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Cloning of the *mgtE* Mg^{2+} Transporter from *Providencia stuartii* and the Distribution of *mgtE* in Gram-Negative and Gram-Positive Bacteria

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The MM281 strain of *Salmonella typhimurium* possesses mutations in each of its three Mg^{2+} transport systems, requires 100 mM Mg^{2+} for growth, and was used to screen a genomic library from the gram-negative bacterium *Providencia stuartii* for clones that could restore the ability to grow without Mg^{2+} supplementation. The clones obtained also conferred sensitivity to Co^{2+}, a phenotype similar to that seen with the *S. typhimurium corA* Mg^{2+} transport gene. The sequence of the cloned *P. stuartii* DNA revealed the presence of a single open reading frame, which was shown to express a protein with a gel molecular mass of 37 kDa in agreement with the deduced size of 34 kDa. Despite a phenotype similar to that of *corA* and the close phylogenetic relationship between *P. stuartii* and *S. typhimurium*, this new putative Mg^{2+} transporter lacks similarity to the CorA Mg^{2+} transporter and is instead homologous to MgtE, a newly discovered Mg^{2+} transport protein from the gram-positive bacterium *Bacillus firmus* OF4. The distribution of *mgtE* in bacteria was studied by Southern blot hybridization to PCR amplification products. In contrast to the ubiquity of the *corA* gene, which encodes the dominant constitutive Mg^{2+} influx system of bacteria, *mgtE* has a much more limited phylogenetic distribution.

Gram-negative bacteria possess at least two distinct classes of Mg^{2+} transport systems: the inducible P-type ATPases exemplified by the MgtA and MgtB systems of *Salmonella typhimurium* and *Escherichia coli* (4, 17) and the constitutive CorA Mg^{2+} transporter, which is ubiquitous in gram-negative bacteria (4, 5, 12–14, 17). Since CorA also mediates transport of Co^{2+} and Ni^{2+}, *corA* mutations confer a distinctive phenotype of Co^{2+} and Ni^{2+} resistance. Mg^{2+} uptake mutants with a CorA-like phenotype have also been observed in other bacteria, notably *Bacillus subtilis* (6, 8, 10, 11). During studies involving cloning of additional CorA transporters, we recently characterized *mgtE*, a putative Mg^{2+} transporter from the gram-positive alkaliphile *Bacillus firmus* OF4 (16). Expression of *mgtE* in a *corA* background restores Mg^{2+} and Co^{2+} uptake and relieves the CorA-like phenotype, but the deduced protein sequence and topology of MgtE bear no resemblance to those of CorA or to any other currently known protein, thus identifying a new class of Mg^{2+} transporter. We report here the cloning of an MgtE Mg^{2+} transporter from *Providencia stuartii*, a gram-negative opportunistic pathogen of humans and penguins closely related to *Proteus vulgaris* as well as to *S. typhimurium* and *E. coli*, thus demonstrating the presence of *mgtE* in gram-negative organisms. However, additional studies of the distribution of *mgtE* and *corA* indicate that while *corA* appears ubiquitous, *mgtE* has a more limited distribution.

Cloning and sequence of *mgtE* from *P. stuartii*. The *S. typhimurium* mutant MM281 [*mgtB10*::MudJ *corA45*::MudJ *mgtA21*::MudJ *zjh1628*::Tn*10*(*cam*) Δ*leuBCD485*] lacks Mg^{2+} transport activity because the CorA, MgtA, and MgtB Mg^{2+} transport systems have been genetically ablated (17); MM281 grows only in media supplemented with 100 mM $MgSO_4$, thus providing a sensitive screen for putative Mg^{2+} transport systems. A pACYC184-based genomic library of *P. stuartii*, provided by P. Rather, was electroporated into MM281 and scored for restoration of Mg^{2+}-independent growth and restoration of Co^{2+} sensitivity (4, 5). Of five similar overlapping clones, pRB6 containing a 3.0-kb insert was selected for further study; the complementing insert DNA was localized to a 2.2-kb *Sph*I restriction fragment and subcloned into the *Sph*I site of pBS KS+ to create pDT3.

