Under nitrogen starvation conditions, Corynebacterium glutamicum was found to take up methylammonium at a rate of $20 \pm 5$ nmol min$^{-1}$ (mg dry weight)$^{-1}$. The specific activity of this uptake was 10-fold lower when growing the cells under sufficient nitrogen supply, indicating a tight regulation on the expression level. The methylammonium uptake showed Michaelis-Menten kinetics with an $K_m$ of $44 \pm 7 \mu M$ and was completely inhibited by the addition of $10 \mu M$ ammonium. This finding and the fact that methylammonium was not metabolized by C. glutamicum strongly suggests that the uptake carrier actually represents an ammonium uptake system. Methylammonium uptake was strictly dependent on the membrane potential. From the pH optimum and the accumulation of methylammonium in equilibrium, it could be deduced that only one net charge is transported and, thus, that methylammonium is taken up in its protonated form via an unipolar mechanism. The $amt$ gene encoding the (methyl)ammonium uptake system was isolated and characterized. The predicted gene product of $amt$ consists of 452 amino acids ($M_r = 47,699$) and shows 26–33% identity to ammonium transporter proteins from Saccharomyces cerevisiae and Arabidopsis thaliana. According to the hydrophobicity profile, it is an integral membrane protein containing 10 or 11 membrane-spanning segments.

Most microorganisms use ammonium ($NH_4^+$) as the preferred nitrogen source. Although ammonia ($NH_3$) is highly membrane-permeable, energy-dependent transport systems for $NH_4^+$ have been found in many bacterial species (1). Usually these carriers are inhibited by glutamine, and their expression is regulated by the availability of $NH_4^+$ itself (2). At high $NH_4^+$ concentrations in the culture medium, i.e. when the nitrogen supply via $NH_3$ diffusion is sufficient, the transport systems are repressed. In general, $NH_4^+$ transport is catalyzed by secondary transport systems that depend on the membrane potential as driving force. As an exception in Escherichia coli, $NH_4^+$ is taken up in antiport against a potassium ion, and thus the potassium gradient is used as energy source for uptake (3).

Despite the large number of carriers characterized functionally, only little information is available concerning genes coding for $NH_4^+$ transport proteins. Up to now, no gene for a procaryotic $NH_4^+$ carrier has been isolated. By complementation of an E. coli mutant deficient in $NH_4^+$ transport, Fabiny et al. (4) identified a gene, designated $amtA$, that codes for a soluble protein and which thus is discussed to represent a cytoplasmic component of an $NH_4^+$ uptake system. The first genes coding for $NH_4^+$ transport proteins ($MEP1$ and $MEP2$) were isolated from Saccharomyces cerevisiae (5) (GenBank number P41948). Expression of cDNA from Arabidopsis thaliana in a transport-deficient mutant of S. cerevisiae allowed cloning of the gene for a high affinity $NH_4^+$ transporter from this plant (6). When searching for similarity of the yeast carrier $MEP1$ to other proteins, Marini et al. (5) found that the N-terminal region of $MEP1$ showed 28% identity to a polypeptide of 148 amino acid residues, encoded by a truncated open reading frame (ORF) downstream of the phosphoethanolamine carbamoylase gene (ppc) of Corynebacterium glutamicum.

Since the ppc gene of C. glutamicum has been found by us (7), the observation of Marini et al. (5) prompted us to isolate the complete ORF downstream of the ppc gene. In this study, we describe the functional characterization of the $NH_4^+$ carrier of C. glutamicum and the isolation and sequencing of the first procaryotic gene encoding such a transport protein.

