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# Regulation of Nitrogenase-2 in *Azotobacter vinelandii* by Ammonium, Molybdenum, and Vanadium

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**Under diazotrophic conditions in the absence of molybdenum and in the presence of vanadium, *Azotobacter vinelandii* reduces $N_2$ to $NH_4^+$ by using nitrogenase-2, a V-containing enzyme complex encoded by *vnfH* (the gene for dinitrogenase reductase-2), and *vnfDGK* (the genes for dinitrogenase-2 subunits). Accumulation of the *vnfHorfFd* and *vnfDGK* transcripts occurred under Mo-deficient conditions in the presence and absence of V; however, in the case of *vnfDGK*, the protein products only accumulated in the presence of V. This suggests that V is required for translation of the *vnfDGK* transcripts. In addition, expression of *vnfH-lacZ* and *vnfD-lacZ* transcriptional fusions was only partially repressed in the presence of $NH_4^+$. Transcripts hybridizing with *vnfH* (1.4 and 1.0 kb), *vnfDG* (3.4 and 1.8 kb), and *vnfK* (3.4 kb) were detected in RNA extracted from wild-type cells cultured with $NH_4^+$ in the presence or absence of V. However, nitrogenase-2 subunits were not detected in extracts of cells derepressed for nitrogenase-2 in the presence of $NH_4^+$. These results indicate that this nitrogen source acts at the posttranscriptional level as well as at the transcriptional level. *vnf* transcripts were not detected in the presence of Mo (with or without $NH_4^+$).**

*Azotobacter vinelandii* harbors three genetically distinct nitrogenases (4). The expression of these nitrogenases is regulated by the availability of ammonium, molybdenum, and vanadium in the growth medium. Nitrogenase proteins do not accumulate in the presence of $NH_4^+$. Nitrogenase-1 (the conventional Mo-containing nitrogenase) is made in the absence of a fixed nitrogen source when Mo is available, whereas nitrogenase-2 (an alternative V-containing nitrogenase) accumulates under Mo-deficient conditions in the presence of V. In the absence of both Mo and V, an alternative nitrogenase that does not appear to contain either Mo or V (nitrogenase-3) accumulates (4; for a review, see reference 5). The regulatory genes *nifA*, *vnfA*, and *anfA* are required for the expression of nitrogenase-1, -2, and -3, respectively (14). NifA binds to an upstream activator sequence (5′-TGT-$N_{10}$-ACA-3′) and activates transcription of *nif* operons preceded by this sequence (6). The binding sites for VnfA and AnfA are unknown. An additional regulatory gene designated *nfrX* has been identified and is required for diazotrophic growth in the presence or absence of Mo (26). The nucleotide sequence of *nfrX* has been determined, and its predicted protein product has an N terminus that is highly homologous to the uridylyltransferase-uridylyl-removing enzyme encoded by *glnD* of *Escherichia coli* (10).

In contrast to the structural genes for nitrogenase-1 (*nifHDK*) and nitrogenase-3 (*anfHDGK*), the structural genes for nitrogenase-2 are organized into two separate transcriptional units, *vnfHorfFd* and *vnfDGK* (16). This allows dinitrogenase reductase-2 (the *vnfH* gene product) and dinitrogenase-2 (encoded by *vnfDGK*) to be expressed independently of each other. Thus, dinitrogenase reductase-2 is expressed under Mo-deficient conditions in the presence or absence of V, whereas dinitrogenase-2 is expressed only in the presence of V (3, 8, 24). Recently it was shown that dinitrogenase reductase-2 is required for expression of nitrogenase-3 (18).

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In the present study we investigate the effects of $NH_4^+$, Mo, and V on the transcription of *vnfHorfFd* and *vnfDGK* in wild-type and mutant strains of *A. vinelandii*.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The strains of *E. coli* and *A. vinelandii* used in this study are listed in Table 1. *E. coli* K-12 71-18, S17-1, and JC5466 were cultured in Luria-Bertani medium. *E. coli* C-600 was grown in Y broth (10 g of Bacto-Tryptone [Difco Laboratories], 2.5 g of NaCl, and 0.1 g of yeast extract per liter). Maltose was added to a concentration of 0.2%. *Azotobacter* strains were grown in modified Burk medium (29) as previously described (2). Ampicillin and tetracycline, when required, were added to final concentrations of 50 and 20 μg/ml, respectively. When required, $Na_2MoO_4$ and $V_2O_5$ were added, each at a final concentration of 1 μM. Fixed N was added as ammonium acetate (final concentration, 28 mM).

