

Menaquinone (Vitamin K₂) Biosynthesis: Nucleotide Sequence and Expression of the *menB* Gene from *Escherichia coli*

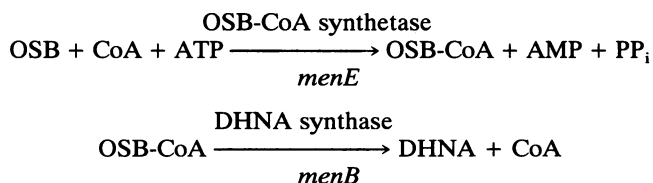
V. SHARMA, K. SUVARNA, R. MEGANATHAN,* AND M. E. S. HUDSPETH

Department of Biological Sciences and the Plant Molecular Biology Center,
Northern Illinois University, DeKalb, Illinois 60115-2861

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In *Escherichia coli*, the biosynthesis of the electron carrier menaquinone (vitamin K₂) involves at least seven identified enzymes. One of these, naphthoate synthase, forms the bicyclic ring system by catalyzing the conversion of *o*-succinylbenzoyl-coenzyme A to 1,4-dihydroxy-2-naphthoic acid. The gene for this enzyme has been previously identified as *menB*. By genetic and biochemical tests, a 1.349-kb DNA fragment from the *E. coli men* locus complements *menB* mutants. This fragment contains a single 285-codon open reading frame (ORF). Recombinant plasmids containing deletions of either the amino or the carboxy region of the ORF fail to complement the mutants. The ORF is preceded by consensus sequences for a ribosomal binding site and a sigma 70 promoter. *menB* transcription sufficient to complement the *menB* mutant in vivo and in vitro can be initiated from the identified putative promoter, and that in the constructs, *menB* expression, can be made independent of read-through transcription from the *lac* promoter. However, multicopy plasmids containing *menB* fail to generate the expected levels of enzymatic activity.

Menaquinone (MK) (vitamin K₂) plays an essential role in several electron transport systems by serving as the major electron carrier during anaerobic growth (9, 21). The biosynthetic pathway for MK has been recently reviewed (2, 3). A major step in MK biosynthesis, in which the bicyclic ring system is introduced, is the enzymatic conversion of the benzenoid compound *o*-succinylbenzoic acid (OSB) [4-(2'-carboxyphenyl)-4-oxobutyric acid] to the naphthalenoid compound 1,4-dihydroxy-2-naphthoic acid (DHNA). This conversion of OSB to DHNA involves OSB-coenzyme A (CoA) and is mediated by the enzymes OSB-CoA synthetase and DHNA synthase (11). Consistent with this observation has been the identification in both *Escherichia coli* and *Bacillus subtilis* (13, 19) of two groups of mutants, designated *menE* and *menB*. The overall conversion of OSB to DHNA can thus be represented as follows:



Of the five identified genes encoding the MK biosynthetic enzymes, four are clustered at 48.5 min on the *E. coli* chromosome (1, 8). These genes have been cloned (8, 20), and the nucleotide sequence of one, *menD*, has been reported previously (16). In this article, we report the nucleotide sequence, organization, and expression of *menB*. A preliminary report of some of these findings has appeared previously (18).

MATERIALS AND METHODS

Bacterial strains, growth conditions, and preparation of extracts. The *E. coli* strains and primary plasmids used are listed in Table 1. Additional plasmid constructs are shown in Fig. 1 and 2. Cultures were routinely stored at -80°C in glycerated L broth and grown on L agar. Recombinant clones containing inserts in pUC18 or pUC19 and pQF50 vectors were selected on L agar plus 0.004% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and 50 μg of ampicillin per ml. Plasmid complementation assays with *menB* mutants were performed anaerobically on glycerol minimal media containing 50 μg of ampicillin per ml and by using trimethylamine *N*-oxide as the electron acceptor as described previously (6). Enzymatic complementation assays were performed with cell extracts from Trypticase soy broth-grown cultures harvested and prepared as previously described (12).