**EXPERIMENTAL PROCEDURES**

Strains, Plasmids, and Culture Conditions—All strains and plasmids used are listed in Table I. Luria Bertani (LB) medium (13) was used as complex medium for both E. coli and C. glutamicum. 2% glucose was added for growth of C. glutamicum. For nitrogen starvation of C. glutamicum, a nitrogen-free minimal medium containing 0.5 g/liter KH$_2$PO$_4$, 0.5 g/liter K$_2$HPO$_4$, 0.25 g/liter MgSO$_4$ $\times$ 7 H$_2$O, 40 g/liter MOPS, 10 mg/liter CaCl$_2$, 10 mg/liter FeSO$_4$ $\times$ H$_2$O, 10 mg/liter MnSO$_4$ $\times$ H$_2$O, 1 mg/liter ZnSO$_4$ 0.2 mg/liter CuSO$_4$, 0.02 mg/liter NiCl$_2$, 0.2 mg/liter biotin, and 30 mg/liter protocatechuate, pH 6.7. As carbon source, 0.5% glucose was added. Where appropriate, carbenicillin (50 mg/liter) or kanamycin (20 or 50 mg/liter) were added to the medium. Cells were grown aerobically on a rotary shaker at 120 rpm at 30°C (C. glutamicum) or 37°C (E. coli). Optical density was measured at 600 nm ($A_{600}$). An $A_{600}$ of 1 corresponded to 0.36 mg dry weight ml$^{-1}$.

Determination of Methylammonium Uptake—After overnight growth on LB medium, C. glutamicum cells were harvested, washed once with nitrogen-free minimal medium, and resuspended in the same medium to an $A_{600}$ of 3–4. If not indicated otherwise, cells were harvested after 3 h of incubation at 30°C and washed once with cold potassium phosphate buffer (50 mM, pH 7.0). Cells were then resuspended in the same buffer to an $A_{600}$ of 3–4. After prewarming at 30°C for 3 min in the presence of 10 mM glucose, uptake was initiated by the addition of $[^{14}C]$methylammonium (Amersham Corp.) (1 $\mu$M to 3 mM; 5.5–800 Bq/ml). A methylammonium concentration of 100 $\mu$M was used, if not indicated otherwise. Within the first 2–5 min, uptake was terminated by rapidly filtering aliquots of the cell suspension on glass fiber filters (pore size, 0.45 $\mu$m; Millipore, Eschborn, Germany). Filters were washed twice with 2.5 ml of ice-cold LiCl (0.1 M). Initial uptake rates were calculated from the linear part of the obtained kinetics (at least four data points).

Measurement of the Cytoplasmic Volume and the Membrane Potential—Determination of the cytoplasmic volume and the membrane potential was determined using the fixed membrane potential technique (9). The cytoplasmic volume was measured using the $[^{3}H]$choline method (10). Membrane potentials were calculated from the ionic activities of the external and internal ions, where the ionic activities of the internal ions were corrected for the membrane potential using the Henderson equation (11). Membrane potentials were measured under conditions of nitrogen-fixing and nitrogen-starved cells. The data given represent the average of at least four experiments.

The abbreviations used are: ORF, open reading frame; MOPS, 3-(N-morpholino)propanesulfonic acid; kb, kilobase pair(s).
Ammonium Uptake in C. glutamicum

### Table 1

| Strain or plasmid | Relevant genotype or characteristics | Source or reference |
|-------------------|-------------------------------------|---------------------|
| E. coli K12       | mcrA, Δ(mrr – hsdRMS – mcrBC)       | Ref. 8              |
| DH5αΔMCR         | thiI, DsR1 hsdR17 (r -m') supE44 pro | Ref. 9              |
| C. glutamicum ATCC 13032 (wild-type) | Amt<sup>-</sup>, BetP<sup>-</sup> | This work           |
| pEM1             | gdh::pSUP301                        | Peter, H., Burkovski, A., and Krämer, R., submitted for publication |
| ppc42            | C. glutamicum DNA                   | Ref. 7              |
| pUC19            | Ap<sup>+</sup> ori of ColE1         | Ref. 12             |
| pUC-dpcp         | pUC19 containing a 2.9 kb insert of chromosomal C. glutamicum DNA | This work           |

### Notes
- Ap<sup>+</sup>, ampicillin resistance
- Km<sup>+</sup>, kanamycin resistance

Potential was carried out as described previously (14). Cells were separated from the surrounding medium by silicone oil centrifugation (15). The internal volume was determined by use of [14C]inulin as nonpenetrating probe for the extracellular space and [3H<sub>2</sub>O] as probe for the total volume (16). A cytoplasmic volume of 1.8 μl (mg dry weight)<sup>-1</sup> was obtained for C. glutamicum and used for all calculations. The membrane potential was measured according to Rottenberg (16) by determining the distribution of the permeant cation [14C]tetraphenylphosphonium across the cytoplasmic membrane.

