Enoyl-[acyl-carrier-protein] reductase FabI and FabL from Bacillus subtilis*

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Enoyl-[acyl-carrier-protein] (ACP) reductase is a key enzyme in type II fatty-acid synthases that catalyzes the last step in each elongation cycle. The FabI component of Bacillus subtilis (bsFabI) was identified in the genomic data base by homology to the Escherichia coli protein. bsFabI was cloned and purified and exhibited properties similar to those of E. coli FabI, including a marked preference for NADH over NADPH as a cofactor. Overexpression of the B. subtilis fabI gene complemented the temperature-sensitive growth phenotype of an E. coli fabI mutant. Triclosan was a slow-binding inhibitor of bsFabI and formed a stable bsFabI-NAD⁺triclosan ternary complex. Analysis of the B. subtilis genomic data base revealed a second open reading frame (ygaA) that was predicted to encode a protein with a relatively low overall similarity to FabI, but contained the Tyr-Xaa₆-Lys enoyl-ACP reductase catalytic architecture. The purified YgaA protein catalyzed the NADPH-dependent reduction of trans-2-enoyl thioesters of both N-acetyl cysteamine and ACP. YgaA was reversibly inhibited by triclosan, but did not form the stable ternary complex characteristic of the FabI proteins. Expression of YgaA complemented the fabI(ts) defect in E. coli and conferred complete triclosan resistance. Single knockout of the ygaA or fabI gene in B. subtilis were viable, but double knockouts were not obtained. The fabI knockout was as sensitive as the wild-type strain to triclosan, whereas the ygaA knockout was 250-fold more sensitive to the drug. YgaA was renamed FabL to denote the discovery of a new family of proteins that carry out the enoyl-ACP reductase step in type II fatty-acid synthases.

Fatty acid synthesis in bacteria is carried out by a collection of individual enzymes that are known as the type II, or disso-
ciated, fatty-acid synthases (for reviews, see Refs. 1 and 2). The trans-2-enoyl-[acyl-carrier-protein] (ACP)¹ reductase component of the type II system catalyzes the last step in each elongation cycle and plays a key role in the regulation of the pathway (3, 4). In Escherichia coli, there is a single, NADH-de-
pendent isoform of this enzyme that catalyzes all of the steps in the pathway and that is essential to cell survival (3, 5, 6). FabI is a distant member of an extended superfamily of proteins termed the short-chain alcohol reductases/dehydrogenases (7). Members of this family are small (~30 kDa) proteins with ~20–30% over-
all identity and utilize nicotinamide cofactors (7, 8). Most short-
chain alcohol reductases/dehydrogenases contain a conserved catalytic triad of Ser-Xaa₄₉-Tyr-Xaa₃-Lys (9). The Lys hydrogen bonds to the 2'-hydroxyl of the cofactor, whereas the Tyr and/or the Ser acts as a proton donor (8). FabI is an atypical short-chain alcohol reductase/dehydrogenase in that the key residues are a diad consisting of the motif Tyr-Xaa₃-Lys (7, 10). Genes encoding homologs of FabI are easily identified in most of the available bacterial genomic data bases based on a high degree of overall identity and the conserved spacing in the Tyr-Lys diad.

The FabI protein is the target of the broad-spectrum anti-
bacterial agent triclosan (11–13). Triclosan acts as a product mimic and inhibits the enzyme by forming a tight, noncovalent complex with the oxidized cofactor in the active site (13–16). The hydroxyl of triclosan forms a strong hydrogen bond with the catalytic Tyr¹⁵⁶ and the 2'-hydroxyl of NAD⁺; the hydroxy-
chlorophenyl ring stacks with the nicotinamide ring of the cofac-
tor; and the 4'-chlorine accepts a hydrogen bond from the back-
bone amide of Ala¹⁶⁵. A key feature of the interaction is that there is a conformational change in the protein that brings the hydro-
phobic residues Ile²⁰⁰ and Phe²⁰₃ in the flexible substrate-bind-
ing loop close enough to the drug to form additional van der Waals interactions, further stabilizing the complex. Mutations in the active site of FabI that lead to triclosan resistance sterically interfere with the ability of the drug to optimally interact with the protein and cofactor (11, 13). The InhA protein of Mycobacterium tuberculosis, which is 40% identical to FabI and catalyzes the identical reaction, is the target of the anti-tubercular drug isoniazid (17–19), which, when activated, forms a covalent bisubstrate complex that tightly binds to InhA (19).

