Cloning and Characterization of MgtE, a Putative New Class of Mg^{2+} Transporter from *Bacillus firmus* OF4

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The MM281 strain of *Salmonella typhimurium* which possesses mutations in each its three known Mg^{2+} transport systems and requires 100 mM Mg^{2+} for growth was used to screen a genomic library from the gram-positive alkaliphilic bacterium *Bacillus firmus* OF4 for clones that could restore the ability to grow without Mg^{2+} supplementation. Of the clones obtained, five also conferred sensitivity to Co^{2+}, similar to the phenotype of mutants with mutations in the *S. typhimurium corA* Mg^{2+} transport locus. All five contained identical inserts by restriction analysis. Using $^{63}Ni^{2+}$ as a surrogate for the unavailable $^{28}Mg^{2+}$, the plasmid insert was shown to restore cation uptake with properties similar but not identical to those of the *S. typhimurium* CorA Mg^{2+} transporter. Sequence analysis of one clone identified a single open reading frame with multiple possible initiation sites. Deletion and mutation analysis identified a minimum open reading frame of 939 bp encoding a polypeptide with a predicted molecular mass of 34 kDa. Disruption of the open reading frame eliminated cation influx activity and restored resistance to Co^{2+}. This putative transporter, designated MgtE, has no sequence similarity to any known protein including CorA and appears to represent a new class of Mg^{2+} transport system.

Salmonella typhimurium and *Escherichia coli* possess three distinct Mg^{2+} transport systems: CorA, MgtA, and MgtB (7, 24). MgtA and MgtB are influx-only systems which are induced in response to Mg^{2+} deprivation. The third system, CorA, is constitutively expressed and mediates both influx and efflux of Mg^{2+}. Under normal growth conditions, CorA is the dominant transporter (7, 8), mediating as much as 99% of the total Mg^{2+} accumulated. The CorA system can also facilitate the uptake of Co^{2+} and Ni^{2+}. Mutant strains lacking a functional CorA transporter demonstrate a significant reduction in the ability to take up Mg^{2+} as well as increased resistance to the cytotoxic effects of Co^{2+} in the culture medium. This phenotype is not unique to *S. typhimurium* and *E. coli*. Mg^{2+} transport mutants of *Bacillus subtilis* and *Rhodobacter capsulatus* that also exhibit resistance to Co^{2+} have been described (11, 13, 19, 20). This phenotype implies the presence of a CorA-like system in these organisms and has prompted the hypothesis that CorA is widely distributed throughout prokaryotes (22). As part of studies to examine the structure and distribution of CorA-like systems, an Mg^{2+} transport-deficient strain of *S. typhimurium* was used to screen a plasmid library from the gram-positive alkaliphile *Bacillus firmus* OF4 (9, 10) for clones that could complement the *corA* mutation. In this study, we describe the isolation and characterization of the MgtE transport system, which complements the Mg^{2+} uptake mutations in *S. typhimurium* and restores sensitivity to Co^{2+}. Sequence analysis, however, indicates that the MgtE locus encodes a 34-kDa protein quite unlike CorA and appears to represent a new class of Mg^{2+} transport system.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in this study are listed in Table 1 with their sources. Plasmids were maintained in either *E. coli* DH5α or *S. typhimurium* JR501 (3). Plasmid complementation assays employed *S. typhimurium* MM281 as the host (7, 8, 24).

Culture media and reagents. Luria-Bertani (LB) broth was routinely used as the complex growth medium. N-minimal broth was used as the minimal growth medium (16). Antibiotics were added to complex and minimal culture media, respectively, at the following concentrations (in micrograms per liter): sodium ampicillin, 100 and 30; tetracycline hydrochloride, 20 and 10; kanamycin sulfate, 50 and 100; chloramphenicol, 25 and 10. Growth of MM281 requires $MgSO_4$ supplementation at a final concentration of 100 mM in both complex and minimal culture media. Restriction endonucleases, T4 DNA ligase, calf intestinal alkaline phosphatase, Klenow fragment of DNA polymerase I, S1 nuclease, and T4 polynucleotide kinase were obtained from Life Technologies, Inc. (Gibco-BRL, Gaithersburg, Md.). Sequencing enzymes and associated biochemicals were obtained from U. S. Biochemicals Inc. (Cleveland, Ohio). $^{57}Co^{2+}$ was obtained from Amersham (Arlington, Ill.). Synthetic oligodeoxynucleotide primers were obtained from Oligo's Etc. (Wilsonville, Oreg.). Additional chemicals were obtained from standard suppliers.

