Effects of Oxygen Levels on the Transcription of *nif* and *gln* Genes in *Bradyrhizobium japonicum*

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The transcription of genes that function in N₂ fixation (*nif*) and nitrogen assimilation (*glnII*) in *Bradyrhizobium japonicum* is coordinately induced in response to O₂ limitation as well as symbiotic development. We have determined the relative steady-state mRNA levels for the *nifH*, *nifDK* and *glnII* transcription units in bradyrhizobial cells grown under a variety of levels of aerobiosis and in cells isolated from soybean root nodules. All three transcripts are found in cells grown in a rich medium sparged with O₂ concentrations of 5% (v/v) or less. This expression is qualitatively similar to that observed for *Bradyrhizobium* during symbiotic development. Potential physiological mechanisms for the coordinate control of these genes are discussed.

INTRODUCTION

Rhizobia and bradyrhizobia reduce atmospheric N₂ in symbiotic association with their leguminous host plants. Development of symbiotic N₂ fixation is a complex process requiring the coordinate differentiation of both plant and bacterial cells. This differentiation results in the formation of morphologically and functionally distinct N₂-fixing endosymbiont bacteria termed 'bacteroids'. Very little of the ammonia produced from N₂ reduction by bacteroids is used to support bacterial growth (Brown & Dilworth, 1975; Stripf & Werner, 1978; Werner *et al.*, 1980); most of it is exported (Bergerson & Turner, 1967) to the plant where it is assimilated (Mifflin & Lea, 1976). The plant supplies reduced carbon compounds to the bacteria to meet the high energy demands of N₂ fixation and cell maintenance.

Nitrogen metabolism during asymbiotic N₂ fixation by bradyrhizobia is similar to that observed in bacteroids, as most of the fixed nitrogen is not used to support growth, but is instead exported (O'Gara & Shanmugan, 1976; Bergerson & Turner, 1978; Ludwig, 1980*a, b*). For at least some bradyrhizobial strains this failure to assimilate fixed nitrogen is partly due to an apparent decrease in glutamine synthetase (GS) activity (Ludwig, 1980*a, b*; Bergerson & Turner, 1978). Regulation of GS activity in rhizobia and bradyrhizobia is complicated by the fact that these bacteria contain two isoforms of the enzyme, GSI and GSII (Darrow & Knotts, 1977). The two distinct nitrogen-assimilatory enzymes are encoded by separate genes (Somerville & Kahn, 1983; Carlson & Chelm, 1986), and transcription of the genes is differentially regulated. Transcription of the gene encoding GSI (*glnA*) is constant under all conditions (Carlson *et al.*, 1985). This enzyme is regulated post-translationally via adenylation, such that activity decreases during growth in nitrogen-rich media (Bishop *et al.*, 1976; Ludwig, 1978). In contrast, no post-translational modification of GSII has been observed. Transcription of the gene encoding GSII (*glnII*) under aerobic growth conditions is controlled by a mechanism that resembles the Ntr system (Carlson *et al.*, 1987) of enteric bacteria (Magasanik, 1982; Gussin *et al.*, 1986); growth conditions that limit nitrogen source activate transcription of the *glnII* gene.

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‡ This paper is dedicated to the memory of Barry Chelm.

Abbreviation: GS, glutamine synthetase.

We are interested in understanding the mechanisms through which *gln* and *nif* gene expression is controlled in *Bradyrhizobium japonicum*, a soybean symbiont, during symbiotic N₂ fixation as well as in free-living cultures. Some bradyrhizobial strains can be induced to fix N₂ in free-living culture under specialized conditions (Keister, 1975; Pagan *et al.*, 1975; Kurz & Larue, 1975; McComb *et al.*, 1975; Tjepkema & Evans, 1975). These conditions include both provision of a source of fixed nitrogen (preferably glutamate) and the presence of O₂ as an electron acceptor for oxidative phosphorylation. The requirement for O₂ in these systems presents a difficulty as the nitrogenase enzyme is inactivated by O₂. The partial pressure of O₂ must be maintained at levels that are high enough to allow the oxidative phosphorylation levels necessary to provide the ATP required for N₂ reduction, and low enough to prevent the irreversible inactivation of nitrogenase (Mortenson & Thornley, 1979). We have chosen to simplify the analysis by directly analysing the effect of microaerobiosis on *nif* transcription. We have determined the relative steady-state mRNA levels for the *nifH*, *nifDK*, *glnA* and *glnII* transcription units in bradyrhizobial cells grown under varying levels of aerobiosis. The results indicate that *nifH*, *nifDK* and *glnII* transcription is coordinately induced in response to both symbiotic development and microaerobic growth.