DHNA synthase assay. Since OSB-CoA, the substrate for DHNA synthase, is unstable and unavailable commercially, it was generated enzymatically from OSB by using an extract from the *menB* mutant. Previous studies (19) have shown that the *menB* mutant used lacks DHNA synthase and that the OSB-CoA synthetase activity is unaltered, consequently forming the OSB-CoA intermediate. The enzymatic incubation mixture, assay conditions, and spectrophotofluorometric determination of DHNA formation were as previously described (11, 19). Protein concentrations were determined according to the procedure of Bradford (5).

DNA isolation. Plasmid DNAs were isolated by the alkaline lysis procedure of Birnboim and Doly (4) and purified in ethidium bromide-CsCl gradients.

Construction of plasmids. Plasmids used in this study consisted of fragments derived from the *men* cluster insert of pGS23 (20) and cloned into pUC18, pUC19, and/or pQF50 as shown in Fig. 1 (pUC derivatives) and 2 (pQF derivatives). The primary plasmids for transformation and enzymatic complementation assays consisted of either the previously defined *menB* complementation region (20) or the *menB*

* Corresponding author.

TABLE 1. Strains of *E. coli* and plasmids used in the study

Strain or plasmid	Genotype	Reference or source
<i>E. coli</i>		
PL2024	<i>gal trpA trpR iclR rpsL</i>	20
JRG962	<i>menB15 gyrA</i>	20
HB101	<i>supE44 hsdS20 (r_B⁻ m_B⁻) recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1</i>	Laboratory stock
JM83	F ⁻ <i>ara Δ(lac-proAB) rpsL (Str^r) [φ80 d (lacZ)M15]</i>	Laboratory stock
Plasmids		
pBR322		19
pUC18		IBI
pUC19		IBI
pQF50		7

region plus 356 bp of 5' flanking sequences. For some constructs, individual fragments were electroeluted from 0.8% or 1.0% agarose gel slices by using an IBI (New Haven, Conn.) unidirectional electroeluter. Ligation and transformation procedures were as described previously (10). Hosts for initial transformations were either HB101 or JM83. Reverse insert orientations relative to *lacZ'* were constructed by the transfer of pUC18 *EcoRI-HindIII* insert fragments to pUC19. The orientations were confirmed by either restriction endonuclease or DNA sequence analysis.

DNA sequencing and sequence analysis. DNA sequences were determined by the dideoxynucleotide chain termination method (17) by using the Sequenase 2.0 kit (U.S. Biochemical Corp., Cleveland, Ohio) and [α -³²P]dATP (ICN, Costa Mesa, Calif.). The DNA sequence of the reported region (1,349 bp) was initially determined from alkaline-denatured double-stranded plasmid DNAs. Compressed regions were