DNA manipulation. Plasmid DNA was prepared from 500-ml cultures with the Wizard Maxiprep DNA isolation system obtained from Promega (Madison, Wis.). Restriction endonuclease digestion, DNA ligation, and transformation of plasmid DNA were performed as described elsewhere (2, 18). Single-stranded DNA was prepared from cultures of MM1098 by using the Wizard M13 DNA purification system obtained from Promega with no modifications.

Isolation of complementing clones. Electrocompetent MM281 was prepared by the method of Miller (16). A genomic library prepared from *B. firmus* OF4 (9, 10) and kindly provided by T. Krulwich was introduced into MM281 by electroporation. Transformant colonies were spread onto LB agar containing ampicillin (100 μg/ml) and incubated at 37°C for 24 h. Individual transformants were colony purified on the same culture medium. The recipient strain MM281 does not grow on unsupplemented LB agar or on N-minimal agar (7, 24). Thus, complementation of the Mg^{2+} uptake deficiency was verified by streaking on N-minimal agar without $MgSO_4$ supplementation and scoring for growth after 24 h of incubation.

Construction of nested deletions. Deletions within the *mgtE* insert were generated by exonuclease III digestion of linearized plasmid DNA (Erase-A-Base nested deletion kit; Promega) according to the instructions of the manufacturer. The endpoints of each deletion were confirmed by sequence analysis. Additional deletion mutants were constructed by excising cassettes with various restriction endonucleases and religating the linear plasmid. Plasmids carrying deletions of *mgtE* were scored for the ability to complement *S. typhimurium* MM281 as described above.

DNA sequencing. DNA sequencing was performed by the dideoxy chain termination method of Sanger (21) as modified by Tabor and Richardson (25) using

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TABLE 1. Bacterial strains and plasmids

Strain[a]	Genotype or description	Location of mutation (bp)[b]	Source or reference
LT2	Wild-type *S. typhimurium*		
DH5α (EC)	F⁻ φ80d*lacZ*ΔM15 *recA1 endA1 gyrA96 thi-1 hsdR17*(r$_K^-$ m$_K^+$) *supE44 relA1 deoR* Δ(*lacZYA-argF*)U169		Gibco-BRL
JR501	*hsdSA29*(r$_{SA}^-$ m$_{SA}^+$) *hsdSB121*(r$_{SB}^-$ m$_{SB}^+$) *hsdL6*(r$_{LT}^-$ m$_{LT}^+$) *galE719 ilv452 metA22 metE551 trpC2 xyl404 rpsL120* H1-b H2-*e,n,x nml* (Fels2)⁻ *fla-66*		3
BMH71-18 (EC)	*thi supE* Δ(*lac-proAB*) *mutS*::Tn*10* (F′ *proAB lacI*q ZΔM15)		Promega
JM109 (EC)	*endA1 recA1 gyrA96 thi hsdR17*(r$_K^-$ m$_K^+$) *relA1 supE44* λ⁻ Δ(*lac-proAB*) (F′ *traD36 proAB lacI*q ZΔM15)		Promega
MM281	DEL*485*(*leuBCD*) *corA45*::MudJ *mgtA21*::MudJ *mgtB10*::MudJ *zjh1628*::Tn*10*Δ16Δ17(Cam)		7
MM387	DEL*485*(*leuBCD*) *corA185*::Tn*10*Δ16Δ17(Tet)		21
MM839 (EC)	DH5α/pBluescript II KS⁻		This study
MM927	MM281/pRS194 (*mgtE⁺*)		This study
MM933	MM281/pRS161 DEL*114*(*mgtE*)		This study
MM934	MM281/pRS162 DEL*115*(*mgtE*)		This study
MM935	MM281/pRS145 DEL*119*(*mgtE*)		This study
MM936	MM281/pRS146 DEL*118*(*mgtE*)		This study
MM1098 (EC)	JM109/pRS181 (*mgtE⁺*) *blaM9*		This study
MM1133	JR501/pRS182 *mgtE106*	783	This study
MM1137	JR501/pRS204 *mgtE97*	461	This study
MM1138	JR501/pRS187 DEL*117*(*mgtE*)		This study
MM1162	JR501/pRS191 *mgtE111*	906	This study
MM1163	JR501/pRS192 *mgtE112*	717	This study
MM1176	MM281/pRS196 DEL*121*(*mgtE⁺*)		This study
MM1177	MM281/pRS197 DEL*122*(*mgtE*)		This study
MM1179	MM281/pRS199 DEL*124*(*mgtE*)		This study
MM1180	MM281/pRS200 DEL*125*(*mgtE⁺*)		This study
MM1182	MM281/pRS202 DEL*127*(*mgtE⁺*)		This study
MM1500	JR501/pRS185 DEL*116*(*mgtE*)		This study
MM1501	JR501/pRS189 *mgtE109*	24	This study
MM1502	JR501/pRS190 *mgtE100*	768	This study
MM1503	JR501/pRS193 *mgtE113*	1344	This study

[a] EC, *E. coli*. All other strains are *S. typhimurium*.
[b] See open reading frame analysis in Fig. 4.