DNA isolation, Manipulation, and Transformation—Plasmid DNA was isolated from E. coli using the plasmid kit from Qiagen (Hilden, Germany). Chromosomal DNA of C. glutamicum was isolated according to Eikmanns et al. (17). E. coli was transformed by the method of Chung et al. (18). Restriction enzymes, DNA polymerase I (Klenow enzyme), shrimp alkaline phosphatase, proteinase K, RNase, and T4 DNA ligase were obtained from Boehringer Mannheim or from Promega and used as instructed by the manufacturers. Restriction analysis was carried out by separation of the fragments in 0.8% agarose gels. Fragments were isolated by use of J. etsorb from Genomed (Bad Oeynhausen, Germany).

DNA Sequence Analysis—By use of the Erase-a-Base<sup>®</sup> System (Promega), unidirectional deletions of the pUC-dpcp insert were constructed. Appropriate subclones were sequenced by use of the dyechain termination method (19). Sequencing was performed with the AutoRead sequencing kit from Pharmacia Biotech Inc., and electrophoresis was performed with an automated laser fluorescence DNA sequencer from Pharmacia. The sequence of the second strand was determined by use of appropriate unlabeled primers, and synthesis of short strands containing fluoro-dATP (Pharmacia). Sequence data were compiled and analyzed with the HUSAR program package (European Molecular Biology Laboratory, Heidelberg, Germany).

Southern Blot Analysis—About 10 μg of chromosomal C. glutamicum DNA was digested with BamHI and SalI, size-fractionated by agarose gel electrophoresis (0.8% agarose), and transferred onto a nylon membrane Nytran 13 (Schleicher und Schuell, Dassel, Germany) by vacuum-supported diffusion using the Vancogen system (Pharmacia). A 1-kb BamHI-SalI fragment isolated from pUC-pcdp1A (7) was labeled with digoxigenin-dUTP and used as a probe. Labeling, hybridization, washing, and detection were performed by use of the nonradioactive DNA labeling and detection kit (Boehringer Mannheim).

Gene Disruption—Gene-directed mutagenesis of the amt gene was performed by the method described by Schwarzer and Pühler (20). A 0.27-kb internal Sphl-amp fragment was ligated into vector pEM1, which cannot replicate in C. glutamicum. The plasmid was transferred from E. coli S71-1 to C. glutamicum by conjugation as described by Schäfer et al. (21). Transconjugants, in which integration of the vector had occurred at the chromosomal Amr locus, were selected on LB agar plates containing kanamycin (25 mg/liter) and nalidixic acid (50 mg/liter<sup>-1</sup>).

RESULTS

In order to detect transport of NH<sub>4</sub><sup>-</sup> in C. glutamicum, [14C]methylammonium as an NH<sub>4</sub><sup>-</sup> analog was employed, since no suitable nitrogen isotope is available. C. glutamicum cells, incubated in nitrogen-free medium, were found to take up methylammonium at a rate of 15 nmol-min<sup>-1</sup>-(mg dry weight)<sup>-1</sup>. When NH<sub>4</sub><sup>-</sup> was added to the transport assay, uptake of methylammonium was completely inhibited (data not shown). Inhibition was competitive, since cells were observed to resume methylammonium uptake at uninhibited rate after some time, i.e. when the added NH<sub>4</sub><sup>-</sup> was consumed. This suggests that the carrier analyzed is not specific for methylammonium but accepts both NH<sub>4</sub><sup>-</sup> and methylammonium as substrate.

