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Cloning and Characterization of the *metE* Gene Encoding *S*-Adenosylmethionine Synthetase from *Bacillus subtilis*

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The *metE* gene, encoding *S*-adenosylmethionine synthetase (EC 2.5.1.6) from *Bacillus subtilis*, was cloned in two steps by normal and inverse PCR. The DNA sequence of the *metE* gene contains an open reading frame which encodes a 400-amino-acid sequence that is homologous to other known *S*-adenosylmethionine synthetases. The cloned gene complements the *metE1* mutation and integrates at or near the chromosomal site of *metE1*. Expression of *S*-adenosylmethionine synthetase is reduced by only a factor of about 2 by exogenous methionine. Overproduction of *S*-adenosylmethionine synthetase from a strong constitutive promoter leads to methionine auxotrophy in *B. subtilis*, suggesting that *S*-adenosylmethionine is a corepressor of methionine biosynthesis in *B. subtilis*, as others have already shown for *Escherichia coli*.

S-Adenosylmethionine (SAM) functions as a methyl or methylene donor for many biochemical reactions, as an amino propyl donor for polyamine synthesis, and as an amino donor in biotin biosynthesis (5, 10, 12). SAM is also involved in the activation of certain free radical enzymes and is the corepressor of the methionine biosynthetic pathway in *Escherichia coli* (7, 28). SAM is chemically unstable and is reported not to be taken up by enteric bacteria (31). Thus, the enzyme that produces SAM, SAM synthetase (ATP:L-methionine *S*-adenosyltransferase; EC 2.5.1.6), appears to be an essential enzyme for enteric bacteria and probably for all other organisms as well (29).

The best-studied SAM synthetase is that of *E. coli*. The enzyme has been purified, characterized kinetically, and crystallized (11, 22). The corresponding gene, *metK*, has been cloned and sequenced (21), and genetic studies have been done to corroborate and extend the biochemical studies (13, 14, 25). Several mutant *metK* alleles have been selected as having a phenotype of resistance to methionine analogs, such as ethionine and α-methylmethionine. The generally accepted hypothesis is that *metK* mutants have SAM synthetase activity that has been reduced but not eliminated. The reduced activity leads to a reduced intracellular concentration of SAM, which in turn leads to induction of the methionine biosynthetic pathway, resulting in methionine overproduction and analog resistance. All *metK* mutants isolated so far still produce SAM and by inference still have at least some residual SAM synthetase activity.

For *Bacillus subtilis*, mutants with greatly reduced SAM synthetase specific activity have been isolated as ethionine resistant (1, 26, 36). The gene corresponding to ethionine resistance in this bacterium was named *metE*. Little is known about regulation of the methionine biosynthetic genes in *B. subtilis* (27). Here we report the cloning and characterization of a *B. subtilis* gene that encodes SAM synthetase. The cloned gene corresponds to the previously described *metE* gene.

MATERIALS AND METHODS

Bacterial strains. Plasmids were constructed in *E. coli* DH5α and YMC9, which were grown in Luria-Bertani medium containing 250 μg of ampicillin or 100 μg of spectinomycin per ml as needed for plasmid selection (3, 19). The plasmids and *B. subtilis* strains used in this study are listed in Table 1. *B. subtilis* was grown in 0.5% yeast extract plus 2.0% veal infusion broth or tryptone blood agar broth (Difco) for a rich medium. Antibiotic selection was done in rich medium containing 5 μg of chloramphenicol per ml or 1 μg of erythromycin plus 25 μg of lincomycin per ml. Minimal medium contained 1× Spizizen salts (32), 0.5% glucose, and 0.04% sodium glutamate, with or without 0.25 mM L-methionine or L-tryptophan as required.

PCR. Reagents for normal PCR were purchased as a kit from Boehringer-Mannheim. Conditions for the thermal cycling were as described previously for low-stringency or high-stringency annealing (4). Inverse PCR was done with the XL PCR kit from Perkin-Elmer under conditions recommended by the manufacturer for 5-kb templates. The primers used in this study were custom synthesized by Amitof BioTech, Inc.

