Isolation of a Glutamate Synthase (GOGAT)-Negative, Pleiotropically N Utilization-Defective Mutant of *Azospirillum brasilense*: Cloning and Partial Characterization of GOGAT Structural Gene

ASIM KUMAR MANDAL AND SUDHAMOY GHOSH*

Department of Biochemistry and Centre for Plant Molecular Biology, Bose Institute, AICB Centenary Building, Calcutta 700 054, India

Received 6 July 1993/Accepted 5 October 1993

An *Azospirillum brasilense* mutant (N12) pleiotropically defective in the assimilation of nitrogenous compounds (Asm-) was isolated and found lacking in the glutamate synthase (GOGAT-). The *glt* (GOGAT) locus of *A. brasilense* was identified by isolating a broad-host-range pLAFR1 cosmid clone from a gene library of the bacterium that rectified Asm- and GOGAT- defects (full recovery of activities of the nitrogenase, the assimilatory nitrate and nitrite reductases, and the glutamate synthase). A 7.5-kb EcoRI fragment of the cosmid clone that also complemented N12 was partially sequenced to identify the open reading frame for the α-subunit of GOGAT. The amino acid sequences deduced from the partial nucleotide sequences of the *glt* locus of *A. brasilense* showed considerable homology with that of the α-subunit of GOGAT coded by the *gltB* gene of *Escherichia coli*. The genetic lesion of N12 was found within the *gltB* gene of *A. brasilense*. The *gltB* promoter of *A. brasilense* showed the presence of a consensus sigma-70-like recognition site (as in *E. coli*) in addition to potential NtrA-RNA polymerase, IHF, and NifA binding sites.

In *Azospirillum brasilense*, a nitrogen-fixing aerobic soil bacterium well known for its ability to colonize roots of grass plants of economic importance and to increase plant productivity (8, 25), two enzymatic pathways exist for ammonia assimilation: the glutamate dehydrogenase (GDH; EC 1.4.1.3) pathway in which ammonia is available at a high level (16, 35) and the glutamine synthetase (GS; EC 6.3.1.2)-glutamate synthase (GOGAT [glutamine 2-oxoglutarate amidotransferase]; EC 1.4.1.13) pathway in which ammonia availability is low, for example, during N₂ fixation (2, 4, 7, 8, 35). The GS-GOGAT pathway is particularly important for all free-living N₂-fixing bacteria for growth under ammonia-deficient conditions (3, 25), but for symbiotic bacteria, e.g., *Rhizobium* spp. within root nodules, the pathway may remain repressed to help ammonia export (11, 24). The importance of the GS-GOGAT pathway was first demonstrated by Nagatani et al. (19), who observed that GOGAT-deficient mutants of *Klebsiella pneumoniae* showed an ammonia assimilation (Asm-) defect; they failed to fix N₂ (Nif-) and utilize a number of amino acids as N sources. GOGAT-deficient mutants of *N₂*-fixing bacteria showing the Asm- phenotype have been described for *A. brasilense* (2), *Bradyrhizobium japonicum* (21), *Rhizobium meliloti* (13, 15, 24), and *Azorhizobium caulinodans* ORS571 (11). It is not yet very clear why GOGAT deficiency would lead to pleiotropic N-assimilation defects (Asm-) in the mutants. An abnormally high concentration of glutamine may cause adenylylation of GS, and repression of sensitive operons in GOGAT- mutants has been suggested (2). The GOGAT structural gene of *Escherichia coli* has been cloned (5) and sequenced (23), and it showed that the α- and β-subunits of GOGAT are determined by the *gltB* and *gltD* genes of the *gltBDF* operon (5). Recent studies with the *glt* operon of *E. coli* (5) and that of *K. pneumoniae* (14) indicate that the pleiotropic Asm- defect may be due to the *gltF* product, which plays an undefined role in ntr regulation. Any role of a *gltF*-like gene in nonenteric bacteria remains unknown. GOGAT of *A. brasilense* has already been well characterized (26, 34), and its α- and β-subunits are very similar to those of *E. coli* in size. Isolated α-subunits of GOGAT from *A. brasilense* and *E. coli* also showed a high degree of homology in their N-terminal sequences (34), but the same deduced sequences from their structural genes could not be compared, as the sequence of the *glt* gene of *A. brasilense* is not known. In our study with *A. brasilense* RG, for an understanding of regulation of N₂ fixation and N metabolism, we isolated a number of Nif- and Asm- mutants. One of these mutants (N12) showing a Nif- Asm- phenotype also lacked GOGAT activity. We report here the cloning of the GOGAT structural gene (glt) of *A. brasilense* and partial sequencing of the *gltB* gene to determine its promoter structure, deduced N-terminal amino acid sequence, and the locus of the mutation in N12.

