(Methyl)ammonium Transport in the Nitrogen-Fixing Bacterium Azospirillum brasilense

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An ammonium transporter of Azospirillum brasilense was characterized. In contrast to previously reported putative prokaryotic NH₄⁺ transporter genes, A. brasilense amtB is not part of an operon with glnB or glnZ which, in A. brasilense, encode nitrogen regulatory proteins P₁₁ and P₁₂, respectively. Sequence analysis predicts the presence of 12 transmembrane domains in the deduced AmtB protein and classifies AmtB as an integral membrane protein. Nitrogen regulates the transcription of the amtB gene in A. brasilense by the Ntr system. amtB is the first gene identified in A. brasilense whose expression is regulated by NtrC. The observation that ammonium uptake is still possible in mutants lacking the AmtB protein suggests the presence of a second NH₄⁺ transport mechanism. Growth of amtB mutants at low ammonium concentrations is reduced compared to that of the wild type. This suggests that AmtB has a role in scavenging ammonium at low concentrations.

Azospirillum species are nitrogen-fixing organisms (diazotrophs), capable of forming an associative relationship with the roots of several economically important cereals (68). Many studies have indicated that Azospirillum promotes plant growth, but the exact mechanism of growth promotion has not been fully characterized. Like most organisms, Azospirillum uses ammonium salts as a preferred nitrogen source (53). In the absence of combined nitrogen and under microaerobic conditions, the nitrogenase enzyme complex is synthesized and converts atmospheric N₂ to NH₄⁺.

Unprotonated NH₃ is predicted to diffuse out of bacterial cells due to a concentration gradient across the plasma membrane (36). The pH gradient (generally slightly more alkaline inside the bacteria) enhances this process. Therefore, an active ammonium uptake system is required to retain the intracellular fixed nitrogen, acquired at high energy cost by the nitrogenase. Hartmann and Kleiner (24) have shown that ammonium uptake in Azospirillum spp. is energy dependent, follows the Michaelis-Menten kinetics, and is repressed by ammonium. No functional characterization of genetic components of this system has yet been reported.

Recently, genes encoding ammonium transporter proteins and putative ammonium transporter proteins have been reported for Saccharomyces cerevisiae (43), Arabidopsis thaliana (52), and Lycopersicon esculentum (tomato) (39). In Bacillus subtilis the nrgA gene, whose corresponding amino acid sequence is homologous to those of NH₄⁺ transporter proteins, is part of the dicistronic nrgAB operon (76). nrgB possibly encodes a nitrogen regulatory protein homologous to P₁₁ proteins, but the biochemical functions of the nrgA and nrgB gene products in B. subtilis have not been reported. The nrgAB operon is highly expressed during nitrogen-limited growth.

More recently, Siewe et al. (61) characterized the first reported prokaryotic NH₄⁺ transporter gene (amt) in Corynebacterium glutamicum. However, in 1986, Jayakumar et al. (28) had already reported on an amtA gene in Escherichia coli which complemented a mutant with less than 10% of the parental CH₃NH₂⁺ uptake activity. The complete amtA sequence was published by Fabiny et al. (19). An analysis of the deduced amino acid sequence of the product of amtA, AmtA, predicted that the protein was a cytoplasmic component of an ammonium transport system. In 1992, however, Neuwald et al. (51) reported that the amtA gene corresponded to cysQ, a gene needed for cysteine synthesis in E. coli. Later on, Van Heeswijk et al. (70) isolated an amtB gene in E. coli K-12. The amtB gene product is homologous to transmembrane NH₄⁺ transporters, but its functional characterization has not yet been reported. The E. coli amtB gene is cotranscribed with glnK, located upstream of amtB. glnK encodes a second P₁₁-like protein (6, 70). The glnK gene product and the glnB gene product (P₁₂) are known to play a role in the reversible adenylylation of glutamine synthetase (GS) in response to the nitrogen status of the cells. In addition, P₁₂ stimulates the kinase-phosphatase enzyme, NtrB, to dephosphorylate the phosphorylated transcriptional activator NtrC. Phosphorylated NtrC is necessary to activate transcription from several RpoN-dependent promoters (reviewed in reference 65; 41, 46).