**Construction of *vnfA-lacZ*, *vnfH-lacZ*, and *vnfD-lacZ* transcriptional fusion strains.** Plasmids containing *vnfA*, *vnfH*, and *vnfD* were transformed into the suppressor-negative strain *E. coli* S17-1 and mutagenized with Tn*5*-B21 (27) as described by Joerger et al. (18). Plasmids containing Tn*5*-B21 insertions were linearized and transformed into *A. vinelandii* (23); then tetracycline-resistant ($Tc^r$) transformants were selected. $Tc^r$ transformants resulting from double-crossover events were identified by screening for ampicillin sensitivity. Strains containing *vnfA-lacZ*, *vnfH-lacZ*, or *vnfD-lacZ* fusions were grown in Mo- and V-deficient modified Burk medium to a cell density of approximately 150 Klett units. Cells were harvested by centrifugation, washed once with potassium phosphate buffer (pH 7.0, 7 mM), and resuspended in the appropriate media. These media were Mo-deficient Burk medium containing ammonium acetate (28 mM), N-free Burk medium containing 1 μM $Na_2MoO_4$ or 1 μM $V_2O_5$, Burk medium containing ammonium acetate plus Mo, and Mo- and V-deficient Burk medium. The resuspended cells were derepressed for β-galactosidase for 6

TABLE 1. Bacterial strains and plasmids

| Strain, plasmid, or phage | Relevant characteristics or genotype | Reference or source |
|---|---|---|
| *E. coli* | | |
| K-12 71-18 | Δ(*lac-pro*) F′ *lac-9 lac-7* M15 *pro*$^+$ *supE* | 20 |
| C600 | F$^-$ *thi-1 thr-1 leuB6 lacYI tonA21 supE44* lambda$^-$ | 25 |
| S17-1 | *thi pro* HsdR$^-$ HsdM$^+$ *recA* RP4 2-TC::Mu-*kan*::Tn7 (integrated plasmid) | 28 |
| JC5466 | *trp his recA56 rplE* | C. Kennedy |
| *A. vinelandii* | | |
| CA | Wild type | 7 |
| DJ143 | Δ*nifA* | D. Dean |
| CA11 | Δ*nifHDK* | 1 |
| CA11.81 | Δ*nifHDK*, *vnfH81*::Tn5-B21 | This work |
| CA11.82 | Δ*nifHDK*, *vnfD82*::Tn5-B21 | This work |
| CA46 | *vnfA46*::Tn5 | 14 |
| CA46.81 | *vnfA46*::Tn5, *vnfH*::Tn5-B21 | This work |
| CA46.82 | *vnfA46*::Tn5, *vnfD82*::Tn5-B21 | This work |
| CA66 | Δ*anfA66*::*kan* | 14 |
| CA66.81 | Δ*anfA66*::*kan*, *vnfH81*::Tn5-B21 | This work |
| CA66.82 | Δ*anfA66*::*kan*, *vnfD82*::Tn5-B21 | This work |
| CA71 | Δ*anfDGK71*::*kan* | 15 |
| CA71.81 | Δ*anfDGK71*::*kan*, *vnfH81*::Tn5-B21 | This work |
| CA71.82 | Δ*anfDGK71*::*kan*, *vnfD82*::Tn5-B21 | This work |
| CA30 | *nifB30*::Tn5 | 17 |
| CA30.81 | *nifB30*::Tn5, *vnfH81*::Tn5-B21 | This work |
| CA30.82 | *nifB30*::Tn5, *vnfD82*::Tn5-B21 | This work |
| CA81 | *vnfH81*::Tn5-B21 | This work |
| CA82 | *vnfD82*::Tn5-B21 | This work |
| CA85 | *vnfA85*::Tn5-B21 | This work |
| Plasmids | | |
| pVASJ1 | pUC18 containing 2.1-kb *Sma*I fragment of *vnfA* | This work |
| pVHSJ1 | pUC9 containing 0.6-kb *Pst*I-*Eco*RI fragment of *vnfH* | This work |
| pVDSJ1 | pUC9 containing 1.4-kb *Pst*I-*Sal*I fragment of *vnfDG* | This work |
| pVKSJ1 | pUC18 containing 0.8-kb *Sal*I-*Pst*I fragment containing *vnfK* | This work |
| Phage | | |
| λ::Tn5-B21 | *b*221 *c*I857 *P*am80 | 28 |

h. Samples (50 μl) were taken from these cultures, and the accumulation of β-galactosidase activity was determined as described by Miller (21). The cells were made permeable by adding 50 μl of 0.1% sodium dodecyl sulfate and 30 μl of chloroform to the assay buffer.