METHODS

Bacterial strains, media and growth conditions. All of the experiments in this paper used a small-colony derivative of *B. japonicum* 311b110, which we term BJ110, isolated as described previously (Kuykendall & Elkan, 1976; Meyer & Pueppke, 1980). Bacteria were grown in YEM (0.04% yeast extract, 1% mannitol, 3 mM-K₂HPO₄, 0.8 mM-MgSO₄, 1.1 mM-NaCl), YEMN (YEM with 10 mM-KNO₃), or YEMNA (YEMN with 10 mM-NH₄Cl) media. Ten-litre cultures of *B. japonicum* were grown using Microferm fermenters (New Brunswick Scientific) agitated at 200 r.p.m. and sparged with mixtures of O₂ and N₂ at rates of 500 ml min⁻¹. Gas flow rates were controlled using thermal mass flowmeters (Brooks Instruments, model 5850 C) to provide the atmospheric O₂ concentrations reported. Inoculation was with 100 ml of a stationary-phase aerobic culture in YEM medium.

Nucleic acid techniques. Methods for RNA isolation were as described previously (Adams & Chelm, 1984).

mRNA abundances were measured by a quantitative S1 nuclease protection method in which multiple mRNAs could be simultaneously quantified from a single reaction. Single-stranded 5' end-labelled probes were synthesized by primer extension as follows. A gene-specific oligonucleotide (80 ng) was labelled by reaction with T4 polynucleotide kinase at 37 °C for 1 h in 100 mM-Tris/HCl, pH 7.5, 10 mM-MgCl₂, 6 mM-dithioerythritol and 100 µCi [γ -³²P]ATP (approx. 3000 Ci mmol⁻¹, 111 TBq mmol⁻¹). In the experiments with mixed probes, the specific activity of each 5' end-labelled oligomer was adjusted to approximately 250 d.p.m. pg⁻¹ with unlabelled oligonucleotide prior to the elongation reaction. The labelled primers were combined with 20 µg of the appropriate single-stranded M13 recombinant phage template DNA, ethanol-precipitated, suspended in 50 µl 10 mM-Tris/HCl, pH 8.5, and 10 mM-MgCl₂, heated for 5 min at 90 °C, and allowed to hybridize for 1 h at 37 °C. The hybridized oligonucleotide primer was extended using 3 units (Bethesda Research Laboratories) of the large fragment of *Escherichia coli* DNA polymerase (Klenow fragment) for 1 h at 37 °C in 100 µl of the same buffer plus 0.6 mM each of dATP, dCTP, dGTP and dTTP. The reaction was stopped by heating at 65 °C for 5 min. The partially double-stranded DNA was ethanol-precipitated and resuspended in the appropriate restriction endonuclease buffer. To prepare the probes specific for the *nifH* and *nifDK* promoters, the product of the extension reaction was digested with *Bst*NI for 3 h at 58 °C, ethanol-precipitated, and suspended in 200 µl 80% (v/v) formamide with tracking dyes. The *glnA* and *glnII* probes were prepared by digestion with *Sal*I for 3 h at 37 °C. After denaturation by incubation at 100 °C and purification by electrophoresis on 8.4 M-urea, 4% (w/v) polyacrylamide gels, the labelled, single-stranded probe was detected by autoradiography and eluted by the crush-and-soak method (Maxam & Gilbert, 1980.)