The nucleotide sequence of this DNA insert (Fig. 1) contains a single open reading frame between nucleotides 343 and 1419, predicting a protein with a size of about 38 kDa. An additional ATG codon is present at bp 469, predicting a protein with a size of 34 kDa and 314 amino acids (Fig. 1). There is no Shine-Dalgarno site near the ATG at bp 343, and there is only a relatively poor one at bp 469. Comparison of the predicted amino acid sequence against sequences contained in GenBank by using the BLAST program (1) identified no proteins that shared significant similarity, including the CorA Mg^{2+} transporter. This laboratory recently reported the cloning of a CorA-like Mg^{2+} transporter with a length of 312 amino acids from the gram-positive alkaliphile *B. firmus* OF4 by similar functional complementation of the Mg^{2+} transport defect of MM281 (16). Comparison of the deduced protein sequences of MgtE from *B. firmus* OF4 with the newly cloned locus from *P. stuartii* shows 55% overall similarity (Fig. 1) to the smaller of the two open reading frames. The larger open reading frame in the *P. stuartii* insert potentially encodes an additional 42 amino acids at the N terminus, but there is no corresponding sequence in the *B. firmus* OF4 protein. The start site for the *B. firmus* OF4 *mgtE* has been established and corresponds well to the start codon in *P. stuartii* at bp 469. The lack of any consensus ribosome binding site near bp 343 also upholds assignment of the start codon at bp 469. The *P. stuartii* insert DNA supports expression of a 37-kDa protein from the

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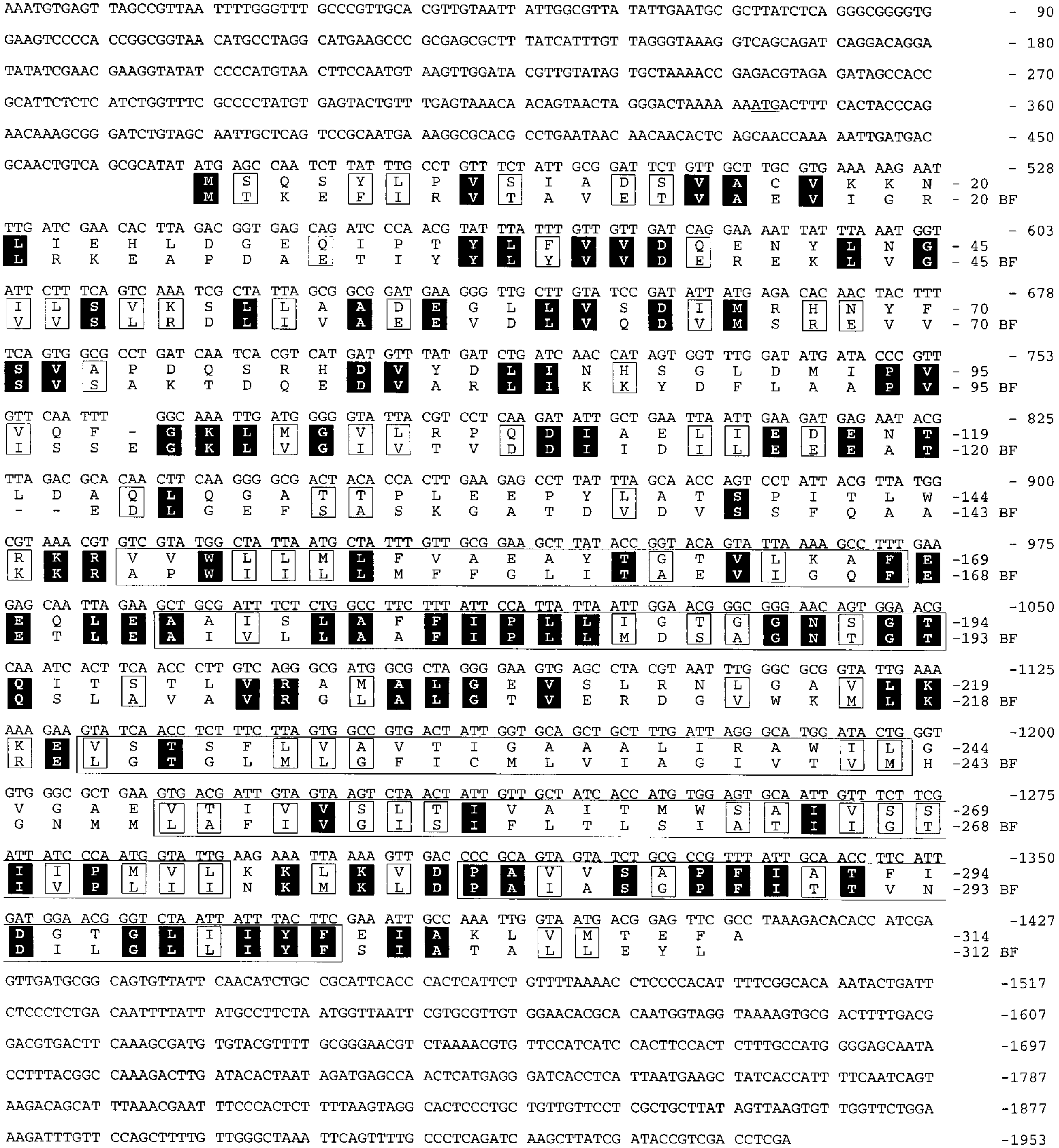

