competitive in nodulation than was CE3Δ*rosR*, the *rosR* parent. One mutant that contained an insertion in a gene with similarity to *exsH* of *Sinorhizobium meliloti* did not nodulate the plant host *Phaseolus vulgaris* without *rosR*. These results indicate that RosR directly or indirectly influences expression of diverse genes in *R. etli*, some of which affect the cell surface and nodulation competitiveness.

We further characterized the *virC1*- and *virDC*-containing region to determine whether other genes with similarity to *vir* genes are present, and to determine whether RosR, like Ros in *A. tumefaciens*, directly regulates some of these genes, which are important for interactions with the plant host in *A. tumefaciens*. We found the region contains ORFs with similarity to *virB9*, *virB10*, *virB11*, *virG*, *virC1*, *virC2*, *virE1*, *virE2*, and *virE3* of *A. tumefaciens*. The region with similarity to *virD3* is not contained within a single ORF and is adjacent to a region likely to be an insertion element. *In vitro* gel mobility shift assays demonstrated that purified RosR protein bound DNA upstream of the *virC* operon from *R. etli*, indicating that RosR was directly involved in transcriptional repression of this region. Southern blot analyses revealed that the *vir* genes were located on the smallest megaplasmid of *R. etli*, plasmid pa, which was self-transmissible between *R. etli* and *A. tumefaciens*, while the *rosR* gene was located on the *R. etli* chromosome. Site-directed mutations in the *virG* and *virE2* ORFs did not affect the symbiotic behavior of *R. etli* on the plant host *Phaseolus vulgaris*.