To test whether C. glutamicum is able to metabolize methylammonium, cells were incubated for 2 h in the presence of 1.7 mM methylammonium, and the methylammonium concentration inside the cells and in the medium was determined as a function of time by use of high-pressure liquid chromatography. No decrease of total methylammonium in the reaction mixture was observed (data not shown), indicating that C. glutamicum is not able to metabolize this NH<sub>4</sub><sup>-</sup> analog.

In many microorganisms, the expression of genes encoding NH<sub>4</sub><sup>-</sup> uptake systems has been found to increase when the nitrogen source in the medium is depleted (1). To check whether the same pattern of expression exists in C. glutamicum, the methylammonium transport activity in cells transferred from complex medium to nitrogen-free minimal medium was measured as a function of time (Fig. 1). Uptake activity was found to increase 10-fold during the first 2–3 h of incubation in the absence of a nitrogen source. No increase of uptake activity was observed when protein synthesis was inhibited by chloramphenicol. For further characterization of (methyl)ammonium uptake, cells were employed after 3 h of incubation in nitrogen-free minimal medium.

Characterization of Methylammonium Uptake in C. glutamicum—Methylammonium uptake was measured as a function of the substrate concentration. Uptake showed Michaelis-Menten kinetics with a K<sub>m</sub> of 44 ± 7 μM and a V<sub>max</sub> of 20 ± 5 nmol-min<sup>-1</sup>-(mg dry weight)<sup>-1</sup> (data not shown). It was not possible to determine a K<sub>i</sub> value for NH<sub>4</sub><sup>-</sup> since complete suppression of methylammonium transport by NH<sub>4</sub><sup>-</sup> was observed even at NH<sub>4</sub><sup>-</sup> concentrations of 10 μM or lower.

To determine the driving forces of methylammonium uptake, the effect of ionophores on transport activity was studied. Methylammonium uptake was found to be inhibited completely when the proton-motive force was destroyed by the protonophor carbonyl cyanide m-chlorophenylhydrazone, indicating that uptake is catalyzed by a secondary transport system depending on components of the proton-motive force for energization. The role of the membrane potential (∆Ψ) in methylammonium transport was analyzed in detail by setting potassium diffusion potentials in the presence of valinomycin (Fig. 2). While the external potassium concentration had no influence on uptake activity in the absence of valinomycin, uptake activity decreased with increasing potassium concentrations, i.e.
with decreasing $\Delta \Psi$, when valinomycin was present (Fig. 2A). Plotting uptake activity as a function of the membrane potential revealed a linear dependence of transport activity on $\Delta \Psi$ for membrane potentials between 30 and 150 mV (Fig. 2B).

The observed $\Delta \Psi$ dependence of methylammonium transport could result from uniport of protonated methylammonium or from cotransport of either unprotonated methylamine or protonated methylammonium with cations such as H$^+$ or Na$^+$. Transport of unprotonated methylamine is unlikely because methylamine is almost completely protonated at physiological pH due to its high $pK_a$ value of 10.7. When methylammonium influx was measured as a function of the extracellular pH, the rate was found to increase with rising pH (data not shown). Since the concentration of the highly permeable unprotonated methylamine also increases at alkaline pH, these data had to be corrected for methylamine influx driven by diffusion and trapping of protonated methylammonium in the cells due to the pH-gradient (pH$_{in}$ < pH$_{ex}$). This diffusion-driven influx, measured as the residual influx after carrier-mediated uptake had been inhibited by 250 $\mu$M p-chloromercuribenzoic acid, was also quantified as a function of the external pH. The true pH dependence of carrier-mediated methylammonium transport, obtained by subtraction of influx in the absence and in the presence of p-chloromercuribenzoic acid, yielded a pH optimum of 7.0, which again argues for protonated methylammonium being the transported species.