**Northern blot analysis.** *A. vinelandii* CA was grown under diazotrophic conditions in the presence of 1 μM $V_2O_5$ to a cell density of 150 Klett units. The cells were harvested by centrifugation, washed once with phosphate buffer, and resuspended in medium containing V or Mo in the presence or absence of $NH_4^+$, as described above. After incubation for 6 h, total RNA was isolated as described by Krol et al. (19). RNA samples (approximately 60 μg) were glyoxylated and electrophoresed on a 1.1% agarose gel in 10 mM sodium phosphate buffer (pH 7.2). RNAs were transferred from gels to GeneScreen hybridization membranes and hybridized as suggested by the suppliers of GeneScreen (NEN). Probes (a 0.6-kb *Pst*I-*Eco*RI fragment containing *vnfH*, a 1.4-kb *Pst*I-*Sal*I fragment containing *vnfDG*, and a 0.8-kb *Sal*I-*Pst*I fragment containing *vnfK*) were labeled with $^{32}P$ by random-primed labeling as described by the supplier of the probes (Amersham). Marker RNAs of different sizes (0.24 to 9.5 kb) were blotted onto GeneScreen and stained with 0.1% methylene blue dissolved in 0.5 M sodium acetate (pH 5.2).

TABLE 2. Activities of *vnfH-lacZ* and *vnfD-lacZ* fusions in *A. vinelandii*

| Strain | Pregrowth conditions[a] | Derepression time (h) | % β-Galactosidase activity[b] | | | |
|---|---|---|---|---|---|---|
| | | | −Mo+N | +Mo−N | +V−N | −Mo−N |
| CA81 | $NH_4^+$ | 0 | 30 | ND[c] | ND | ND |
| | | 6 | 50 | 31 | 100[d] | 92 |
| | | 24 | 67 | 8 | 112 | 64 |
| | Mo | 0 | ND | 10 | ND | ND |
| | | 6 | 7 | 7 | 100[e] | 53 |
| | | 24 | 34 | 2 | 132 | 75 |
| CA82 | $NH_4^+$ | 0 | 13 | ND | ND | ND |
| | | 6 | 16 | 12 | 100[f] | 58 |
| | | 24 | 47 | 13 | 133 | 92 |
| | Mo | 0 | ND | 7 | ND | ND |
| | | 6 | 19 | 14 | 100[g] | 29 |
| | | 24 | 21 | 8 | 142 | 46 |

[a] Cells were precultured in the presence of 28 mM $NH_4^+$ acetate or 100 nM $Na_2MoO_4$.
[b] Percent β-galactosidase activity for cells cultured in the presence of $NH_4^+$ and in the absence of Mo (−Mo+N), in the presence of Mo and the absence of $NH_4^+$ (+Mo−N), in the presence of 1 μM $V_2O_5$ and the absence of $NH_4^+$ (+V−N), or in the absence of Mo, V, and $NH_4^+$ (−Mo−N). Each value represents the average of three or more experiments.
[c] ND, not determined.
[d] 100% = 9,835 ± 695 Miller units.
[e] 100% = 10,960 ± 1,911 Miller units.
[f] 100% = 15,007 ± 1,163 Miller units.
[g] 100% = 9,696 ± 548 Miller units.

**Two-dimensional protein gel electrophoresis.** *A. vinelandii* CA, CA81, and CA82 were derepressed for nitrogenase-2 for 6 h under the conditions described above. Cell extracts were prepared as previously described by Bishop et al. (2). Isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins in cell extracts were conducted as described by O'Farrell (22) with the modifications of Bishop et al. (2).