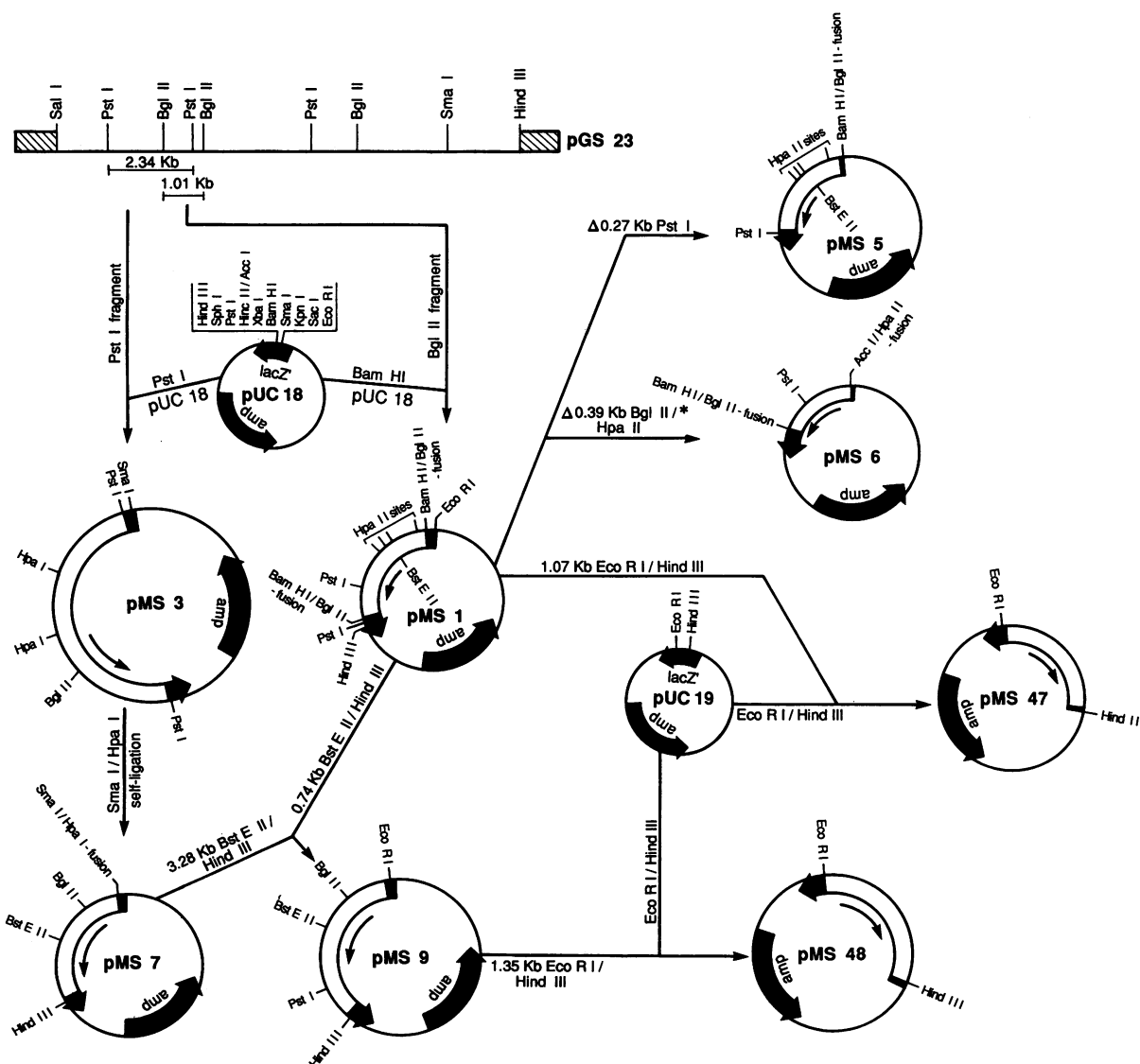


FIG. 1. Construction of *menB* recombinant plasmids based on the pUC18 and pUC19 vectors. All pUC plasmids were constructed as indicated from the original pGS23 insert (20). Only restriction sites relevant to each pMS construct are indicated. Δ, deletion of the specified fragment; *, pMS6 was constructed indirectly by ligation of the *HindIII-HpaII* pMS1 insert-pUC18 fragment into the *HindIII* and *AccI* sites of pUC18.

