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# *Bacillus subtilis* Acyl Carrier Protein Is Encoded in a Cluster of Lipid Biosynthesis Genes

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**A cluster of *Bacillus subtilis* fatty acid synthetic genes was isolated by complementation of an *Escherichia coli fabD* mutant encoding a thermosensitive malonyl coenzyme A-acyl carrier protein transacylase. The *B. subtilis* genomic segment contains genes that encode three fatty acid synthetic proteins, malonyl coenzyme A-acyl carrier protein transacylase (*fabD*), 3-ketoacyl-acyl carrier protein reductase (*fabG*), and the N-terminal 14 amino acid residues of acyl carrier protein (*acpP*). Also present is a sequence that encodes a homolog of *E. coli plsX*, a gene that plays a poorly understood role in phospholipid synthesis. The *B. subtilis plsX* gene weakly complemented an *E. coli plsX* mutant. The order of genes in the cluster is *plsX fabD fabG acpP*, the same order found in *E. coli*, except that in *E. coli* the *fabH* gene lies between *plsX* and *fabD*. The absence of *fabH* in the *B. subtilis* cluster is consistent with the different fatty acid compositions of the two organisms. The amino acid sequence of *B. subtilis* acyl carrier protein was obtained by sequencing the purified protein, and the sequence obtained strongly resembled that of *E. coli* acyl carrier protein, except that most of the protein retained the initiating methionine residue. The *B. subtilis fab* cluster was mapped to the 135 to 145° region of the chromosome.**

Fatty acid biosynthesis in the eubacteria is catalyzed by the action of discrete cytoplasmic proteins. Much has been learned of the fatty acid biosynthetic (*fab*) pathway of *Escherichia coli* in recent years. Most of the genes involved have been cloned and sequenced, and their gene products have been characterized biochemically. Although about half of the *fab* genes are dispersed around the chromosome, a cluster of *fab* genes has been described (14, 15, 25). Subsequent work has extended and delimited the cluster. The clustered *E. coli fab* genes are in the order *fabH fabD fabG acpP fabF* and encode 3-ketoacyl-acyl carrier protein synthase III, malonyl coenzyme A-acyl carrier protein (ACP) transacylase, 3-ketoacyl-ACP reductase, ACP, and 3-ketoacyl-ACP synthase II, respectively. A sixth gene, *plsX*, which plays a poorly understood role in phospholipid synthesis (13) is located immediately upstream of the *fabH* gene (21). The cluster is delimited by two genes involved in other cellular processes, a ribosomal protein gene (*rpmF*) upstream of *plsX* and a gene (*pabC*) involved in *p*-aminobenzoic acid synthesis downstream of *fabF* (14, 21).

Subsequent work has shown the presence of *fab* gene clusters in other proteobacteria, i.e., *Haemophilus influenzae* Rd (*fabH fabD fabG acpP*) (6), *Vibrio harveyi* (*fabD fadG acpP fabF*) (31), and *Rhodobacter capsulatus* (*plsX fabH*) (4). In each case, one end of the cluster is known to be delimited by either a ribosomal protein gene or a *pabC* homolog. This sequence conservation is not unexpected, since the mechanisms and products of straight-chain fatty acid biosynthesis are well conserved within the proteobacteria. In gram-positive bacteria, our knowledge is limited to the high-G+C organisms and was obtained mainly as a byproduct of studies of the biosynthesis of polyketide antibiotics by *Streptomyces* spp. Polyketides are formed by a pathway similar to that of fatty acid biosynthesis (8). *Streptomyces glaucescens* contains a cluster of genes with products homologous to the *E. coli fab* gene products in the order *fabD fabH acpP fabB*(*F*) (*acpP* was called *fabC* in this organism [32] to distinguish the gene from those encoding ACPs involved in polyketide synthesis), and *S. coelicolor* has adjacent *fabD*, *fabH*, and *acpP* genes (26). The closely related organism *Saccharopolyspora erythraea* has also been shown to have a gene that resembles *fabF-fabB* located downstream of an *acpP* homolog (27).

Fatty acid synthesis by members of the genus *Bacillus* and other low-G+C gram-positive bacteria has attracted little attention, although *Bacillus* species have unusual fatty acid compositions (5, 11). Thus, while the membrane lipids of proteobacteria contain mostly straight-chain fatty acids, the lipids of *Bacillus* species contain mainly branched-chain fatty acids, which can account for >90% of the total fatty acid content of the cytoplasmic membrane (11). Although the fatty acid compositions of many *Bacillus* species are known (11), only the first step of the biosynthetic pathway, synthesis of the priming molecule, has been studied in detail. Oku and Kaneda (22) reported the purification and characterization of a branched-chain 2-ketoacid decarboxylase from *Bacillus subtilis* which plays a major role in the generation of the primer molecules by decarboxylation of branched-chain amino acids.