*A. brasilense* RG, a wild-type strain (sp. 81 from the stock of N. R. Krieg, Blacksburg, Va.) was used for this study. The bacterium was grown, as described previously (16), in succinate-salt medium with no N source (SSM), with 0.1% NH₄Cl (SSM), or with 0.2% of another fixed N source when necessary (adding 1.6% agar for plates). Mutagenesis of the bacterium by *N*-methyl-*N*-nitro-*N'*-nitrosoguanidine was carried out essentially by the procedure of Miller (17). For enrichment of the Nif- mutants, aliquots from the stocks of mutants were added to SSM (optical density, 0.3), the cells were allowed to grow microaerobically (optical density, 0.6), and ampicillin and cycloserine were added to the growing culture at final concentrations of 2 mg and 50 μg ml⁻¹, respectively. After lysis of the wild-type Nif+ cells (in about 4 h of further microaerobic incubation), enriched Nif- cells were collected from the lysed culture, washed, resuspended in saline, and spread on SSM plates containing 0.003% yeast extracts (SSM-0.003% YE). Tiny colonies (Nif-) were selected from large colonies (Nif+) and replica plated on SSM.

* Corresponding author.
TABLE 1. Specific activities of ammonia, nitrate, and dinitrogen assimilatory pathway enzymes in *A. brasilense* RG, its mutants, and complemented N12

<table>
<thead>
<tr>
<th><em>A. brasilense</em> straina</th>
<th>N sourceb</th>
<th>Nitrogenasec</th>
<th>GS Sp act (nmol min⁻¹ mg of protein⁻¹)d</th>
<th>GOGAT Sp act (nmol min⁻¹ mg of protein⁻¹)d</th>
<th>GDH Sp act (nmol min⁻¹ mg of protein⁻¹)d</th>
<th>NAR Sp act (nmol min⁻¹ mg of protein⁻¹)d</th>
<th>NIR Sp act (nmol min⁻¹ mg of protein⁻¹)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG</td>
<td>NH₄Cl</td>
<td>0.01</td>
<td>1.621</td>
<td>814</td>
<td>113</td>
<td>246</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>Glutaminate</td>
<td>6.4</td>
<td>2,626</td>
<td>1,696</td>
<td>60</td>
<td>18</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>Glutamate</td>
<td>169</td>
<td>3,046</td>
<td>1,918</td>
<td>38</td>
<td>16</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>No fixed N</td>
<td>175</td>
<td>837</td>
<td>200</td>
<td>5.2</td>
<td>147</td>
<td>46</td>
</tr>
<tr>
<td>N12</td>
<td>NH₄Cl</td>
<td>NT</td>
<td>1,643</td>
<td>817</td>
<td>108</td>
<td>203</td>
<td>36.3</td>
</tr>
<tr>
<td></td>
<td>Glutaminate</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>No fixed N</td>
<td>189</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>N12(pMG1-2)</td>
<td>NH₄Cl</td>
<td>NT</td>
<td>1,662</td>
<td>831</td>
<td>101</td>
<td>242</td>
<td>35.6</td>
</tr>
<tr>
<td></td>
<td>Glutaminate</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>No fixed N</td>
<td>187</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