Sequenase modified T7 DNA polymerase obtained from U. S. Biochemicals Inc. Initial reactions were performed with T7 and SP6 promoter primers obtained from Gibco-BRL. Subsequent reactions employed synthetic oligodeoxynucleotide primers complementary or identical to segments within a previously sequenced segment. DNA sequences were determined for both strands and verified by at least two separate determinations for each strand.

Mutagenesis. Site-directed mutations were constructed on single-stranded DNA prepared from MM1098 with an Altered Sites II in vitro mutagenesis system (Promega) and synthetic oligodeoxynucleotide primers. A 25:1 picomole ratio of primer to template was used for each reaction. Newly synthesized DNA was recovered in recipient strain BMH-71 and subsequently transformed into either DH5α or JR501. Mutations were identified by restriction fragment analysis and verified by sequence analysis.

⁵⁷Co²⁺ transport. Accumulation of ⁵⁷Co²⁺ was assayed as described previously (6, 8, 24) for uptake of ²⁸Mg²⁺ and ⁵⁷Co²⁺. All transport assays were performed at 37°C. Time of incubation was 20 min. Uptake is linear for at least 30 min. The amount of isotope retained on the filters was quantitated in a Beckman LS7000 scintillation counter with a counting efficiency of about 80%. Results shown are from single experiments, with each experiment being representative of at least three separate experiments; each point was determined in triplicate. Error bars are not shown but were less than 10% of the absolute value of the average at each point.

Disk diffusion assays for cation sensitivity. Test strains were inoculated into N-minimal broth supplemented with 10 μM MgSO$_4$ and incubated overnight at 37°C. One hundred microliters from each culture was spread onto the surface of an N-minimal agar plate and allowed to dry. A 6-mm-diameter filter paper disk was placed in the center of the plate and loaded with 15 μl of the cation solution (100 mM CoCl$_2$, 100 mM NiCl$_2$, 100 mm ZnCl$_2$, and 100 mm CaCl$_2$). After 24 h of incubation the inhibition zones were measured and compared against positive and negative controls (7, 21).

Nucleotide sequence accession number. The GenBank accession number of the sequence reported in this article is U18744.

RESULTS

Phenotypic characterization. A genomic library from the gram-positive bacterium *B. firmus* OF4 was screened for recombinant plasmids capable of restoring growth to a mutant strain of *S. typhimurium* lacking any functional Mg²⁺ transport system. The recipient strain, MM281, carries insertion mutations in each of the three Mg²⁺ transport loci (7, 24). These mutations abolish the primary Mg²⁺ influx pathways and render the strain incapable of growth on medium containing less than 100 mM Mg²⁺. Introduction of a wild-type allele of any of the three known Mg²⁺ transport systems allows growth on unsupplemented medium. Thus, MM281 is an extremely sensitive detector of the presence of a functional Mg²⁺ uptake system. This genetic screen was used to identify independent complementing clones capable of sustaining growth on both complex and minimal media without Mg²⁺ supplementation. Positive colonies were individually scored for sensitivity to divalent cations by disk diffusion assay. Five clones demonstrated sensitivity to Co²⁺, Ni²⁺, and Zn²⁺ and resistance to Ca²⁺ and Mn²⁺ (Table 2). These cation sensitivity profiles were similar to those for strains of MM281 containing plasmids that encode the CorA Mg²⁺ transport system (7, 24). In contrast to a *corA* mutation, however, which has no greater resistance to Zn²⁺ than that of the wild type, the complementing plasmids from *B. firmus* OF4 conferred a relatively high degree of sensitivity to this cation (Table 2).

TABLE 2. Cation sensitivity of MgtE versus CorA

Strain	Relevant genotype	Zone of inhibition[a] (mm^2)		
		Zn^{2+}	Ni^{2+}	Co^{2+}
LT2	Wild type	314	3,220	3,630
MM387	corA::Tn10Δ16Δ17(Tet)	314	1,260	1,520
MM927	corA::Tn10Δ16Δ17(Tet)/pRS197	1,520	4,540	4,300

[a] No inhibition was observed for Ca^{2+} or Mn^{2+}.