Two P₁₁ homologs have been identified in Azospirillum brasilense (14, 15). glnB is part of the nitrogen-regulated, but NtrC-independent, glnBA4 operon, and its product is required for nitrogen fixation. In contrast to what is found for other species, P₁₁ (glnB gene product) is not involved in the ammonium control of GS activity by adenylylation. The level of glnA expression is, however, lower in glnB mutant strains than in the wild-type strain (14, 15). The second P₁₁-like protein of A. brasilense, P₁₂, is not functionally equivalent to P₁₁. glnB-null mutants exhibit a Nif⁻ phenotype that is not complemented by structural gene glnZ. The two-component regulatory system NtrB-NtrC and the σ₅₄ factor (RpoN) in A. brasilense have been characterized (40, 48). NtrC is involved in nitrate utilization (40) and (methyl)ammonium uptake (69). Notably, the rpoN mutant has a pleiotropic effect: nitrogen fixation, nitrate assimilation, ammonium uptake, and flagellar biosynthesis are impaired (48).

We report here the isolation and characterization of a nitrogen-regulated (methyl)ammonium transporter gene from A. brasilense.
Strains were grown in Luria-Bertani (LB) medium (57) at 37°C. Plasmids were used for plasmid isolation, chromosomal DNA preparation, restriction analysis, RNA work, minimal K medium (18) supplemented with sodium lactate (5 g per liter). Hybridization filter service, Eupen, Belgium). For Southern hybridization, DNA was transduced into E. coli HB101, and selected for isolation of tetracycline-resistant colonies.

An EcoRI recognition site had been added to the 5'-end of each primer to facilitate cloning of the amplified PCR product into a vector. PCR products were performed in a thermocycler (TRIO-Thermoblock; Biometra, Göttingen, Germany) in a reaction volume of 25 μl containing 0.5 U of Taq DNA polymerase (Boehringer Mannheim) and each of the primers at 1 mM concentration. A PCR cycle consisting of 1 min of denaturation at 94°C, 1 min of primer annealing at 50°C, and 1 min of primer extension at 72°C was applied 30 times. These 30 cycles were preceded by 5 min of denaturation (94°C) and were followed by 7 min of primer extension (72°C).

DNA sequencing and analysis. The chain-terminating deoxynucleotide triphosphate method (58) was performed by using an automated sequencer (ALF; Pharmacia Biotech Benelux, Roosendaal, The Netherlands) with the Auto-read sequencing kit (Pharmacia Biotech Benelux). The PALING, CLUSTAL, PROSITE, SOAP, NOVOTNY, BETATURN, HELIXMEM, and PSIGNAL programs of the P.C. Gene software package (Intelligenetics) were used to process and analyze the sequencing data. Potential coding regions were identified.

Materials and Methods

Bacterial strains, plasmids, media, and growth conditions. The E. coli and A. brasilense strains used are listed in Table 1. Plasmids mentioned in the text are also described in this table. Sequencing constructs and intermediate constructs are not given. A genomic library of A. brasilense Sp7 was constructed by ligation of fragments generated by partial EcoRI digestion of total DNA into cosmid pLAFR1. These constructs were packed into phage particles, transferred to E. coli HB101, and selected for isolation of tetracycline-resistant colonies. E. coli strains were grown in Luria-Bertani (LB) medium (57) at 37°C. A. brasilense was grown in LB medium supplemented with 2.5 mM CaCl₂ and 2.5 mM MgSO₄ (LB* medium) at 30°C. For solid media, 15 g of agar per liter was added. Conjugal transfers of recombinant plasmids, derived either from pLAFR1 or pFAJ314 pUC18 containing the 14-kb EcoRI fragment cloned in pUC18, were performed in a thermocycler (TRIO-Thermoblock; Biometra, Göttingen, Germany) in a reaction volume of 25 μl containing 0.5 U of Taq DNA polymerase (Boehringer Mannheim) and each of the primers at 1 mM concentration. A PCR cycle consisting of 1 min of denaturation at 94°C, 1 min of primer annealing at 50°C, and 1 min of primer extension at 72°C was applied 30 times. These 30 cycles were preceded by 5 min of denaturation (94°C) and were followed by 7 min of primer extension (72°C).