a The strains N12(pMG1-2) and N12(pMG3-3) are described in Fig. 1; N12(pMG1) (GOGAT- nitrate ) is an isolate of N12 growing faster on SSM-0.2% glutamate plates and showing nearly normal-size colonies like those of N12 (see the text).
b Cells were grown aerobically in SSM supplemented with nitrogen sources (weight/volume): NH₄Cl (0.1%), glutamate (0.2%), and glutamic acid (0.2%). No fixed, N cells grown microaerobically for nitrogenase assays only, as described in footnote c.
c For nitrogenase assays, cells were pregrown aerobically with 0.1% NH₄Cl as the N source followed by microaerobic growth for 18 h from an initial optical density of 0.5 in 20 ml of SSM containing 0.05% agar in a 150-ml conical flask (27). Results are given in nanomoles of C₂H₄ formed per hour per milligram of cell protein. d GS (3), GOGAT (18), GDH (16), nitrate reductase (NAR) (32), and nitrite reductase (NIR)(39), and nitrogenase (27) assays were performed, following the published methods. For NAR and NIR induction, 10 mM NaNO₃ was added to exponentially growing cells at an optical density of about 1.0 for the former and 0.5 mM NaNO₂ was added for the latter and further incubation was continued (aerobically) for 3 h at 32°C before collection of cells for assay. When RG (wild-type) cells were grown aerobically in SSM plus 0.01% NH₄Cl, low ammonium, the specific activity of GOGAT was reduced to 25; when grown in SSM plus 0.1% KNO₃, the specific activities of nitrate and nitrite reductases were 16.7 and 2.1, respectively.

and SSM-0.003% YE agar plates. Nif− mutants yielded normal colonies on SSM but only tiny colonies on SSM-0.003% YE. All Nif− mutants were further screened for their ability to grow on a mixture of arginine plus proline as the N source (i.e., on SSM-ArgPro plates). Mutants pleiotropically defective in N assimilation were selected by their normal-size colonies on SSMN but by very tiny colonies on SSM-ArgPro and SSM-0.003% YE plates.

*A. brasilense* RG can assimilate various inorganic and organic nitrogenous compounds as the sole source of N for growth (31). By our selection procedure, a pleiotropic N assimilation-defective mutant, N12, that failed to utilize any of these compounds (e.g., glutamine, asparagine, glutamate, aspartate, arginine, proline, alanine, urac, nitrate, dinitrogen, or a low concentration of ammonium [<0.01% NH₄Cl]) as the sole N source for growth was isolated. This mutant (N12) showed a phenotype very similar to that of Asm− mutants of *K. pneumoniae* (19), *E. coli* (25), and *A. brasilense* (2) described previously that had very little or no GOGAT activity. Therefore, ammonia assimilation pathway enzymes in *A. brasilense* RG (wild type) and N12 were assayed. As shown in Table 1, the GOGAT activity of N12 was 22-fold less than that of the wild type. The specific activity of GOGAT in RG was maximal when grown with 0.1% NH₄Cl; lower activities were observed when it was grown with 0.01% NH₄Cl, glutamate, or glutamine as the sole nitrogen source (Table 1). Compared with activities in the wild type, some reductions of the GS and cold-labile GDH activities were observed in N12; lower activities of these two enzymes were also noticed with other Asm− mutants (2).

GOGAT mutants of *A. brasilense* described earlier by Bani et al. (2) showed N assimilation defects (Asm− phenotype), but unlike N12, they could assimilate glutamine and glutamate as an N source for growth. In fact, GOGAT− mutants of nitrogen-fixing bacteria do show diverse abilities to utilize N sources for growth. GOGAT− mutants of *R. meliloti* (13, 15, 24) and *B. japonicum* (21) obtained by chemical or UV mutagenesis could use histidine, arginine, or proline but not ammonia at any concentration for growth. On the other hand, *glt* insertion mutants of *R. meliloti* (33) could grow with proline or asparagine but not with ammonium, aspartate, arginine, or histidine. In the case of stem-nodulating symbiotic *A. sesbaniae* ORS571, well-defined *Tn5*-induced GOGAT− mutants showed a Nif− Asm− phenotype with the ability to grow on glutamine, asparagine, or aspartate as the N source (11). Therefore, one would tend to believe that the physical location of the mutation in the *glt* locus might be responsible for this variable Asm− phenotype.