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TATATCGAAC GAAGGTATAT CCCCATGTAA CTTCCAATGT AAGTTGGATA CGTTGTATAG TGCTAAAACC GAGACGTAGA GATAGCCACC        - 270
GCATTCTCTC ATCTGGTTTC GCCCCTATGT GAGTACTGTT TGAGTAAACA ACAGTAACTA GGGACTAAAA AAATGACTTT CACTACCCAG        - 360
AACAAAGCGG GATCTGTAGC AATTGCTCAG TCCGCAATGA AAGGCGCACG CCTGAATAAC AACAACACTC AGCAACCAAA AATTGATGAC        - 450

GCAACTGTCA GCGCATAT ATG AGC CAA TCT TAT TTG CCT GTT TCT ATT GCG GAT TCT GTT GCT TGC GTG AAA AAG AAT        - 528
                     M   S   Q   S   Y   L   P   V   S   I   A   D   S   V   A   C   V   K   K   N  - 20
                     M   T   K   E   F   I   R   V   T   A   V   E   T   V   A   E   V   I   G   R  - 20 BF

TTG ATC GAA CAC TTA GAC GGT GAG CAG ATC CCA ACG TAT TTA TTT GTT GTT GAT CAG GAA AAT TAT TTA AAT GGT        - 603
 L   I   E   H   L   D   G   E   Q   I   P   T   Y   L   F   V   V   D   Q   E   N   Y   L   N   G  - 45
 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  - 45 BF

ATT CTT TCA GTC AAA TCG CTA TTA GCG GCG GAT GAA GGG TTG CTT GTA TCC GAT ATT ATG AGA CAC AAC TAC TTT        - 678
 I   L   S   V   K   S   L   L   A   A   D   E   G   L   L   V   S   D   I   M   R   H   N   Y   F  - 70
 V   V   S   L   R   D   L   I   V   A   E   E   V   D   L   V   Q   D   V   M   S   R   E   V   V  - 70 BF

TCA GTG GCG CCT GAT CAA TCA CGT CAT GAT GTT TAT GAT CTG ATC AAC CAT AGT GGT TTG GAT ATG ATA CCC GTT        - 753
 S   V   A   P   D   Q   S   R   H   D   V   Y   D   L   I   N   H   S   G   L   D   M   I   P   V  - 95
 S   V   S   A   K   T   D   Q   E   D   V   A   R   L   I   K   K   Y   D   F   L   A   A   P   V  - 95 BF

GTT CAA TTT     GGC AAA TTG ATG GGG GTA TTA CGT CCT CAA GAT ATT GCT GAA TTA ATT GAA GAT GAG AAT ACG        - 825
 V   Q   F   -   G   K   L   M   G   V   L   R   P   Q   D   I   A   E   L   I   E   D   E   N   T  -119
 I   S   S   E   G   K   L   V   G   I   V   T   V   D   D   I   I   D   I   L   E   E   E   A   T  -120 BF

TTA GAC GCA CAA CTT CAA GGG GCG ACT ACA CCA CTT GAA GAG CCT TAT TTA GCA ACC AGT CCT ATT ACG TTA TGG        - 900
 L   D   A   Q   L   Q   G   A   T   T   P   L   E   E   P   Y   L   A   T   S   P   I   T   L   W  -144
 -   -   E   D   L   G   E   F   S   A   S   K   G   A   T   D   V   D   V   S   S   F   Q   A   A  -143 BF

CGT AAA CGT GTC GTA TGG CTA TTA ATG CTA TTT GTT GCG GAA GCT TAT ACC GGT ACA GTA TTA AAA GCC TTT GAA        - 975
 R   K   R   V   V   W   L   L   M   L   F   V   A   E   A   Y   T   G   T   V   L   K   A   F   E  -169
 K   K   R   A   P   W   I   I   L   L   M   F   F   G   L   I   T   A   E   V   I   G   Q   F   E  -168 BF

GAG CAA TTA GAA GCT GCG ATT TCT CTG GCC TTC TTT ATT CCA TTA TTA ATT GGA ACG GGC GGG AAC AGT GGA ACG        -1050
 E   Q   L   E   A   A   I   S   L   A   F   F   I   P   L   L   I   G   T   G   G   N   S   G   T  -194
 E   T   L   E   A   I   V   L   L   A   A   F   I   P   L   L   M   D   S   A   G   N   T   G   T  -193 BF

CAA ATC ACT TCA ACC CTT GTC AGG GCG ATG GCG CTA GGG GAA GTG AGC CTA CGT AAT TTG GGC GCG GTA TTG AAA        -1125
 Q   I   T   S   T   L   V   R   A   M   A   L   G   E   V   S   L   R   N   L   G   A   V   L   K  -219
 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  -218 BF

AAA GAA GTA TCA ACC TCT TTC TTA GTG GCC GTG ACT ATT GGT GCA GCT GCT TTG ATT AGG GCA TGG ATA CTG GGT        -1200
 K   E   V   S   T   S   F   L   V   A   V   T   I   G   A   A   A   L   I   R   A   W   I   L   G  -244
 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  -243 BF