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cision. The resulting plasmid was conjugated to *A. brasilense* Sp7 with 3 ml of nitrogen-free MMAB medium by replacing the 77-ml air pocket. Hybridization with the radiolabeled probe was performed for 6 h at 68°C in the presence of Rapid-hyb Buffer from Amersham. Primer extension. Primer extension was performed as described by Ausubel et al. (2) with two primers labeled with 32P-dATP: AMT8-15 (5'-CAGACCGGGCGAAGCCATCCGCGCAT-3') and AMT8-17 (5'-GCGCCGCTTCTCCTGGGGGAAGGGCGG-3'). Ten picomoles of each primer added to 20 μl of total RNA was heated in the hybridization buffer with 80% formamide for 10 min at 85°C, and the mixture was subsequently incubated overnight at 30°C. The primer extension was performed for 90 min at 42°C with 50 U of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim). The extension products were run on a sequencing gel adjacent to the DNA sequence obtained by using the same oligonucleotide as the primer.

**Construction of an antI::EcoRI fusion and β-glucuronidase assay.** The 1.8-kb Smal-terminating cassette from pKW117 (74) containing the gusA gene was cloned as a 1.6-kb Smal-I fragment, and sequence analysis showed the presence of a Rho-independent transcription terminator (56) (Fig. 1).

**RESULTS**

Cloning of an *A. brasilense* Sp7 gene encoding a putative NH₄⁺ transporter. Degenerate PCR primers were used to amplify a 344-bp internal fragment of the putative NH₄⁺ transporter gene (see Materials and Methods). This amplification product was used to screen a pLAFR1-derived genomic library of *A. brasilense* Sp7, made by cloning partial EcoRI-digested genomic DNA (see Materials and Methods). The 1.4-kb EcoRI fragment of a positively hybridizing cosmid clone was subcloned as a 1.6-kb EcoRI-SalI fragment, and sequence analysis identified the first 1.249 bp of a putative *A. brasilense* NH₄⁺ transporter gene. Cloning the original 14-kb EcoRI fragment in vector pUC18 (pFAJ314) permitted the sequencing of the remainder of the gene by using primer walking from the 5'-terminal part of the known sequence. Figure 1 shows the organization of the sequenced DNA region.

The G+C content of the entire open reading frame (ORF) is 67.2%. This is consistent with the high G+C content of *A. brasilense* DNA. The G+C content in the third position of the codons is 91.9%. The potential ATG start codon is preceded by a putative ribosome-binding site. Immediately downstream of the ORF there is a G+C-rich sequence with interrupted dyad symmetry (AG[25]T[25] = −21.6 kcal) followed by a T-rich region. This suggests the presence of a Rhoindependent transcription terminator (56) (Fig. 1).

DNA sequence analysis did not reveal other ORFs in the 330-bp region immediately downstream of the putative NH₄⁺ transporter gene or in the 350 bp preceding the ORF.

**Analysis of the deduced amino acid sequence.** A comparison of the deduced amino acid sequence of the product of the *A. brasilense* antI gene with those of reported putative NH₄⁺ transporter proteins revealed that the sequence of this protein showed the highest level of similarity (approximately 50%) to the deduced amino acid sequences of the products of the *E. coli* antI gene and the *B. subtilis* nrgA gene (data not shown). In Fig. 2 a phylogenetic tree built by multiple sequence alignment of reported putative NH₄⁺ transporter proteins is shown. Besides the proteins included in this figure, proteins belonging to the Mep/Amt family (44) have been reported for *Mycobacterium leprae* (accession no. L78818), *Rhodobacter capsulatus* (accession no. X12359), and *Methanobacterium thermooautrophicum* (accession no. AE000846). However, no NH₄⁺ transporter homolog was found by examining the complete genomic sequences of *Haemophilus influenzae* and *Mycoplasma genitalium*, two bacteria whose natural environment is human tissues (44).

**Analysis of the promoter region and the transcription of the antI gene.** To localize the transcription start site of antI, primer extension analysis was performed. RNA was isolated from wild-type (Sp7) cells grown in the presence of different nitrogen sources. The complementary sequence of nucleotides between positions 379 and 408 was used as a primer (AMT8-15). Figure 3 shows that the P2 transcription start site, located 80 to 90 nucleotides upstream of the translation start site of antI, is preferentially used when 10 mM aspartate is the nitrogen source. In conditions of ammonium excess, a weak but significant signal was obtained at the P2 transcription start site. Another weak signal also appeared on the autoradiogram at the P1 transcription start site (94 to 95 nucleotides upstream of the translation start site).
of the translational start site of amtB). Under conditions of nitrogen fixation the transcription of amtB starts at the P2 transcription start site, but less efficiently than in the presence of aspartate (data not shown). Using another oligonucleotide (AMTB-17; see Materials and Methods) allowed the detection of the same transcription start sites (P1 and P2) (data not shown). The results obtained under conditions of nitrogen fixation or with 10 mM aspartate as the nitrogen source were the same for a glnB-null mutant strain (7606) (data not shown).