SAM synthetase assay for *B. subtilis*. The assay for SAM synthetase in crude cell extracts was adapted from a published protocol (26). The two changes from the published protocol were the use of a different growth medium and the use of phosphocellulose spin columns (Pierce Chemical Co.) instead of phosphocellulose paper. Strains to be assayed were grown in 1× Spizizen salts (32) plus, per liter, 5 ml of 0.1% thiamine, 1 ml of Micronutrients (0.15 g of $Na_2MoO_4 \cdot 2H_2O$, 2.5 g of H_3BO_3, 0.7 g of $CoCl_2 \cdot 6H_2O$, 0.25 g of $CuSO_4 \cdot 5H_2O$, 1.6 g of $MnCl_2 \cdot 4H_2O$, and 0.3 g of $ZnSO_4 \cdot 7H_2O$ per liter), 5 ml of iron citrate (0.2 g of $FeSO_4$ and 20 g of trisodium citrate per 100 ml), 1 ml of 1 M $CaCl_2$, 40 ml of 50% glucose, 10 ml of 40% sodium glutamate, and 20 ml of 0.4% tryptophan. For each time point in the assay, a 25-μl aliquot of stopped-reaction mixture was applied to a phosphocellulose spin column and the column was rinsed twice with 0.5 ml of 75 mM phosphoric acid. Conversion of [^{35}S]methionine to [^{35}S]SAM was calculated as the percent counts per minute that bound to the phosphocellulose after subtracting the value for the zero time point. The assay was linear with respect to time for 20 min and was linear with respect to protein concentration in the range of 0 to 4 mg/ml in the final assay solution. A wild-type strain typically gave a background (zero time point) of about 1,000 cpm, with an additional ~1,000 cpm incorporated in 20 min. This translated to a specific activity of about 200 pmol min^{-1} mg of $protein^{-1}$.

Miscellaneous. Plasmid construction, Southern blotting, Northern (RNA) blotting, and DNA sequencing were done by standard procedures (19). Purification of genomic DNA (8) and RNA (2) and transformation (9) and transduction (15) of *B. subtilis* were done by published methods. DNA fragments were purified by preparative agarose gel electrophoresis and use of Qiagen spin columns as directed by the manufacturer. β-Galactosidase was assayed in toluene-treated cells as previously described (23). SAM was purchased as the HCl salt (Sigma) and stored as a 100 mM solution at −70°C. Protein was assayed with a bicinchoninic acid protein assay kit (Pierce) with bovine serum albumin as the standard. Manipulation of DNA sequences and homology searches of GenBank were performed with DNASTAR software.

Nucleotide sequence accession number. The DNA sequence of the *metE* gene was submitted to GenBank and was assigned accession number U52812.

RESULTS

Cloning of the *B. subtilis* gene encoding SAM synthetase. On the basis of a homology alignment of several amino acid sequences of SAM synthetases from a variety of eukaryotic and prokaryotic organisms (29), we designed a pair of degenerate

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TABLE 1. *B. subtilis* strains and plasmids

Strain or plasmid	Characteristic or relevant insert	Source or reference
Strains		
BI9	Δ*bio*::*neo*	27a
CU4435	*trpC2 ilvB*Δ*1 zghJ*::Tn*917*	34
PY79	Prototroph; SPβ^c	40
SA19	ZB493 with Φ*metE*::*lacZ*(Hyb)	This study
SA21	PY79 with Φ*metE*::*lacZ*(Hyb)	This study
SA22	63278 with Φ*metE*::*lacZ*(Hyb)	This study
SA28	1A1 with Φ*metE*::*lacZ*(Hyb)	This study
SA29	62378 with *sacB*::ΦP_{veg}*metE*$^+$	This study
ZB493	*trpC2 pheA1 abrB703* SPβ *c2 del2*::Tn*917*::pSK10Δ6	20
1A1	*trpC2*	Bacillus Genetic Stock Center
62378	*trpC2 metE1*	1
Plasmids		
pSAM420	0.75 kb of *metE*$^+$ coding	This study
pSAM429	pSAM420 plus *cat* gene	This study
pSAM437	3.8 kb, including all of *metE*$^+$	This study
pSAM446	Φ*metE*::*lacZ*(Hyb)	This study
pSAM39	ΦP_{veg}*metE*$^+$	This study
pCL1920	Low-copy-number vector	18
pXI15	P_{veg} expression vector	M. Hümbelin

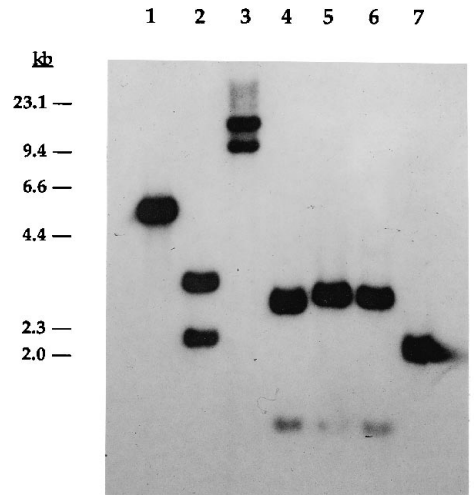

FIG. 1. *metE*$^+$-specific restriction fragments detected by Southern blotting. Various restriction enzyme digests of *B. subtilis* PY79 genomic DNA (5 μg) were separated by electrophoresis in 1% agarose and transferred to a nitrocellulose membrane filter. The filter was hybridized with PCR-synthesized *metE* DNA labeled with [^{32}P]dCTP by using primers SAM4A-5′ and SAM8-3′. Lanes: 1, *Eco*RI; 2, *Eco*RI and *Pst*I; 3, *Pst*I; 4, *Pst*I and *Hin*dIII; 5, *Hin*dIII; 6, *Hin*dIII and *Eco*RI; 7, *Pst*I, *Bam*HI, and *Eco*RI. The signal detected in lane 7 represents two fragments, one of 2.2 kb and one of 2.1 kb.