GTG GGC GCT GAA GTG ACG ATT GTA GTA AGT CTA ACT ATT GTT GCT ATC ACC ATG TGG AGT GCA ATT GTT TCT TCG        -1275
 V   G   A   E   V   T   I   V   V   S   L   T   I   V   A   I   T   M   W   S   A   I   V   S   S  -269
 G   N   M   M   L   A   F   I   V   G   I   S   I   F   L   T   L   S   I   A   T   I   I   G   T  -268 BF

ATT ATC CCA ATG GTA TTG AAG AAA TTA AAA GTT GAC CCC GCA GTA GTA TCT GCG CCG TTT ATT GCA ACC TTC ATT        -1350
 I   I   P   M   V   L   K   K   L   K   V   D   P   A   V   V   S   A   P   F   I   A   T   F   I  -294
 I   V   P   L   I   I   N   K   M   K   L   D   P   A   I   A   S   G   P   F   I   T   T   V   N  -293 BF

GAT GGA ACG GGT CTA ATT ATT TAC TTC GAA ATT GCC AAA TTG GTA ATG ACG GAG TTC GCC TAAAGACACACC ATCGA         -1427
 D   G   T   G   L   I   I   Y   F   E   I   A   K   L   V   M   T   E   F   A                      -314
 D   I   L   G   L   L   I   Y   F   S   I   A   T   A   L   L   E   Y   L                          -312 BF

GTTGATGCGG CAGTGTTATT CAACATCTGC CGCATTCACC CACTCATTCT GTTTTAAAAC CTCCCCACAT TTTCGGCACA AATACTGATT         -1517
CTCCCTCTGA CAATTTTATT ATGCCTTCTA ATGGTTAATT CGTGCGTTGT GGAACACGCA CAATGGTAGG TAAAAGTGCG ACTTTTGACG         -1607
GACGTGACTT CAAAGCGATG TGTACGTTTT GCGGGAACGT CTAAAACGTG TTCCATCATC CACTTCCACT CTTTGCCATG GGAGCAATA          -1697
CCTTTACGGC CAAAGACTTG ATACACTAAT AGATGAGCCA ACTCATGAGG GATCACCTCA TTAATGAAGC TATCACCATT TTCAATCAGT         -1787
AAGACAGCAT TTAAACGAAT TTCCCACTCT TTTAAGTAGG CACTCCCTGC TGTTGTTCCT CGCTGCTTAT AGTTAAGTGT TGGTTCTGGA         -1877
AAGATTTGTT CCAGCTTTTG TTGGGCTAAA TTCAGTTTTG CCCTCAGATC AAGCTTATCG ATACCGTCGA CCTCGA                        -1953
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FIG. 1. Nucleotide and deduced amino acid sequence of *mgtE* from *P. stuartii*. The insert DNA sequence of *mgtE* from *P. stuartii* was determined by the dideoxy chain termination method of Sanger et al. (19) as modified by Tabor and Richardson (18) with Sequenase modified T7 DNA polymerase (U.S. Biochemicals, Cleveland, Ohio). Initial sequence was obtained from deletion derivatives of pDT3 obtained by exonuclease III treatment with T7 promoter primers. Subsequent reactions used synthetic oligodeoxynucleotide primers complementary to and identical to segments within a previously sequenced segment. Complete sequence was obtained in both strands. The GenBank accession number is U23806. The deduced amino acid sequence of MgtE is presented immediately underneath in the single-letter code and is compared with that previously determined from *B. firmus* OF4 (BF [16]). Identical residues are boxed in black with white letters, while conservative substitutions are indicated by clear boxes and black letters. Substitution of an amino acid was considered to be conservative according to the following groupings: G, A, S, T; I, L, V, M; H, R, K; E, D, N, Q; and W, F, Y. No amino acid substitution for proline or cysteine was considered conservative. Putative membrane segments are indicated by the large boxes around both amino acid sequences. The GenBank accession number for the *B. firmus* OF4 sequence is U18744. An additional in-frame ATG start codon (underlined) is present in the *P. stuartii* sequence at bp 343. This is unlikely to be the actual start site, however, as discussed in the text.