Although N12 failed to assimilate many N sources for growth, we tested only the enzymes needed for the metabolism of two of these compounds: dinitrogen and nitrate. No nitrogenase activity could be detected in N12 under conditions of derepression that expressed the enzyme in the wild type (Table 1). Reduction of nitrate in *A. brasilense* RG can be carried out by two enzymes—assimilatory and dissimilatory nitrite reductases (20). Growth of *A. brasilense* with nitrate as the sole N source depends on both the assimilatory nitrate and nitrite reductases. Presence of the dissimilatory nitrite reductase in *A. brasilense* is strain dependent; the enzyme is absent in *A.
**FIG. 1.** Physical map of *A. brasilense* RG *glt* locus, cloned *glt* regions, and their abilities to complement N12. The topmost line indicates the 27.1-kb *EcoRI* insert of cosmid clone pMG1-198 in which various restriction sites and approximate distances (in kilobases) are shown. The lines below indicate the various regions of the 27.1-kb insert cloned. On the right-hand side of each line, the name of the plasmid clone and the ability of the cloned DNA fragment to complement N12 are indicated. Restriction fragments were subcloned in pLAFR1 (pMG1 series) or in pLAFR3 (pMG3 series) with *E. coli* S17.1 as the host for their conjugal transfer to N12 for complementation analysis. Subcloning in pUC19 vector (pMG19 series) was done for nucleotide sequencing. The small arrows under PB and ES DNA fragments indicate the direction and extent of the sequence determined. Sequencing was done in both directions by the dideoxy chain termination method using a Sequenase version 2.0 kit supplied by U.S. Biochemical Corp., Cleveland, Ohio. Ordered deletion subclones were made by using a double-stranded nested deletion kit supplied by Pharmacia P-L Biochemicals, Inc., Milwaukee, Wis., following the manufacturer's protocol. The approximate limits of the analogous *glt* gene of *E. coli* (23) are shown (hatched arrow). Restriction sites: *EcoRI*; B, BamHI; P, PstI; S, SalI. +, complementation by some recombinational events (see the text); −, no complementation when transferred as an insert in pLAFR3; NT, not tested.

*brasilense* RG (15a) but present in *A. brasilense* Sp7 (36). The assimilatory nitrate and nitrite reductases are repressed by the growth of the bacterium in high levels of ammonia, but the dissimilatory nitrate reductase is not susceptible to ammonia repression; assimilatory and dissimilatory enzymes are all induced to high levels by nitrate in the presence of glutamine in *A. brasilense* RG (Table 1). N12 and *A. brasilense* RG grown on SSMN with nitrate as the inducer showed nearly the same low levels of synthesis of dissimilatory nitrate reductase (Table 1), and both of them also showed the same degree of chlorate sensitivity (data not shown). In the wild-type *A. brasilense* RG, growth in the presence of glutamine makes it possible for the nitrate to induce fully the assimilatory nitrate reductase and for the nitrite to induce the assimilatory nitrite reductase (Table 1); the inability of N12 to grow with glutamine as an N source makes such induction experiments unfeasible. It seems that the inability of the mutant N12 to grow with nitrate as the N source is mainly due to failure of the nitrate or nitrite to induce any assimilatory nitrate or nitrite reductase that converts nitrate to ammonia, on which cell growth depends. To confirm the status of the nitrite reductase in N12, we isolated a few colonies of N12 with the ability to grow faster on SSM-glutamate. After purifying a number of these colonies (N12Glu1), they were also found to grow well in glutamine but were GOGAT− and nitrate negative. When the nitrate and nitrite reductase activities of N12Glu1 were measured after the bacteria had grown in SSM-glutamine in the presence of appropriate inducers, the former was found in the cell extracts at the same level as the dissimilatory nitrate reductase and the latter at the uninduced basal level of the assimilatory nitrite reductase in the wild-type bacteria (Table 1).