PCR primers that corresponded to amino acid sequences that were highly conserved among the known SAM synthetases. The 5′ primer, called SAM4A-5′ (CCCGGATCCATGTTYG GNTAYGC), corresponds to amino acids 139 through 144 of the murine SAM synthetase sequence, and the 3′ primer, called SAM8-3′ (CCCGAATTCCCRAARTGNCCRTA), corresponds to amino acids 377 through 382 of the same sequence (29). The primers introduced a *Bam*HI site at the 5′ end and an *Eco*RI site at the 3′ end. PCR was performed under low-stringency annealing conditions with *B. subtilis* PY79 DNA or *E. coli* W3110 DNA as the template. Both templates produced a PCR product of the expected size, 750 bp.

The purified 750-bp PCR product from the *B. subtilis* template was cleaved with *Bam*HI and *Eco*RI and was cloned into pUC19 (37) to yield pSAM420. The DNA sequence of the 750-bp fragment of pSAM420 was then determined. The fragment coded for an open reading frame (ORF) that spanned the entire fragment and was homologous to genes for the known SAM synthetases.

A Southern blot of *B. subtilis* PY79 DNA cleaved with various restriction enzymes was probed with the 750-bp cloned fragment (Fig. 1). The results of the blotting allowed the construction of a rough restriction map of the region (Fig. 2). In particular, the cloned fragment hybridized to a single 5-kb *Eco*RI fragment. The *Eco*RI fragment was cleaved once with *Pst*I near the middle of the fragment, and this *Pst*I site was found near the middle of the sequenced region, suggesting that the SAM synthetase gene lies near the middle of the 5-kb *Eco*RI fragment. However, attempts to clone this *Eco*RI fragment from a plasmid library by colony hybridization with the same *metE*-specific probe were unsuccessful. Therefore, we chose to clone the gene and flanking sequences by inverse PCR, using the 5-kb genomic fragment as the template.

B. subtilis PY79 DNA was cleaved with *Eco*RI, and DNA from 4.5 to 5.5 kb was purified by preparative agarose gel electrophoresis. The purified DNA was self-ligated at a low concentration (5 μg/ml) to favor circle formation. The circularized DNA was then used as a template for PCR with back-to-back primers, named RY45 (CCCCTGCAGCTTATATGC GGCAAGATACGTTGCGA; hybridizes to coordinates 1875 to 1906 of Fig. 3) and RY46 (CCCCTGCAGAACGGTCTA CCTTCGTCGCGTCC; hybridizes to coordinates 1880 to 1852 of Fig. 3), both of which included the unique *Pst*I site that had been found near the middle of the sequenced 750-bp cloned fragment of pSAM420. The expected 5-kb linear PCR product was obtained and purified. This product was assumed to contain an inside-out version of the SAM synthetase gene.

The restriction map based on the Southern blot described above suggested that the gene of interest lay on a 3.8-kb *Bam*HI-to-*Eco*RI fragment containing the unique *Pst*I site near the middle of the fragment (Fig. 2). Therefore, the inside-

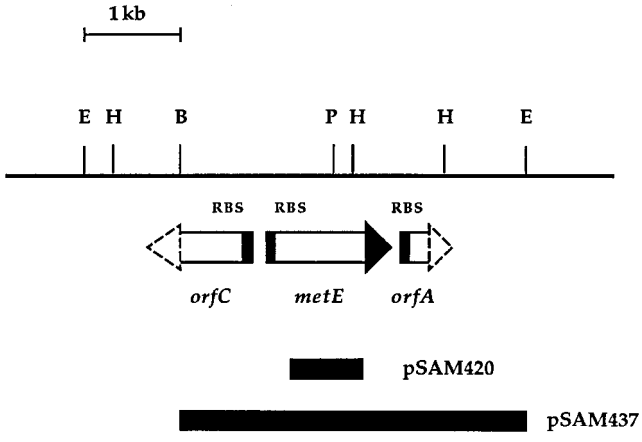

FIG. 2. Chromosomal map of the *metE* locus. The top diagram represents the chromosome of *B. subtilis*, showing the tentative order of restriction fragments determined from the Southern blot shown in Fig. 1. The middle diagram shows the locations of the possible structural genes (▭) and putative ribosome binding sites (RBS [▮]) determined from the nucleotide sequence shown in Fig. 3. The regions cloned in plasmids (▬) are indicated at the bottom of the diagram. E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; P, *Pst*I.