## RESULTS

**Expression of *vnfH-lacZ* and *vnfD-lacZ* transcriptional fusions.** Table 2 summarizes the β-galactosidase activities observed after 6 h of derepression of the *vnfH-lacZ* (strain CA81) and *vnfD-lacZ* (strain CA82) transcriptional fusion strains. Activities for strain CA81 were highest after derepression in the presence of V with cultures that were pregrown in Mo-deficient medium containing $NH_4^+$ or in medium containing Mo. High activities were also observed after derepression in Mo- and V-deficient media, conditions under which nitrogenase-3 is expressed. Surprisingly, β-galactosidase activity also accumulated in the presence of $NH_4^+$. Cells grown in the presence of Mo and subsequently derepressed in Mo-deficient medium in the presence or absence of $NH_4^+$ showed reduced β-galactosidase activities (Table 2). These reduced activities probably resulted from carryover of previously accumulated Mo, even though the concentration of $Na_2MoO_4$ (100 nM) in the preculture growth medium was 10-fold lower than that normally used for Mo-dependent diazotrophic growth. For strain CA82, the

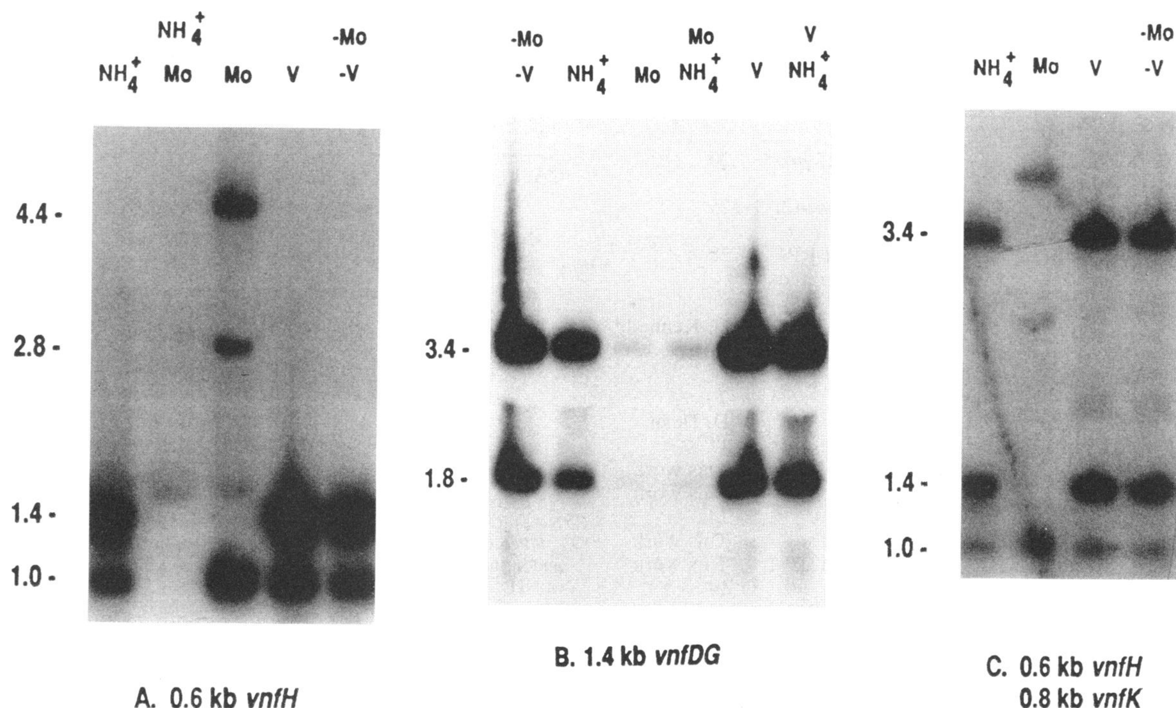


FIG. 1. Identification of *A. vinelandii* CA transcripts hybridizing to *vnfH* (A), *vnfDG* (B), and *vnfH* and *vnfK* (C). For the experiment shown in panel C, hybridization was conducted with the *vnfH* probe first and then with the *vnfK* probe.

highest β-galactosidase activities were also observed under V-sufficient conditions and lower activities were seen in the absence of V. Again, as with CA81, these activities were not repressed by $NH_4^+$. The fusions carried by strains CA81 and CA82 were also expressed at low levels when cells were derepressed in the presence of Mo. After preculture in the presence of Mo, the β-galactosidase activities for strains CA81 and CA82 were reduced to 2 and 8%, respectively, after a 24-h derepression period.