In this report, we describe the cloning of a gene cluster encoding genes involved in fatty acid metabolism in *B. subtilis*. Analysis of the cloned DNA showed that the insert contained three open reading frames (ORFs) that encode proteins with high amino acid sequence similarity to PlsX, FabD, FabG, and ACP of *E. coli*. We also report the complete amino acid sequence of *B. subtilis* ACP (only the 5′ end of the gene was cloned). With this work, the presence of *fab* gene clusters has been reported in the two main groups of gram-positive bacteria (both the low- and high-G+C groups) and the proteobacteria.

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## MATERIALS AND METHODS

**Bacterial strains, plasmids, and phages.** The growth media and growth conditions for *E. coli* and *B. subtilis* and most of the bacterial strains, plasmids, and phages used in this study were described previously (17). *E. coli* DH5α and JM109 and *B. subtilis* 168 and JH642 were used as standard cloning hosts (17). A library made of *B. subtilis* 168 DNA fragments cloned into λZAP was the generous gift of A. Sonenshein (18). The fragments were derived by partial digestion with *Sau*3A and ligated into the *Xho*I site of the vector. In vivo excision of pBluescript plasmid clones from the corresponding λZAP phagemids was done as recommended by the manufacturer (Stratagene). Phage and small-scale plasmid preparations and most other DNA manipulations were done as described previously (17). Large-scale plasmid preparations were done by using Qiagen plasmid purification columns (Qiagen). DNA elution from agarose gels was done with a Qiagex DNA elution kit (Qiagen) as instructed by the suppliers. For PCR-mediated amplification of the *fabD* and *fabG* genes from the *B. subtilis* chromosome, the following primers (given 5′ to 3′) were used: *fabD*, AGAGAATTCAGATGAGTAGTCTGG and CGGGAAGCTTCAGTGACAATAGC; *fabG*, ATGATTAAGCTTACACGCTCTAATG and ACGACTTGAATTCAAATCGGTCC (the sequences underlined are the *Hin*dIII and *Eco*RI restriction sites constructed to facilitate subsequent cloning). The conditions used for PCR were 1 min at 94°C, 20 s at 57°C, and 70 s at 74°C. The PCR products were resolved by agarose gel electrophoresis, eluted, digested with *Eco*RI and *Hin*dIII, and ligated to pGEM7z(+) digested with the same enzymes to give plasmids pRMU119 and pRMU122 containing *fabD* and *fabG*, respectively. These plasmids were moved to strain BL21(λ DE3) to study the expression of the *B. subtilis* FabD and FabG proteins in *E. coli* as described previously (17).

DNA sequencing was done by the dideoxy-chain termination method with a Sequenase version 2.0 kit (Amersham). Primers synthesized by the Genetic Engineering Facility, University of Illinois at Urbana-Champaign, were used for further sequencing of both strands of the *fab* cluster with *Taq* DNA polymerase cycle sequencing of plasmid DNA on an Applied Biosystems 373 DNA sequencer.

**Purification of ACP.** ACP was purified as described for *E. coli* ACP by Rock and Cronan (28) with minor modifications. Briefly, ca. 100 g of cell paste (*B. subtilis* 168 grown in Luria-Bertani medium at 37°C) was resuspended in 100 ml of 50 mM Tris-HCl (pH 8.0). The temperature was 4°C unless otherwise stated, and all buffers contained 10 mM 2-mercaptoethanol. The mixture was frozen and thawed three times and then stirred for 30 min after addition of 30 mg of lysozyme, 1 mg of DNase, 1 mg of RNase, and 25 mg of phenylmethylsulfonyl fluoride. The resulting cell paste was passed through a French pressure cell (18,000 lb/in$^2$) and centrifuged at 48,000 × *g* for 30 min. Cold 2-propanol was added to the supernatant to a 50% final concentration, and the resulting mixture was stirred overnight and then centrifuged at 48,000 × *g* for 30 min. The clear supernatant was brought to pH 6.2 by dropwise addition of glacial acetic acid, after which a DEAE-cellulose slurry (20 g) equilibrated in 50 mM Tris-HCl (pH 6.2) was added and the mixture was stirred gently for 12 h. The resin was collected by filtration and washed at room temperature with 10 bed volumes of 50 mM Tris-HCl (pH 6.2) containing 0.25 M LiCl. The washed resin was then packed into a column, and ACP was eluted at room temperature with 50 mM Tris-HCl (pH 6.2) containing 0.5 M LiCl. Fractions of 50 ml were collected, and the proteins were precipitated by titration to pH 3.0 with acetic acid. After standing overnight, the precipitated proteins were collected by centrifugation and redissolved in 50 mM Tris-HCl (pH 7.4). The samples with the highest content of ACP (as judged by gel electrophoresis) were pooled and applied to a Mono Q HR 5/5 column (Pharmacia). ACP was eluted with a 0 to 0.5 M linear gradient of NaCl in 50 mM Tris-HCl (pH 7.4). The ACP content of each fraction was assessed by urea-polyacrylamide gel electrophoresis (PAGE) (24) (2.5 M urea in 13% polyacrylamide gels), and the fractions were concentrated by acid precipitation as described above and resuspended in 50 mM ammonium bicarbonate. These ACP preparations were 90 to 95% pure by both urea-PAGE and sodium dodecyl sulfate (SDS)-PAGE (28). This value is probably an underestimation of the purity due to the relatively inefficient visualization of ACP by the common protein stains. ACP was transferred from an SDS-PAGE gel to a polyvinylidene difluoride membrane and located by Ponceau red staining by standard protocols. N- and C-terminal protein sequencing was done by Peter Harrsch of Hewlett-Packard on Hewlett-Packard HP G1005A and HP G1009A instruments, respectively. The ACP preparation sequenced was almost entirely unprocessed by methionine aminopeptidase (see below), thus allowing N-terminal sequencing to proceed for the length of the molecule. Mass spectrometry was done as previously described (12).