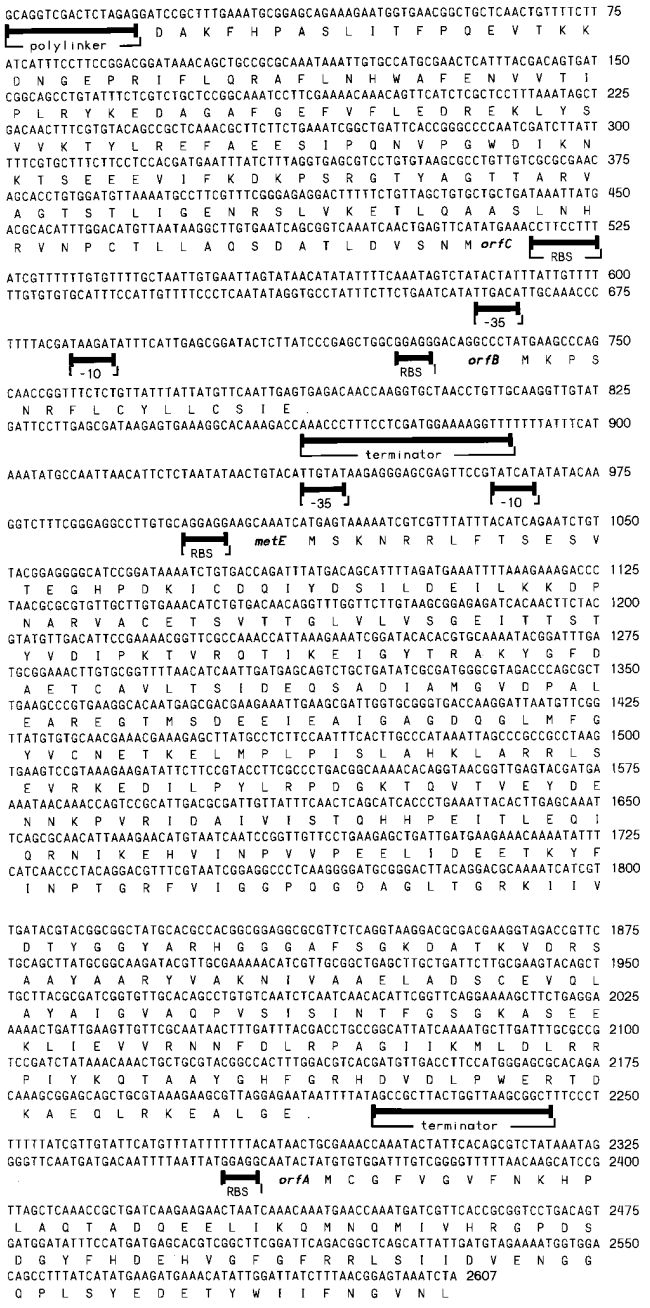

FIG. 3. Nucleotide and deduced amino acid sequences of the *B. subtilis metE* gene (GenBank accession number U52812). RBS, putative ribosome binding site; terminator, putative *rho*-independent transcription terminator; −35 and −10, putative promoter sites recognized by the *Bacillus* vegetative RNA polymerase (σ^A). The first 18 bases of the sequence are derived from a polylinker and are not part of *orfC*.

out PCR product was cleaved with *Pst*I, *Bam*HI, and *Eco*RI. The predicted 2.0- and 1.8-kb fragments were obtained, purified, and ligated together with *Eco*RI- and *Bam*HI-cleaved pSAM146A. pSAM146A is a derivative of pCL1920 (18) that contains a gene from pC194 conferring chloramphenicol resistance on gram-positive organisms (17). The resulting plasmid, pSAM437, had the expected structure and was hypothesized to contain a reconstituted copy of the SAM synthetase gene.

DNA sequence of the SAM synthetase gene. The DNA sequence was determined for the middle 2.6-kb region of the 3.8-kb cloned insert of pSAM437 (Fig. 3). The sequence contained an ORF encoding a 400-amino-acid sequence; the ORF begins with an ATG start codon and has a favorable ribosome binding site, AGGAGG. The ORF product is similar in size to, and is homologous to, the published sequence of the *E. coli* SAM synthetase encoded by *metK* (21), with 57% identical amino acids (Fig. 4). The homology of the *B. subtilis* sequence to the unpublished amino acid sequence of a putative second SAM synthetase from *E. coli* encoded by *metX* (30) (GenBank accession number M98266) is even greater (61% identity).