The number of charges transported in each transport cycle can be deduced from the accumulation of methylammonium ($c_{in}/c_{ex}$) in thermodynamic equilibrium. This could be measured in C. glutamicum, since no degradation of methylammonium took place. As shown in Fig. 3, a constant maximum methylammonium accumulation of 350-fold was found at external concentrations between 6 and 60 $\mu$M. A membrane potential of $-160$ mV was measured for the cells used in this experiment. Assuming that only one charge is transported in each transport cycle, i.e. that the carrier catalyzes uniport of protonated methylammonium, the membrane potential of $-160$ mV could be used to build up a maximum accumulation of about 460-fold, according to the equation $c_{in}/c_{ex} = \exp(-\Delta \Psi/Z) (Z = 2.3 RT/F)$. The fact that the observed accumulation was somewhat lower argues against another ion being transported together with methylammonium since then a much higher accumulation should be reached. In accordance with this conclusion, we observed that the external Na$^+$ concentration had no influence on the rate of methylammonium uptake (data not shown).

Isolation of the Downstream Region of the ppc Gene from C. glutamicum—Recently, the MEP1 gene encoding the low affinity, high capacity NH$_4^+$ transport system of S. cerevisiae has been cloned and sequenced. The authors noted that the N-terminal region of the MEP1 gene product showed 28% identity to a polypeptide (148 amino acid residues) encoded by an ORF, which starts downstream of the phosphoenolpyruvate carboxylase gene (ppc) of C. glutamicum and which has not been sequenced completely. The ppc gene was also cloned by us (7). We obtained this gene on cosmid ppc42 and, after subcloning, on a 3.3-kb HindIII fragment in pUC8 (pUC-ppcIIA). To identify a genomic fragment 3' adjacent to the ppc gene, the BamHI-Sall fragment of pUC-ppcIIA containing the 3'-region of the ppc gene and 0.4 kb downstream of it was digoxigenin-
and determined the effect on NH4 after sequencing.

This clone (Amt gants was tested for uptake of methylammonium (Table II).

Isolated chromosomal DNA of C. glutamicum was obtained, suggesting that integration of the vector at the chromosomal ppc Gene—function is shown in Fig. 4.

In order to prove whether the gene encoding the ammonium transport system of C. glutamicum is in fact located downstream of the ppc gene, we destroyed the ORF in the chromosome of C. glutamicum by gene-directed mutagenesis and determined the effect on NH4 transport. For gene disruption, the 0.27-kb SplI fragment, known from sequence data of O'Regan et al. (22) to be located in the ORF, was chosen. The fragment was cloned into vector pEM1, and the vector was introduced into C. glutamicum. Kanamycin-resistant clones were obtained, suggesting that integration of the vector at the chromosomal am locus had occurred. One of these transconjugants was tested for uptake of methylammonium (Table II). This clone (Amt−) showed a 20-fold reduced uptake activity as compared with the wild-type, while methylammonium uptake of the C. glutamicum strains BetP− and EB1, carrying defined mutations in the genes for the glycinebetaine carrier and for the glutamate dehydrogenase, respectively, was not affected.

We thus conclude (i) that in the transconjugant the ORF is in fact inactivated (ii) that the ORF (amt gene) indeed codes for the NH4 uptake system (Amt protein), and (iii) that this gene is located on the cloned 3-kb BamHI fragment.

Analysis of the amt Gene and the Deduced Amt Protein—The nucleotide sequence of the 2.0-kb SalI-BamHI fragment of the pUC-dppc insert was determined from both strands by the dideoxy-chain termination method (GenBank accession number X93513). Computer analysis for potential coding regions revealed an ORF extending from nucleotides 353-1720 of the sequenced fragment. Assuming that translation starts from the first ATG at base pair 365 the ORF encodes a polypeptide of 452 amino acids with a molecular weight of 47,699. All other potential translation start sites in the ORF (assuming AUG-, GUG- or UUG-directed initiation) lie within or downstream of regions coding for amino acids already showing high identity to the ammonium transport carriers of other organisms.