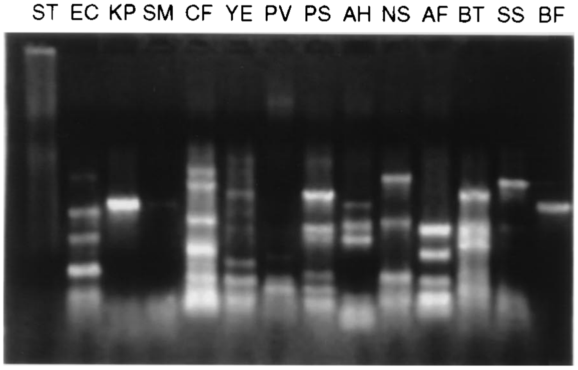
TABLE 1. Southern blot hybridization and PCR analysis of the phylogenetic distribution of *mgtE*[a]

Species[b]	PCR amplification from genomic samples		Southern hybridization against PCR products	
	B. firmus OF4 primers	*P. stuartii* primers	*B. firmus* OF4 products with *B. firmus* OF4 Probe	*P. stuartii* products with *P. stuartii* probe
Salmonella typhimurium	−	+	−	−
Escherichia coli	+	+	+	−
Klebsiella pneumoniae	+	+	+	−
Serratia marcescens	+	+	+	+
Citrobacter freundii	+	+	+	−
Yersinia enterocolitica	+	+	−	−
Proteus vulgaris	−	+	−	−
Providencia stuartii	+	+	−	+
Alteromonas haloplanktus	+	+	+	−
Neisseria sicca	+	+	+	−
Alcaligenes faecalis	+	+	−	−
Shigella flexneri	−	−	−	−
Bacillus thuringiensis	+	+	−	+
Streptococcus salivarius	+	+	−	+
Bacillus firmus OF4 (plasmid insert)	+	−	+	−

[a] Reaction conditions are described in the legends to Fig. 2 and 3.

[b] *S. typhimurium* LT2 and *E. coli* K-12 were grown as previously described (4, 17). *S. flexneri*, *S. faecalis*, and *N. sicca* were obtained from Carolina Biological Supply Co. (Burlington, N.C.). *E. coli* DH5α and HB101 were obtained from Gibco-BRL. Sources for other strains have been previously described (13). Bacto marine broth 2216 (Difco) was used to cultivate *A. haloplanktus*. Bacto brain heart infusion medium (Difco) was used to cultivate the remaining strains.

Genomic Amplification Products using *B. firmus* CX11/CX12 Primers



Genomic Amplification Products using *P. stuartii* PS1/PS2 Primers

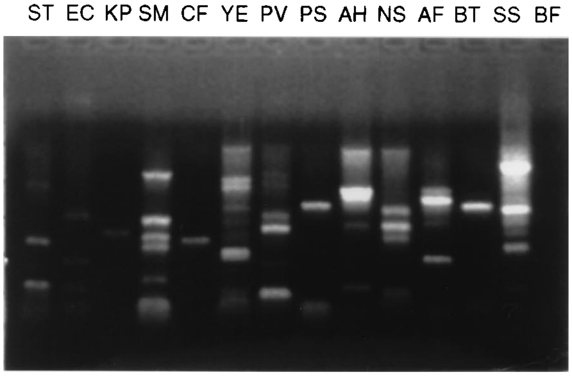


FIG. 2. PCR amplification products from genomic DNA with primers to *B. firmus* OF4 *mgtE* (top panel [16]) or *P. stuartii mgtE* (bottom panel). The *B. firmus* OF4 primers corresponded to the sequence from bp 483 to 502 (sense strand) and bp 1374 to 1355 (antisense strand). The *P. stuartii* primers corresponded to the sequence from bp 498 to 517 (sense strand) and bp 1411 to 1392 (antisense strand). Genomic DNA (0.25 to 0.5 μg) was added to a 100-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 200 μM (each) deoxynucleoside triphosphate, and 2.5 U of AmpliTaq DNA polymerase. Primers were added at a final concentration of 1.0 μM. Reactions were carried out in HotStart 100 tubes ($M_\beta P$, San Diego, Calif.) to eliminate oil overlay. Reaction mixtures were initially incubated for 5 min at 95°C followed by 2 min at 45°C. The reaction mixtures were then subjected to 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min in a Perkin-Elmer DNA thermal cycler. Reaction mixtures containing plasmid DNA from pRS194 (*B. firmus* OF4) and pRB6 (*P. stuartii*) were used as positive controls. Mixtures containing primers with λ DNA template, primers with no added DNA, and plasmid DNA without primer pairs served as negative controls. No amplification products were observed in the negative control reactions. Controls for PCR amplification have been previously discussed (13) in our study of the distribution of *corA* in gram-negative bacteria. Bands were visualized by ethidium bromide staining. The abbreviations for the organisms tested are as follows: ST, *S. typhimurium*; EC, *E. coli*; KP, *K. pneumoniae*; SM, *S. marcescens*; CF, *C. freundii*; YE, *Y. enterocolitica*; PV, *P. vulgaris*; PS, *P. stuartii*; AH, *A. haloplanktus*; NS, *N. sicca*; AF, *A. faecalis*; BT, *B. thuringiensis*; SS, *S. salivarius*; and BF, *B. firmus* OF4. Gels were scanned at 300 dots per in. on a Hewlett-Packard Scanjet IIcx. Digitized data were contrast adjusted with Adobe Photoshop, version 3.0.

