

TABLE S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant characteristic(s) ^a	Source or reference
<i>A. vinelandii</i>		
strains		
UW136	Rif ^r derived from strain UW (ATCC 13705)	(1)
UW1	<i>nifA</i> ⁻	(6)
AVW552	UW136 <i>vnf::Φ(nasA</i> ^{-316 to +297} <i>-lacZ)</i>	This study
AVW627	UW136 <i>vnf::Φ(nasA</i> ^{-316 to +297} <i>-lacZ)</i> with +60 ATG→TAG mutation	This study
AVW643	UW136 <i>vnf::Φ(nasA</i> ^{-316 to +297} <i>-lacZ)</i> with +14 to +100-nt deletion	This study
AVW650	UW136 <i>vnf::Φ(nasA</i> ^{-316 to +297} <i>-lacZ)</i> with +14 to +25-nt deletion	This study
AVW663	UW136 <i>vnf::Φ(nasA</i> ^{-316 to +297} <i>-lacZ)</i> with -24/-12 GG/GC → AA/AT mutation	This study
AVW664	UW136 <i>vnf::Φ(nasA</i> ^{-316 to +297} <i>-lacZ)</i> with +68 to +100-nt deletion	This study
AVW668	UW136 <i>vnf::Φ(nasA</i> ^{-316 to +297} <i>-lacZ)</i> with +53 to +58-nt deletion	This study
AVW672	UW136 <i>vnf::Φ(nasA</i> ^{-131 to +297} <i>-lacZ)</i> with +14 to +100-nt deletion	This study

AVW677	UW136 <i>vnf::Φ(nasA^{-43 to +297}-lacZ)</i> with +14 to +100-nt deletion	This study
AVW678	UW136 <i>vnf::Φ(nasA^{-108 to +297}-lacZ)</i> with +14 to +100-nt deletion	This study
AVW693	UW136 <i>vnf::Φ(nasA^{-168 to +297}-lacZ)</i> with +14 to +100-nt deletion	This study
AVW853	UW136 $\Delta nasC::Gm^r$	This study
AVW816	UW136 $\Delta nasC::Gm^r$ <i>vnf::Φ(nasA^{-316 to +297}-lacZ)</i>	This study
AVW908	UW136 <i>vnf::Φ(nasA^{-316 to +297}-lacZ)</i> with +68 to +100-nt deletion	This study
AVW960	UW136 <i>vnf::Φ(P_{nasA}-lacZ)</i>	This study
AVW961	UW136 $\Delta nasC::Gm^r$ <i>vnf::Φ(P_{nasA}-lacZ)</i>	This study
AVW962	UW1 <i>vnf::Φ(P_{nasA}-lacZ)</i>	This study
<i>E. coli</i> strain		
DH5 α	<i>supE44 ΔlacU169 hsdR17 recA1 endA1</i> <i>gyrA96 hi-1 relA1</i>	GIBCO-BRL
MC1061	F ⁻ $\Delta(lacI-lacY)74 galE15 galK16 relA1$ <i>rpsL150 spoT1 hsdR2 λ⁻</i>	(2)
Plasmids		
pBluescript II KS(+)	Car ^r , cloning vector	Stratagene

pBT	Cm ^r ; bait vector of BacterioMatch® II two-hybrid system	Stratagene
pBTW	Cm ^r ; derivative of pBT, expression vector	This work
pBlue-lacZ	Car ^r ; pBluescript II KS(+) containing <i>lacZ</i> from pSUP102::Tn5-B21 at <i>BamHI-XhoI</i> region	This study
pDK6	Km ^r ; protein expression vector	(4)
pDK6-S	Km ^r ; pDK6 carrying <i>nasS</i> at <i>EcoRI-BamHI</i> region	This work
pDK6-T	Km ^r ; pDK6 carrying <i>nasT</i> at <i>EcoRI-BamHI</i> region	This work
pICT4	Car ^r ; pIC20H carrying four tandem copies of <i>rrnB1</i> cloned at the <i>SphI</i> site	This study
pJW1	Car ^r ; pTZ19 carrying <i>vnf</i> sequence	(8)
pKT2-lacZ	Km ^r ; expression vector	(Pierson, unpublished data)
pPROBE-NT	Promoter-probe vector	(5)
pSUP102::Tn5-B21	Cm ^r Tet ^r , Transposable promoter probe; B21 in pSUP102	(7)
pTnMod-OGm	Gm ^r ; pMB1, <i>mob_ Tn5 tnp</i>	(3)
pVnflacZa	Car ^r p15A; translational <i>lacZ</i> fusion probe vector	This study
pVnflacZb	Car ^r p15A; transcriptional <i>lacZ</i> fusion	This study