Plasmid DNA was prepared from each of these five colonies and analyzed by restriction endonuclease digestion. The complementing plasmids were found to contain genomic inserts which were identical with respect to size and restriction fragment length polymorphism. Strain MM927, containing plasmid pRS194, was selected for additional characterization. The plasmid locus conferring these characteristics was designated *mgtE*.

Transport characteristics. The transport system encoded by the *S. typhimurium corA* locus mediates the influx of Mg^{2+}, Co^{2+}, and Ni^{2+}, with the highest affinities for Mg^{2+} and Co^{2+}. As would be expected on the basis of their mutual transport, each of these cations competitively inhibits uptake of the other cations via the CorA system. This feature is fortuitous given the lack of adequate techniques for measuring Mg^{2+} flux across biological membranes. Since all of the previously characterized prokaryotic Mg^{2+} transport systems also transport Co^{2+} and Ni^{2+}, these radioisotopes can be used for initial characterization of novel transport systems. In such experiments Mg^{2+} uptake is inferred from Mg^{2+} inhibition of ^{57}Co^{2+} or ^{63}Ni^{2+} uptake (6, 8, 24). Studies using ^{57}Co^{2+} indicate that the *mgtE* locus encodes a transporter that mediates Co^{2+} uptake with a V_{max} of about 350 pmol min^{-1} 10^8 cells^{-1} and a K_m for Co^{2+} of about 80 μM (Fig. 1). Co^{2+} uptake was competitively inhibited by Mg^{2+} with an apparent K_i of 50 μM (Fig. 2 and data not shown). The apparent affinity of MgtE for Mg^{2+} is slightly poorer than CorA's affinity (Fig. 2). Co^{2+} uptake via MgtE was also inhibited by Ni^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, and Sr^{2+} but not Ba^{2+} (Fig. 2 and Table 3). Mg^{2+}, Zn^{2+},

FIG. 2. Effects of Mg^{2+}, Ni^{2+}, and Zn^{2+} on Co^{2+} uptake via MgtE. ^{57}Co^{2+} uptake in strain MM927 (closed symbols) was measured as described in Materials and Methods. MM927 harbors an insertion mutation in *corA* and, thus, cannot transport Co^{2+}. Mg^{2+} inhibition of ^{57}Co^{2+} uptake via the CorA Mg^{2+} transport system in a *corA* wild-type strain (open circles) is shown for comparison. Cation uptake was measured for 20 min at 37°C. The data reflect a single experiment representative of three independent assays.

and Ca^{2+} were the most potent inhibitors. The slopes of the inhibition curves with Zn^{2+} and Mn^{2+} were consistent with competitive inhibition, although this was not tested directly. Ni^{2+} inhibition could not be tested completely, since it is quite toxic at the very high concentrations of Ni^{2+} dictated by its relatively poor apparent K_i for MgtE. Experiments with ^{63}Ni^{2+} evidenced a very low level of uptake that could not be accurately characterized kinetically. This is in contrast to the CorA Mg^{2+} transport system, which has good affinity and capacity for Ni^{2+} uptake (24).

Physical structure of the *mgtE* locus. The nucleotide sequence of the pRS194 insert was determined and is presented in Fig. 3. A large open reading frame encompassing 1359 of the 1399 nucleotides in the insert was identified. Sequence data alone could not unambiguously identify the correct initiation codon, since six potential start sites are present in the first 447 bp of this reading frame. To identify the correct initiation codon, a series of deletion and point mutants were constructed and scored for the ability to complement MM281 (Fig. 4). Deletions at or 3' to nucleotide 582 failed to complement the Mg^{2+}-dependent growth phenotype of MM281 and the mutants no longer transported ^{57}Co^{2+} (data not shown), indicating that the start site lies 5' to this nucleotide. The deletion mutations at or before nucleotide 435 were all able to comple-
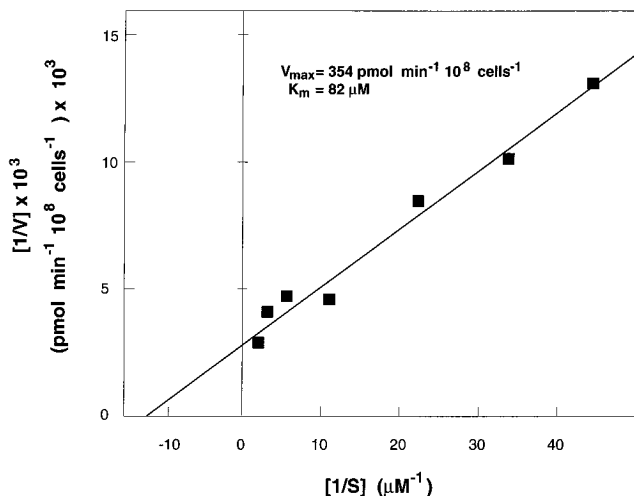

