**Research Note**

**Rhizobium etli CE3 Carries vir Gene Homologs on a Self-Transmissible Plasmid**

M. A. Bittinger,1,2 J. A. Gross,1 J. Widom,3 J. Clardy,3 and J. Handelsman1

1Department of Plant Pathology, University of Wisconsin-Madison, Madison 53706, U.S.A.; 2Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison 53706, U.S.A.; 3Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, U.S.A.

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RosR is a transcriptional regulator important for determining cell-surface characteristics and nodulation competitiveness in *Rhizobium etli* CE3. We identified a 15-kb region that contains genes with similarity to members of the *virB*, *virC*, *virG*, and *virE* operons of *Agrobacterium tumefaciens* and demonstrated that RosR directly regulates one operon in this region. These genes were located on plasmid pa of *R. etli* CE3, which is self-transmissible between *R. etli* and *A. tumefaciens*.

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**Corresponding author:** Jo Handelsman, Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Drive, Madison 53706, U.S.A. Telephone: 1-608-263-8783; Fax: 1-608-262-8643; E-mail: joh@plantpath.wisc.edu
on plasmid pa, was mixed with *A. tumefaciens* GMI9023, spectinomycin-resistant derivatives of GMI9023 arose at a frequency of $4.4 (\pm 0.4) \times 10^{-4}$, which is similar to previously reported frequencies of transfer (Noel et al. 1984). The presence of plasmid pa in the transconjugants was confirmed by Southern analysis (Fig. 2). Additionally, GMI9023 carrying only plasmid pa transmitted spectinomycin resistance back to *R. etli* (data not shown), indicating that plasmid pa is self-transmissible between members of this bacterial family.

Purified RosR protein specifically bound DNA sequences in the region upstream of the *virC1* ORF, further demonstrating the functional similarities between RosR and Ros. The 162-bp region immediately upstream of the *virC* ORF was generated by PCR (corresponding to nucleotides 6035 to 6196 of GenBank no. AF176227), labeled with digoxigenin, and used as a substrate for in vitro gel mobility shift assays with the DIG Luminescent Detection Kit (Roche, Indianapolis, IN) (Bittinger 1999). The mobility of this 162-bp fragment decreased in the presence of purified RosR and an excess of unlabeled competitor DNA, and the amount of the shifted DNA fragment was dependent on the amount of RosR added to the binding reaction (Fig. 3).

To determine whether these *vir* genes are involved in the symbiosis of *R. etli* CE3 with common bean, we constructed mutants of CE3 with either the *virG* or *virE2* genes disrupted, and screened those mutants for altered symbiotic phenotypes.

A promoterless *gusA* gene was introduced into the ORF of each of these genes by marker exchange with the counter-selectable suicide vector pJQ200KS (Quandt and Hynes 1993) (Fig. 1). Southern analysis of genomic DNA from these mutants confirmed the gene disruptions (Bittinger 1999). Both mutants formed nitrogen-fixing nodules on *Phaseolus vulgaris* cv. Black Turtle, and neither mutant displayed altered nodulation competitiveness when coinoculated in a 1:1 ratio with *R. etli* CE3013, a kanamycin-resistant derivative of CE3 unaffected in competitiveness (Beattie and Handelsman 1993; Bittinger 1999).

The findings of this study reinforce the similarity between the role of RosR in *R. etli* CE3 and that of Ros in *A. tumefaciens*.
ens. Additionally, this is the first report of a *Rhizobium* species carrying homologs of the *vir* genes. Although it is surprising to find homologs of the *vir* genes in a rhizobial species, they may no longer be involved in pathogenesis due to the transposition of the large insertion element. Because plasmid pa is self-transmissible, it seems likely that it is present as a result of horizontal transfer from other members of the *Rhizobiaceae*; however, we do not know if this plasmid provides a selective advantage to the bacterium in its saprophytic life in the soil. It would be interesting to identify the other genes present on this plasmid and ultimately determine whether the rhizobial *vir* homologs can functionally complement *vir* mutants of *A. tumefaciens*.

**LITERATURE CITED**