Several putative promoters, ribosome binding sites, and transcription terminators were found by visual inspection and computer-assisted searches of the DNA sequence. These features are shown in Fig. 3. In particular, two *metE*-oriented σ^A promoter-like sequences, with a *rho*-independent terminator-like sequence between them, were found at the 5′ end of *metE*. Another *rho*-independent terminator-like sequence was found just downstream from the *metE* coding sequence.

Genetic mapping of the *metE* gene on the *B. subtilis* chromosome. The 750-bp insert of pSAM420 was ligated into pUC21 (35) together with the chloramphenicol resistance gene from pC194 (17) to yield pSAM429. We attempted to integrate pSAM429 into the chromosome of competent *B. subtilis* PY79 by DNA transformation, but no chloramphenicol-resistant transformants were obtained. A control plasmid known to be able to accomplish integrative transformation yielded thousands of transformants with the same batch of competent PY79. Since pSAM429 contains an incomplete gene, integrative transformation by pSAM429 would be expected to disrupt the putative *metE* gene. We concluded that disruption of the putative *metE* gene is lethal.

In contrast to pSAM429, pSAM437, which contains the entire putative *metE* gene with about 1 kb of flanking sequence on each side, was successfully integrated into PY79 by DNA transformation to yield strain SA18. Since the only homology to the *B. subtilis* chromosome was on the 3.8 kb of cloned DNA, we hypothesized that pSAM437 had integrated at or adjacent to the *metE* locus. The *metE1* mutation had been reported to map near *leuS* at 272° (34, 36). To determine the map position of the integrated pSAM437 plasmid, a tranducing lysate was made from SA18 by using PBS1, and the lysate was used to infect CU4435 (*zghJ*::Tn*917*) and BI9 (Δ*bio*::*neo*), selecting for chloramphenicol resistance. Analysis of the transductants for the unselected markers (erythromycin sensitivity in the case of CU4435 and Bio^+ in the case of BI9) indicated that pSAM437 had integrated at a locus about 92% linked to *leuS* (*zhg*::Tn*917*) and about 69% linked to *bio* (262°). These linkages imply a map location for integrated pSAM437 that is consistent with the published map location of the *metE1* mutation.

Functional integrity of the cloned *metE* gene. To test whether the cloned gene in pSAM437 encoded an active SAM synthetase, pSAM437 was integrated by DNA transformation into the chromosome of strain 62378 (*metE1*), which has low-level SAM synthetase activity. The SAM synthetase specific activity in a transformant was compared with that in the untransformed parent and a wild-type strain. The integrated plasmid restored the wild-type SAM synthetase level to the *metE1* mutant (Table 2). The pSAM437 transformant also became sensitive to 1 mM ethionine, like the wild-type strain PY79. The parent strain, 62378, is resistant to 1 mM ethionine. We concluded that pSAM437 contained a functional copy of the *metE* gene, or at least a copy that could recombine with the *metE1* allele to yield a functional gene.

Sequences flanking the *metE* gene. Two ORFs were found flanking *metE*, one upstream and one downstream (Fig. 2).

