JOURNAL OF BACTERIOLOGY, Feb. 2008, p. 872–878 Vol. 190, No. 3
0021-9193/08/$08.00+0 doi:10.1128/JB.01505-07

Identification of a Spermidine Excretion Protein Complex (MdtJI) in *Escherichia coli*[▽]

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Received 19 September 2007/Accepted 2 November 2007

A spermidine excretion protein in *Escherichia coli* was looked for among 33 putative drug exporters thus far identified. Cell toxicity and inhibition of growth due to overaccumulation of spermidine were examined in an *E. coli* strain deficient in spermidine acetyltransferase, an enzyme that metabolizes spermidine. Toxicity and inhibition of cell growth by spermidine were recovered in cells transformed with pUC*mdtJI* or pMW*mdtJI*, encoding MdtJ and MdtI, which belong to the small multidrug resistance family of drug exporters. Both *mdtJ* and *mdtI* are necessary for recovery from the toxicity of overaccumulated spermidine. It was also found that the level of *mdtJI* mRNA was increased by spermidine. The spermidine content in cells cultured in the presence of 2 mM spermidine was decreased, and excretion of spermidine from cells was enhanced by MdtJI, indicating that the MdtJI complex can catalyze excretion of spermidine from cells. It was found that Tyr[4], Trp[5], Glu[15], Tyr[45], Tyr[61], and Glu[82] in MdtJ and Glu[5], Glu[19], Asp[60], Trp[68], and Trp[81] in MdtI are involved in the excretion activity of MdtJI.

Polyamines (putrescine, spermidine, and spermine) are essential for normal cell growth (3, 11, 12), and their content in cells is regulated by biosynthesis, degradation, uptake, and excretion (5, 9, 10, 26). With regard to transport, the properties of three polyamine transport systems were characterized in *Escherichia coli* (15, 16, 40). They include spermidine-preferential and putrescine-specific uptake systems, which belong to the family of ATP binding cassette transporters, and a protein, PotE, involved in the excretion of putrescine by a putrescine-ornithine antiporter activity. Furthermore, it has been reported that cadaverine and aminopropylcadaverine function as compensatory polyamines for cell growth (13), and CadB, a cadaverine-lysine antiporter, is strongly involved in cell growth at acidic pH, like PotE (23, 33, 34, 41). Analogous to the *speF-potE* operon (18), *cadB* is one component of the *cadBA* operon, in which *cadA* encodes lysine decarboxylase (22, 41) and is induced by acidic pH and lysine (23). The *cadBA* and *speF-potE* operons contribute to an increase in the pH of the extracellular medium through excretion of cadaverine and putrescine, the consumption of a proton, and a supply of carbon dioxide during the decarboxylation reaction (33, 38), so the expression of these two operons is important for cell growth at acidic pH.

Although PotE and CadB excrete putrescine and cadaverine at acidic pH, they function as uptake proteins for putrescine and cadaverine at neutral pH (16, 33). Thus, no polyamine excretion proteins that function at neutral pH have been identified to date. Overaccumulated spermidine is either metabolized by acetylation of spermidine, in a reaction catalyzed by spermidine acetyltransferase (6), or neutralized by the increase in L-glycerol 3-phosphate (27). In this study, we looked for spermidine excretion proteins among putative drug exporters comprising five families (the major facilitator family, the small multidrug resistance [SMR] family, the resistance-nodulation-cell division family, the ATP binding cassette family, and the multidrug and toxic compound extrusion family) (25) and found that the MdtJI complex in the SMR family can catalyze excretion of spermidine at neutral pH.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *E. coli* CAG2242 (*speG putE44 hsdR thi thr leu lacY1 tonA21*), a spermidine acetyltransferase-deficient mutant (2), was kindly supplied by E. W. Gerner (University of Arizona Health Science Center). The cells were grown in Luria-Bertani (LB) medium (10 g of tryptone per liter, 5 g of yeast extract per liter, and 10 g of NaCl per liter). Where indicated, 2 or 12 mM spermidine was added at the onset of cell growth. Cell growth was monitored by measuring the A_{540}. Cell viability was determined by counting colonies on 1.5% agar plates containing LB medium at 37°C. Thus, the definition of viable cells was cells that were able to grow on the agar plate. Ampicillin (100 μg/ml), tetracycline (15 μg/ml), and/or chloramphenicol (30 μg/ml) was added to the medium, if necessary. *E. coli* CAG2242 *mdtJ*::Km^r or *mdtI*::Km^r was constructed by means of P1 transduction, in which *E. coli* W3110 *mdtJ*::Km^r or *mdtI*::Km^r was used as a donor. *E. coli* W3110 strains were kindly supplied by H. Mori, Nara Institute of Science and Technology (1).