FIG. 1. Cobalt uptake by the MgtE Mg^{2+} transport system. ^{57}Co^{2+} uptake kinetics of strain MM927 encoding the *mgtE* Mg^{2+} transport locus. Cation uptake was measured for 20 min at 37°C as described in Materials and Methods. The data are a single experiment representative of three similar assays.
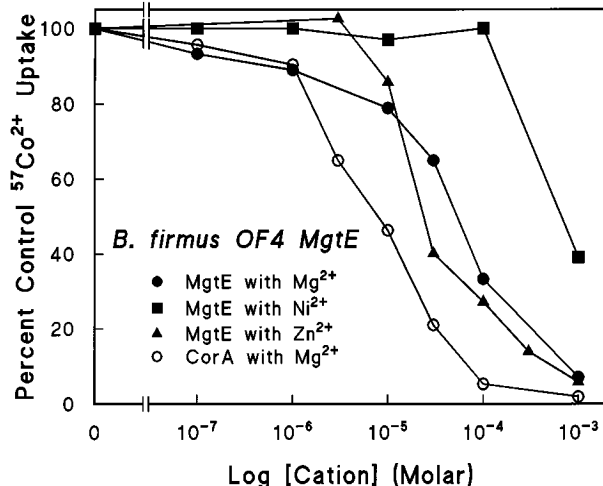
TABLE 3. Cation inhibition of ^{57}Co^{2+} uptake

Cation	Apparent K_i (μM)
Mg^{2+}	50
Ca^{2+}	50
Sr^{2+}	80
Ba^{2+}	—[a]
Ni^{2+}	>200
Co^{2+}	100
Mn^{2+}	70
Zn^{2+}	20

[a] —, no inhibition.

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TCCCATAATA GGGGGTCTAC AACATGGTAA AATTAAACGA ACAAAAAAGA TTGACGTATG CAAAGCAAAT ATATGATTTA TTAAAAAGTG GAGATGTTGA        100

ACAGTTTCGT GAGTGGTTTC TTGCGCTCCA CCCAACAGAC CAAGCAGATA TTTTCATTGA GCTAGCAATT GAAGCGCGCC AAAAAGTGTA TCAATTCCTA        200

TCTCCGAATG AATTTGCCGA AGTGTTTGAA GGACTTGAAA AGAAAGAGCA GATGTATGTG TTTAAAGAGC TTGATCCAAC ATACGGATCA GCAATGTTAA        300

ATGATATGTA TGCCGATGAC GTCGTAACTT TTTTATCTTT TTTGAGTGAA AAGGAAGTTA CAGCCATCTT ACATTCAATG GAAGAATCAG AGGCAAGTGA        400
                                                                                                  -35
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                                           Shine-Dalgarno  M  T  K  E  F   I  R  V   T  A  V  E   T  V  A   E  V  I
AGTCCGGGAA TTATTATCCT ATGAGCCTGA GACTGCCGGA GCGATTATGA CTAAAGAGTT TATTCGCGTT ACTGCTGTAG AGACGGTAGC TGAAGTCATT        500
        -10
```

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G  R  L  R   K  E  A   P  D  A   E  T  I  Y   Y  L  Y   V  V  D   E  R  E  K   L  V  G   V  V  S   L  R  D  L
GGCAGGCTTA GAAAAGAAGC ACCTGATGCG GAGACGATCT ACTATTTATA TGTAGTAGAT GAGCGAGAGA AACTAGTCGG GGTTGTTTCT TTACGAGATT        600
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   I  V  A   E  E  V   D  L  V  Q   D  V  M   S  R  E   V  V  S  V   S  A  K   T  D  Q   E  D  V  A   R  L  I
TAATTGTTGC TGAGGAGGTC GATTTAGTAC AGGATGTTAT GAGCCGGGAA GTCGTTTCAG TTTCGGCTAA AACCGACCAA GAAGATGTAG CTCGCTTAAT        700
```