Using the amino acid sequence deduced from the analyzed ORF, the GenBank and SwissProt data base were searched for related proteins. As expected, a significant degree of identity of the C. glutamicum protein to the three known NH4 transporters of other organisms was found, extending over the whole sequence. The Mep2 (GenBank entry P41948) and Mep1 (5) proteins of S. cerevisiae show 33.4 and 28.1% identical amino acids, respectively, and the NH4 carrier of the plant A. thaliana (6) shows 25.9% identical amino acids. In addition, the C. glutamicum protein shares a high identity of 41.6% with the NrgA protein of Bacillus subtilis, a protein of yet unknown function (23).
Since the Amt protein of *C. glutamicum* is a membrane protein, it was analyzed with respect to the distribution of hydrophobic and hydrophilic amino acid residues. The hydrophathy plot (Fig. 5) shows highly hydrophobic stretches and a hydrophilic C-terminal region. Computer analysis predicts 10 or 11 membrane-spanning, helical segments. When comparing the distribution of hydrophobic regions in the corynebacterial protein with the distribution in the Mep1 and Mep2 proteins of *S. cerevisiae* (Fig. 4), membrane-spanning regions and hydrophilic loops were found to be located in corresponding protein stretches with the exception of helix 8 in the Mep1 protein, which is not clearly predicted for the Mep2 protein and the Amt protein of *C. glutamicum* (Fig. 6).

**DISCUSSION**

In this communication, we describe the functional characterization of the NH$_4^+$ uptake system of *C. glutamicum* and the identification of the corresponding gene. The carrier was found to be synthesized only under conditions of nitrogen starvation. It functions as a secondary transport system. The gene encoding the carrier protein, the first bacterial gene for an NH$_4^+$ transporter available so far, is located downstream the phosphoenolpyruvate carboxylase (*ppc*) gene.

For analysis of NH$_4^+$ transport, methylammonium was used, a compound shown to be accepted as alternative substrate by the NH$_4^+$ transporters of numerous other organisms. NH$_4^+$ and methylammonium have quite similar physical properties. Both compounds are predominantly protonated at neutral pH due to their high pK values (9.25 for NH$_4^+$, 10.7 for methylammonium). In unprotonated, i.e., uncharged form (NH$_3$ and methylamine), both compounds can easily diffuse across biological membranes, while these membranes are virtually impermeable for the protonated forms. Nevertheless, under the conditions employed here, i.e., neutral pH and low external substrate concentration (100 μM), diffusion can be neglected. Several properties of methylammonium influx argue against mediation by diffusion. (i) Uptake was saturable. (ii) Uptake rates varied by a factor of 20 depending on the growth conditions. And (iii) uptake resulted in significant intracellular accumulation of the substrate.

In several methylotrophic bacteria, which can use methylammonium as nitrogen and/or carbon source, transport systems specific for this compound have been described (25, 26). This possibility can be ruled out in case of the transport system of *C. glutamicum* since (i) the organism was found not to be able to metabolize methylammonium and (ii) methylammonium uptake was competitively inhibited by addition of NH$_4^+$. In fact, inhibition by NH$_4^+$ was so effective that it was impossible to determine a Kᵢ value, indicating that the affinity of the carrier for NH$_4^+$ is much higher than for methylammonium. It can therefore be concluded that NH$_4^+$ represents the natural substrate of the carrier analyzed.

Regulation of NH$_4^+$ uptake in *C. glutamicum* is similar to
that in many other organisms (2). The carrier protein itself was found to be inhibited by internal glutamine (data not shown). Since the intracellular pool of this compound immediately reflects the nitrogen supply, this regulation ensures that uptake activity corresponds to the requirements of the organism. In addition, the expression of the uptake carrier of C. glutamicum was repressed by high NH₄⁺ concentration in the culture medium, i.e. when supply via NH₄⁺ diffusion is sufficient. As has been pointed out by Kleiner (27), intracellular NH₄⁺ accumulation by an uptake system may create an energy wasting futile cycle, during which energy-dependent uptake is counteracted by diffusion of NH₃ out of the cells. Thus, repression of the carrier under conditions of sufficient nitrogen supply ensures that energy is expended for NH₄⁺ uptake only when cells are starving for nitrogen. In enterobacteria repression of the carrier along with that of numerous other operons encoding enzymes of nitrogen catabolism is mediated by the “nitrogen control” (Ntr) system, which coordinates carbon and nitrogen metabolism. Our results suggest that a similar control system exists in C. glutamicum.