Plasmids and site-directed mutagenesis of *mdtJI*. Plasmids encoding 33 putative drug transporters in pUC119 (Takara Shuzo) were prepared as described previously (25). These plasmids included the original promoters for the drug transporter genes. Since the functions of some proteins among the 33 putative drug transporters were identified, new genetic names (Geno Base [http://ecoli.naist.jp/GB6/search.jsp]) were also attached (Fig. 1). The plasmids pMW*cusA*, pMW*mdtABC*, pMW*acrD*, pMW*mdtJI* (*ydgFE*), pMW*sugE*, pMW*mdtG* (*yceE*), pMW*ydiM*, pMW*yieO*, pMW*ybjYZ*, pMW*yddA*, and pMW*yojIH* were prepared by inserting the corresponding genes in pUC119 into pMW119 (Nippon Gene), using the same restriction enzymes. For preparation of pMW*acrAB*, pMW*emrE*, and pMW*mdfA*, PCRs were performed using pUC*acrAB*, pUC*emrE*, and pUC*mdfA* as templates, with the following primers: acrAB (SphI), 5′-ATTTTG

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▽ Published ahead of print on 26 November 2007.

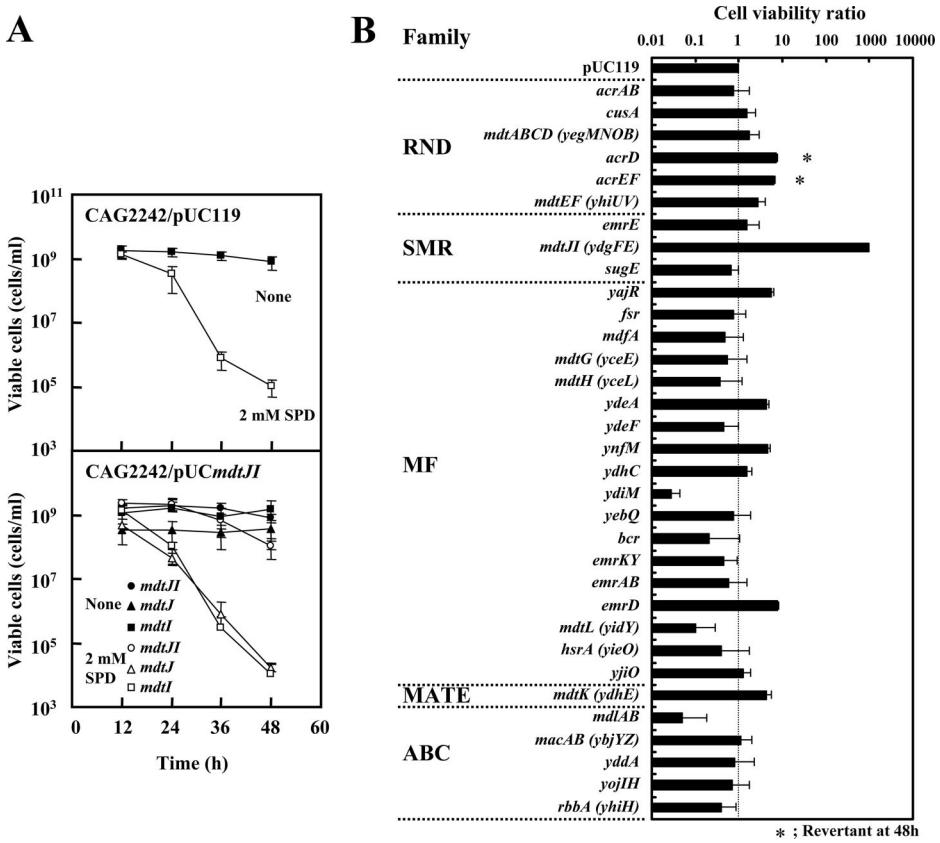

FIG. 1. Effect of spermidine on cell viability of *E. coli* CAG2242 transformed with pUC119 encoding putative drug transporters. Cells were cultured in the presence and absence of 2 mM spermidine, and viable cells were counted at the designated times as described in Materials and Methods. (A) Effects of 2 mM spermidine, *mdtJI*, *mdtJ*, and *mdtI* on cell viability. Symbols shown in the figure indicate the transformed genes. Numbers of colonies (viable cells) are shown in the figure. SPD, spermidine. (B) Effects of 2 mM spermidine and 33 putative drug transporters on cell viability at 48 h. The number of colonies obtained from cells transformed with pUC119 was defined as 1, and cell viability ratios are shown in the figure, in which genes are classified into five families of putative drug exporters. *, a revertant appeared at 48 h. Values are the means ± standard deviations (SD) for three samples.

CATGCGTATGTACCATAGCACGACG-3′; acrAB (BamHI), 5′-ATTAGGATCCACTCCTTAATGTTCGTAGGT-3′; emrE (EcoRI), 5′-CAGAGAATTCCGATGAAACGGGTATTGAGG-3′; emrE (SphI), 5′-TATTGCATGCTTCTTACGCCATAATCTTGA-3′; mdfA (EcoRI), 5′-GTAAGAATCGCTTAACCGTGGTTTCAGCT-3′; and mdfA (HindIII), 5′-GAGAAAGCTTGATCGCACAAAGCAGTCAGG-3′. The PCR products thus obtained were digested with SphI and BamHI for *acrAB*, EcoRI and SphI for *emrE*, and EcoRI and HindIII for *mdfA*, and the fragments were inserted into the same restriction sites of pMW119.