The following recombinant M13 phages were constructed for use in synthesizing single-stranded DNA hybridization probes: M13H has a 704 bp *Bam*HI–*Hind*III fragment from *nifH* and its upstream region (Adams & Chelm, 1984) in M13mp19; M13DK contains a 417 bp *Pst*I–*Sph*I fragment from the promoter region of the *nifDK* operon (Adams & Chelm, 1984) in M13mp19; M13glnII has the 2.1 kbp *Sal*I fragment of pBJ196 containing the *glnII* promoter region (Carlson & Chelm, 1986) in M13mp18; and M13glnA has a 391 bp *Sal*I fragment from the promoter region of *glnA* (Carlson *et al.*, 1985) in M13mp18. Oligonucleotides for primer extension were synthesized and purified as described previously (Carlson & Chelm, 1986). The sequences of each primer were as follows: 5'-TGCTCTCCATCAACCGA (*nifH*); 5'-CTTCACCCCGCAGTCCG (*nifDK*); 5'-CCCCTTTGGCTCTGCG (*glnA*); and 5'-CGACGCGAATTCCTTGA (*glnII*). The partially protected fragments from S1 analyses with each of the probes generated by elongation of these primers are 150, 200, 125, and 170 nucleotides long, respectively. For mixed-probe experiments, approximately 6 × 10⁻¹⁵ mol of each 5' end-labelled single-stranded fragment was included in the hybridization. For the driver RNA quantities used in these

experiments, this proved to be an excess of probe since increasing RNA concentrations resulted in a proportional increase in the amount of hybrid detected (data not shown). Hybridization, S1 digestion, and identification of partially protected products were as described previously (Adams & Chelm, 1984).

Isolation of bacteria from soybean root nodules. Total bacterial populations from frozen soybean nodules were prepared as described previously (Adams & Chelm, 1984). Separation of these bacteria into the three developmental fractions, as first described by Ching *et al.* (1977), was done by two rounds of centrifugation through discontinuous sucrose gradients using a zonal ultracentrifuge rotor (Beckman 14Ti) as described by Carlson *et al.* (1985).

RESULTS

Effects of O₂ concentration on B. japonicum gene expression

To study the role of O₂ limitation in regulating *nif* and *gln* gene expression for *B. japonicum*, we examined total cellular RNA isolated from *B. japonicum* cultures grown in standard nitrogen-rich media at a variety of O₂ concentrations. The results for one such set of experiments are shown in Fig. 1. The expression levels of the *nifH*, *nifDK*, *glnII* and *glnA* genes were monitored using a quantitative S1 protection assay system. Equal quantities of four single-stranded, 5' end-labelled DNA probes specific for the *nifH*, *nifDK*, *glnII* and *glnA* promoters were mixed and hybridized to total cellular RNA under conditions where the probes were in excess to the respective RNAs (see Methods). After hybridization, the mixtures were treated with S1 nuclease and the residual undigested DNA fragments then separated by gel electrophoresis. The hybridization probes were designed such that the protected fragments would be well resolved on the gel, allowing for the simultaneous measurement of transcript abundance for all four genes using a single RNA sample. The radioactive content for each band on the gel was determined directly (see Methods). RNA abundance is reported as c.p.m. protected DNA probe per µg driver RNA and is taken from a point within the linear range of the RNA:probe titration.

When *B. japonicum* was grown in a standard rich medium (YEMN) continuously sparged with air, no transcript for the *nifH*, *nifDK* or *glnII* genes was observed (Fig. 1, lane 1, and Table 1). In contrast, the transcript from the *glnA* gene was present, as reported previously (Carlson *et al.*, 1985). We have observed only small variations in *glnA* transcript levels between any two culture conditions. If *B. japonicum* was cultured in this same medium but continuously sparged with a mixture of N₂ and O₂ such that O₂ availability limited growth (5% O₂ or less in the YEMN experiments), then expression of all four transcription units was observed (Fig. 1, Table 1). Similar cultures were grown either with (YEMN) or without (YEM) added KNO₃. Under microaerobic conditions, KNO₃ helps to stimulate growth by serving as an alternative electron acceptor (Daniel *et al.*, 1982; M. L. Guerinot & B. K. Chelm, unpublished). A similar pattern for the relative levels of transcripts for any of the genes monitored was observed but the oxygen concentration dependence was shifted (Table 1). A three- to fourfold decrease in mRNA abundance for *nifH*, *nifDK* and *glnII* was observed as O₂ levels were increased above 1% in YEMN medium or above 0.1% in YEM medium. Despite the high levels of the two *nif* mRNAs observed in these cultures, no nitrogen-fixation activity, as measured by the acetylene reduction assay (Hardy *et al.*, 1968), was detectable, in agreement with previous reports (e.g. Scott *et al.*, 1979). Transcription of the *nifB* and *fixA* genes was also analysed (data not shown) and results identical to those obtained for *nifH*, *nifDK* and *glnII* were obtained.