***vnfH*-, *vnfDG*-, and *vnfK*-hybridizing mRNA species in the wild-type strain CA.** Since the *lacZ* fusion strains CA81 and CA82 are inactivated for *vnfH* and *vnfD*, respectively, we considered the possibility that repression by $NH_4^+$ requires the products of these structural genes. We therefore used Northern RNA blot analysis to look for the presence or absence of *vnf* transcripts in RNA from the wild-type strain CA. With *vnfH* as a probe, two mRNA species (1.4 and 1.0 kb) were detected after growth in Mo-deficient medium in the presence or absence of V or $NH_4^+$ (Fig. 1A). The amounts of these species were somewhat reduced in RNA from cells cultured in the presence of $NH_4^+$ as compared with those in RNA from cells cultured in the absence of $NH_4^+$. The 1.0-kb mRNA species contains the structural *vnfH* gene without the ferredoxinlike *orf*, whereas the 1.4-kb mRNA species represents the full-length *vnfHorfFd* transcript (12, 16). A third mRNA species of about 1.3 kb was also visible in RNA from cells cultured in the presence of $NH_4^+$. However, the band representing the 1.4-kb mRNA species from cells cultured under N-free conditions was very intense, making it difficult to resolve the 1.3-kb mRNA species. After growth in the presence of Mo, mRNA species of 4.4, 2.6, and 1.0 kb hybridized with the *vnfH* probe. Based on the similarity between *vnfH* and *nifH* sequences (88.5% identity [16]), the origin of these mRNA species is likely to be the *nifHDK* operon (12). After growth in the presence of $NH_4^+$ plus Mo, no *vnfH*-hybridizing mRNA species were detected (Fig. 1A). *vnfD*-hybridizing mRNAs of 3.4 and 1.8 kb were abundant in RNA from cells cultured in Mo-deficient medium in the presence or absence of V (Fig. 1B). The amounts of *vnfD*-hybridizing mRNA species in RNA from cells cultured in the presence of $NH_4^+$ and the absence of V were slightly reduced. These mRNA species were very faint in RNA from cells grown in the presence of Mo (Fig. 1B).

With a *vnfK*-specific probe, a single mRNA species (3.4 kb) was observed in RNA from cells cultured in Mo-deficient medium in the presence or absence of V (Fig. 1C). Again this species was reduced in amount, but still present, in RNA from cells cultured in medium containing $NH_4^+$.

Cells were cultured for 24 h in the presence or absence of V and $NH_4^+$. *vnfH* and *vnfDGK* transcripts were present in cells grown in the absence of Mo, V, and $NH_4^+$ and in the presence of V with or without $NH_4^+$. However, in the presence of $NH_4^+$, transcripts were not detected in the absence of V (data not shown). This indicates that prolonged growth in the absence of V and in the presence of $NH_4^+$ results in the disappearance of *vnf* transcripts.

**Presence of nitrogenase-2 proteins.** From the results presented above, it is apparent that transcription of the *vnfHorfFd* and *vnfDGK* operons is only partially inhibited by the presence of $NH_4^+$, whereas little or no accumulation of transcripts occurs in the presence of Mo. To determine whether the expression of nitrogenase-2 is posttranscriptionally regulated by $NH_4^+$, we used two-dimensional protein gel electrophoresis to determine the presence or absence of dinitrogenase reductase-2 and dinitrogenase-2 subunits in cell extracts. Subunits of dinitrogenase-2 and dinitrogenase reductase-2 were absent when strain CA was incubated in the presence of Mo with or without $NH_4^+$ (Table 3). These subunits were present in extracts of cells incubated in

TABLE 3. Presence of protein subunits of nitrogenase-2 under different conditions of derepression[a]

| Strain | Addition(s) | Presence of nitrogenase-2 subunits: | | |
|---|---|---|---|---|
| | | α | β | γ |
| CA | $NH_4^+$, Mo | – | – | – |
| | $NH_4^+$ | – | – | – |
| | Mo | – | – | – |
| | V | + | + | + |
| | None | – | – | + |
| CA81 | $NH_4^+$, Mo | – | – | – |
| | V | + | + | – |
| CA82 | $NH_4^+$, Mo | – | – | – |
| | V | – | – | + |

[a] Cells were grown in Mo-free Burk medium containing 28 mM ammonium acetate, harvested, resuspended in Burk medium containing 28 mM ammonium acetate, 1 μM $Na_2MoO_4$, or 1 μM $V_2O_5$, and derepressed for nitrogenase-2 for 6 h.