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K  K  Y   D  F  L  A   A  P  V   I  S  S   E  G  K  L   V  G  I   V  T  V   D  D  I   I  D  I  L   E  E  E
TAAGAAATAT GATTTCTTGG CAGCACCTGT TATTTCAAGT GAAGGAAAGC TGGTCGGGAT TGTTACGGTG GATGACATCA TCGATATTTT AGAAGAGGAA        800
```

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A  T  E  D   L  G  E   F  S  A   S  K  G  A   T  D  V   D  V  S   S  F  Q  A   A  K  K   R  A  P   W  I  I  L
GCAACGGAGG ACTTAGGAGA ATTTTCTGCA TCTAAAGGAG CGACAGATGT GGATGTGTCT TCTTTTCAAG CAGCTAAAAA GCGCGCACCT TGGATCATTT        900
```

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   L  M  F   F  G  L   I  T  A  E   V  I  G   Q  F  E   E  T  L  E   A  I  V   L  L  A   A  F  I  P   L  L  M
TATTGATGTT TTTTGGACTT ATTACGGCTG AAGTGATCGG CCAGTTTGAA GAAACATTAG AAGCGATTGT TCTGTTGGCT GCCTTTATCC CGCTGTTAAT       1000
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D  S  A   G  N  T  G   T  Q  S   L  A  V   A  V  R  G   L  A  L   G  T  V   E  R  D  G   V  W  K   M  L  K
GGACTCTGCG GGCAATACTG GTACACAGTC ACTGGCTGTA GCGGTTCGTG GTCTAGCGCT TGGCACTGTT GAGCGAGATG GTGTCTGGAA GATGTTAAAG       1100
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R  E  L   G  T  G  L   M  L  G   F  I  C  M   L  V  I   A  G  I   V  T  V  M   H  G  N   M  M  L   A  F  I  V
CGTGAACTTG GGACAGGTTT GATGCTTGGG TTCATCTGTA TGCTTGTTAT CGCAGGGATT GTGACAGTGA TGCACGGAAA TATGATGCTT GCATTCATAG       1200
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   G  I  S   I  F  L   T  L  S  I   A  T  I   I  G  T   I  V  P  L   I  I  N   K  M  K   L  D  P  A   I  A  S
TTGGTATCTC TATCTTTCTT ACATTAAGCA TCGCTACAAT TATTGGTACA ATTGTGCCTC TCATTATTAA TAAAATGAAA CTCGACCCAG CCATCGCATC       1300
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G  P  F   I  T  T  V   N  D  I   L  G  L   L  I  Y  F   S  I  A   T  A  L   L  E  Y  L   ***
TGGTCCATTT ATTACAACTG TTAATGATAT ATTAGGATTG CTCATTTACT TTTCCATCGC AACAGCATTA CTAGAGTATT TATAGAAAGT ATTATTAGA        1399
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FIG. 3. Nucleotide sequence and inferred protein sequence of MgtE. The nucleotide sequence was determined as described in Materials and Methods. The potential −10, −35, and Shine-Dalgarno sequences and the open reading frame determined as described in the text are shaded. The predicted amino acids are positioned above the first base pair of the corresponding codon. Charged amino acids are indicated (+ or −).

ment MM281, suggesting that the most likely start site was at nucleotide 447. Point mutations were also constructed to introduce a stop codon within the open reading frame. The mutation at nucleotide 24 at the first possible initiation codon had no effect on growth or cation resistance profiles of MM281 carrying the corresponding plasmid (Fig. 4). Together with the lack of effect of the deletions prior to nucleotide 435, the ATG at nucleotide 447 is most likely the correct start codon. This is
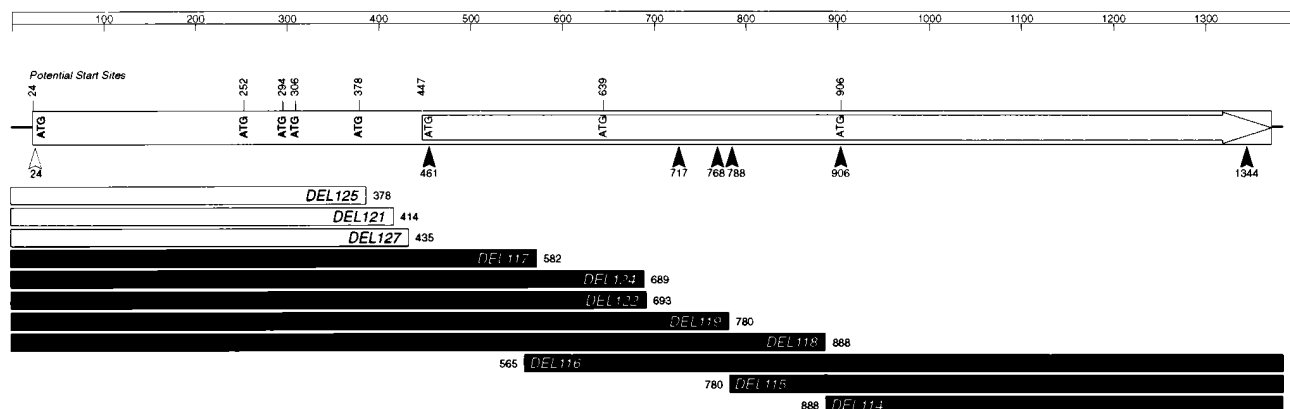