Effect of ammonia on microaerobic growth and gene expression

Under aerobic growth conditions, *glnII* transcription is induced when growth is limited by a poor nitrogen source to carbon source balance (Carlson *et al.*, 1987). Presumably this activation is an attempt by the cells to maximize their capacity to assimilate nitrogen. Conversely, limitation of growth by carbon source does not induce *glnII* transcription, presumably because increased nitrogen assimilation would not increase growth. This control is similar to the Ntr control system which operates in the Enterobacteriaceae (Magasanik, 1982). To determine whether the microaerobic induction of *glnII* and *nif* gene transcription described above is under Ntr-like control, *B. japonicum* grown under microaerobic conditions in YEMN medium was tested for inhibition of *nif* and *glnII* transcription by the addition of 10 mM-NH₄Cl (YEMNA

Fig. 1. A typical abundance measurement for the *nifH*, *nifDK*, *glnII* and *glnA* transcripts. *B. japonicum* was grown under a variety of atmospheric O_2 concentrations. Quantitative analyses were by the S1 protection method described in Methods. The migration positions of the four protected fragments are indicated by P_D (*nifDK*), P_{II} (*glnII*), P_H (*nifH*) and P_A (*glnA*). The other major radioactive bands observed represent residual undigested probe DNA. The total cellular RNAs used in the hybridization reactions ($2 \mu\text{g}$ RNA in each) were isolated from *B. japonicum* cultured under the following conditions: 20% (v/v) O_2 in YEMN (lane 1); 0.2% O_2 in YEMNA (lane 2); 5% O_2 in YEMN (lane 3); 2% O_2 in YEMN (lane 4); 1% O_2 in YEMN (lane 5); 0.4% O_2 in YEMN (lane 6); 0.2% O_2 in YEMN (lane 7); and 0.1% O_2 in YEMN (lane 8). All media and growth conditions are described in Methods.

medium). The final cell density of microaerobic cultures grown in YEMNA media is more than twice that of cultures grown in YEMN (data not shown). Microaerobic induction of *glnII*, as well as *nifH* and *nifDK*, could therefore be due, directly or indirectly, to an Ntr-like response similar to aerobic *glnII* induction. When total cellular RNA was isolated from YEMNA cultures and assayed for *glnII*, *nifH*, *nifDK* and *glnA* expression, transcript levels for all but *glnA* were significantly and reproducibly reduced by the addition of ammonia (Table 1).

Table 1. *Effects of O₂ concentration on transcription of nif and gln genes*

Medium*	Probe	mRNA abundance†							
		O ₂ concentration (% v/v):							
		0.1	0.2	0.4	1.0	2.0	5.0	10.0	20.0
YEM	<i>nifDK</i>	380	56	36	78	—	—	—	—
	<i>nifH</i>	295	22	20	23	—	—	—	—
	<i>glnII</i>	101	27	9	45	—	—	—	—
	<i>glnA</i>	44	12	11	18	—	—	—	—
YEMN	<i>nifDK</i>	161	175	231	211	110	59	0	0
	<i>nifH</i>	133	72	157	151	59	75	0	0
	<i>glnII</i>	51	43	72	63	24	25	0	0
	<i>glnA</i>	34	23	34	37	29	35	31	18
YEMNA	<i>nifDK</i>	—	62	—	—	—	—	—	—
	<i>nifH</i>	—	42	—	—	—	—	—	—
	<i>glnII</i>	—	11	—	—	—	—	—	—
	<i>glnA</i>	—	21	—	—	—	—	—	—

* Media and growth conditions were as described in Methods. Carbon and nitrogen sources are abbreviated as follows: YE, yeast extract; M, mannitol; N, nitrate; A, ammonia.

† C.p.m. of hybridization probe protected from S1 nuclease digestion per µg of input total cellular RNA. This indicates the relative steady-state mRNA level for each gene. Data represent a mean value determined from two separate experiments. Variations between experiments were less than 20%. —, Not determined; 0, transcript levels were below the limit of detection of the assay.