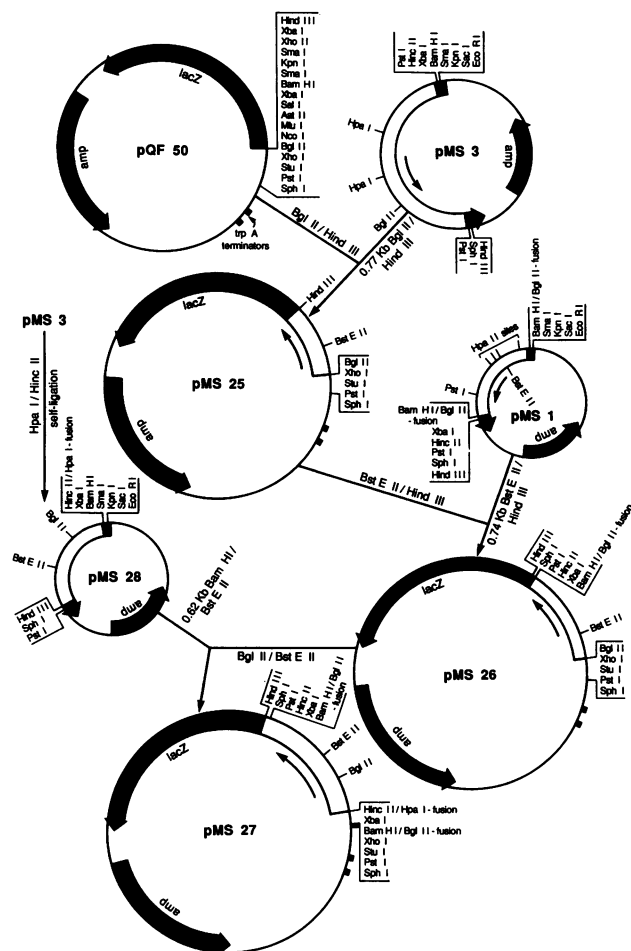


FIG. 2. Construction of *menB* recombinant plasmids based on the pQF50 vector. All inserts were derived from pUC plasmids pMS1 and pMS3 (Fig. 1).

resolved by single-stranded sequencing from the equivalent M13 clones by using dITP. Sequencing reactions were primed with universal, reverse, and sequence-generated synthetic oligonucleotide primers synthesized on an Applied Biosystems (Foster City, Calif.) 391 DNA synthesizer. All nucleotide positions were confirmed by sequencing of the complementary strands. Nucleic acid and deduced protein sequences were analyzed by using the Pustell DNA sequence analysis program (IBI Inc.) and the Genetics Computer Group program (University of Wisconsin Biotechnology Center, Madison, Wis.).

Nucleotide sequence accession number. The 1,349-bp *Hpa*I-*Bgl*II sequence data reported in this article appear in the EMBL, GenBank, and DDBJ nucleotide sequence data bases under accession number M93421.

RESULTS

Cloning of the *menB* locus. The original cloning of the *E. coli men* gene cluster in pBR322 as pGS23 and the identification of various *men* complementing regions were previously described (20). For DNA sequencing, the *Bgl*II fragment of pGS23, previously shown to complement *menB* mutants in pBR322 (20), was fused into the *Bam*HI site of pUC18 (Fig. 1) and designated pMS1. This region is approx-

imately 1.25 kb downstream from the termination of *menD* (16). The nucleotide sequence of the pMS1 insert (Fig. 3) contains a single ATG-initiated and TGA-terminated open reading frame (ORF) of 285 codons that is oriented in the direction of the vector *lacZ'* transcription. Five base pairs preceding the translational initiation are a putative ribosomal binding site containing four of six consensus bases (AG GACA), but no obvious promoter consensus sequences are present. A search of the GenBank data base revealed an identity of 35.5% in a 138-amino-acid overlap with rat mitochondrial enoyl-CoA hydratase. The homology presumably reflects the presence of CoA in both substrates.

***menB* deletion clones.** To confirm the identified ORF as the legitimate *menB* gene, the deletion plasmids pMS5 and pMS6 and the pMS1 reverse orientation plasmid pMS47 were constructed (Fig. 1) and transformed into the *menB* mutant. Plasmid complementation of the *menB* mutant was assayed for the restoration of anaerobic growth on glycerol-trimethylamine *N*-oxide media. Growth of pMS1-transformed cells was restored, while both pMS5 transformants, which lack the downstream *Pst*I-*Bgl*II region of the insert (including a portion of the carboxy terminus of the ORF), and pMS6 transformants, which are deleted upstream from the *Bgl*II through the *Hpa*II regions of the insert (including part of the ORF amino terminus), failed to complement the mutant. Thus, the *menB* coding region minimally encompasses the *Hpa*II-*Pst*I region of the insert present in the defined ORF. Strains carrying plasmids capable of restoring anaerobic growth were subsequently assayed for DHNA synthase activity (Table 2). Of the three types of transformants, only pMS1-transformed cells restored detectable DHNA synthase activity. The enzymatic activity of this type of transformant, however, was only about eight times that of wild-type strains. Given a pUC18 copy number of about 30, the absence of more highly elevated DHNA synthase levels was consistent with the absence of effective promoter sequences in the pMS1 insert DNA sequence. Further supporting evidence for the lack of *menB* promoter elements was obtained when the pMS1 insert was placed in the opposite orientation (pMS47; Fig. 1). With this plasmid, growth on trimethylamine *N*-oxide was restored, but the DHNA synthase activity was reduced to about 1.6-fold that of the wild type when assayed for *in vitro* biochemical complementation.