*B. subtilis* ACP was labeled by growing strain 168 in minimal medium in the presence of [β-$^3$H]alanine (5 μCi/ml of culture; 92 Ci/mmol), whereas *E. coli* ACP was labeled by first starving strain SJ16 (a β-alanine auxotroph) in minimal medium (10) and then adding [β-$^3$H]alanine as described above. In both cases, the cultures were grown for 8 to 10 h at 37°C with agitation and then centrifuged. Following resuspension of the cells in water, the proteins were precipitated by addition of trichloroacetic acid to a final concentration of 6% and this precipitate was resuspended in 50 mM Tris-HCl (pH 8.0). Analysis of the radiolabeled proteins was done by urea-PAGE.

## RESULTS

**Cloning of the *B. subtilis fabD* gene.** Our approach to the cloning of genes involved in fatty acid biosynthesis in *B. subtilis* was complementation of an *E. coli* mutant deficient in malonyl coenzyme A-ACP transacylase activity. We assumed that the overall genetic organization previously reported for the *E. coli fab* gene cluster (14) is present in *B. subtilis*. Within the *E. coli* gene cluster, only mutations in *fabD* and *fabF* have been reported (14). One of the mutants with a clearly defined phenotype suitable for complementation screening (15) was strain LA2-89, which is a phage λ lysogen and carries an amber mutation in the *fabD* gene plus a *supE* tRNA suppressor (34). The combination of the latter two characters results in an amino acid substitution that gives a malonyl coenzyme A-ACP transacylase with greatly increased thermosensitivity (34). Several libraries of *B. subtilis* DNA fragments in various plasmid and lambda vectors were screened with negative results. Finally, the use of a library made in λZAP (the generous gift of A. Sonenshein [18]) yielded five colonies that grew at 42°C. Lysates of each of the candidate clones were made by mitomycin induction and used to transduce *E. coli* LA2-89 to temperature insensitivity again, verifying that the lysates complemented the enzyme deficiency of the *E. coli* strain. In vivo excision of the pBluescript plasmid moiety present in the λZAP vector gave five corresponding plasmid clones. Restriction analysis of the clones showed that all of these plasmids carried the same 2.6-kbp insert, and thus, only plasmid pRMU100a was further studied. In plasmid pRMU100a, the *B. subtilis* genes were oriented such that they could be properly transcribed from the *lac* promoter of the vector. Repeated propagation of plasmid pRMU100a in standard *E. coli* host strains gave low plasmid yields and accumulation of altered plasmids that carried deletions which included portions of the insert DNA. Thus, we routinely prepared plasmid pRMU100a in strain LA2-89 at 42°C to select against such deletions. Alternatively, frozen stocks of strain JM109 or DH5α carrying plasmid pRMU100a were grown only briefly (<10 h) before use.

**DNA sequence of the *B. subtilis fabD* gene and adjacent ORFs.** DNA sequence analysis showed that the insert in pRMU100a contained three complete ORFs plus an incomplete ORF, all encoded on the same DNA strand (Fig. 1). A database search identified the ORFs as homologs of the *E. coli plsX*, *fabD*, *fabG*, and *acpP* genes, and thus, we have given the *B. subtilis* genes the same designations. The antisense strand starts with the *plsX* sequence followed by complete *fabD* and *fabG* genes (Fig. 1). The *fabG* gene is followed by a sequence encoding the first 14 N-terminal residues of ACP (Fig. 1). Each of the *B. subtilis* gene products deduced from the DNA sequence has essentially the same size as the analogous *E. coli* protein, except PlsX, which is only three-fourths of the size of the *E. coli* protein. The deduced amino acid sequences were readily aligned with those of the homologous *E. coli* proteins, with (excluding PlsX) only a few small gaps (Fig. 2). The *B. subtilis* and *E. coli* proteins had 39, 47, and 47% identical residues in the amino acid sequences deduced from the *plsX*, *fabD*, and *fabG* genes, respectively. Although only the 5′ end of the *acpP* gene was obtained, the amino acid sequence of the purified *B. subtilis* ACP was determined (see below) and showed 61% residue identity with *E. coli* ACP (Fig. 2). All of the residues known to be involved in catalysis in *E. coli* FabD, for which a high-resolution crystal structure is available (29), are strictly conserved in the *B. subtilis* protein (many of these are located in the large block of conserved residues centered at residue 93). Transformation of *plsX plsB* mutant strain