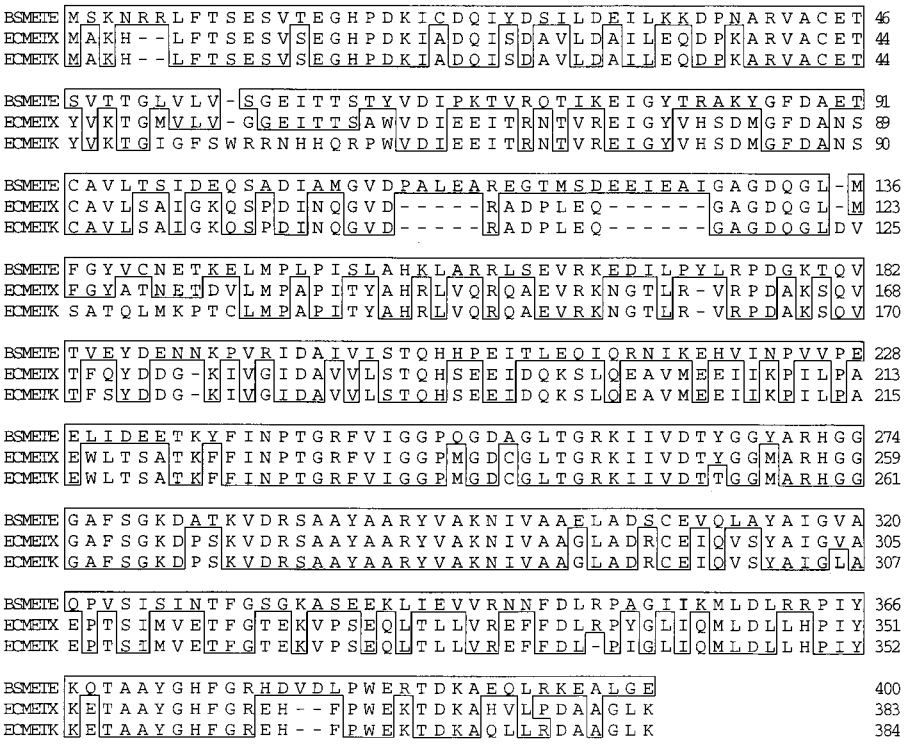

FIG. 4. Comparison of deduced amino acid sequences of SAM synthetases from *B. subtilis* and *E. coli*. Sequences were aligned by using the Megalign program of DNASTAR with the Clustal method. BSMETE, *B. subtilis* MetE; ECMETX, *E. coli* MetX; ECMETK, *E. coli* MetK. Amino acids identical to those of *B. subtilis* MetE are enclosed in boxes. Gaps introduced to maximize the fit are shown as dashes.

Neither of the ORFs was sequenced completely because both ran off the ends of the cloned fragment. The upstream ORF, provisionally named *orfC*, is transcribed in the direction opposite to that of *metE* and is homologous to a number of phosphoenolpyruvate carboxykinases (data not shown), including those from *Trypanosoma* spp. (*pepck*, 36% similarity), *Saccharomyces cerevisiae* (*ppcl* or *pepc*, 41% similarity) and *E. coli* (*pckA*, 32% similarity). The *orfC* gene was preceded by several plausible σ^A promoters and a putative ribosome binding site (AAAGGAAGG).

The downstream ORF, provisionally named *orfA*, is transcribed in the same direction as *metE*. At least the first 80 amino acids of OrfA are homologous to the amino-terminal ends of several asparagine synthetases in GenBank (data not shown), including those from *Lotus japonicus* (encoded by the AS gene; 28% similarity) and *E. coli* encoded by *asnB*; 33% similarity). Interestingly, this gene product is also 41% similar to an asparagine synthetase-like product from a gene named VE7AR near the *B. subtilis gnt* operon (39). The *orfA* gene is also preceded by a plausible σ^A promoter and a ribosome binding site (GGAGG).

TABLE 2. Levels of SAM synthetase in crude extracts of various *B. subtilis* strains

Strain	Relevant genotype	SAM synthetase sp act (pmol min^{-1} mg of $protein^{-1}$)
62378	*metE1*	<20
PY79	*metE*$^+$	140
62378/pSAM437	*metE1-metE*$^+$	180
SA29	*metE1-sacB*::ΦP_{veg}*metE*$^+$	870

Transcription of the *metE* gene. Transcription of the *metE* gene was examined by performing Northern blotting of total RNA extracted from strain PY79, a Met$^+$ strain, grown in minimal medium in the presence or absence of methionine (Fig. 5). The probe was made by PCR from the 750-bp fragment of pSAM420 (see above). The major transcript migrated at 1.6 kb, which matches the distance between the putative

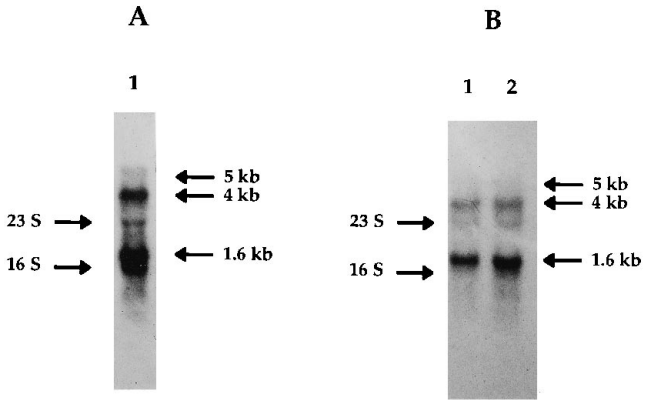

FIG. 5. *metE*-specific RNA transcripts detected by Northern blotting. *B. subtilis* PY79 total RNA (5 μg) was separated on a 1.2% agarose-formaldehyde gel and transferred to a nitrocellulose membrane filter, and the filter was hybridized with PCR-synthesized *metE* DNA labeled with [^{32}P]dCTP. (A) Lane 1, total RNA isolated from PY79 grown in minimal medium with 0.12% vitamin-free Casamino Acids. (B) Lane 1, total RNA isolated from PY79 grown in minimal medium without methionine; lane 2, total RNA isolated from PY79 grown in minimal medium with 0.25 mM methionine. In addition to the specific transcripts shown, the probe hybridized nonspecifically to the 16S and 23S RNA, as indicated.