Location of the *menB* promoter elements. To determine whether promoterlike sequences were present directly upstream of pMS1, we constructed pMS3 (Fig. 1), which contains sequences overlapping the 5' region of pMS1. DNA sequence analysis of part of this insert (Fig. 3 from *Hpa*I to *Bgl*II) located several potential -10 and -35 promoter consensus sequences for sigma 70 within a 110-bp region upstream of the pMS1 *menB* ORF initiation codon. All promoter elements contained matches at four of six positions for the *E. coli* -10 and -35 consensus sequences of TATAAT and TTGACA. None of the intervals between promoter elements fell within the commonly accepted 16- to 18-bp range for *E. coli* promoters, but one fell within the expanded 15- to 21-bp range (15). The combination with the most appropriate interval (19 bp) is indicated in Fig. 3.

Expression of *menB* in pUC plasmids. As a potential polycistronic message with internal promoters has been suggested for the *B. subtilis men* loci (14), and as the interval between the identified *menB* promoter elements falls outside of the commonly accepted *E. coli* 16- to 18-bp range (15), we assayed the 5' *menB* flanking sequences for promoterlike activity. For this assay, an insert of the chromosomal region

1 GTTAACGGCGGTATTTGCCGACTGGTATCAACAGCCTGTTTTGCCTCACTCAATGACGATCAACGCCGGGAGCTGGTGGCGCTGCGCAGCAACAATAAT
HpaI
 101 GGC~~GCA~~ACC~~GGT~~GCCGCCATGCTGGAGCGACTTCTCTCGCCGTCCAGCCTGATTTACGTGCTAACCTTAGCGCCCGCACATTTGCGTTTTATTATTATG
 201 TGGTGAACGTGACAGCAAATTCGCGCCCTGGCGGGGAACTGGCTGCCGACTGCCATGTCATTCTCGCGCCGGACATAACGCGCATCGGGAAAAATCCC
 - 35 - 10
 M I Y P D E A M L Y A P V E W
 301 GCTGCGGTAATCGCAAGTCTGGCGCAGATCTTGC~~GT~~TTCTGACTAAAGGACACAATATGATTTATCCTGATGAAGCAATGCTTACGCACCCGGTGAATG
BglII RBS
 H D C S E G F E D I R Y E K S T D G I A K I T I N R P Q V R N A F
 401 GCACGACTGCTCCGAAGGTTTCGAGGACATTGTTATGAAAAATCCACCGACGGTATCGCAAAAAATCACCATTAATCGTCCGACGGTGGC~~CAAT~~GCCTC
 R P L T V K E M I Q A L A D A R Y D D N I G V I I L T G A G D K A F
 501 CGTCTCTGACGGTAAAAGAGATGATCCAGGCGCTGGCAGATGCCGTTATGACGACAACATCGGCGTGATCATTCTGACTGGTGCAGGGGATAAAAGCGT
 C S G G D Q K V R G D Y G G Y K D D S G V H H L N V L D F Q R Q I
 601 TCTGCTCCGGTGGTACCAGAAAAGTGC~~GT~~GGTATTACGGCGGTATAAAGATGATTCGGCGGTACATCACCTGAATGTGCTGGACTTCCAGCGTCAGAT
 R T C P K P V V A M V A G Y S I G G G H V L H M M C D L T I A A D
 701 CCGTACCTGTCCGAAACCGGTTGTCGCGATGGTGGCTGGCTACTCCATCGGCGGGGTACCGTTCTGCACATGATGTGCGACCTGACTATCGCGGCAGAT
 N A I F G Q T G P K V G S F D G G W G A S Y M A R I V G Q K K A R E
 801 AATGCCATCTTCGGTCCAGACTGGCCCGAAAAGTCCGTTCTTCGACGGCGGCTGGGGCGCTTCTACATGGCTCGCATCGTCCGGCAGAAAAAAGCGCGT
 I W F L C R Q Y D A K Q A L D M G L V N T V V P L A D L E K E T V
 901 AAATCTGGTTCCTGTGCCCTCAGTACGACGCAAAACAGGCGCTGGATATGGGCCTTGTGAACACCGTGGTACCCTGGCGGATCTGAAAAAAGAAACCGT
 R W C R E M L Q N S P M A L R C L K A A L N A D C D G Q A G L Q E
 1001 CCGTTGGTGGCGGAAATGCTGCAAAACAGCCCGATGGCGCTGCGCTGCCTGAAAAGCTGCACTGAACGCCGACTGTGACGGGCAGGCGGGGCTGCAAGGAG
 L A G N A T M L F Y M T E E G Q E G R N A F N Q K R Q P D F S K F K
 1101 CTGGCGGGCAACGCCACCATGCTGTTCTACATGACGGAAGAAGGTGAGGAAGGTGCAACGCCTTCAACCAGAAACGTGAGCCTGACTTACGCAAAATCA
 R N P *
 1201 AACGGAATCCGTAATGCGTAGCGCGCAGGTATACCGCTGGCAGATCCCCATGGACGCGGGGGTGGTCTGCGCGACAGGCGGTTAAAAACCCGCGACGGG
 1301 CTGTATGTTTGCCTGCGTGAAGCGGAGCGCGAAGGGTGGGGGAGATCT
BglII