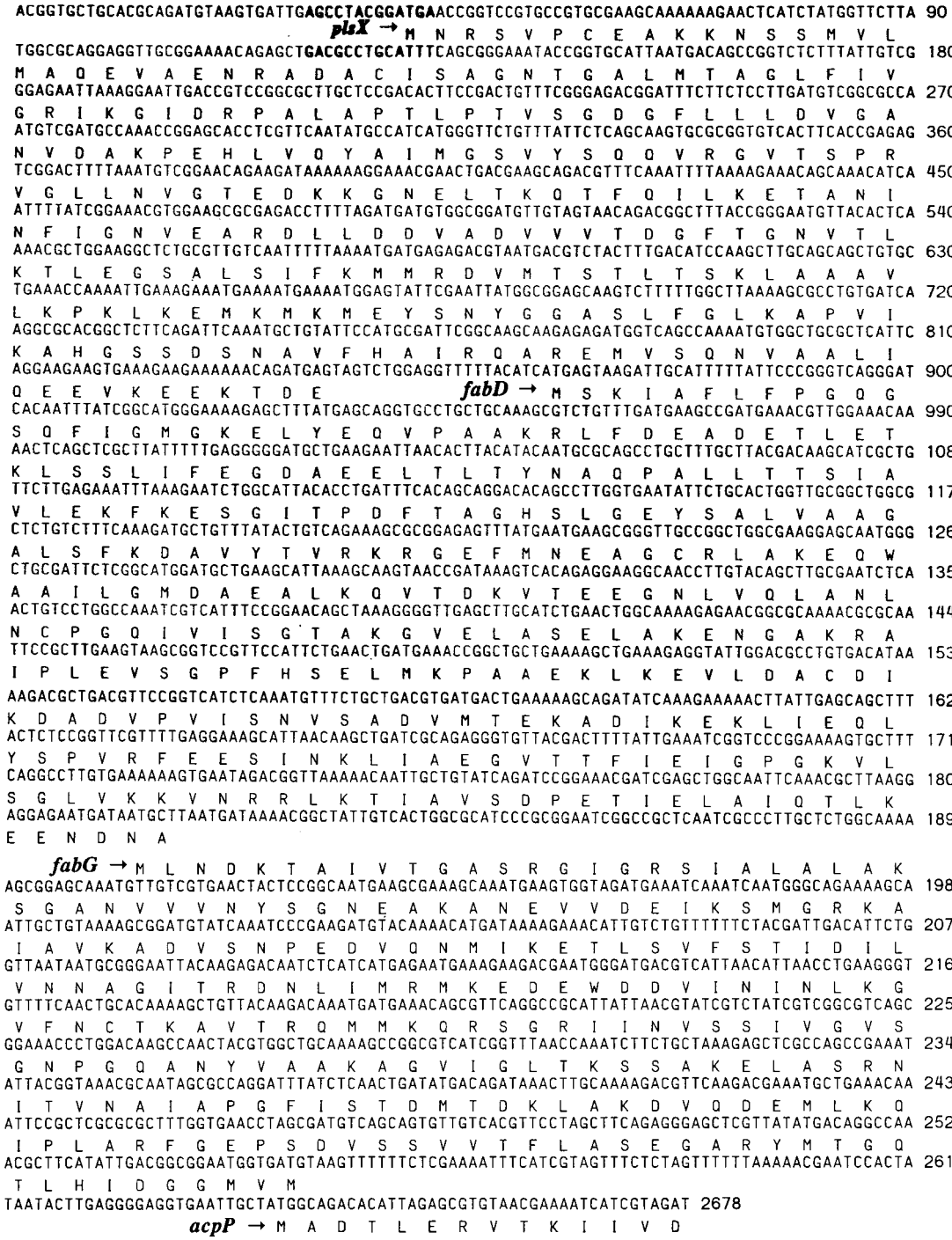


FIG. 1. DNA sequence of the *B. subtilis fab* gene cluster. The entire sequence of the insert of plasmid pRMU100a and the deduced amino acid sequences of the encoded proteins are given.

BB26-36 (13) with plasmid pRMU100a gave ampicillin-resistant recombinants which grew in the absence of *sn*-glycerol-3-phosphate. However, growth of the recombinant colonies was very slow (a period of several days was required for visible colony formation), indicating either poor expression of the *B. subtilis plsX* gene (consistent with a poor ribosome-binding site) or that the *B. subtilis* PlsX protein could only partially replace the function of the *E. coli* protein (or both).

Except *plsX*, each of the ORFs of the insert of pRMU100a is preceded by an appropriately spaced sequence having a reasonable match to the consensus ribosome-binding site of *B. subtilis* (30). The intergenic region between *plsX* and *fabD* is only 18 bp long, whereas *fabD* and *fabG* overlap by at least 8 bp (another possible initiation codon is located six bases upstream of the initiation codon assigned by alignment to the *E. coli* sequence), suggesting that these three genes may be co-