For preparation of pUC*mdtJ* (*ydgF*) and pUC*mdtI* (*ydgE*), PCRs were performed using pUC*mdtJI* (*ydgFE*) as the template, with the following primers: P-J1, 5′-GGTTTCGCTGGATCCAGCGAAAATTAA-3′; P-J2, 5′-CAAAAAGACGTTAGCAACGAATTCCAGCAC-3′; P-I1, 5′-CAGGTACCCGGATCCCGCGTAAACCTGAAC-3′; and P-I2, 5′-AAAATAGAATTCAAACGCTGCCCGACAGCG-3′. The PCR products thus obtained were digested with BamHI and EcoRI, and the fragments were inserted into the same restriction site of pUC119. Plasmids pUC*mdtJ* (*ydgF*) and pUC*mdtI* (*ydgE*) were under the control of the *lacUV5* promoter instead of the original promoter.

Plasmid YEp-HA_3 with 170 bp of the 3′-untranslated region (3′-UTR) of the *UGA4* gene (YEp-HA_3-3′-UTR · UGA4) (39) was used for preparation of YEp-*mdtJ*-HA_3. The BamHI site of the HA_3 region of YEp-HA_3-3′-UTR · UGA4 was deleted by site-directed mutagenesis, and then the *mdtJ* gene, containing the promoter region and the open reading frame lacking the termination codon, was inserted into the SalI and BamHI sites of the plasmid to fuse to the HA_3 tag. pUC*mdtJ*-HA_3 was prepared by inserting the 0.7-kb SalI-EcoRI fragment of YEp-MdtJ-HA_3 into the same restriction site of pUC119. pCA24N-*mdtI* with an IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible promoter, pT5/lac, and a His tag in the NH_2 terminus of MdtI was kindly supplied by H. Mori, National BioResource Project (NIG, Japan) (20). Site-directed mutagenesis for the construction of mutated *mdtJ* (*ydgF*) and *mdtI* (*ydgE*) genes was performed using pUC*mdtJI*, encoding both MdtJ and MdtI in an operon, by overlap extension using PCR (8) or with a QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer's protocol. The nucleotide sequences of the plasmids were confirmed with a CEQ8000 DNA genetic analysis system (Beckman Coulter).

Dot blot analysis. Total RNA was prepared from *E. coli* CAG2242 transformed with pUC*mdtJI* (*ydgFE*) or pMW*mdtJI* (*ydgFE*) according to the method of Emory and Belasco (4). Dot blot analysis was performed using various amounts of total RNA and a ^{32}P-labeled probe consisting of 400 bp of *mdtI*, which was labeled with [α-^{32}P]dCTP by use of a BcaBEST labeling kit (Takara Shuzo) (30).

Western blot analysis. Western blotting was performed by the method of Nielsen et al. (24), using 40 μg of cell lysate and ECL Western blotting reagents (GE Healthcare). Mouse monoclonal anti-hemagglutinin (anti-HA) antibody (clone HA-7) and rabbit anti-six-His antibody were purchased from Sigma and Bethyl Laboratories, respectively.

Measurement of polyamines. Polyamine levels in *E. coli* were determined by high-performance liquid chromatography, as described previously (13), after homogenization and extraction of the polyamines with 5% trichloroacetic acid and centrifugation at 27,000 × *g* for 15 min at 4°C. The retention times for putrescine, spermidine, and spermine were 6.7, 13, and 25 min, respectively. Protein content in the precipitate was determined by the method of Lowry et al. (21).

Assay for spermidine excretion from cells. *E. coli* CAG2242 cells transformed with either pUC119 or pUC*mdtJI* were cultured as described above and harvested at an A_{540} of 0.5. After cells were washed with buffer 1, containing 0.4%

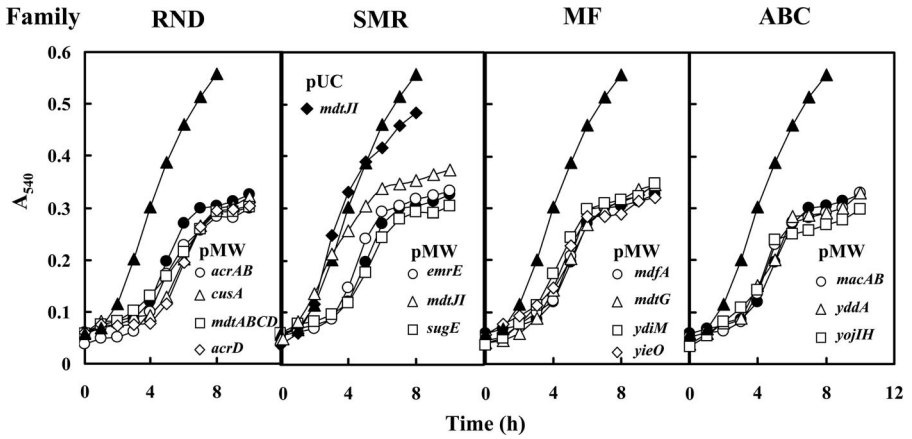

FIG. 2. Effect of spermidine on cell growth of *E. coli* CAG2242 transformed with pMW119 encoding putative transporters. Cells were cultured in the presence and absence of 12 mM spermidine, and cell growth was monitored by measuring the A_{540}. ▲, cell growth of *E. coli* CAG2242/pMW119 without spermidine; ●, cell growth of *E. coli* CAG2242/pMW119 with 12 mM spermidine. Data were shown by dividing the transporters into families. Symbols shown in the figure indicate the genes transformed with pMW119. As a control, cell growth of *E. coli* CAG2242/pUC*mdtJI* (◆) was monitored. Values are the averages of duplicate determinations.