FIG. 3. Nucleotide and deduced amino acid sequences of the *E. coli menB* gene. The noncoding strand is shown with the *menB* ORF originating at nucleotide 357. Amino acids (single-letter code) are indicated above the codons. Putative promoter (-10 and -35) and ribosomal binding site (RBS) sequences are underlined. Asterisk indicates the termination codon.

was reconstructed by fusion of the 325-bp *HpaI*-*BglII* promoterlike region of pMS3, with pMS1 as plasmid pMS9 (Fig. 1). This construct was then assayed for DHNA synthase activity by using cell extracts of pMS9-transformed *menB* mutants. The results (Table 2) demonstrated an 18.5-fold increase in the synthase activity relative to that of the wild-type strain and a 2.4-fold increase relative to that of the pMS1-transformed *menB* mutant. When the insert orientation was reversed by transfer into pUC19 as pMS48, however, the enzymatic activity was reduced to only 10 times that of the wild type but still sixfold higher than that of the

equivalent promoterless pMS47. These data suggested that, while the putative promoter region was indeed functional in vivo, our assays were affected by transcriptional read-through from the upstream *lacZ'* promoter.

Expression of *menB* in pQF50. The effects of transcriptional read-through of pUC plasmids on the presumptive *menB* promoter were subsequently confirmed by using constructs of the promoterless vector pQF50 (Fig. 2). In this vector, two tandem *trpA* transcriptional terminators precede the multiple cloning site and reportedly prevent read-through transcription from upstream promoters (7). Two *menB* plas-

TABLE 2. DHNA formation in the presence of various *menB* plasmids^a

Strain	Plasmid	OSB-CoA synthetase ^b (<i>menB</i> extract)	Amt of DHNA formed (nmol/h/mg of protein)
PL2024		–	3.0
		+	3.7
JRG962		–	ND ^c
		+	ND
	pMS1	–	5.6
		+	29.0
	pMS9	–	11.7
		+	68.3
	pMS47	–	0.6
		+	6.1
	pMS48	–	9.0
		+	36.8
	pMS26	–	0.3
		+	0.3
	pMS27	–	6.7
+		30.4	

^a The plasmid-containing strain for enzymatic assays was the *menB* mutant JRG962. The assays were done in 0.1 M potassium phosphate buffer, pH 8.0.

^b OSB-CoA synthetase (> 100 U) was added in the form of *menB* extract for each assay.