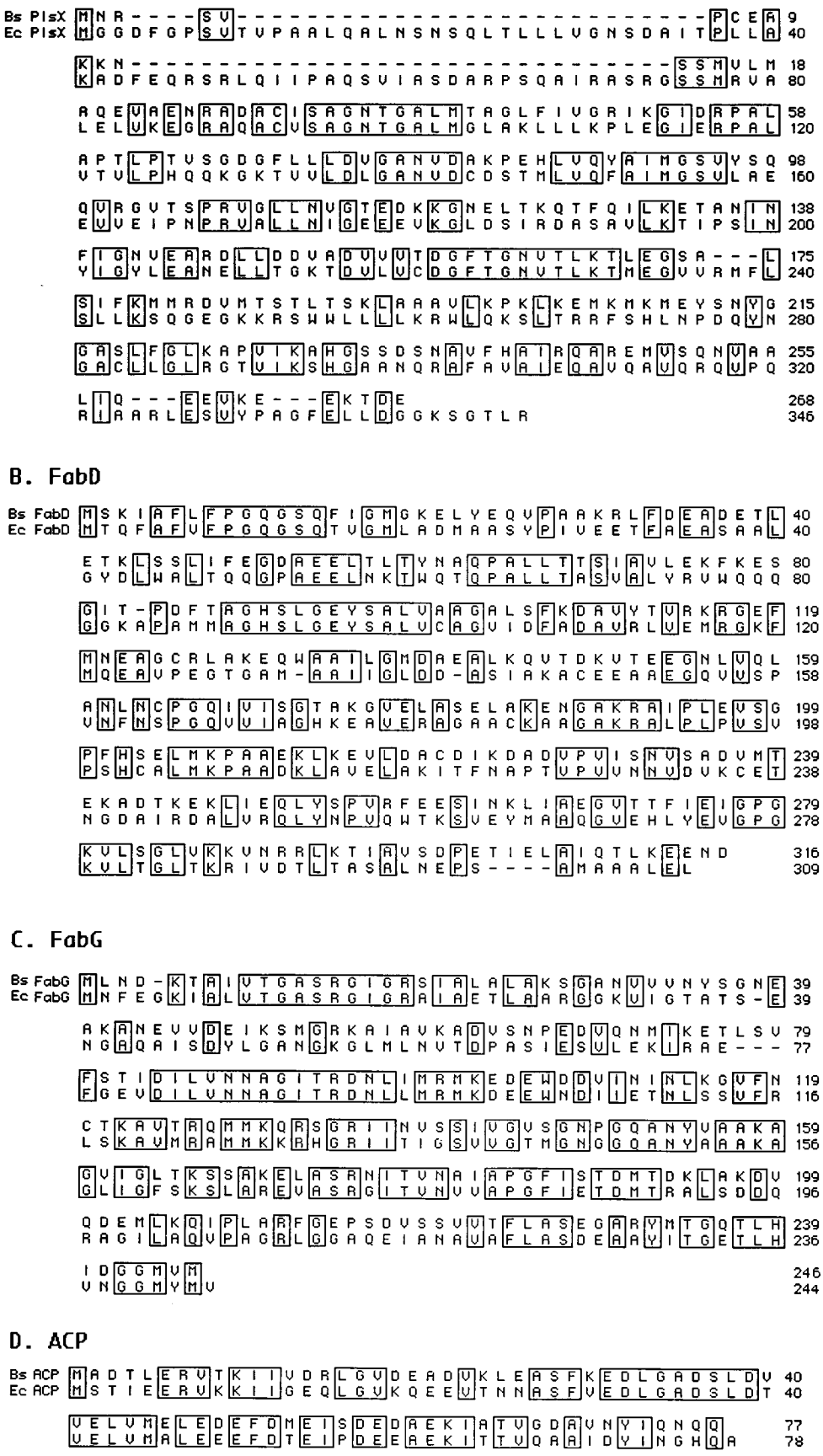


FIG. 2. Alignments of the deduced amino acid sequences of the *B. subtilis* (Bs) proteins with those of *E. coli* (Ec). Identical residues are boxed, and gaps are shown by dashes.

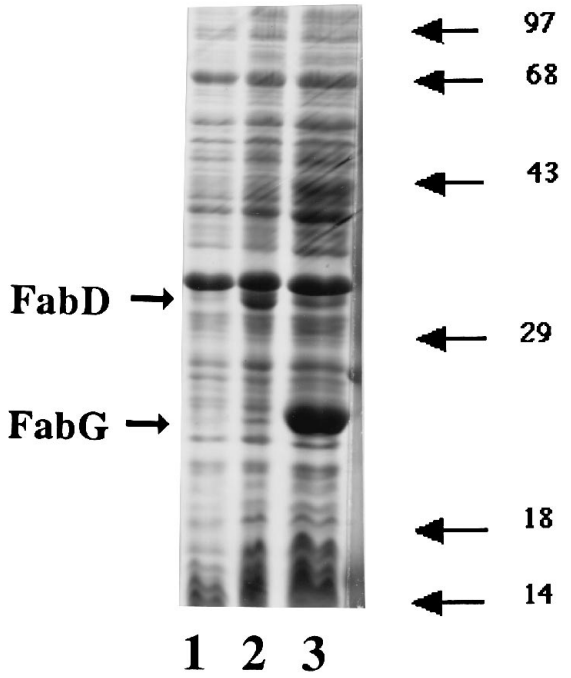


FIG. 3. Expression of *B. subtilis fabD* and *fabG* gene products in *E. coli*. Gene expression from the T7 promoters of various plasmids was induced in strain BL21(λDE3) (see Materials and Methods). The cellular proteins were then separated by SDS-PAGE and stained with Coomassie blue. Lanes: 1, vector plasmid pGEM7z(+); 2, plasmid pRMU119 (*fabD*), 3, plasmid pRMU122 (*fabG*). The migration positions of the molecular mass standards (values in kilodaltons) and the expressed proteins are given.