glucose, 62 mM potassium phosphate, pH 7.0, 1.7 mM sodium citrate, 7.6 mM $(NH_4)_2SO_4$, and 0.41 mM $MgSO_4$, [^{14}C]spermidine was preloaded by the incubation of cells (0.2 mg protein/ml buffer 1) with 1 mM [^{14}C]spermidine (37 MBq/mmol) for 90 min. After cells were washed with buffer 1, spermidine excretion from cells was measured by incubating cells (0.2 mg/ml buffer 1) at 37°C for the designated times. The cells were then removed by centrifugation at 17,000 × *g* for 5 min at 4°C, and the radioactivity of the supernatant (0.5 ml) was counted by a liquid scintillation counter. When excreted polyamines were analyzed by high-performance liquid chromatography, nonlabeled spermidine was used instead of [^{14}C]spermidine.

RESULTS

Identification of spermidine excretion protein. We previously reported that spermidine toxicity is increased due to overaccumulation of spermidine when spermidine acetyltransferase is deficient (6). To look for a spermidine excretion protein(s), cell viability was estimated using *E. coli* CAG2242, which is deficient in spermidine acetyltransferase (2), after transformation with a candidate gene potentially encoding a spermidine excretion protein. First, recovery of cell viability, i.e., spermidine excretion activity, was examined using cells transformed with 33 kinds of drug transporters (25). As shown in Fig. 1A, the viability of *E. coli* CAG2242 cultured in the presence of 2 mM spermidine was reduced to <0.1% compared with that of cells cultured without spermidine. When the *mdtJI* gene was transformed into *E. coli* CAG2242, cell viability during culture with 2 mM spermidine increased >1,000-fold (Fig. 1A and B). Essentially the same results were obtained when *E. coli* CAG2242 *mdtJ*::Km^r or *mdtI*::Km^r was used instead of *E. coli* CAG2242 (data not shown). When genes for the other 32 drug transporters were transformed, the viability of *E. coli* CAG2242 did not increase significantly (Fig. 1B). It has been reported that *mdtJ* and *mdtI* are coexpressed (25). When either *mdtJ* or *mdtI* was transformed alone, the cell viability of *E. coli* CAG2242 did not increase significantly (Fig. 1A), indicating that both the MdtJ and MdtI proteins are required to rescue cell viability during culture with 2 mM spermidine.

Among 33 genes for drug transporters, the expression of 13 of these genes by the multicopy vector pUC119 significantly inhibited cell growth in the absence of spermidine. Thus, the effect of expression of these genes on cell viability was examined using the low-copy-number vector pMW119. However, there was no significant effect of these genes on viability of *E. coli* CAG2242 cultured with 2 mM spermidine (data not shown). We then tested whether growth of *E. coli* CAG2242 cultured with a higher concentration of spermidine (12 mM) was influenced by the expression of these genes inserted in the low-copy-number pMW119 vector. As shown in Fig. 2, growth of *E. coli* CAG2242 was significantly inhibited by 12 mM spermidine. Expression of the same 13 genes as those studied previously did not influence the growth of *E. coli* CAG2242 cultured with 12 mM spermidine, whereas cell growth was rescued by the expression of *mdtJI*. The degree of the rescue of cell growth was greater with pUC*mdtJI* than with pMW*mdtJI*. The results strongly suggest that *mdtJI* can enhance cell viability and growth through excretion of spermidine when spermidine overaccumulates in cells.

Excretion of spermidine by MdtJI. It was then determined whether spermidine can be excreted from *E. coli* CAG2242 by MdtJI. When the spermidine and putrescine contents were measured in *E. coli* CAG2242 cultured with or without 2 mM spermidine, overaccumulation of spermidine, but not putrescine, was observed in cells cultured with spermidine (Table 1). When *mdtJI* was transformed into *E. coli* CAG2242, accumulation of spermidine in *E. coli* CAG2242 cultured with 2 mM spermidine was greatly diminished in parallel with the recovery of cell viability (Table 1 and Fig. 1). Next, excretion of [^{14}C]spermidine by MdtJI was examined. As shown in Fig. 3A, excretion of accumulated [^{14}C]spermidine was observed in cells transformed with pUC*mdtJI* but not in cells carrying a vector. Excretion of spermidine from cells was confirmed by measuring the level of polyamines in the reaction mixture after removal of cells by centrifugation. As shown in Fig. 3B, the level of spermidine in the reaction mixture at 40 min increased significantly when pUC*mdtJI* was transformed into cells. These