An examination of the DNA region upstream from these transcription start sites reveals a putative RpoN-binding consensus sequence at positions 226 to 214 relative to the P2 transcription start site (Fig. 1). This sequence differs by two nucleotides from consensus sequence CTGGYAYR-N4-TTGCA (four positions previously defined as invariant are underlined) (3) for RpoN-dependent promoters. The occurrence of A instead of C at position 214 is also found in the Rhizobium leguminosarum nifH promoter sequence (55) and in the R. leguminosarum biovar phaseoli nifH1, nifH2, and nifH3 promoter regions (47). A partially conserved NtrC-binding consensus sequence is present at positions 212 through 269 (relative to the P2 transcription start site; the consensus sequence is TGCACCA-N3-TGGTGCA) (41) (Fig. 1).

Northern blot analyses were performed with RNA extracted from wild-type Sp7 and glnB::kan mutant (7606) cells grown under different nitrogen conditions. Figure 4 shows the hybridization of total RNA with an amtB probe. A single transcript of 1.5 kb is detected under all physiological conditions, indicating that amtB is transcribed as a monocistronic unit. This is consistent with the absence of ORFs in the vicinity of amtB and the presence of a putative downstream Rho-independent terminator close to the 3' end of amtB (Fig. 1).

As was also observed in the primer extension experiment, the level of the amtB mRNA signal is high in cells grown in aspartate-containing minimal medium, reduced in cells grown under conditions of nitrogen fixation, and very low in cells grown in a nitrogen-free medium. The results obtained under conditions of nitrogen fixation or with 10 mM aspartate as the nitrogen source were the same for a glnB-null mutant strain (7606) (data not shown).
 grown in the presence of 20 mM NH₄⁺. This is in agreement with negative regulation by ammonium.

Northern blot analysis of a gltB::kan mutant strain shows that the absence of PI does not affect the transcription of the amtB gene under conditions of nitrogen fixation or when 10 mM aspartate is the nitrogen source. Thus, in contrast to what is found for the regulation of nif gene expression (14), the transduction of the nitrogen signal for amtB gene expression does not require PI under the conditions tested.

Expression of an amtB-gusA fusion in wild-type and regulatory mutant strains. To investigate the regulation of amtB transcription in A. brasilense, an amtB: gusA translational fusion was constructed in vector pLAFR3, resulting in pFAJ302 (Fig. 1D). As NH₄⁺ transport is reported to be regulated by the Ntr system in E. coli (27, 29) and Klebsiella pneumoniae (35), the pFAJ302 vector was conjugated into wild-type A. brasilense and three different A. brasilense ntr mutants: 7194 (ntrB:: Tn5-194), 7148 (ntrC:: Tn5-148), and FAJ301 (rpoN::kan). Expression of the amtB-gusA fusion is maximal in the presence of 10 mM aspartate or 2 mM NH₄⁺ and is reduced eightfold in the presence of 20 mM NH₄⁺ (Table 2), in agreement with the results of the Northern blot analysis. This confirms that the nitrogen status of the cell influences amtB transcription. amtB: gusA expression levels were significantly lowered in the ntr mutants under all physiological conditions tested. This suggests that the Ntr system is involved in the nitrogen regulation of amtB transcription. The slightly higher amtB: gusA expression levels observed in the ntr mutants grown on 20 mM NH₄⁺ could be explained by assuming that in these circumstances the first promoter, located upstream of the P2 transcription start site (Fig. 1 and 3), participates in the transcription of ammonium.

Ammonium uptake in ntr mutants. In line with the nearly absent expression of the amtB-gusA fusion in ntr mutants, these mutants do not show any [¹⁴C]methylammonium uptake (48). In order to measure the transport of the natural substrate for the AmtB transporter protein, a selective ammonium electrode was used to measure the uptake of NH₄⁺ added to a cell suspension of wild-type Sp7 cells, rpoN:kan mutant cells (FAJ301), and ntrB:: Tn5 mutant cells (7194). In contrast to the results of the [¹⁴C]methylammonium uptake studies, no significant difference between wild-type cells and ntr mutant cells was observed (Fig. 5).