FIG. 4. Open reading frame analysis of the *B. firmus* OF4 insert. Sequence analysis identified a large open reading frame between nucleotides 24 and 1374 (shaded bar). This reading frame contains eight possible start codons (ATG). The correct initiation codon and resulting open reading frame (see the text) are indicated by the open bar, with the arrow indicating the direction of transcription. Deletion mutants generated as described in Materials and Methods are shown below. Allele numbers of mutations are given within the bars, and the nucleotide positions of the deletion endpoints are given outside the bars (see Table 1). Arrowheads, point mutations which introduce stop codons (see Materials and Methods). Open and solid symbols, mutations that rescued growth of MM281 and mutations that didn't, respectively.
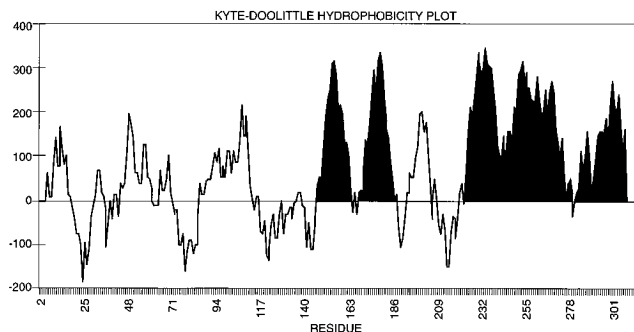
FIG. 5. Hydropathy profile of the MgtE protein sequence. The hydropathy profile was determined by the method of Kyte and Doolittle (12) using a sliding window of 15 amino acids. Other algorithms gave similar results. Filled segments have a peak hydrophobicity and length sufficient to form a transmembrane domain. Other peaks either are not of sufficient hydrophobicity or are not of sufficient length to form a membrane segment.

consistent with the effect of similar point mutations at and 3′ to nucleotide 717, all of which eliminate the ability of the insert DNA to complement MM281. Translation beginning at nucleotide 447 predicts a polypeptide 312 amino acid residues in length with a predicted molecular mass of 33.7 kDa. This open reading frame is preceded by a putative Shine-Dalgarno sequence (GAGG) that is 7 bp 5′ to the start codon and a tentative −10 and −35 promoter sequence (Fig. 3). The other possible initiation sites have poorer possible Shine-Dalgarno and −10 sequences.

MgtE protein. Both the nucleotide and the amino acid sequences were compared against the current databases with the BLAST algorithm (1). Neither had homology to any previously described entry. Specifically, there is no homology to the CorA proteins from *S. typhimurium* or *E. coli*. MgtE is highly charged for a membrane protein. The majority of the 22% of the amino acid residues predicted to be charged are situated in the N-terminal third of the protein (Fig. 3). The hydropathy profile determined for the predicted amino acid sequence by the method of Kyte and Doolittle (12) predicts MgtE to be an integral membrane protein with at least four and probably five transmembrane segments (Fig. 5). The orientation of the protein in the membrane cannot be directly inferred from the hydropathy profile; however, consideration of the location of positively charged residues immediately before or after a putative membrane segment (27–29) suggests that the N terminus is situated in the cytosol.