T7 promoter of the plasmid, in agreement with either open reading frame (data not shown). Likewise, the predicted hydropathy profiles (7) of the *P. stuartii* and *B. firmus* OF4 MgtE proteins are virtually identical and predict a protein with a predominantly hydrophilic character at the N terminus and with five potential membrane-spanning regions towards the C terminus. The two regions with the highest level of similarity occur in hydrophobic domains M2 and M5. In contrast, there is virtually no similarity between the M3 domains of the two MgtE proteins, although the M3 domain is sufficiently hydrophobic in both to form a potential membrane-spanning region.

$^{57}Co^{2+}$ transport by MgtE. The *B. firmus* OF4 MgtE transporter is capable of mediating influx of Mg^{2+} and Co^{2+} but transports little or no Ni^{2+} (16). These properties are shared by the MgtE system of *P. stuartii*. Since $^{28}Mg^{2+}$ is extremely costly and rarely available, $^{57}Co^{2+}$ was used to characterize transport by methods previously described (3, 17). The K_m of the *P. stuartii* transporter is about 65 μM, similar to the 80 μM K_m for the *B. firmus* OF4 system (16). The activity of the *P. stuartii* system also is comparable to that of the *B. firmus* OF4 system, with respective V_{max} values of 0.70 versus 0.35 nmol min^{-1} 10^8 $cells^{-1}$. Nevertheless, these V_{max} values for MgtE, expressed from a multicopy plasmid, are significantly lower than that for CorA derived from expression of a single chromosomal copy of *corA* (5, 17). Mg^{2+} and several other divalent cations had similar effects on MgtE-mediated $^{57}Co^{2+}$ uptake in either *B. firmus* OF4 (16) or *P. stuartii* (data not shown), further indicating the functional identity of the transporters.

Phylogenetic studies. We have previously shown that *corA*, encoding the dominant Mg^{2+} influx system in *S. typhimurium* and *E. coli*, is ubiquitous within the gram-negative bacteria and is present in at least one gram-positive organism, *Streptococcus faecalis* (14). Consequently, we investigated the distribution of *mgtE* and, in parallel studies, the presence of *corA* within additional gram-positive organisms. Twelve strains (excluding *P. stuartii*) were tested for the presence of *mgtE* homologs by a combination of Southern blot hybridization and PCR amplifi-