Recently, we characterized the NADPH-dependent FabI from Staphylococcus aureus (20). This reductase is 48% iden-
tical to the E. coli protein, functionally replaces FabI in E. coli, and forms a high-affinity FabI-NAD⁺-triclosan complex (20). The genome of Bacillus subtilis also contains a gene with very high similarity to the E. coli and S. aureus FabI proteins. We cloned this open reading frame (yjbW, B. subtilis fabI) to de-
terminate if the NADPH cofactor preference was a common feature of Gram-positive enoyl-ACP reductases. Furthermore, S. aureus and B. subtilis display a 10-fold difference in their susceptibilities to triclosan, and we wished to establish if this difference in drug sensitivity was an intrinsic property of the FabI proteins. We also discovered a new gene that encodes an NADPH-dependent enoyl-ACP reductase. This unique protein product is termed FabL, and its expression confers triclosan resistance to type II fatty-acid synthases.

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¹ The abbreviations used are: ACP, acyl carrier protein; NAC, N-acetyl cysteamine; bsFabI, Bacillus subtilis FabI; ecFabI, Escherichia coli FabI; PCR, polymerase chain reaction; MIC, minimal inhibitory concentration.
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EXPERIMENTAL PROCEDURES

Materials—Trans-2-Enoyl-NAC derivatives were the generous gift of Rocco Gogliotti and John Domagala (Parke-Davis Pharmaceutical Research). Sigma supplied ACP, NADH, NAD+, NADPH, NADP+, and crotonyl-CoA. Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) was the gift of KIC Chemicals (Aramonk, NJ). [2-14C]Malonyl-CoA (specific activity of 55 mCi/mmol) was from Amersham Pharmacia Biotech. All other reagents were of the highest available quality.

Cloning B. subtilis fabI and fabL—The entire predicted open reading frame (yjbW, GenBankTM/EBI Data Bank accession number Z99110) for the B. subtilis fabI gene was amplified from genomic DNA using primers bs-xho (5'-CTCGAGGCTCTTTATTAAATATAGGAGGC) and bs-bam (5'-GGATCCTTTAACAGGCAGTAGGCTTCGCCTCCGTCGTAAC) and ts-cloning into the XhoI and BamHI sites of the pET-15b vector (Novagen). The resulting plasmid, pET-YjbW, was then digested with NdeI and religated to remove the coding region for the first 11 residues of non-homologous sequences and to fuse the His tag to the proposed initiator methionine (Met1 of accession number Z99110). This plasmid, pETsFabL, expresses the predicted bsFabI protein with an NH2-terminal His tag. The ygaA open reading frame was amplified from B. subtilis chromosomal DNA with primers ygaA-nde (5'-CCATATGGAACAAAATAAATGTGCACTCGTAAC) and ygaA-bam (5'-GGATCCTTTAACAGGCAGTAGGCTTCGCCTCCGTCG) and cloned into the NdeI and BamHI sites of the pET-15b vector (Novagen). The resultant plasmid, pET-YjbW, was then digested with NdeI and religated to prepare a fusion protein possessing a 10-15 amino acid extension at the N-terminus of the YgaA protein.

Identification of the Reductases—A BLAST search of the B. subtilis genome (27) using the ecFabI sequence (Swiss-Prot accession number P29132) as the bait revealed an open reading frame (yjbW, GenBankTM/EBI Data Bank accession number Z99110) that was predicted to encode a FabI homolog (bsFabI). The predicted protein product was 51% identical to FabI from E. coli and 48% identical to FabL from the Gram-positive S. aureus (20). Two potential start codons were present in yjbW, the second of which (encoding Met12 of the predicted YjbW protein) aligned with that of other FabL proteins (Fig. 1). The open reading frame encoding both the long and shorter (first 11 codons deleted) versions of the protein were fused to the pET-15b vector for expression as NH2-terminal His-tagged proteins, which were purified to homogeneity by metal chelation affinity chromatography as described under “Experimental Procedures.” The two proteins had identical activities in the spectrophotometric assay with 8:1-NAC as substrate (specific activities of 0.08 and 0.14 ± 0.004 nmol/min/μg for the long and short versions, respectively), and we have utilized the short version of the protein for our studies on bsFabI.