Cloning of the GOGAT structural gene of *A. brasilense* RG and its partial sequencing were carried out, following the standard DNA recombinant techniques as described in reference 28. For this purpose, a genomic library of *A. brasilense* RG DNA was constructed with tetracycline-resistant broad-host-range cosmid pLAFR1 (1) at the *EcoRI* site with *E. coli* S17.1 (30) as the host that can directly transfer the recombinant pLAFR1 cosmid clone into *A. brasilense* by mating. A cosmid clone, pMG1-198, containing a 27.1-kb insert of DNA that could rectify Asm− and Nif− defects when conjugally transferred into N12 (Fig. 1) was identified from the library. The complemented N12(pMG1-198) strain also showed GOGAT activity like that of the wild type (Table 1). A restriction site map of the 27.1-kb DNA insert is shown in Fig. 1. Cloning of the *EcoRI*-digested DNA fragments (in pLAFR1) followed by complementation analysis with N12 identified the locus of the GOGAT structural gene (glt) within
FIG. 2. (A) Nucleotide sequence of the 0.863-kb EcoRI-Sall fragment of the gltB gene of A. brasilense RG. The coding strand of the DNA is presented in a 5’-to-3’ direction. Amino acids deduced from the nucleotide sequence and specified by standard one-letter abbreviations are indicated above the first base of each codon. The putative Shine-Dalgarno sequence upstream of the ATG codon is underlined. The potential NtrA-dependent promoter (−12 and −24 sequences) and sigma-70 promoter (−10 and −35 sequences) are overlined. ***, potential NifA binding site (TGT-N_{12}-ACA); **, IHF binding site. (B) N-terminal amino acid sequence homology between gltB of A. brasilense RG (Ab) and that of E. coli (Ec), as deduced from their nucleotide sequences. The N-terminal amino acid sequences (residues 1 to 151 of A. brasilense RG and 1 to 154 of E. coli) are aligned; the residues connected by vertical lines are identical, and dashes in the sequences represent gaps introduced to improve sequence homology. The predicted cleavage site for generating the mature α-subunit of GOGAT (34) is indicated by vertical arrows. There is about 43.5% identity in the N-terminal amino acids of the two mature α-subunits of GOGAT.

The 7.5-kb insert DNA of pMG1-2 (Fig. 1). As shown in Table 1, N12(pMG1-2) was GOGAT* and Nif* like the wild type, and it could also use all normal N sources for growth (Asm*).

The 7.5-kb DNA fragment was recloned in tetracycline-resistant IncP1 cosm pLAFR3 (obtained from N. Keen, Riverside, Calif.) containing multiple cloning sites, and a new restriction site map of the DNA fragment was constructed. As shown in Fig. 1, the two small PstI-PstI 1.18-kb and BamHI-PstI 0.9-kb DNA fragments subcloned in pLAFR3 also could complement the mutant N12 by mating although at a low frequency. Only a few among the tetracycline-resistant transconjugants obtained after mating of S17.1(pMG3-2) or S17.1(pMG3-3) with N12 could grow on SS-0.003% YE or SSM-ArgPro plates, and they showed an Asm* GOGAT* phenotype. [The enzyme activities of only N12(pMG3-3) are shown in Table 1.] The N12(pMG3-2) and N12(pMG3-3) transconjugants showing the wild-type characteristics are extremely unlikely to be wild-type revertants of N12, since the Asm* GOGAT* reversion frequency is very low (<10^-7) and we failed to detect any such revertants in control plates. Moreover, the Asm* GOGAT* transconjugants were very specifically obtained only when the donor’s plasmid DNA contained the 0.9-kb BamHI-PstI sequence. Since GOGAT subunits of A. brasilense are known to be 140 and 56.5 kDa (34), the observed complementation by the small DNA fragment (0.9-kb PstI-BamHI fragment) could only be due to some recombinational rectification of the mutant gene in N12. This was confirmed by curing of the resident plasmid of the GOGAT* Asm* N12(pMG3-2) and N12(pMG3-3) transconjugants by the RP4 plasmid (a kanamycin- and tetracycline-resistant IncP1, broad-host-range plasmid). The cured kanamycin-resistant N12 strains did show the expected Asm* GOGAT* phenotype where RP4 was the only detectable resident plasmid. These results most probably mean that the locus of the genetic lesion that caused the GOGAT* Asm* phenotype in N12 is within the 0.9-kb BamHI-PstI fragment.

To confirm that the GOGAT structural gene is in the 7.5-kb DNA fragment, the small 0.28-kb PstI-BamHI fragment was sequenced and compared with the homologous gltB region of E. coli. The nucleotide sequences and the deduced amino acid sequences of the two gltB genes have very high homology in this region. A 50% identity at the level of amino acids and 56.7% identity at the level of nucleotides were observed in these two partial gene sequences. This homology enabled us to align the promoter and the translation start site of the gltB gene of A. brasilense RG (in the 0.863-kb EcoRI-Sall DNA fragment) with the corresponding gltB region of E. coli (23).