	probe vector	
pWB30	Car ^r ; pBluescript II KS(+) carrying 7.8-kb <i>XhoI-XhoI</i> DNA fragment containing the 5' end of <i>nasA</i> and its upstream sequence	This study
pWB552	Car ^r ; pVnflacZa carrying <i>nasA'</i> -' <i>lacZ</i>	This study
pWB555	Cm ^r , pBTW carrying <i>lacUV5-nasA'</i> -' <i>lacZ</i> fusion	This work
pWB627	Car ^r ; pWB552 with ATG→TAG mutation in the <i>nasAB</i> leader region	This study
pWB643	Car ^r ; pWB552 with +14 to +100-nt deletion in the <i>nasAB</i> leader region	This study
pWB650	Car ^r ; pWB552 with +14 to +25-nt deletion in the <i>nasAB</i> leader region	This study
pWB663	Car ^r ; pWB552 with -24/-12 GG/GC → AA/AT mutation at the <i>nasAB</i> promoter	This study
pWB664	Car ^r ; pWB552 with +53 to +58-nt deletion in the <i>nasAB</i> leader region	This study
pWB668	Car ^r ; pWB552 with +68 to +100-nt deletion at the leader region	This study
pWB672	Car ^r ; pWB643 with the 5' end upstream of the <i>nasAB</i> promoter at -131	This study
pWB677	Car ^r ; pWB643 with the 5' end upstream of the <i>nasAB</i> promoter at -43	This study

pWB678	Car ^r ; pWB643 with the 5' end upstream of the <i>nasAB</i> promoter at -108	This study
pWB680	Car ^r ; pGEM-T carrying the <i>ntrC</i> gene	This study
pWB685	Gm ^r Car ^r ; pWB680 with $\Delta ntrC::Gm^r$	This study
pWB693	Car ^r ; pWB643 with the 5' end upstream of the <i>nasAB</i> promoter at -168	This study
pWB700	Car ^r ; pVnflacZa carrying <i>nirB'</i> -' <i>lacZ</i>	This study
pWB901	Cm ^r ; pBTW carrying <i>lacUV5-nasA'</i> -' <i>lacZ</i> fusion with the 5' stem of hairpin I deleted	This work
pWB902	Cm ^r ; pBTW carrying <i>lacUV5-nasA'</i> -' <i>lacZ</i> fusion with the part of loop and 3' stem of hairpin II deleted	This work
pWB910	Cm ^r ; pBTW carrying <i>lacUV5-nasA'</i> -' <i>lacZ</i> fusion with hairpin III deleted	This work
pWhite	Car ^r ; cloning vector	This study
pWvnf	Car ^r ; pWhite carrying 2.8-kb <i>vnf</i> sequence	This study

^a Abbreviations: Car, carbenicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Rif, rifampicin; Tet, tetracycline; *nasA'*, DNA sequence -316 to +297 around the *nasAB* promoter.

References

1. **Bishop, P. E., and W. J. Brill.** 1977. Genetic analysis of *Azotobacter vinelandii* mutant strains unable to fix nitrogen. *J. Bacteriol.* **130**:954-6.
2. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. *J. Mol. Biol.* **138**:179-207.
3. **Dennis, J. J., and G. J. Zylstra.** 1998. Plasposons: Modular Self-Cloning Minitransposon Derivatives for Rapid Genetic Analysis of Gram-Negative Bacterial Genomes. *Appl. Environ. Microbiol.* **64**:2710-2715.
4. **Kleiner, D., W. Paul, and M. J. Merrick.** 1988. Construction of multicopy expression vectors for regulated over-production of proteins in *Klebsiella pneumonia* and other enteric bacteria. *J. Gen. Microbiol.* **134**:1779-84.
5. **Miller, W. G., J. H. J. Leveau, and S. E. Lindow.** 2000. Improved *gfp* and *inaZ* Broad-Host-Range Promoter-Probe Vectors. *Molecular Plant-Microbe Interactions* **13**:1243-1250.
6. **Shah, V. K., J. L. Pate, and W. J. Brill.** 1973. Protection of nitrogenase in *Azotobacter vinelandii*. *J. Bacteriol.* **115**:15-7.
7. **Simon, R., J. r. Quandt, and W. Klipp.** 1989. New derivatives of transposon *tn5* suitable for mobilization of replicons, generation of operon fusions and induction of genes in Gram-negative bacteria. *Gene* **80**:161-169.
8. **Walmsley, J., and C. Kennedy.** 1991. Temperature-Dependent Regulation by Molybdenum and Vanadium of Expression of the Structural Genes Encoding Three Nitrogenases in *Azotobacter vinelandii*. *Appl. Environ. Microbiol.* **57**:622-4.