TABLE 4. Activities of *vnfH-lacZ* and *vnfD-lacZ* fusions in different mutant background *A. vinelandii*

| Strain | Relevant genotype | % β-Galactosidase activity[a] | | | |
|---|---|---|---|---|---|
| | | −Mo+N | +Mo−N | +V−N | −Mo−N |
| CA81[b] | | 70 | 25 | 100[c] | 117 |
| CA46.81 | *vnfA46*::Tn*5* | 2 | 1 | 2 | 2 |
| CA66.81 | Δ*anfA66*::*kan* | 19 | 10 | 47 | 55 |
| CA71.81 | Δ*anfDGK71*::*kan* | 66 | 13 | 104 | 94 |
| CA11.81 | Δ*nifHDK* | 78 | 25 | 108 | 71 |
| DJ143.81 | Δ*nifA* | 72 | 29 | 57 | 60 |
| CA30.81 | *nifB30*::Tn*5* | 73 | 20 | 74 | 85 |
| CA82[d] | | 32 | 15 | 100[e] | 51 |
| CA46.82 | *vnfA46*::Tn*5* | 2 | 1 | 8 | 10 |
| CA66.82 | Δ*anfA66*::*kan* | 29 | 6 | 46 | 19 |
| CA71.82 | Δ*anfDGK71*::kan | 28 | 16 | 77 | 45 |
| CA11.82 | Δ*nifHDK* | 34 | 17 | 67 | 31 |
| DJ143.82 | Δ*nifA* | 34 | 18 | 58 | 46 |
| CA30.82 | *nifB30*::Tn*5* | 35 | 20 | 63 | 63 |

[a] Percent β-galactosidase activity for cells precultured in the presence of 28 mM ammonium acetate and resuspended in the presence of $NH_4^+$ and the absence of Mo (−Mo+N); in the presence of 1 μM $Na_2MoO_4$ and the absence of $NH_4^+$ (+Mo−N); in the presence of 1 μM $V_2O_5$ and the absence of $NH_4^+$ (+V−N); or in the absence of Mo, V, and $NH_4^+$ (−Mo−N). The resuspended cells were incubated for 6 h.
[b] CA81 mutant strains contain *vnFH*::Tn*5*B21.
[c] 100% = 9,068 ± 720 Miller units. Percentages represent the averages of two to five independent experiments.
[d] CA82 mutant strains contain *vnfD*::Tn*5*B21.
[e] 100% = 9,596 ± 696 Miller units. Percentages represent the averages of two to five independent experiments.

Mo-deficient medium in the presence of V. The dinitrogenase reductase-2 subunit was the only subunit present in the absence of V, and nitrogenase-2 subunits were not detected in extracts of cells incubated in Mo-deficient medium containing $NH_4^+$. As expected, strain CA81 (*vnfH-lacZ*) did not accumulate dinitrogenase reductase-2 and strain CA82 (*vnfD-lacZ*) did not accumulate dinitrogenase-2. However, each strain accumulated the complementary nitrogenase component not affected by the Tn*5*-B21 insertion.

**Expression of *vnfH-lacZ* and *vnfD-lacZ* transcriptional fusions in different mutant backgrounds.** *vnfH-lacZ* and *vnfD-lacZ* fusions in the $VnfA^-$ strains CA46.81 and CA46.82 were not expressed under any condition tested (Table 4). In the $AnfA^-$ strain CA66.81, the level of *vnfH-lacZ* expression in the presence of $NH_4^+$ was much lower than that under the same conditions in the $AnfA^+$ strain CA81. Expression was also reduced in N-free Mo-deficient medium with and without V. However, Northern blot analysis of RNA from strain CA66 indicated the presence of large amounts (comparable to those found for the wild type) of the *vnfH*-hybridizing mRNA species (1.4 and 1.0 kb) in cells grown in Mo-deficient medium in the presence or absence of V or $NH_4^+$ (data not shown). The $NifA^-$ strains (DJ143.81 and DJ143.82) and strains containing deletions in the structural genes for nitrogenase-1 and -3 (strains CA11.81, CA11.82, CA71.81, and CA71.82) were relatively unaffected in the accumulation of β-galactosidase activity (Table 4). These results indicate that regulatory and structural genes required for nitrogenase-1 and nitrogenase-3 are not significantly involved in the expression or repression of nitrogenase-2. Finally, *nifB*, a gene that is essential for diazotrophic growth under all conditions (13, 17) and is known to be involved in FeMo cofactor synthesis, does not appear to be required for transcription of the *vnfHorfFd* and *vnfDGK* operons (strains CA30.81 and CA30.82) (Table 4).