transcribed. The relatively large 54-bp intergenic space between *fabG* and *acpP* contains a putative sigma A promoter which has a consensus −10 region at 2609 to 2614 but lacks a consensus −35 sequence. Since ACP is a very abundant protein in *B. subtilis* cells (as it also is in *E. coli*), an *acpP*-specific promoter might be expected. Indeed, in preliminary Northern (RNA) blotting experiments, *acpP* mRNA was readily detectable whereas the *fabD* and *fabG* transcripts were at the limit of detection (data not shown).

The deduced molecular masses of the *fabD* and *fabG* gene products were verified by synthesis of the encoded proteins in *E. coli* under control of a phage T7 promoter. Apparent molecular masses of 33 and 24 kDa were determined for FabD and FabG, respectively, by SDS-PAGE (Fig. 3). These values are within 10% of those calculated from the DNA sequences. In all of these experiments, *fabG* was much more efficiently expressed than was *fabD*. The codon usage in both genes is characteristic of low-to-moderate-expression genes (30), but in *fabD* the bias towards rarely used codons was more pronounced.

**The ACP of *B. subtilis*.** To confirm the identification of the partial *acpP* ORF, we purified the ACP of *B. subtilis* to homogeneity (Fig. 4A) and determined the amino acid sequence by use of an improved automated Edman sequencing system that allowed the entire sequence to be determined from the native amino terminus. The sequence obtained (Fig. 2) was 61% identical to that of *E. coli* ACP and apparently ended with a single glutamine residue. Carboxyl-terminal sequencing also gave glutamine. However, electrospray mass spectrometry of the C-terminal cyanogen bromide peptide gave a mass 130 ± 4 Da larger than that calculated from the amino acid sequence, and thus we deduced that the protein ends with two glutamine residues and carryover from the previous sequencing cycle had masked the second residue.

Attachment of the 4′-phosphopantetheine group to serine 37 was detected by (i) release of an amino acid derivative in cycle 37 which failed to cochromatograph with any standard amino acid (no serine was detected); (ii) biosynthetic labeling of the protein with [β-$^3$H]alanine (a precursor of 4′-phosphopantetheine) (Fig. 4B); (iii) the presence of β-alanine in acid hydrolysates of the protein; (iv) acylation of the protein by *E. coli* acyl-ACP synthetase (Fig. 5), an enzyme that specifically acylates the 4′-phosphopantetheine sulfhydryl group of ACPs (28); and (v) mass spectrometry (Fig. 6), which gave the molecular mass calculated for the 4′-phosphopantetheine-modified protein.

Protein chemistry analysis also showed that *B. subtilis* ACP is an unusual protein in that a significant (albeit variable) portion of the protein retained the N-terminal methionine resulting from translational initiation. The first indication of partial processing of the amino terminus was given by automated N-terminal sequencing of a preparation of the intact protein. Two residues were released in each cycle of sequencing, one of which was that released in the cycle just completed. Partial N-terminal processing was confirmed by electrospray mass spectrometry of the intact protein, which gave two major peaks with masses of 8,875 ± 8 and 9,004 ± 8 Da, differing by 129 Da (a Met residue is 131 Da) (Fig. 6), and a similar result was obtained by laser-assisted matrix desorption mass spectrometry (data not shown). These values indicated that both forms of the protein were modified by attachment of 4′-phosphopantetheine (present as a mixed disulfide with 2-mercaptoethanol [23]). Partial processing at the ACP amino terminus was shown for ACP preparations from six independently grown cell lots,