TABLE 1. Effect of spermidine addition to the medium on the level of polyamines in cells[a]

Strain	Time of incubation (h)	Addition of 2 mM spermidine	Polyamine concn (mM)	
			Putrescine	Spermidine
CAG2242/pUC119	12	−	4.86 ± 1.12	5.27 ± 0.88
	12	+	0.19 ± 0.14	26.2 ± 2.34
	24	−	3.03 ± 1.14	5.73 ± 1.04
	24	+	0.17 ± 0.10	30.9 ± 2.28
	36	−	1.66 ± 0.16	4.58 ± 0.58
	36	+	0.07 ± 0.04	32.3 ± 3.06
CAG2242/pUC*mdtJI*	12	−	0.96 ± 0.52	5.95 ± 1.42
	12	+	0.15 ± 0.14	11.8 ± 0.66
	24	−	3.40 ± 1.26	4.71 ± 1.06
	24	+	0.24 ± 0.10	12.1 ± 2.06
	36	−	3.92 ± 0.46	2.83 ± 1.00
	36	+	0.33 ± 0.26	15.5 ± 1.34

[a] *E. coli* CAG2242/pUC119 and CAG2242/pUC*mdtJI* cells were cultured in the presence and absence of 2 mM spermidine for the indicated times, and the polyamine content in cells was measured as described in Materials and Methods. The concentrations of polyamines were calculated based on the data for 5 μl of cell volume per mg protein (19, 29). Values are means ± SD for three samples.

results indicate that MdtJI can catalyze the excretion of spermidine.

Increase in the level of *mdtJI* mRNA by spermidine. We examined whether the level of *mdtJI* mRNA was increased by spermidine. The level of *mdtJI* mRNA was measured by dot blot analysis. The level of *mdtJI* mRNA expressed from host genes was very low (Fig. 4). Expression of *mdtJI* mRNA was clearly observed only when pMW*mdtJI* or pUC*mdtJI* was transformed into cells. As shown in Fig. 4A, the level of *mdtJI* mRNA was increased about 1.5- to 2.0-fold by 2 mM spermidine during culture from 12 h to 36 h when pUC*mdtJI* was transformed into cells. When cells were cultured in the presence of 12 mM spermidine, the level of *mdtJI* mRNA was greatly increased in cells transformed with either pMW*mdtJI* or pUC*mdtJI* 2 h after the onset of cell growth (Fig. 4B). However, when pMW*mdtJI* was transformed into cells, the level of *mdtJI* mRNA decreased 4 h after the onset of cell growth (Fig. 4B), in parallel with the slowdown of cell growth (Fig. 2). The results indicate that the increase in the level of *mdtJI* mRNA by spermidine is important for the decrease in spermidine toxicity.

Identification of functional amino acids in MdtJI. We previously identified the functional amino acids which recognize spermidine on the substrate binding protein PotD in the spermidine-preferential uptake system (17, 36). They were acidic amino acids (Glu and Asp) and aromatic amino acids (Trp and Tyr). To determine the functional amino acids in MdtJ and MdtI, Asp, Glu, Trp, and Tyr were replaced by Asn, Gln, Leu, and Leu, respectively, and the viability of cells carrying mutated pUC*mdtJI* cultured with 2 mM spermidine was measured 48 h after the onset of cell growth. As shown in Fig. 5A, mutation of Tyr^4, Trp^5, Glu^{15}, Tyr^{45}, Tyr^{61}, and Glu^{82} in MdtJ and Glu^5, Glu^{19}, Asp^{60}, Trp^{68}, and Trp^{81} in MdtI decreased cell viability >100-fold compared to that of the wild-type MdtJI complex. The activity of spermidine excretion also decreased in these mutants, judging from the increased level of spermidine in cells (Table 2). The levels of the mutated proteins were nearly equal to those of wild-type MdtJ and MdtI (Fig. 5B). The results indicate that these amino acid residues are important for the activity of spermidine excretion by MdtJI. The results are in accordance with the idea that NH_2