^c ND, not detectable.

mid constructs of pQF50, pMS26 and pMS27 (the equivalents of pMS1 and pMS9), were used to transform mutant cells. In the absence of the putative *menB* promoter region (pMS26), the DHNA synthase activity in the transformed mutant was more than 90-fold lower than in the equivalent pMS1 strain. In similar assays with the promoter-containing pMS27 insert (the equivalent of pMS9), synthase activity increased about 100-fold relative to that in pMS26 and about 8-fold compared with that in the wild type. Thus, the identified promoter sequences functioned in *menB* expression. The levels of DHNA synthase activity, however, still remained at a lower level than anticipated for a multicopy plasmid.

DISCUSSION

The conversion of OSB-CoA to DHNA is mediated by the enzyme DHNA synthase, which is encoded by *menB* (3). Mutants blocked in this reaction have been identified in *B. subtilis* and *E. coli* (13, 19). This locus in *E. coli* has been previously cloned, and recombinant plasmids have been isolated (20). In this study, we have determined the nucleotide sequence of the *menB* locus and shown it to encode a 285-residue 31.6-kDa polypeptide. The legitimacy of the ORF has been genetically verified by the absence of both anaerobic growth and DHNA synthase activity when either the amino or carboxy terminus is deleted.

The nucleotide sequence data of the *menB* 5' flanking region identified putative promoter and ribosomal binding site sequences. The promoter region, based on established sigma 70 *E. coli* consensus sequences, contains a –10 sequence of CATAAC and a –35 sequence of CTGCCA (in both, four of six match), but with the expanded 19-bp (15) interval between the elements. An acceptably positioned ribosomal binding site of AAGGACA (four of six match) is also present 5 bp upstream from the initiation codon. When mutant cells were transformed with multicopy *menB* plasmids devoid of the upstream flanking sequences containing the identified promoter elements, anaerobic growth was visually restored and DHNA synthase activities were in-

creased to 1.6-fold (pMS47) or 7.8-fold (pMS1) of wild-type levels. These results are consistent with prior studies showing poor complementation of *menB* mutants by this fragment in the multicopy plasmid pBR322 (20). Even the low level of DHNA synthase observed in plasmids containing the insert in reverse orientation is sufficient to provide functional MK concentrations. When the putative promoter region was added to the plasmid constructs (pMS9 and pMS48), growth and enzymatic activity were significantly increased, suggesting that some combination of the promoter elements is functional. However, the *in vitro* complementation was dramatically affected by the insert orientation, indicating read-through from the *lacZ'* promoter.

To eliminate the effects of transcriptional read-through, the same fragments were cloned into pQF50 (as pMS26 and pMS27), which blocks read-through by the presence of two tandem transcriptional terminators 5' to the insert. A strain containing pMS26 showed about 100-fold-lower activity than a strain containing the equivalent pMS1. On the other hand, the presence of the putative promoter region (pMS27) showed an 8-fold-higher activity relative to the wild type and about a 100-fold-higher activity versus that in the presence of pMS26. Thus, the 5' flanking sequences of the insert do significantly influence the transcription of *menB*.

The eightfold elevation of synthase activity in the presence of the *menB* promoter is significantly lower than that expected for a gene on a multicopy plasmid. Although we could attribute this result to inefficient expression resulting from the expanded 19-bp spacing (15) between the *menB* promoter elements, we cannot currently determine whether the identified promoter is the primary *menB* promoter or an internal promoter. Several lines of evidence paralleling our result, however, suggest that *men* gene expression does not necessarily require optimal transcriptional levels. Complementation of *men* mutants with a multicopy plasmid containing the entire *men* cluster produces only moderately elevated enzymatic activities (20). The *menD* gene under the control of the *tac* promoter, also on a multicopy plasmid, generates only a 40-fold elevation of 2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase even upon induction by isopropyl-β-D-thiogalactopyranoside (16); and the presence of a "leaky" mutation producing 0.07 nmol of OSB-CoA/h/mg of protein in a *menE* mutant does not adversely affect the anaerobic growth of the mutant (19), suggesting that either low or suboptimal enzyme activity is enough to provide concentrations of MK sufficient for anaerobic growth. These observations are consistent with either the presence of inefficient promoters or read-through from upstream promoters. On the other hand, the possibility of a single polycistronic message with internal (less efficient) promoters has already been postulated for the *men* genes in *B. subtilis* (14). *E. coli menB* transcription data may allow us to distinguish between the alternatives of primary or internal promoter function for the identified *menB* promoter.