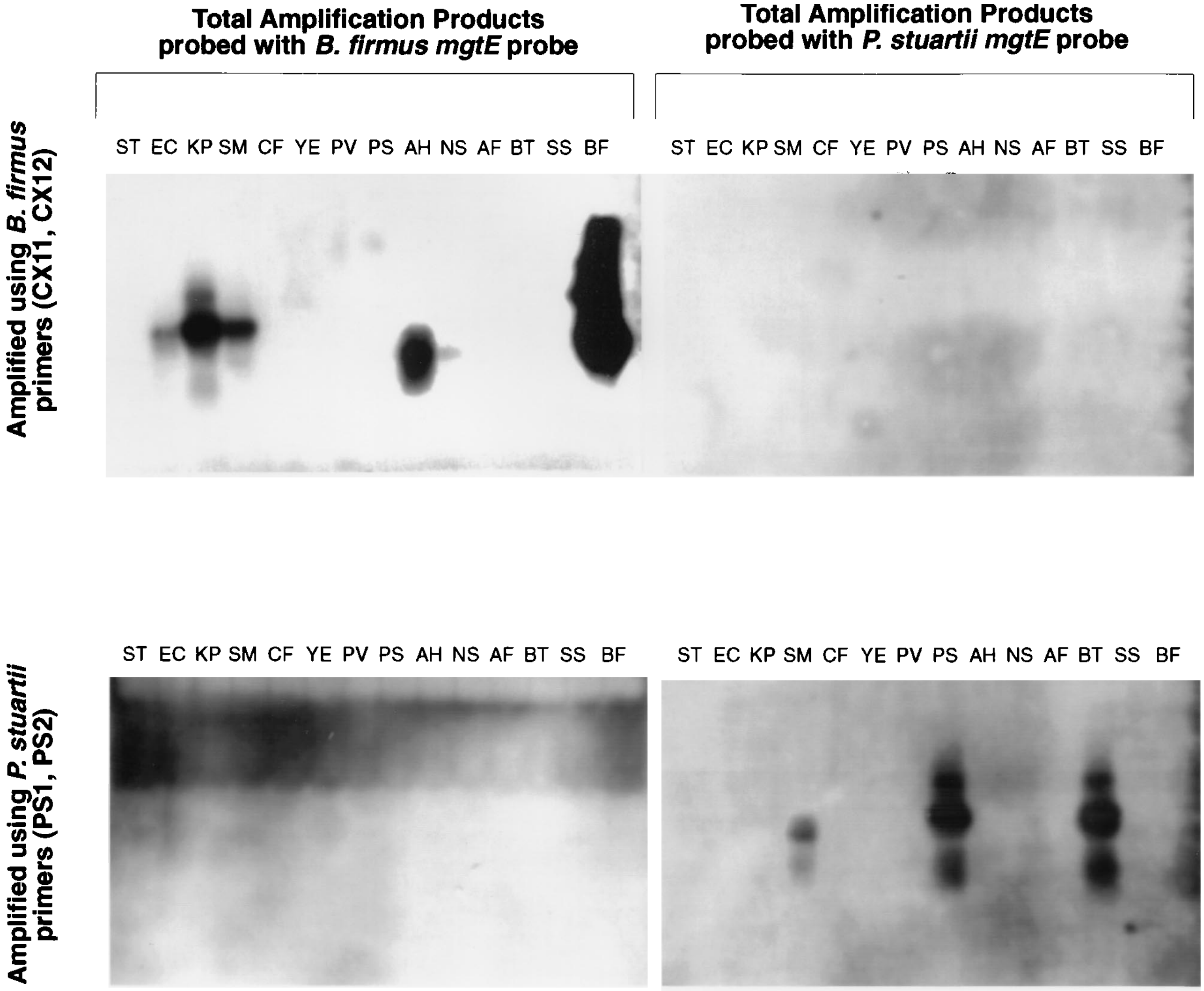


FIG. 3. Southern blot analysis of total PCR amplification products. Genomic DNA (data not shown) or PCR amplification products from either *B. firmus* OF4 or *P. stuartii* were hybridized against nucleic acid probes for the *mgtE* homologs from *B. firmus* OF4 and *P. stuartii*. Probes were prepared from PCR cassettes. The *B. firmus* OF4 probe was amplified from plasmid pRS194 as an 892-bp fragment (bp 483 to 1374 [16]) representing 90% of the coding sequence. The *P. stuartii* probe was amplified from plasmid pRB6 as a 914-bp fragment (bp 498 to 1411 [Fig. 1]) representing 97% of the coding sequence. Amplification products from each reaction were gel purified and labeled with biotin 14-dCTP by using a Bioprime labeling kit from Gibco-BRL. Against genomic DNA, the *B. firmus* OF4 probe did not hybridize to any genomic DNA sample except that from *B. firmus* OF4, and similarly, the *P. stuartii* probe failed to hybridize against any genomic DNA sample except that from *P. stuartii* (data not shown, see text). Except with the *S. marcescens* PCR amplification product, neither the *P. stuartii* probe nor the *B. firmus* OF4 probe hybridized to any PCR product amplified with primers from the alternate species. For hybridization, genomic DNA (15 to 20 μg) was digested to completion with *Eco*RI or *Bam*HI and separated on a 0.8% agarose gel (13). PCR products (1 to 2 μg) were separated on a 1% agarose gel. After electrophoresis, DNA fragments from either the genomic DNA or PCR amplification preparations were transferred to neutral nylon membranes (Schleicher & Schuell, Keene, N.H.) as described by Ausubel et al. (2) and covalently bound by UV cross-linking at 12,000 μJ/cm^2. Membranes were preincubated in hybridization solution (13) containing 50% formamide for 4 h at 42°C. Biotinylated probe was denatured by boiling and added to hybridization buffer containing 5% dextran sulfate, 50% formamide, and 20 mM sodium phosphate buffer (pH 6.5). Membranes were incubated overnight at 42°C. Membranes were washed twice in 5× SSC (1 × SSC is 150 mM NaCl plus 15 mM sodium citrate) plus 0.5% sodium dodecyl sulfate (SDS) at 65°C (high stringency) or 42°C (low stringency) for 5 min followed by two washes at 35°C in 0.1× SSC plus 1% SDS for 30 min followed by two washes at room temperature in 2× SSC for 5 min; wash volume was 2 ml/cm^2 of membrane. Detection of the biotinylated probe was achieved through chemiluminescence with streptavidin-conjugated alkaline phosphatase and the Photogene nucleic acid detection system (Gibco-BRL). The upper panels show the PCR products obtained with *B. firmus* OF4 primers probed with *mgtE* from *B. firmus* OF4 (left panel) or *P. stuartii* (right panel). The lower panels show the PCR products obtained with *P. stuartii* primers probed with *mgtE* from *B. firmus* OF4 (left panel) or *P. stuartii* (right panel). Gels were scanned at 300 dots per in. on a Hewlett-Packard Scanjet IIcx. Digitized data were contrast adjusted with Adobe Photoshop, version 3.0.

cation techniques (Table 1). Initially, nucleic acid probes complementary to *B. firmus* OF4 and *P. stuartii mgtE* alleles were constructed and used to screen samples of genomic DNA from each organism by Southern hybridization. In contrast to the comparable experiments with *corA* (14), no hybridization to *mgtE* probes was observed in any additional organism under either high- or low-stringency hybridization conditions (data not shown). Of particular interest was the lack of cross-hybridization between *B. firmus* OF4 and *P. stuartii* samples.