TABLE 3. Expression of a *metE-lacZ* translational fusion in *B. subtilis*

Strain	β-Galactosidase sp act (Miller units) [a]	
	With methionine (1 mM)	Without methionine
SA21 [*metE*$^+$ SPβ::Φ*metE-lacZ*(Hyb)]	12 ± 3	42 ± 10
SA22 [*trpC2 metE1* SPβ::Φ*metE-lacZ*(Hyb)]	57 ± 6	100 ± 7
SA28 [*trpC2 metE*$^+$ SPβ::Φ*metE-lacZ*(Hyb)]	46 ± 1	62 ± 2

[a] Average of two isolates; calculated according to reference 23.

terminator near the 3′ end of *metE* and the more upstream of the two *metE*-oriented σ^A promoter-like sequences between the 5′ end of *metE* and the 5′ end of *orfC* (Fig. 3). It is also possible that the 1.6-kb transcript initiates from the downstream putative promoter, which is 1.3 kb upstream from the putative terminator, and that the transcript migrated anomalously under our conditions of electrophoresis. A short ORF, named *orfB*, having a putative ribosome binding site, lies just downstream from the upstream putative promoter but upstream from the *metE* ORF, and a transcription terminator-like sequence lies between *orfB* and the putative downstream promoter. It is possible that the sequence features found at the 5′ end of *metE* are involved in the regulation of expression of *metE*, but further studies will have to be done to ascertain the functions and relationships of these features.

Two longer but much less abundant transcripts of about 4 and 5 kb in length were also detected. These suggest that *metE* may be in an operon that perhaps includes *orfA* (which encodes an asparagine synthetase homolog).

The absolute levels of the three transcripts and their relative abundance were approximately the same from cells grown with or without methionine, suggesting that transcription of *metE* is not tightly regulated in response to exogenous methionine. In order to further examine the possibility of methionine regulation, a *metE-lacZ* fusion was constructed to obtain a more precise quantitative assessment.

Construction and evaluation of a *metE-lacZ* fusion. Wabiko et al. (36) have reported that *B. subtilis* SAM synthetase levels are reduced twofold by the addition of methionine to a minimal medium. To test whether this regulation was at the level of expression, we constructed a translational fusion between *metE* and *E. coli lacZ*.

A DNA fragment containing the 800 bp upstream from *metE* and the first 50 bp of the *metE* coding region was generated by PCR and ligated to a version of the *E. coli lacZ* gene contained on a 3-kb *Bam*HI-to-*Bgl*II fragment (38) in a pBR322-derived vector designed to integrate *lacZ* fusions in a single copy into the SPβ prophage (20). The primers used to amplify the *metE* promoter and start codon region were named NOTPLCC (GG CCGCTTCGCGAAGCTT) and RY50 (CCCAGATCTGTCA GAAATCTGGTCACA). The resulting plasmid, pSAM446, was used to transform strain ZB493, which harbors the modified prophage SPβ *c2 del2*::Tn*917*::pSK10Δ6, to yield SA19. SA19 was heat induced to obtain a specialized transducing lysate, which was then used to infect PY79, resulting in a chloramphenicol-resistant transductant, SA21. SA21 was grown in minimal medium with or without methionine, and cell extracts were assayed for β-galactosidase (Table 3). Expression of β-galactosidase was low but was repressed 3.5-fold by exogenous methionine. Slightly lower levels of repression (1.4- to 2.0-fold) were observed when the fusion was introduced into another *metE*$^+$ strain containing the *trpC2* allele or into a *metE1* mutant. Taken together, these results are consistent with the earlier report on regulation of SAM synthetase (36). If sequences more than 800 bp upstream of *metE* are also involved in its regulation, this experiment would not have detected that regulation. However, since *orfC* lies just upstream from *metE*, reading in the opposite direction, it is unlikely that *metE* regulatory sequences would be found more than 800 bp upstream.

Overproduction of SAM synthetase leads to methionine auxotrophy. To overproduce SAM synthetase in *B. subtilis*, the *metE* gene was placed under the control of the σ^A promoter of the *veg* gene of *B. subtilis*, P_{veg} (24). The vector used, pXI15, was specifically designed for placing genes under P_{veg} control and integrating at the *sacB* locus (17a). Just downstream from the promoter are unique *Nde*I and *Bam*HI sites, between which genes of interest can be cloned.

The *metE* gene was generated as an *Nde*I-to-*Bam*HI fragment by PCR with primers SAM/ATG-5′ (CCCCCTCGAGC ATATGAGTAAAAATCGTCGTTTATTTACA) and SAM/TAA-3′ (CCCGGATCCAAATTATTCTCCTAACGCTTCT TTACG). Insertion of this PCR product into pXI15 yielded plasmid pSAM39. To prepare the plasmid for transformation, pSAM39 was cleaved with *Sac*I to remove the 40-bp "stuffer" fragment located between the −10 and −35 regions of the promoter, and the resulting 5.6-kb fragment was purified. The linear DNA was ligated into circles, and the ligated DNA was purified on a Qiagen column. The circularized DNA was then cleaved with *Kas*I, which cleaves once in the pBR322 backbone, and transformed into PY79, selecting for the erythromycin resistance gene carried on the vector.