Growth phase dependence of microaerobic nif and gln transcription

Previous studies on the role of microaerobiosis for nonsymbiotic *nif* gene induction in bradyrhizobia have relied upon the expression of nitrogenase enzyme activity. Those studies indicate that the presence of nitrogenase activity in free-living cultures of bradyrhizobia usually requires that the cells have stopped growing (e.g. Ludwig, 1984). For this reason, the cultures used for the experiments described above were grown for 2 d into stationary phase prior to harvesting (with the exceptions of the 1, 2, and 5% O₂ cultures, which were harvested earlier due to more rapid growth). To determine whether the microaerobic transcriptional induction of *glnII* and *nif* genes has a growth phase requirement like that for nitrogenase activity, cells were harvested at different times during microaerobic growth and the abundances of the *nif* and *gln* transcripts were determined. The *nifH*, *nifDK* and *glnII* transcripts were all found at near maximal levels within 24 h of inoculation and expression remained at high levels throughout the 120 h growth period analysed (data not shown). These cultures do not enter stationary growth phase for at least 72 h (not shown).

Transcription of nif and gln genes during symbiotic development

In order to evaluate the role of bacteroid development in *nif* and *gln* transcription, RNA was isolated from the bacteria within *B. japonicum* induced soybean root nodules and mRNA levels were determined. Two weeks after sowing and inoculation, transcript levels for both *nif* operons ranged up to four-fold higher in the nodule bacteria population than under any of the microaerobic growth conditions tested (Table 2). Transcript abundances for the *nifH* and *nifDK* genes were approximately equal under the microaerobic conditions. In contrast, bacterial RNA from soybean root nodules had an increased ratio of *nifH* to *nifDK* message, with *nifH* transcript about twice as abundant as that for *nifDK*. This was accomplished mainly through an increase in the steady-state mRNA levels for *nifH*. Whether these differences represent changes in transcription rate or changes in mRNA stability has not yet been determined. The *glnII* transcript levels in the nodule bacterial cell populations were approximately equal to those seen in microaerobic culture.

A method for fractionating the mixed populations of bacteria isolated from within soybean root nodules has been described (Ching *et al.*, 1977; Carlson *et al.*, 1985). This technique relies

Table 2. *mRNA abundance of nif and gln genes during symbiotic development*

RNA source	mRNA abundance*			
	<i>nifH</i>	<i>nifDK</i>	<i>glnII</i>	<i>glnA</i>
Bacteria from 14-d-old nodules	498	243	63	29
Bacteria from 28-d-old nodules	636	205	66	15
'Nodule bacteria'†	579	181	34	23
'Transforming bacteria'†	767	166	34	25
'Bacteroids'†	442	171	19	31

* C.p.m. of hybridization probe protected from S1 nuclease digestion per µg of input total cellular RNA. Data represent mean values determined from duplicate experiments. Variation between experiments was less than 20%.

† 'Developmental' cell types isolated from the total population of bacterial cells within a soybean root nodule as described in Methods.

on the change in buoyant density of bacteroids which occurs due to their accumulation of polyhydroxybutyrate (PHB) granules. The expression of certain other 'bacteroid-specific' properties (including nitrogenase enzyme activity) parallels this PHB accumulation (Ching *et al.*, 1977). mRNA levels were analysed from the three bacterial populations distinguished by this method (see Methods). No significant differences in *nifH*, *nifDK* or *glnII* gene expression patterns were observed between the three bacterial fractions (Table 2).

DISCUSSION

Under specialized cultural conditions, several bradyrhizobial strains, including *B. japonicum*, are able to reduce N₂ to ammonia in free-living culture (Keister, 1975; Pagan *et al.*, 1975; Kurz & Larue, 1975; McComb *et al.*, 1975; Tjepkema & Evans, 1975). Optimal conditions for asymbiotic N₂ fixation include the presence of organic acids as carbon and energy source, a growth-promoting nitrogen source, and extreme microaerobiosis. Microaerobic conditions are required for N₂ fixation because the nitrogenase enzyme is inactivated by intracellular O₂. Some O₂ is required for growth, however, as rhizobia are obligate aerobes. The highest free-living N₂ fixation rates by bradyrhizobia are achieved at extremely low O₂ tensions (Ludwig, 1984). We have directly studied the effect of O₂ limitation of growth on transcription. The requirements for the transcriptional induction of the genes encoding the nitrogenase structural polypeptides were found to be less complex than those for achieving nitrogenase enzyme activity. High levels of several *nif* mRNAs were found when O₂ levels were rate-limiting for growth. Extreme microaerobiosis was not required, nor were organic acids or any of the other specialized conditions previously reported (see references above) as necessary for the induction of nitrogenase activity.