Genomic DNA samples were also tested for the ability to facilitate amplification of products with *mgtE* primers from both species. Unlike results with direct Southern blot analysis, PCR products were observed with most species tested (Fig. 2). In reactions with *mgtE* primers from the gram-positive *B. fir-*

mus OF4, strong amplification was observed in *E. coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *P. stuartii*, *Alteromonas haloplanktus*, *Neisseria sicca*, *Alcaligenes faecalis*, *Bacillus thuringiensis*, and *Streptococcus salivarius*. Weaker bands were observed in some other species. Several additional experiments under a variety of reaction conditions failed to detect *mgtE* in *S. typhimurium*. The specificity of the PCR amplification products was tested by Southern blot analysis with the nearly full-length *B. firmus* OF4 *mgtE* probe; significant reactions were seen with *E. coli*, *K. pneumoniae*, *Serratia marcescens*, and *A. haloplanktus* in addition to the *B. firmus* OF4 control (Fig. 3 and Table 1). A weaker reaction was seen with *N. sicca.*

The converse experiment, with *mgtE* primers from *P. stuartii*, also showed amplification in most species tested (Fig. 2). Only *B. thuringiensis* gave a product of similar size. When tested by Southern blot analysis with the *P. stuartii* probe, bands were obtained under high-stringency conditions in *S. marcescens*, *B. thuringiensis*, and the control, *P. stuartii* (Fig. 3 and Table 1). The results from the cross-hybridization experiments, involving Southern blot analyses of PCR products obtained with primers from one species and a probe from the other species, were completely negative (Fig. 3). That is, for example, the *P. stuartii* probe did not react with any PCR product obtained with the *B. firmus* OF4 primers.

Discussion. From the current data, it cannot be determined whether the MgtE transporter primarily mediates influx or efflux or indeed whether it is a physiologically relevant Mg^{2+} transporter. While it can clearly mediate the uptake of Mg^{2+}, it is possible that its true function is the efflux of Mg^{2+} or of another cation. Regardless of its physiological role, *mgtE* is present in a variety of organisms. *mgtE* was first demonstrated in the gram-positive organism *B. firmus* OF4 (16). This report describes the identification of *mgtE* in a gram-negative organism. This might suggest therefore that the *mgtE* class of transporter would be widespread in bacteria; nevertheless, *mgtE* could only be detected in a relatively small number of species. In addition to the two cloned genes, *mgtE* was detected only in *B. thuringiensis* among the gram-positive organisms tested. Among the gram-negative organisms, *mgtE* was detected within the γ division in *E. coli*, *K. pneumoniae*, *S. marcescens*, and *A. haloplanktus*. In the β division, *mgtE* was detected in *N. sicca* but not *A. faecalis*. Of interest is the fact that the probes derived from the *B. firmus* OF4 gene and the *P. stuartii* gene detected distinct sets of homologs with virtually no overlap (Fig. 3 and Table 1). Only in *S. marcescens* did both probes detect *mgtE*. This is not necessarily surprising, since the two *mgtE* genes are not remarkably similar at the nucleotide level and are only 31% identical at the amino acid level. What is somewhat surprising is that the probe from the gram-negative *P. stuartii* detected *mgtE* in a gram-positive organism, *B. thuringiensis*, and, conversely, that the probe from the gram-positive *B. firmus* OF4 detected *mgtE* only in gram-negative organisms. While a small number of *mgtE* homologs may have been missed by these experiments, it seems very unlikely that all of the organisms that tested as negative actually carry *mgtE*. Identical protocols, with several controls to verify specificity of the reactions, have detected *corA* homologs in all species tested (14). In addition, PCR experiments for several species were performed under as many as 12 different reaction conditions, and Southern blots were performed under both high- and low-stringency hybridization conditions to optimize the possibility of detection of *mgtE*. No additional *mgtE* homologs were detected in any species by these further experiments (data not shown).

These results for *mgtE* are in sharp contrast to the ubiquity of distribution exhibited by the *corA* Mg^{2+} transporter. Our previous report (14) demonstrated that *corA* was ubiquitous within the gram-negative bacteria. *corA* is also present in several gram-positive species, notably *S. faecalis* (14), *B. thuringiensis*, and *Micrococcus luteus* (1). Thus *corA* is likely ubiquitous within the eubacteria. On the basis of its physiology in *S. typhimurium* and *E. coli* (4–6, 12, 17), its activity in *B. subtilis* (6, 10), and the ubiquity of its distribution, *corA* likely forms the dominant Mg^{2+} transporter in most if not all bacteria, while *mgtE* has a significantly more limited distribution.

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