Four of five erythromycin-resistant transformants required methionine for growth on minimal medium. We hypothesized that the methionine auxotrophy is caused by overexpression of SAM synthetase, which in turn leads to a higher level of SAM, which acts as a corepressor of genes involved in methionine biosynthesis. This hypothesis was based on the case of *E. coli*, in which SAM binds to the aporepressor encoded by *metJ* to repress the methionine regulon (7).

To further explore this hypothesis, we assayed SAM synthetase activity in extracts from representative 62378/pSAM39 transformants and the untransformed parent strain grown in minimal medium containing methionine (Table 2). Strains containing integrated pSAM39 produced about six times more SAM synthetase than a wild-type strain, proving that SAM synthetase was in fact being overproduced. The one aberrant transformant that did not require methionine for growth did not overproduce SAM synthetase (data not shown). These results support the hypothesis that SAM is the corepressor of the methionine regulon in *B. subtilis*, but more direct experimentation will be required to substantiate this hypothesis. Another possibility is that increased SAM synthetase activity simply leads to depletion of the methionine pool.

DISCUSSION

Cloning of the *B. subtilis metE* gene. The complete *metE* gene and flanking sequences were cloned by inverse PCR on a low-copy-number plasmid, pSAM437. The cloned *metE* gene on pSAM437 complemented a *metE1* mutation in *B. subtilis* and mapped at or near the *metE1* locus, suggesting that we had cloned a functional copy of the *metE* gene, without any PCR-induced errors.

Overexpression of the *metE* gene in *B. subtilis* by a factor of about 6 leads to methionine auxotrophy. The simplest interpretation of this result is that a higher level of SAM synthetase

leads to a higher concentration of SAM in the cell, which in turn acts to repress or inhibit the methionine biosynthetic pathway.

***metE* appears to be an essential gene.** The failure to integrate pSAM429, which contains only a portion of the *metE* gene, into PY79 suggested that the integration is a lethal event. If this interpretation is correct, then either *metE* is essential or *metE* exists in an operon upstream from another gene that is essential. On the basis of Northern blotting and sequence homology, it appears that a gene named *orfA* that possibly encodes an asparagine synthetase may lie just downstream from *metE* on the same transcript. However, asparagine synthetase would not be expected to be essential on the rich medium that was used for the selection, and a second potential σ^A promoter sequence was found between *metE* and *orfA*, possibly providing for an independent transcript for *orfA*. Moreover, *B. subtilis* appears to contain a second asparagine synthetase-like gene (see Results). Therefore, we hypothesize that it is the *metE* gene itself that is essential. The Southern blot probed with a *metE* sequence gave only a single band, suggesting that no other close homolog of *metE* exists in *B. subtilis*. This is consistent with *metE* being indispensable for *B. subtilis*. In contrast, *Saccharomyces cerevisiae* is known to contain two SAM synthetase genes (6, 33), and *E. coli* has been reported to contain two closely related SAM synthetase genes (30). In both cases one of the two genes is thought to be dispensable.

MetE is more similar to MetX than to MetK. The *B. subtilis* MetE protein is 57% identical to the *E. coli* MetK protein but 61% identical to the *E. coli* MetX protein. The *metX* gene is reported to be adjacent to the *metK* gene and to encode a second SAM synthetase in *E. coli* (30). In reviewing the sequences of *metK* and *metX*, we noticed that additions or deletions of 1 or 2 bp at a few locations in the *metK* coding sequences would make the two protein products identical. This raised the possibility that the published *metK* DNA sequence (21) contains errors and that *metK* and *metX* could in fact be the same gene. In support of this possibility, a search of the most recent release of GenBank DNA sequences revealed that the *metK* region of the *E. coli* genome has been resequenced (GenBank accession number U28377, coordinates 41101 to 42255). Only one gene that is similar to *metK* exists in that region of the genome, and its sequence is nearly identical to that of *metX* (GenBank accession number M98266).

Properties of *B. subtilis* SAM synthetase. In several aspects, the *B. subtilis metE* gene and its corresponding protein are similar to the well-studied *E. coli metK* gene and its product, SAM synthetase. The two enzymes are homologous at the level of amino acid sequence. Expression of both *metE* and *metK* is repressed by exogenous methionine, albeit to different extents. In *E. coli*, *metK* expression appears to be repressed about 5-fold by exogenous methionine (16), while we found the repression of *metE* expression in *B. subtilis* to be between 1.4- and 3.5-fold. Ethionine-resistant mutants of both bacteria have greatly reduced levels of SAM synthetase activity. SAM is the corepressor of the methionine regulon in *E. coli*. It will be interesting to establish whether SAM is also the corepressor of the methionine regulon in *B. subtilis.*

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