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*Research Note*

# Inhibition of *nod* Gene Expression in *Bradyrhizobium japonicum* by Organic Acids

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**Significant inhibition of *nodD*$_1$ and *nodY* gene expression in *Bradyrhizobium japonicum* occurred when specific organic acids ( i.e., acetate, fumarate, L-malate, succinate, or α–ketoglutarate) were added. Gene expression was inhibited by 91% when acetate and L-malate were added together. Since organic acids are thought to be the primary carbon sources used by rhizobia in planta, these results suggest a mechanism by which *nod* gene expression can be inhibited in bacteroids.**

*Additional keywords:* gene regulation, nodulation.

*Rhizobium*, *Bradyrhizobium*, and *Azorhizobium* species are Gram negative soil bacteria capable of infecting and nodulating the roots of their host leguminous plants. In nodules, the bacteria divide and differentiate into bacteroids. Using carbon sources provided by the host plant, the bacteroids convert atmospheric nitrogen into ammonia, which is supplied to the host as a nitrogen source. The nodulation process requires specific bacterial and plant gene expression controlled via the mutual exchange of diffusible signals.

The bacterial genes required for infection and nodule formation are designated as *nod*, *nol*, or *noe* genes (Stacey 1995). In most cases, induction of *nod/nol* gene transcription requires the presence of a positive regulatory protein, NodD, and host plant–produced flavonoids (Schlaman et al. 1992; Hombrecher et al. 1984; Jacobs et al. 1985; Kondorosi et al. 1984; Downie et al. 1985; Kosslak et al. 1987; Sadowsky et al. 1988; Long 1989). For example, the *Bradyrhizobium japonicum nodD*$_1$ and *nodYABCSUIJnolMNO* operons are induced in the presence of the isoflavones genistein and daidzein (Banfalvi et al. 1988). Induction is mediated by the binding of NodD to a conserved promoter sequence, the *nod* box, and activation of transcription in the presence of the flavonoid inducer (Fisher et al. 1988; Fisher and Long 1992; Hong et al. 1987). An apparently unique situation exists in *B. japonicum* in which *nod* gene induction can occur in the absence of NodD. In this case, *nod* gene expression requires the *nodV* and *nodW* gene products (Sanjuan et al. 1994). NodV and NodW show similarity to membrane-bound sensors and transcriptional regulators of the two-component family of regulatory proteins (Göttfert et al. 1990). In addition, the *B. japonicum nolA* gene product has a repressive effect on the expression of *nodYABCSUIJ* and *nodD*$_1$ operons (Sanjuan et al. 1994).

Earlier reports showed that a low level of *nod* gene expression was sufficient for nodulation (Mulligan and Long 1985), while a high level of *nod* gene expression was detrimental to efficient nodulation (Knight et al. 1986). Therefore, it is not surprising that *nod* gene expression is tightly regulated. Although flavonoid *nod* gene inducers can be isolated from nodules (Karr et al. 1992), *nod* genes are not expressed in planta. Indeed, constitutive expression of the *R. leguminosarum* bv. *viciae nod* genes in planta produced a Fix$^-$ nodule (Knight et al. 1986). Schlaman et al. (1991) showed that the lack of *nod* gene expression in *R. leguminosarum* bv. *viciae* bacteroids was not due to a lack of NodD protein or to a lack of flavonoid inducers. Therefore, the mechanism preventing *nod* gene expression in planta remains to be determined.

The regulation of the *B. japonicum nod* genes has some similarity to *vir* gene regulation in *Agrobacterium tumefaciens* in that both systems involve members of the two-component regulatory family (i.e., *virAG* in *A. tumefaciens*; Stachel and Zambryski 1986). Ankenbauer and Nester (1990) showed that certain sugars can act synergistically with the plant phenolic metabolite, acetosyringone, to induce *A. tumefaciens vir* gene expression to a high level. The research described below was designed to determine if a similar regulation mechanism could also be involved in regulating *B. japonicum nod* gene expression.

The induction of *nodD*$_1$-*lacZ* and *nodY-lacZ* expression was measured in *B. japonicum* strains ZB977 (*nodY-lacZ*), Δ1267 (Δ *nodD*$_1$*D*$_2$*nolA*-; *nodY-lacZ*), SL101 (*nptII-lacZ*), LB100 (*nodY-lacZ*), SW100 (*nodD*$_1$-*lacZ*), and LB101 (*nodD*$_1$-*lacZ*) as previously described (Banfalvi et al. 1988). Strains ZB977, Δ1267, SW100, and SL101 are derived from wild-type strain USDA110, while strains LB100 and LB101 are derived from wild-type strain USDA135. These strains are resistant to tetracycline except for strain Δ1267, which is resistant to kanamycin, spectinomycin, and tetracycline. Cells were grown in *Rhizobium* defined, yeast extract (RDY) broth (So et al. 1987) with suitable antibiotics at 100 μg/ml. Log-phase cultures were diluted into 2 ml of minimal medium (pH 7) (Bergersen 1961) to a final $OD_{600}$ = 0.025. Minimal medium contains 0.4% glycerol as the sole carbon source. Genistein at the concentration indicated in each experiment was added to

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