The deduced N-terminal amino acid sequences of the gltB gene from A. brasilense RG and E. coli (Fig. 2A and B) showed that, starting from the cysteine residue at position 37 in the former and position 43 in the latter, there exists a strong homology between the two. This is expected because it is already known that the first 20 N-terminal amino acid sequences of the α-subunit of GOGAT of the two bacteria are quite similar, and
both the sequences start with cysteine (10, 34). In fact, the 20-amino-acid N-terminal sequence of the α-subunit of *A. brasilense* Sp7 GOGAT reported by Vanoni et al. (34) exactly matches the deduced N-terminal amino acid sequence of *A. brasilense* RG GOGAT, starting with the cysteine residue at position 37, as shown in Fig. 2B.

The promoter sequences of the *gltB* gene of *A. brasilense* and *E. coli* differed considerably. Whereas the *A. brasilense* promoter appears to have sigma-70 as well as sigma-54 recognition sites (Fig. 2A), *E. coli* has only the former. Surprisingly, the *gltB* and *nifH* promoters of *A. brasilense* show considerable similarity. The NtrA recognition domain CTGGGC-CGATG CGAC in the *nifH* promoter of *A. brasilense* (7) is similar to the corresponding domain, CTGGGC-GTGAAG, in the *gltB* promoter. In addition, the presence of TGT-N_1-AACA (NifA consensus: TGT-N_1-AACA) and AAGCAATCCATAA (IHF consensus: WATCAA-N_2-TTR; W = A or T; and R = A or G) sequences in the *gltB* promoter are suggestive of potential NifA and IHF binding sites, respectively (12). Although it is not very unusual to find multiple promoters in highly regulated genes (6), it is not clear why this should be encountered in the *glt* operon of *A. brasilense*, which is not known to be highly regulated. Obviously, we have learned more about the *glt* operon of *A. brasilense*, particularly its interrelationship with other regulatory genes, e.g., *trp* (9) or *gltF* of enterobacteria, that may also control N metabolism in the bacterium. Study in this direction should also take into account our results showing that the *Asm* phenotype is correlated with a mutation within the *gltB* gene of *A. brasilense*.

**Nucleotide sequence accession numbers.** The nucleotide sequences for the 5′-terminal and internal coding regions have been given the accession numbers X71090 and X71632, respectively, by the EMBL Data Bank.

We thank the Council of Scientific and Industrial Research, Government of India, for supporting us with an Emeritus Scientist Fellowship to S.G. and a Senior Research Fellowship to A.K.M.

We are indebted to Sudip Chattopadhyay for supplying us his cosmid pLAFR1 library of *A. brasilense* RG. We thank N. Keen (Riverside, Calif.) for giving us cosmid pLAFR3. Expert technical assistance of Aism Banerjee and A. Ali Molla is gratefully acknowledged. We also thank M. K. Maiti, N. Das, and A. Basu for their helpful suggestions and critical comments.

**References**


AUTHOR’S CORRECTION

Isolation of a Glutamate Synthase (GOGAT)-Negative, Pleiotropically N Utilization-Defective Mutant of Azospirillum brasilense: Cloning and Partial Characterization of GOGAT Structural Gene

ASIM KUMAR MANDAL AND SUDHAMOY GHOSH

Department of Biochemistry and Centre for Plant Molecular Biology, Bose Institute,
AJCB Centenary Building, Calcutta 700 054, India

Volume 175, no. 24, p. 8024–8029: We reported a partial nucleotide sequence of the Azospirillum brasilense RG gltB locus that included 453 bp of the gltB coding region and 410 bp of the 5'-flanking DNA. Unfortunately, we were unaware of a publication by Pelanda et al. that should have been cited (R. Pelanda, M. A. Vanoni, M. Perego, L. Piubelli, A. Galizzi, B. Curti, and G. Zanetti, J. Biol. Chem. 268:3099–3106, 1993). In this publication, the entire sequence of the glt locus from another strain of A. brasilense, Sp7, was reported. This sequence included 852 bp of DNA 5' to the gltD coding sequence, the 1443-bp gltD sequence, and a 141-bp intergenic region followed by the 4545-bp coding sequence for gltB and 295 bp of 3'-flanking DNA. Within the aligned sequences of the corresponding DNA from the two A. brasilense strains there is 98.5% identity in the gltB open reading frames and 84.6% identity in the DNA upstream of gltB.