## DISCUSSION

Expression of the *vnfHorfFd* and *vnfDGK* operons in *A. vinelandii* seems to be regulated at least at two levels. Transcription of both operons is strongly repressed by Mo. On the other hand, the presence of $NH_4^+$ only partially repressed transcription of these operons. This latter observation is in contrast to the situation with the structural gene operons encoding the other two nitrogenases, in which $NH_4^+$ almost completely represses transcription (12, 18; unpublished data). The results of the experiments with *lacZ* fusions and Northern blots indicate that transcription of *vnfHorfFd* and *vnfDGK* in the presence of $NH_4^+$ is at least 50% of that present under V sufficiency in the absence of $NH_4^+$. On the other hand, no accumulation of nitrogenase-2 subunits occurred in the presence of $NH_4^+$. In addition, preliminary results with translational *vnfH-lacZ* and *vnfD-lacZ* fusions indicate that β-galactosidase does not accumulate in the presence of $NH_4^+$ (unpublished data). Transcription of the *vnfENX* operon also takes place in the presence of $NH_4^+$ (30). This indicates that some of the regulation by $NH_4^+$ occurs at the posttranscriptional level in at least three different *vnf* operons (*vnfHorfFd*, *vnfDGK*, and *vnfENX*). VnfA is required for the expression of the *vnfHorfFd*, *vnfDGK*, and *vnfENX* operons; as might be expected, a transcriptional *vnfA-lacZ* fusion also accumulates β-galactosidase in the presence of $NH_4^+$ (unpublished data). In *Klebsiella pneumoniae*, *nifL* is thought to regulate *nif* operons in response to $NH_4^+$ and $O_2$ by destabilizing *nif* transcripts (9) or inactivating NifA by binding of NifL to NifA (11). However, a *nifL*-like gene linked to *vnf* operons has not been found. One might speculate that the absence of a factor comparable to NifL in *K. pneumoniae* could lead to relatively stable *vnf* transcripts in the presence of $NH_4^+$. Lack of translation of these transcripts in cells grown in the presence of $NH_4^+$ suggests that translational repression may occur under these conditions. *vnf* transcripts are absent after a longer (24-h) incubation period in the presence of $NH_4^+$ and the absence of V. *vnfDGK* transcripts are still present after 24 h of incubation in the absence of $NH_4^+$, Mo, and V, whereas no dinitrogenase-2 subunits are present under these conditions. Therefore, the requirement of V for expression

of dinitrogenase-2 seems to be at the level of translation. Alternatively, in the absence of V, dinitrogenase-2 subunits may undergo rapid proteolytic degradation.

In conclusion, regulation of the structural genes encoding nitrogenase-2 in *A. vinelandii* occurs at both the transcriptional and posttranscriptional levels. Molybdenum blocks transcription, whereas $NH_4^+$ appears to act at both the transcriptional and posttranscriptional levels. In the absence of Mo and V, conditions under which nitrogenase-3 is expressed, the *vnfH* gene product is expressed, since dinitrogenase reductase-2 is required for transcription of *anfHDGK* (18). However, dinitrogenase-2 does not appear to accumulate in the absence of V, even though *vnfDGK* transcripts are made in the absence of this metal. One might speculate that regulation of nitrogenase-2 at the posttranscriptional level might give *A. vinelandii* an advantage in a microenvironment (such as a microbial community) with rapidly fluctuating $NH_4^+$ and/or V concentrations. For example, after depletion of $NH_4^+$, *A. vinelandii* cells would be able to express nitrogenase-2 in the presence of V relatively quickly, since transcripts are already present.

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