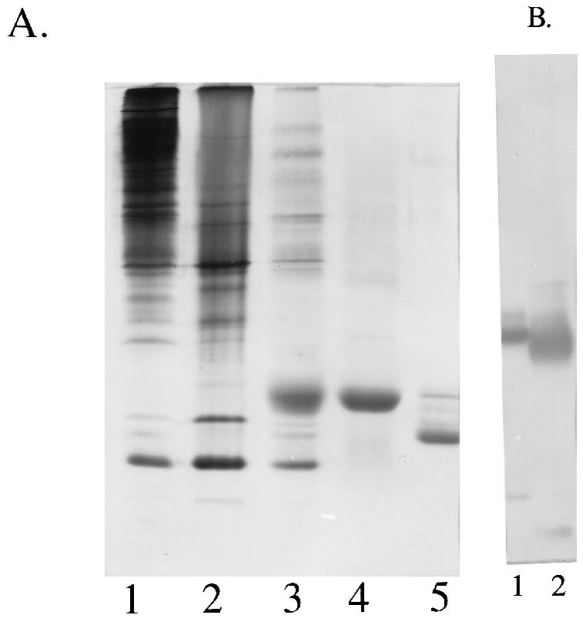


FIG. 4. Purification and [β-$^3$H]alanine labeling of *B. subtilis* ACP. (A) Protein composition of fractions from the various purification steps run on a 13% polyacrylamide gel containing 2.5 M urea (24) and stained with Coomassie blue. Lanes: 1, cell extract; 2, 2-propanol supernatant; 3, peak fractions from DEAE-cellulose chromatography; 4, peak fractions from Mono Q chromatography; 5, *E. coli* ACP. The slower migration of the purer fractions in lanes 3 and 4 is probably due to loss of short-chain acyl groups during the chromatography steps. (B) [β-$^3$H]alanine-labeled cell extracts of *B. subtilis* (lane 1) and *E. coli* (lane 2) were analyzed as for panel A, except that detection was by fluorography rather than staining.

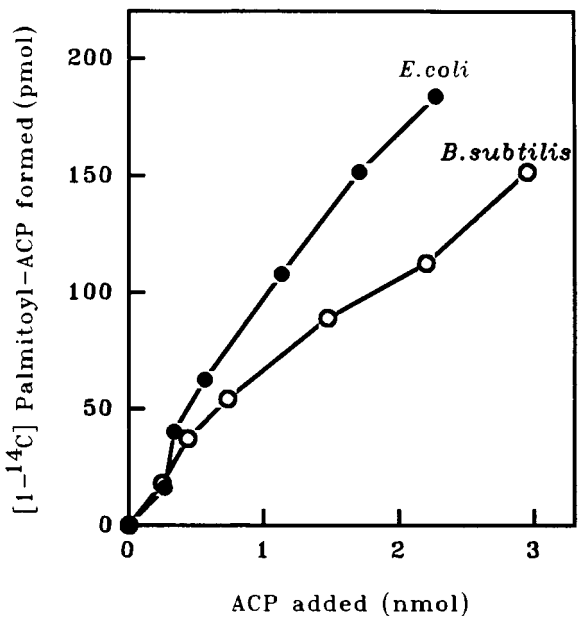


FIG. 5. Activity of *B. subtilis* ACP in the *E. coli* acyl-ACP synthetase reaction. Various amounts of purified preparations of either *B. subtilis* ACP or *E. coli* ACP were added to the acylation reactions, and the reactions were done as previously described (28), except that commercial acyl-ACP synthetase (Sigma Chemical Company, St. Louis, Mo.) was used.

and the unprocessed material ranged from 40 to 90% of the total ACP. It should be noted that SDS-PAGE gave an incorrect molecular mass for *B. subtilis* ACP (17.5 kDa), as expected from prior work with the *E. coli* protein (28).

**Genome location and attempted disruption of the *fab* cluster genes of *B. subtilis*.** To determine the genomic location of the *fab* cluster in *B. subtilis*, we chose an internal fragment of *plsX*. Our choice of *plsX* as the probe was based on the high homology of the amino acid sequences of the *plsX* homolog of *E. coli*, *R. sphaeroides*, and *B. subtilis*. Also, we feared that use of a *fab* gene probe might also detect sequences involved in polyketide synthesis. By using the *plsX* probe, we detected hybridization to only a single YAC clone (clone 10-117) on a mapping membrane containing an ordered array of *B. subtilis* DNA fragments cloned in a YAC vector (1). These data indicated that the *fab* cluster lies within the chromosomal fragment spanning 135 to 145° of the *B. subtilis* genome map. Repeated attempts to disrupt the *plsX* gene were unsuccessful.

## DISCUSSION

The *B. subtilis fab* cluster has important similarities to and interesting differences from the *E. coli* gene cluster. This is the first report of the existence of *plsX* in gram-positive bacteria. This gene affects lipid biosynthesis in *E. coli* in a way that is not understood. The findings that the function, amino acid sequence, and gene location (adjacent to a cluster of fatty acid synthesis genes) of PlsX are conserved between *E. coli* and *B. subtilis* suggests that this protein may play a key role in lipid biosynthesis. One role proposed for the wild-type form of *E. coli* PlsX is elevation of the intracellular levels of *sn*-glycerol-3-phosphate. However, given the marked differences in the regulation of *sn*-glycerol-3-phosphate metabolism in *E. coli* and *B. subtilis* (17), this proposal seems less likely.