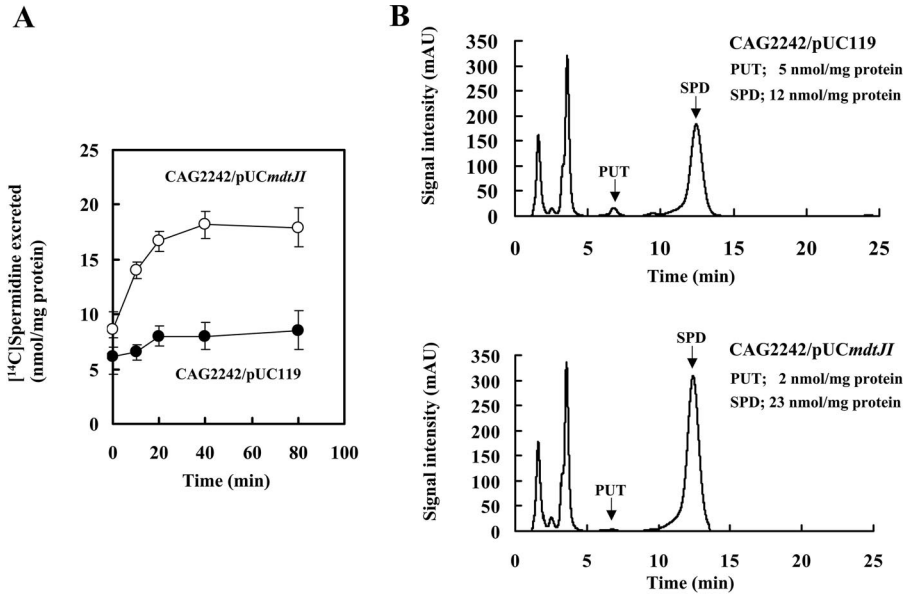

FIG. 3. Excretion of spermidine from cells by MdtJI. (A) Assays for excretion of [^{14}C]spermidine were performed as described in Materials and Methods. The amount of [^{14}C]spermidine accumulated in *E. coli* CAG2242 cells transformed with either pUC119 or pUC*mdtJI* during a 90-min preincubation was 70.1 ± 0.5 or 65.2 ± 7.5 nmol/mg protein, respectively. (B) Assays of excretion of spermidine from cells were performed with nonlabeled spermidine, and excreted spermidine at 40 min was measured by high-performance liquid chromatography after the removal of cells by centrifugation. The retention times for putrescine (PUT) and spermidine (SPD) were 6.7 and 13 min, respectively. Levels of putrescine and spermidine in the supernatant per mg protein of cells in the precipitate are shown in the figure. Experiments were repeated twice, and the results were reproducible. AU, absorbance units.

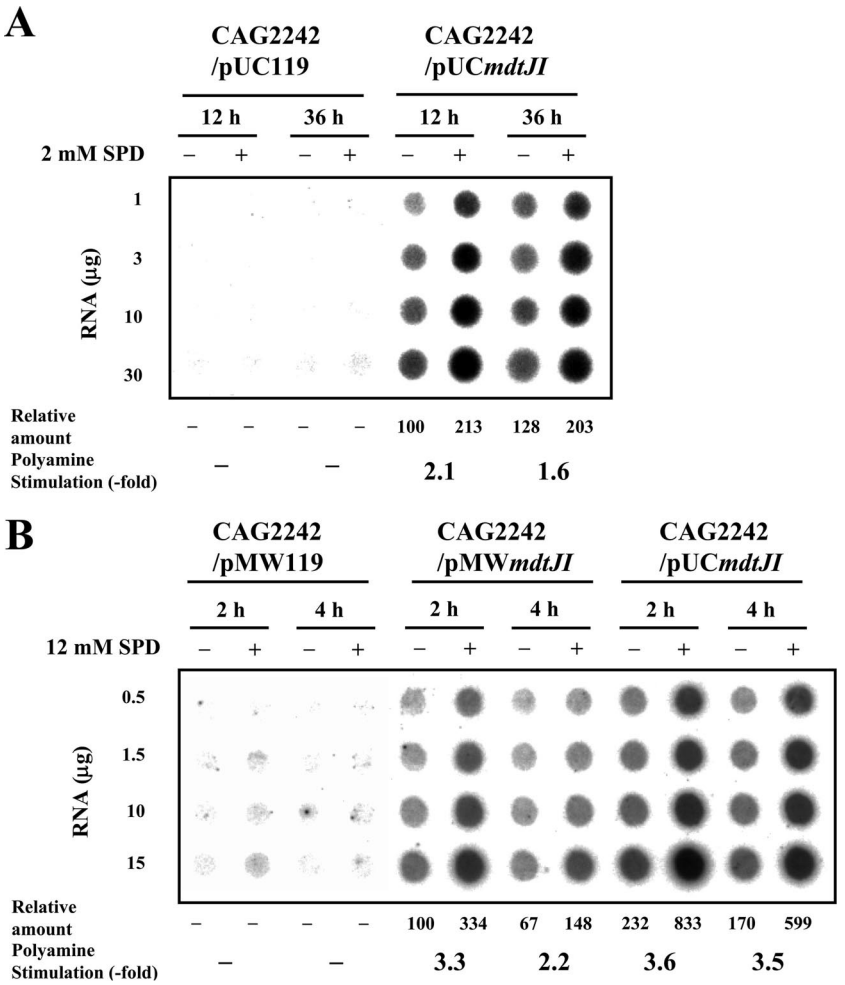

FIG. 4. Dot blot analysis of *mdtJI* mRNA in *E. coli* CAG2242 cells cultured with or without spermidine (SPD). Dot blot analysis was performed as described in Materials and Methods. The radioactivity of each spot was quantified using a Fujix Bas 2000II imaging analyzer, and the averages for four dots are shown as relative amounts. Results were reproducible in two separate experiments. (A) Levels of *mdtJI* mRNA in *E. coli* CAG2242 cells transformed with pUC*mdtJI* or pUC119 and cultured for 12 or 36 h in the presence and absence of 2 mM spermidine. (B) Levels of *mdtJI* mRNA in *E. coli* CAG2242 cells transformed with pMW*mdtJI*, pMW119, or pUC*mdtJI* and cultured for 2 or 4 h in the presence and absence of 12 mM spermidine. Cell growth was started at an A_{540} of 0.05.

and NH groups of spermidine are recognized by Asp and Glu and that propyl and butyl groups of spermidine are recognized by Trp and Tyr in MdtJI proteins (17, 36).