Construction and phenotypic characterization of an A. brasilense amtB:: kan insertion mutant. An Sp7 mutant strain was constructed by inserting a kanamycin resistance gene (kan) into the structural amtB gene (Fig. 1C). This mutant strain is unable to take up [¹³C]methylammonium (Fig. 6). It should be noted that although the activity of the AmtB carrier is measured with radioactively labeled [¹³C]methylammonium, the affinity of this carrier for CH₃NH₂⁺ is considerably less than its affinity for NH₄⁺, and methylammonium cannot serve as a carbon or nitrogen source for A. brasilense (24).

Growth rates on rich medium or on minimal medium with 20 mM NH₄⁺, 2 mM NH₄⁺, 8 mM nitrate, or 10 mM aspartate were similar for both the wild-type and mutant strains. As with the wild-type strain, the amtB:: kan mutant fixes nitrogen in nitrogen-free minimal medium and at low oxygen concentration. No ammonium excretion exceeding the minimum detection level of the assay method (30 mM) could be measured.

At low ammonium concentrations (0.1 mM) growth of the amtB:: kan mutant cells was reduced compared to that of wild-type cells (Fig. 7).

Complementation of an A. brasilense amtB:: kan insertion mutant and overexpression of amtB in wild-type cells. The amtB gene, expressed from its own promoter, was inserted in the broad-host-range vector pLAFR3 (resulting in pFAJ309). This construct was transferred to both wild-type cells and amtB::kan mutant cells. [¹⁴C]methylammonium uptake was restored in the mutant and occurred at even a higher rate than that in wild-type cells (Fig. 6). This could be due to the presence of extra copies of the amtB gene carried on the low-copy-

![FIG. 4. Northern blot analysis of total RNA hybridized with the 1.6-kb EcoRI-SalI amtB probe. Total RNA was prepared from Sp7 (wild-type) and 7606 (gltB::kan mutant) cells grown in minimal medium with 10 mM aspartate (Asp) or 20 mM ammonium (NH₄⁺) as the nitrogen source or grown under nitrogen-fixing conditions (N-fix).](image)

![FIG. 5. Uptake of NH₄⁺ in the wild-type A. brasilense Sp7 strain (○), rpoN:: kan mutant strain FAJ301 (□), and ntrB:: Tn5-194 mutant strain 7194 (•). Uptake was measured as the disappearance of NH₄⁺ in the assay buffer after the addition of ammonium to a final concentration of 0.1 mM at time zero. The detection limit of the selective ammonium electrode used was reached after 25 min. The inset gives a representation of the mean slope values for the wild-type A. brasilense Sp7 strain, rpoN::kan mutant strain FAJ301, and ntrB::Tn5-194 mutant strain 7194. The values shown are the means of two independent replicates. Standard deviations are indicated as vertical bars.](image)
number pLAFR3 vector (4 to 7 copies per cell) (33). Another possibility is that the lacZ promoter adjacent to the multiple-cloning site in pLAFR3 is highly expressed in *A. brasilense*. Since the *amtB* gene is oriented in the direction of expression of the lacZ promoter, this could result in enhanced *amtB* expression. The enhancement of [14C]methylammonium uptake in wild-type strain Sp7 when pFAJ309 was introduced is in line with these hypotheses.

**DISCUSSION**

When *Azospirillum* cells are grown under conditions of nitrogen fixation, no ammonium can be detected in the growth medium. It was postulated that bacteria have an active ammonium uptake system to compensate for the loss of NH₄⁺ by diffusion through the plasma membrane (36). Here we report on the genetic and biochemical characterization of AmtB, a (methyl)ammonium transport protein of *A. brasilense*.

In contrast to that of *E. coli*, the *A. brasilense* *amtB* gene is not part of an operon with a glnB-like gene. The coexistence of two PII-like proteins, encoded by glnB and glnZ, has been established in *A. brasilense* (15). Comparing physical maps of the DNA fragments containing the *A. brasilense* glnB, glnZ, and *amtB* genes confirms that they are located on different restriction fragments. In *E. coli*, *amtB* is cotranscribed with glnK, which encodes a nitrogen regulatory P₇₀-like protein (70). In *B. subtilis* the nrgA gene is also part of an operon with nrgB, encoding a P₇₀-like protein, but nrgA is located upstream of nrgB. In *M. jannaschii* two genes which encode putative NH₄⁺ transporter proteins are located just next to a gene homologous to *glnB* (7). *C. glutamicum* is the only prokaryote for which it has also been reported that *amt* is not located in an operon structure with a gene encoding a P₇₀-like protein (61).