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REFERENCES

- Bachmann, B. J. 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. 54:130–197.
- Bentley, R., and R. Meganathan. 1982. Biosynthesis of vitamin K (menaquinone) in bacteria. Microbiol. Rev. 46:241–280.
- Bentley, R., and R. Meganathan. 1987. Biosynthesis of the isoprenoid quinones ubiquinone and menaquinone, p. 512–520.

- In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
4. Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7**:1513–1523.
 5. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254.
 6. Daruwala, R., and R. Meganathan. 1991. Dimethyl sulfoxide reductase is not required for trimethylamine N-oxide reduction in *Escherichia coli*. *FEMS Microbiol. Lett.* **83**:255–260.
 7. Farinha, M. A., and A. M. Kropinski. 1990. Construction of broad-host-range plasmid vectors for easy visible selection and analysis of promoters. *J. Bacteriol.* **172**:3496–3499.
 8. Guest, J. R., and D. J. Shaw. 1981. Molecular cloning of menaquinone biosynthetic genes of *Escherichia coli* K12. *Mol. Gen. Genet.* **181**:379–383.
 9. Lin, E. C. C., and D. Kuritzkes. 1987. Pathways for anaerobic electron transport, p. 201–221. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
 10. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 11. Meganathan, R., and R. Bentley. 1979. Menaquinone (vitamin K₂) biosynthesis: conversion of *o*-succinylbenzoic acid to 1,4-dihydroxy-2-naphthoic acid by *Mycobacterium phlei* enzymes. *J. Bacteriol.* **140**:92–98.
 12. Meganathan, R., and R. Bentley. 1981. Biosynthesis of *o*-succinylbenzoic acid in a *men*⁻ *Escherichia coli* mutant requires decarboxylation of L-glutamate at the C-1 position. *Biochemistry* **20**:5336–5340.
 13. Meganathan, R., R. Bentley, and H. Taber. 1981. Identification of *Bacillus subtilis men* mutants which lack *o*-succinylbenzoyl-coenzyme A synthetase and dihydroxynaphthoate synthase. *J. Bacteriol.* **145**:328–332.
 14. Miller, P., A. Rabinowitz, and H. Taber. 1988. Molecular cloning and preliminary genetic analysis of the *men* gene cluster of *Bacillus subtilis*. *J. Bacteriol.* **170**:2735–2741.
 15. O'Neill, M. C. 1989. *Escherichia coli* promoters. I. Consensus as it relates to spacing class, specificity, repeat structures, and three dimensional organization. *J. Biol. Chem.* **264**:5522–5530.
 16. Popp, J. L. 1989. Sequence and overexpression of the *menD* gene from *Escherichia coli*. *J. Bacteriol.* **171**:4349–4354.
 17. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
 18. Sharma, V., K. Suvarna, R. Meganathan, and M. E. S. Hudspeth. 1990. Menaquinone (vitamin K₂) biosynthesis: cloning and sequencing of the *menB* region from *Escherichia coli*, abstr. K-116, p. 239. Abstr. 90th Annu. Meet. Am. Soc. Microbiol. 1990. American Society for Microbiology, Washington, D.C.
 19. Shaw, D. J., J. R. Guest, R. Meganathan, and R. Bentley. 1982. Characterization of *Escherichia coli men* mutants defective in conversion of *o*-succinylbenzoate to 1,4-dihydroxy-2-naphthoate. *J. Bacteriol.* **152**:1132–1137.
 20. Shaw, D. J., E. C. Robinson, R. Meganathan, R. Bentley, and J. R. Guest. 1983. Recombinant plasmids containing menaquinone biosynthetic genes of *Escherichia coli*. *FEMS Microbiol. Lett.* **17**:63–67.
 21. Taber, H. 1980. Functions of vitamin K₂ in microorganisms, p. 177–187. In J. W. Suttie (ed.), *Vitamin K metabolism and vitamin K-dependent proteins*. University Park Press, Baltimore.