The NH₄⁺ uptake system of C. glutamicum catalyzes uniport of NH₄⁺ and uses the membrane potential as driving force. Transport of the protonated form, i.e. the form not able to diffuse across the cytoplasmic membrane, is rational from a physiological point of view and could be deduced from the pH optimum of the carrier in the neutral pH range in which the NH₄⁺ ion is the predominant species. Analysis of the maximum accumulation of methylammonium indicates that no other ion is cotransported with ammonium. The fact that the accumulation ratio determined is somewhat lower than that expected for the ΔΨ-driven uptake of a monovalent cation could result from inaccuracies of the ΔΨ determination. The addition of different external methylammonium concentrations (10 to 100 μM) led to a significant variation of the internal concentration and thus to different diffusion rates, but it did not result in a change of the accumulation ratio (Fig. 3). Therefore, underestimation of the accumulation ratio due to methyamine efflux from the cells by passive diffusion can be excluded.

In the second part of this study, we describe the isolation and sequencing of the amt gene encoding the characterized NH₄⁺ uptake system. The isolation was based on the observation of Marini et al. (5) that the N-terminal region of the Mep1 protein of S. cerevisiae shows a significant degree of identity to a polypeptide encoded by a truncated ORF downstream of the ppc gene of C. glutamicum. In S. cerevisiae, two NH₄⁺ transport proteins Mep1 and Mep2 have been studied, which revealed functional properties similar to the transporter of C. glutamicum. We isolated and sequenced the 3′-region of the ppc gene and the complete ORF downstream of it. This ORF consists of 1367 base pairs and encodes a protein of 452 amino acids. Disruption of the ORF resulted in 20-fold reduction of methylammonium uptake activity, demonstrating that it represents indeed the amt gene encoding the NH₄⁺ transport system characterized.

Hydropathy analysis of the Amt protein revealed 10 or 11 membrane-spanning segments. According to the positive inside rule of von Heijne (28) the model predicting 11 membrane spanning helices must be favored because only in this case every second interhelical loop contains a high number of arginine plus lysine residues. These positively charged amino acids have in most membrane proteins been found to be much more abundant in cytoplasmic as compared with periplasmic regions. Applying this rule to the Amt protein results in orientation of its N terminus to the outside and location of the hydrophilic C terminus in the cytoplasm.

The Amt protein of C. glutamicum shows a significant degree of identity to the three other known NH₄⁺ transport systems, i.e. Mep1 and Mep2 of S. cerevisiae (5), and AMT1 of A. thaliana (6). Identical stretches of amino acid residues are spread over the whole protein. However, the Amt protein of C. glutamicum shares the highest identity with the NrgA protein of B. subtilis, a protein of unknown function (23). Therefore we conclude, that the NrgA protein probably is also an NH₄⁺ uptake system. The corresponding nrgA gene of B. subtilis is located in a dicistronic, nitrogen-regulated operon together with nrgB. This latter gene encodes a protein that has significant sequence similarity with the gltB-encoded P₁ protein from E. coli, an important part of the nitrogen regulatory system (Ntr). To determine whether the amt gene of C. glutamicum is also located in an operon, its downstream region was isolated and sequenced. Analysis of a chromosomal 3-kb Sad fragment, containing the 3′-region of the pUCdppc-insert (1.5 kb) and the downstream region, revealed that there is no ORF similar to nrgB (data not shown). The deduced amino acid sequence of the downstream region showed a significant degree of identity to procaroytic sarcosine oxidases. Since downstream of the ppc gene there is a strong stem-loop structure similar to bacterial rho-independent terminators (7, 22), transcription of the amt gene most probably occurs also independent of the ppc gene.

The high similarity of all NH₄⁺ transporters found so far, in fungi (5), plant (6), and, as described in this study, in bacteria, emphasizes the importance of NH₄⁺ transport systems under different environmental conditions.

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