An important difference between the genetic organization of the *fab* cluster of *B. subtilis* and that of the *fab* clusters of *Streptomyces* spp. and the proteobacteria (*E. coli*, *H. influenzae*, and *R. capsulatus*) is the absence of a *fabH* gene in the *B. subtilis* cluster. This gene encodes 3-ketoacyl-ACP synthase III, an enzyme involved in the synthesis of the straight-chain primer used for fatty acid biosynthesis in *E. coli* (14). Since the main difference between straight-chain and branched-chain fatty acid biosynthesis results from the use of different primers, it seems reasonable that the *B. subtilis* cluster lacks a *fabH* homolog. The low levels of straight-chain saturated and unsaturated fatty acids present in *B. subtilis* membrane lipids might be synthesized by the same enzymatic system that carries out the synthesis of branched primers, although with much lower efficiency (3). It should be noted that following submission of the original version of this report, a new entry appeared in Genbank (accession no. D64116) that overlaps the 3′ 182 bp of our sequence (Fig. 1) and contains the complete DNA sequence of *acpP*. Although not so annotated, the sequence of Oguro and coworkers (20) contains the last 56 codons of *fabG* (their ORF1) plus the entire sequence of *acpP* (their ORF2) and continues into a series of downstream genes, the first of which encodes a homolog of *E. coli* RNase III. Thus, the *B. subtilis fab* cluster lacks a homolog of *E. coli fabF* (consistent with the differing fatty acid compositions of the two organisms) and the downstream end of the cluster is delimited. Our DNA sequence also matches the sequence of Oguro et al., except at bp 2612 of our sequence, where we found A rather than the G they reported (the difference would be a silent mutation within *fabG*). Moreover, the ACP amino acid sequence deduced from the DNA sequence of Oguro et al. (20) exactly matches that obtained by protein chemistry analysis. It should be noted that the available data indicate only the absence of *fabH* and *fabF* homologs in the *B. subtilis* gene cluster. It remains possible that homologs of these proteins could be encoded by genes located elsewhere in the chromosome.

It is not surprising that *B. subtilis* ACP strongly resembles the *E. coli* protein. ACPs in general are a well-conserved protein family. Moreover, *E. coli* ACP is known to replace a crude preparation of *B. subtilis* ACP for in vitro fatty acid synthesis by *B. subtilis* cell extracts (3). However, our finding that *B. subtilis* ACP exists in two molecular forms is surprising in view of the similarity of its N-terminal region to that of the *E. coli* protein

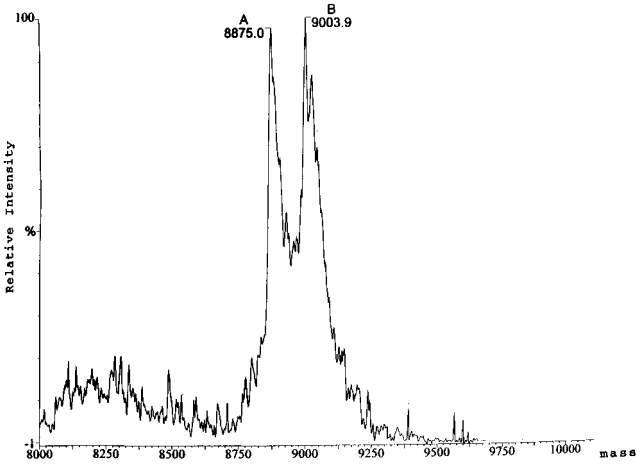


FIG. 6. Major mass spectral peaks from the electrospray ionization analysis of *B. subtilis* ACP. The peak at 8,875.0 is that of the processed protein lacking the N-terminal methionine, whereas the peak at 9,003.9 is that of the unprocessed protein. The minor peaks on the upfield sides of the peaks are monovalent ion adducts due to the strongly acidic nature of the protein.

(Fig. 2). Only upon massive overproduction of *E. coli* ACP does unprocessed protein accumulate; at normal levels of ACP production, no unprocessed protein is observed (12). The cleavage specificity of the methionine aminopeptidase of *B. subtilis* is very similar to that of *E. coli* (2, 7, 9, 33), and thus the partial N-terminal processing observed for ACP cannot be attributed to the N-terminal sequence. Failure to deformylate the initiating *N*-formylmethionine (as occurs for a few bacterial proteins; e.g., see reference 16) can also be excluded, since this would have blocked N-terminal sequencing.

We have repeatedly failed to isolate the intact *B. subtilis acpP* gene, although diverse clone bank screening and PCR protocols, as well as a variety of *E. coli* vectors, were employed. Oguro and coworkers also had difficulties in cloning *acpP*-containing DNA fragments into *E. coli* plasmids and obtained the templates sequenced by inverse PCRs (19). It seems likely that these failures are due to inhibitory effects of *B. subtilis* ACP production on the growth of *E. coli*. Overproduction of *E. coli* ACP inhibits growth of *E. coli* (25), and it has recently been shown that this toxicity is due to accumulation of the apo form of the protein, a potent inhibitor of lipid metabolism (12). Expression of the *acpP* genes of other bacteria is also toxic to *E. coli*. Shen and Byers (31) were unable to clone the intact *V. harveyi acpP* gene, and the *H. influenzae acpP* gene also appears to be toxic to *E. coli*, since none of the five distinct *H. influenzae acpP* clones isolated contained the intact gene (6). The toxicity of a given ACP gene in *E. coli* seems to be related to the likelihood of gene expression from the cloned segment. Successful cloning of ACP genes from *Streptomyces* spp. and related high-G+C organisms (26, 27, 32) is probably due to lack of expression of the native forms of these genes in *E. coli*, whereas the native forms of genes from *B. subtilis*, *H. influenzae*, and *V. harveyi* are often well expressed in *E. coli.*

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