DISCUSSION

Mg²⁺ has the largest hydrated radius of all divalent cations of biological interest. However, the aqueous hydrated Mg²⁺ must lose its water shell in order to be transferred across the hydrophobic barrier of the cell membrane (4, 5). This results in a volume change of almost 400 fold for Mg²⁺, since its atomic cation is the smallest of the divalent cations. Ca²⁺, Na⁺, and K⁺ exhibit only a 4- to 25-fold volume change during passage through the membrane. We have hypothesized that, because of this unique chemistry, Mg²⁺ transport proteins either are unusual members of known transport families or contain unique classes of membrane transporters (14, 21–23). Previous examples of Mg²⁺ transporters have supported this suggestion. The CorA Mg²⁺ transporter lacks homology to known proteins, is very highly charged, and contains only three membrane-spanning domains (21). Although the *S. typhimurium* MgtA and MgtB Mg²⁺ transport systems belong to the P-type ATPase

protein family (23), they are unusual members of that family in that they use the energy of ATP to transport Mg²⁺ into the cell, with rather than against its electrochemical gradient (15, 23). The characteristics of MgtE as a Mg²⁺ transporter continue to support the suggestion that Mg²⁺ transporters are unique or have unusual characteristics. MgtE lacks sequence homology to known proteins. Like CorA, it is highly charged for a membrane protein and contains only five transmembrane segments, rather than the more usual 10 to 12 for most other transporters (17).

The evidence that *mgtE* encodes an Mg²⁺ transport protein is substantial but necessarily indirect, since ²⁸Mg²⁺ is not routinely available for measurement of Mg²⁺ uptake directly. Although it is reasonable to assume that the physiological function of MgtE is Mg²⁺ transport in *B. firmus* OF4, there is no direct evidence of this. Even if the current genetics of *B. firmus* OF4 facilitated chromosomal ablation of the locus, it is unlikely that an *mgtE* mutation would result in an Mg²⁺-dependent phenotype. The organism almost certainly contains additional Mg²⁺ transport systems, and as with *S. typhimurium*, mutation of a single system would not confer an Mg²⁺-specific phenotype (7, 24). Transport of Mg²⁺ can be inferred from the ability of *mgtE* to allow cell growth without addition of supplemental Mg²⁺ to the growth medium. MM281 requires 100 mM Mg²⁺ for growth. N-minimal and LB media contain 15 to 30 μM total Mg²⁺ (7, 8). Thus, the presence of *mgtE* confers a >1,000-fold difference in the Mg²⁺ requirement of the cell. Consistent with this interpretation is the competitive nature of the Mg²⁺ inhibition of ⁵⁷Co²⁺ uptake and the pattern of uptake inhibition by other cations. MgtE is unlikely to be a Ca²⁺ transporter since, although both Ca²⁺ and Sr²⁺ inhibit uptake, Ba²⁺ does not. Physiologically, the K_i values for the transition metal cations are much greater than their expected physiological free concentrations in the extracellular medium. In contrast, a K_i of 50 μM for Mg²⁺ would put the K_m for Mg²⁺ uptake in an appropriate physiological range. An alternative interpretation for the function of MgtE might be that it is actually an efflux system that can leak sufficient Mg²⁺ to allow growth. However, one might expect a poorer apparent K_m for extracellular Mg²⁺ in such a case. Thus, the most probable function of MgtE is likely to be that of a carrier for Mg²⁺.

The phenotype of *mgtE* is similar but not identical to that of *corA*. Both transporters mediate flux of Co²⁺ and Mg²⁺. CorA, however, mediates substantial Ni²⁺ flux (24); MgtE appears to mediate little or no Ni²⁺ uptake even when expressed from a high-copy-number plasmid. Regardless of the level of Ni²⁺ uptake, expression of MgtE does give a high degree of Ni²⁺ sensitivity (Table 2) similar to that for a wild-type allele of *corA*. MgtE also confers a high degree of sensitivity to Zn²⁺ that is also reflected in the potent inhibition of transport by Zn²⁺. This suggests that MgtE might also mediate some degree of Zn²⁺ uptake. The very high Zn²⁺ sensitivity, however, is likely due to expression from a high-copy-number plasmid. Expression of a wild-type *corA* allele from a similar plasmid confers an even greater Zn²⁺ sensitivity on the host strain (data not shown).

Structurally, the CorA and MgtE proteins have gross similarity but none in detail. In both proteins there is a large, highly charged N-terminal region followed by relatively closely spaced membrane-spanning domains. However, MgtE appears to have five transmembrane segments, and its N-terminal domain is likely oriented predominantly towards the cytosol, with little protein exposed extracellularly. CorA has three transmembrane segments and a large N-terminal periplasmic domain with no appreciable cytosolic domain. Neither protein is similar to any other protein currently in sequence databases.

We conclude that MgtE from the gram-positive alkaliphile *B. firmus* OF4 represents a new class of Mg^{2+} transport system. Further, this transporter is not likely limited to *Bacillus* species or to gram-positive organisms. We have preliminary data suggesting that a homologous gene is present in the gram-negative species *Providencia stuartii* (26).

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