The MdtJI proteins belong to the SMR family. Proteins in the SMR family (EmrE, MdtJI, and SugE) are thought to have four transmembrane segments. It has also been reported that the structure of EmrE is a parallel dimer (35). If MdtJ and MdtI have parallel topology, most of the functional amino acid residues would be located in the cytoplasmic side (Fig. 5C), in a situation similar to that of other putrescine and cadaverine excretion proteins, such as PotE and CadB (16, 34).

DISCUSSION

It is known that overaccumulation of spermidine and/or spermine inhibits growth in both *E. coli* (6) and mammalian cells (7). Thus, the enzymes that metabolize spermidine and/or spermine—spermidine acetyltransferase in *E. coli* (6) and spermidine/spermine N^1-acetyltransferase in mammalian cells (7)—are induced when spermidine and/or spermine overaccumulates (6, 7). Once spermidine and/or spermine is acetylated, it cannot interact with RNA, and inhibition of protein synthesis due to overaccumulation of spermidine and/or spermine is relieved (14). The second mechanism to decrease polyamine toxicity is induction of L-glycerol 3-phosphate, which makes a complex with spermidine (27). Accordingly, inhibition of protein synthesis due to overaccumulation of spermidine is relieved. This was observed when AcrD and AcrEF were overproduced (Fig. 1).

The third mechanism to decrease polyamine toxicity is excretion of polyamines from cells. Since spermidine acetyltransferase does not exist in *Saccharomyces cerevisiae*, there are five excretion proteins (TPO1 to TPO5) in yeast (37). There were no previous reports identifying a spermidine excretion protein in *E. coli*. To find the spermidine excretion protein(s), a spermidine acetyltransferase-deficient mutant, CAG2242, was used. In this study, we have shown that the MdtJI protein complex can excrete spermidine. It is likely that the MdtJI