In addition, we found that under these O₂-limited growth conditions transcription of the *glnII* gene, encoding GSII, was induced. Neither the *nif* genes nor *glnII* were transcribed in the same medium under fully aerobic conditions: they were induced only under conditions in which growth was directly affected by availability of O₂. Thus, expression of *glnII* appears to be activated coordinately with that of *nif* genes in response to O₂ limitation. Rao *et al.* (1978) reported that GSII activity decreased during microaerobiosis for some bradyrhizobia. The reported inhibition of GSII activity occurred at the same O₂ tension as that at which nitrogenase activity first became apparent (about 0.4% O₂ in the experiments described by Rao *et al.*, 1978). Since no nitrogenase activity was observed in the experiments we describe, it is possible that at lower O₂ concentrations *glnII* transcription could have been repressed. However, we observed similar levels of *glnII* transcript in total cellular RNA populations isolated from the N₂-fixing bacteria in soybean root nodules and from bacteria grown in microaerobic, non-N₂-fixing culture (Tables 1 and 2). If GSII is inactive in nodules and under microaerobic conditions, it is possible that expression of this enzyme is regulated by an unknown post-transcriptional mechanism.

As shown here, the relationship between O₂ and nitrogen limitation in bradyrhizobia is complex. Addition of ammonia to microaerobic cultures of *B. japonicum* in an amino-acid-based medium (YEMN) increases both growth rate and final cell density. Similar changes in growth properties can be achieved by increasing atmospheric O₂ levels, indicating that the amino acids present in yeast extract do not limit aerobic growth. These data imply that growth limitation during microaerobiosis results from nitrogen limitation brought about by changes in the cells' ability to utilize amino acids as a source of nitrogen. O₂ limitation has been shown to induce an ammonium export system in *Bradyrhizobium* sp. 32H1 (Ludwig, 1980*b*; Gober & Kashkett, 1983). If such a system were induced here, nitrogen limitation could occur even if amino acids were catabolized normally, as active ammonium export would compete with its assimilation. Addition of ammonium to these cultures might then partially relieve nitrogen limitation and stimulate growth by shifting the intracellular/extracellular ammonium balance.

Of primary interest is the mechanism that controls the transcriptional induction of *nif* and *glnII* genes. In aerobic culture, transcription of *glnII* is mediated by an Ntr-like system (Carlson *et al.*, 1987). When *B. japonicum* is grown aerobically under nitrogen-limiting conditions, transcription of *glnII* is induced and when nitrogen nutrition does not limit growth, *glnII* is not transcribed. Transcript levels for the *nif* and *glnII* genes normalized to the level of *glnA* transcript are reduced by the addition of ammonium (Table 1). This result indicates that nitrogen limitation might be responsible in part for causing an Ntr-like induction of *glnII* and/or *nif* genes. Similar results have been observed in studies of ammonia effects on nitrogenase activity (Ludwig, 1980*a, b*; Bergerson & Turner, 1978). Ntr-like control of nitrogenase expression has previously been described for bradyrhizobia (Ludwig, 1980*a, b*; Bergerson & Turner, 1978) and rhizobia (Szeto *et al.*, 1987). The *glnII* gene promoter shares sequence homology in the -10 to -25 region with *B. japonicum nif* promoters (Carlson *et al.*, 1987) and with promoters from other bacteria that are controlled by nitrogen availability (Ntr control; Magasanik, 1982; Ausubel, 1984; Gussin *et al.*, 1986). A gene, termed *nifA*, that is required for both microaerobic and symbiotic induction of *nif* and *glnII* gene expression in *B. japonicum* has been characterized (Fischer *et al.*, 1986; Adams, 1986). The *nifA* gene product acts on a DNA sequence that is found upstream of *nif* genes (Alvarez-Morales *et al.*, 1986) but not upstream of the *glnII* gene (Carlson *et al.*, 1987). Mutations in *nifA* have no effect on the aerobic Ntr-like control of *glnII* expression (unpublished results). Taken together with the partial ammonium repression of *glnII* expression, the implication of these data is that the role of the *nifA* product on the O₂ limitation response of the *glnII* gene is indirect, possibly acting through the Ntr system. Isolation of Ntr mutants of *B. japonicum* defective in the aerobic Ntr-like control of *glnII* will help to clarify the microaerobic Ntr-like response described here and to define the hierarchy of *nifA*- and Ntr-mediated control of *nif* gene expression in the bradyrhizobia.

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