The synthesis of most prokaryotic NH₄⁺ carriers is repressed by NH₄⁺ (37). This is also the case for the yeast *S. cerevisiae* (16, 43), but *Arabidopsis thaliana* AMT1 activity is not lowered in cells grown in the presence of NH₄⁺ (52). Transcription of the *E. coli* glnKamtB operon and the *C. glutamicum* *amt* gene is under nitrogen control in both organisms (61, 70). This is also observed for the *A. brasilense* *amtB* gene. The level of expression of an *amtB::gusA* fusion is significantly lowered in a ntrC mutant (Table 2). This indicates that the Ntr system is involved in the nitrogen-regulated transcription of *amtB*.

The NH₄⁺ uptake rates of *ntr* mutant strains, as well as the observation that an *amtB::kan* mutant is still able to grow on ammonium concentrations as low as 0.1 mM (Fig. 7), suggest that existence of a second NH₄⁺ transport mechanism. The existence of multiple NH₄⁺ transporter proteins has been reported for other organisms. In yeast three genes encoding an NH₄⁺ transporter (*MEP1, MEP2, and MEP3*) (43, 44) were isolated. Evidence also exists for the presence of two transporter systems in *Nostoc muscorum* (32), *Rhodobacter sphaeroides* (12), and *Anacystis nidulans* (4). In these three bacteria, one of the transport systems is constitutive and cannot transport methylammonium, while the other system is repressed in high NH₄⁺ concentrations and is capable of transporting methylammonium.

The ammonium uptake profiles of *ntr* mutants, in K⁺-free assay buffer (Fig. 5), indicate that in these conditions the second ammonium transport mechanism does not correspond to simple diffusion. Indeed, in this assay, the external ammonium concentration, as measured by the ammonium electrode, drops below 42 μM, which is the apparent *K_m* (Michaelis constant) of the GS enzyme of *A. brasilense* (54). The *K_m* value of the main ammonium-assimilating enzyme, which is the GS enzyme in *A. brasilense* (73), can be used to estimate the steady-state internal ammonium concentration (11). K⁺ uptake systems...
have been reported to support NH$_4^+$ uptake in *E. coli* (8), and in *Rhodobacter capsulatus* (22). Since NH$_4^+$ ions are similar both in charge and size to K$^+$ ions, the possibility that a K$^+$ transporter can mediate NH$_4^+$ uptake in K$^+$-free conditions cannot be excluded. Further investigation will be needed to establish the mode of ammonium transport in mutants lacking the nitrogen-regulated (methyl)ammonium transporter.

An analysis of the distribution of hydrophobic and hydrophilic amino acid residues shows that the AmtB amino acid sequence has highly hydrophobic stretches (38). The algorithm of Klein et al. (34) predicted 12 transmembrane domains and classified the protein as an integral membrane protein. The stretches separating the putative transmembrane regions are rich in charged amino acid residues and correspond to potential P$_i$-transport proteins (42). An analysis of the distribution of hydrophobic and hydrophilic amino acid residues shows that the AmtB amino acid sequence has highly hydrophobic stretches (38). The algorithm of Klein et al. (34) predicted 12 transmembrane domains and classified the protein as an integral membrane protein. The stretches separating the putative transmembrane regions are rich in charged amino acid residues and correspond to potential P$_i$-transport proteins (42). An analysis of the distribution of hydrophobic and hydrophilic amino acid residues shows that the AmtB amino acid sequence has highly hydrophobic stretches (38). The algorithm of Klein et al. (34) predicted 12 transmembrane domains and classified the protein as an integral membrane protein. The stretches separating the putative transmembrane regions are rich in charged amino acid residues and correspond to potential P$_i$-transport proteins (42).

In conclusion, we have characterized an *A. brasilense* (methyl)ammonium transporter. The corresponding gene, *amtB*, is the first *A. brasilense* gene known to require NtrC for expression. Indeed, this transcriptional activator is not required for the transcription of *nifA*, *glnA*, or *glnB* in *A. brasilense*. The AmtB NH$_4^+$ transporter is necessary for growth on low NH$_4^+$ concentrations. However, it appears not to be the sole mechanism for NH$_4^+$ uptake in *A. brasilense*.

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