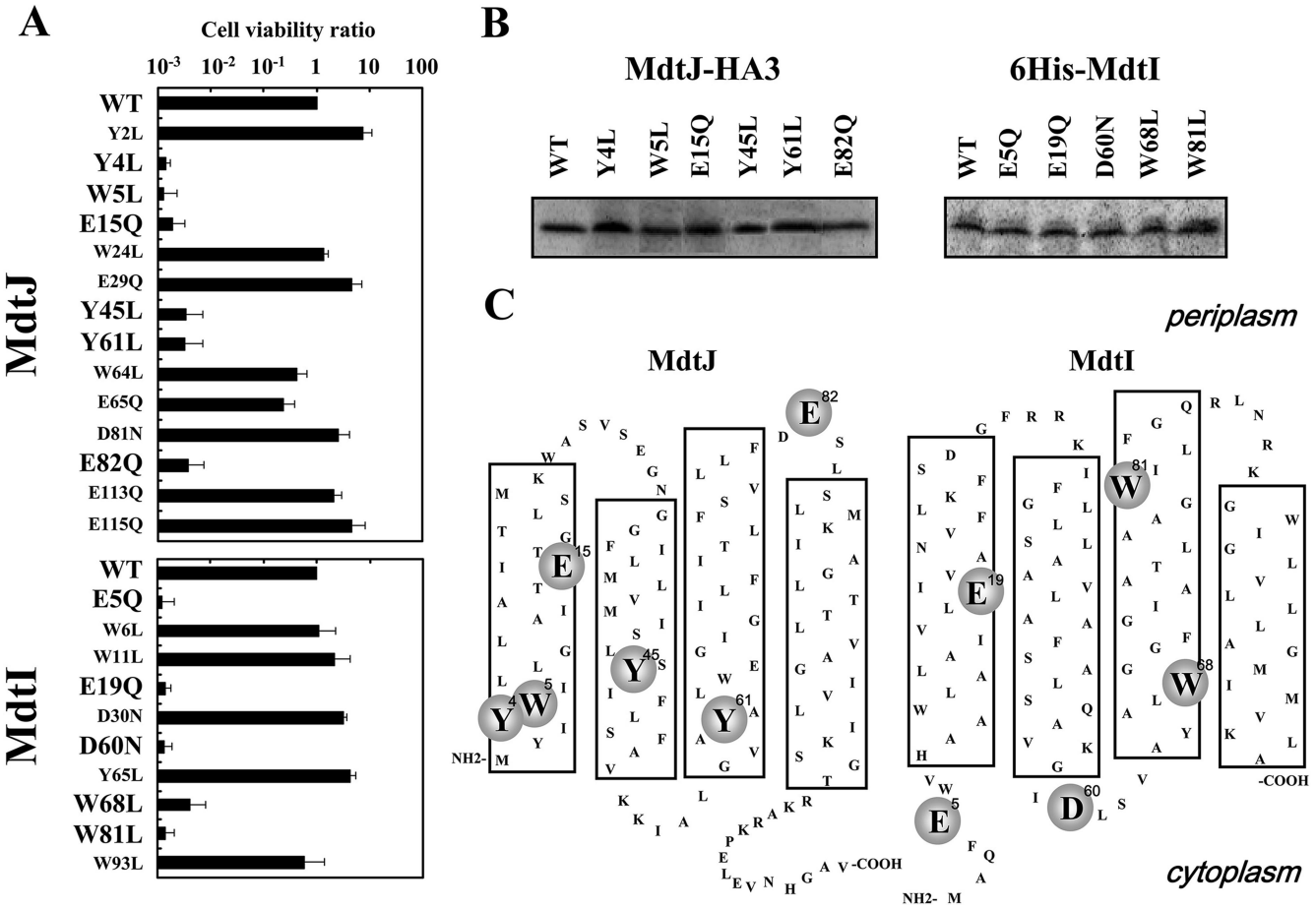

FIG. 5. Cell viability of various mutants of MdtJ and MdtI. (A) Cell viability of various mutants of MdtJ and MdtI was measured as described in Materials and Methods. Values are means ± SD for three samples. (B) *E. coli* CAG2242/pUC*mdtJ*-HA$_3$ and its mutants and *E. coli* CAG2242/pCA24N-*mdtI* and its mutants were cultured for 24 h. *E. coli* CAG2242/pCA24N-*mdtI* and its mutants were cultured further for 2 h in the presence of 1 mM IPTG. Experiments were repeated twice, and the results were reproducible. The levels of mutated MdtJ and MdtI were evaluated as described in Materials and Methods, using antibodies against the HA tag and the His tag, respectively. (C) Amino acid residues of MdtJ and MdtI involved in relief from spermidine toxicity. Models of secondary structures of proteins were constructed according to the average hydropathy profiles obtained with a hydrophilicity/hydrophobicity plot (Genentyx-Mac, version 10). Putative transmembrane segments are shown in large boxes. Amino acid residues involved in relief from spermidine toxicity are shown in circles.

TABLE 2. Levels of polyamines in CAG2242/pUC*mdtJI* and its mutants cultured with 2 mM spermidine[a]

Strain	Polyamine concn (mM)	
	Putrescine	Spermidine
CAG2242/pUC*mdtJI*	0.49 ± 0.12	11.7 ± 0.42
mdtJ(*Y4L*)	0.14 ± 0.05	36.0 ± 1.39
mdtJ(*W5L*)	0.23 ± 0.07	24.1 ± 0.99
mdtJ(*E15Q*)	0.07 ± 0.03	24.7 ± 1.83
mdtJ(*Y45L*)	0.33 ± 0.14	31.1 ± 0.64
mdtJ(*Y61L*)	0.36 ± 0.09	33.0 ± 1.56
mdtJ(*E82Q*)	0.19 ± 0.11	31.9 ± 2.01
mdtI(*E5Q*)	0.36 ± 0.18	30.9 ± 2.30
mdtI(*E19Q*)	0.60 ± 0.24	23.2 ± 1.35
mdtI(*D60N*)	0.65 ± 0.15	20.9 ± 0.49
mdtI(*W68L*)	0.24 ± 0.10	29.4 ± 2.14
mdtI(*W81L*)	0.18 ± 0.13	20.7 ± 0.88

[a] *E. coli* CAG2242/pUC*mdtJI* and its mutants were cultured in the presence of 2 mM spermidine for 24 h, and the polyamine content in cells was measured as described in Materials and Methods. Values are means ± SD for three samples.

protein complex is the major spermidine excretor in *E. coli* and contributes to relief from spermidine toxicity. In connection with this, it is noted that the level of *mdtJI* mRNA is increased in the presence of spermidine. This is most likely due to the enhancement of transcription of *mdtJI* mRNA by spermidine rather than to stabilization of its mRNA. Such an increase in *mdtJI* mRNA by spermidine strongly contributes to the relief of toxicity by overaccumulation of spermidine.

It has been reported that expression of MdtJI causes resistance to sodium dodecyl sulfate and deoxycholate (25). Therefore, this is the first report that the MdtJI complex excretes positively charged substances such as spermidine. In this respect, it is noted that EmrE and SugE, which also belong to the SMR family, can recognize positively charged substances such as tetraphenylphosphonium (TPP^+) (28, 31, 32). The total number of functional amino acid residues (Asp, Glu, Trp, and Tyr) in MdtJI was similar to that in the EmrE homodimer (28, 31). However, as for acidic amino acid residues, five molecules were necessary for excretion of spermidine by MdtJI, although

only two molecules were necessary for excretion of TPP^+ by the EmrE homodimer. This may be due to a difference in the numbers of positive charges in spermidine and TPP^+.

ACKNOWLEDGMENTS

We thank A. J. Michael for his kind help in preparing the manuscript. Thanks are also due to E. W. Gerner and H. Mori for kindly supplying *E. coli* CAG2242, *E. coli* W3110 *mdtJ*::Km^r or *mdt*::Km^r, and pCA24N-*mdtI* plasmid.

This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science, Sports, and Culture, Japan.

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