RESULTS

Bacterial Growth and Inhibition—E. coli cells were grown on LB medium, with ampicillin (100 μg/ml) where appropriate, at 37 °C, except the fabI/fts mutant strain RJH13 (22), which was cultivated at 30 °C for normal growth or at 42 °C for the nonpermissive conditions. B. subtilis cells were grown on LB medium at 37 °C. MIC testing in E. coli was performed by spotting multiple individual colonies onto plates containing triclosan. Experiments were repeated several times with similar results. MIC testing in B. subtilis was performed by adding 5 μl of a dilution series of triclosan in 10% Me2SO to the wells of a sterile 96-well microtiter plate. Next, 95 μl of a cell suspension (5 × 104 cells/ml) in LB medium was added to the plates, which were incubated for 48 h. The MIC was the most dilute concentration that resulted in no growth of the strain of interest.

Gene Deletions—Nonpolar, precise, in-frame deletions of fabI (yjbW), yrgB (a fabB-like gene), and ygaA (fabL) were generated in B. subtilis P7418 by PCR primer sets similar to that described for Saccharomyces cerevisiae (24). Briefly, two sets of oligonucleotide primers were designed to PCR-amplify ~1 kilobase of sequence immediately upstream and downstream of the target gene (see Table I). PCR amplification was carried out using the Expand High Fidelity PCR system (Roche Molecular Biochemicals) according to the manufacturer’s instructions. Two primers, B/C, anneal to just outside of the 5’- and 3’-ends, respectively, of the predicted fragment and serve to amplify DNA proximal and distal to the target gene. PCR amplification of the primers in each set, at/and, anneal to ~1 kilobase upstream and downstream of primers B/C, respectively, and allow polymerization toward the target gene. In initial PCRs with strain PY79 genomic DNA as the template, products are formed using the A/B and C/D primer combinations. Because primers B/C also contain “tails” that anneal to a variety of antibiotic resistance cassettes (see below), the A/B and CD PCR products can be used as primers in a secondary PCR with an antibiotic resistance cassette. The resulting second PCR product thus encodes sequence upstream of the target gene, an antibiotic resistance gene in place of the target gene, and sequence downstream of the target gene. These PCR products were used to transform B. subtilis strain PY79 to antibiotic resistance by standard techniques (25). Nonessential genes can be replaced in this way with the antibiotic resistance marker by double crossover recombinations. Typical transformation yields at least 20 antibiotic-resistant colonies. The genotypes of transformants were verified by PCR of genomic DNA from antibiotic-resistant clones.

Antibiotic resistance cassettes were synthesized in PCRs using universally “tailed” primers and the Expand Long Template PCR system (Roche Molecular Biochemicals). These 5’- and 3’-tails are complementary to the tails of primers B and C, respectively, and allow the AB and CD PCR products to act as primers for amplification of the cassette. Two different cassettes were used: spectinomycin resistance (the spc gene, GenBankTM/EBI Data Bank accession number U46203) and chloramphenicol resistance (cat, accession number V01277). Spectinomycin was used at 150 μg/ml and chloramphenicol at 5 μg/ml (both from Sigma) in LB medium.

yjaA, the other hand, did not utilize NADH and catalyzed the reduction of the 8:1-NAC substrate only in the presence of...
The apparent $K_m$ value of bsFabI for NADH was 7 μM, whereas YgaA had a $K_m$ of 16 μM for NADPH. Neither protein exhibited the cooperative binding characteristic of S. aureus FabI for NADPH (20). The proteins were further characterized by measuring their specific activities with the 8:1-NAC substrate (Fig. 3A). The bsFabI and YgaA proteins had activities of 0.14 ± 0.004 and 0.18 ± 0.01 nmol/min/mg, respectively. Under identical assay conditions, ecFabI was more active (1.02 ± 0.006 nmol/min/mg). These data establish that both bsFabI and YgaA are enoyl reductases.

Intermediates in fatty acid biosynthesis are carried in the cell as thioesters of ACP. The 8:1-NAC is thus an analog of the true substrate for FabI, and so we also tested the ability of the bsFabI and YgaA proteins to reduce a trans-2-enoyl-ACP. 4:1-ACP was generated in situ by incubating purified E. coli enzymes of fatty acid synthesis with acetyl-CoA, malonyl-CoA, E. coli ACP, and cofactors as described under “Experimental Procedures.” Enoyl reductase protein was added to initiate the reaction, and the products were separated by conformationally sensitive gel electrophoresis. Using these assay conditions (Fig. 3B), bsFabI was as active as ecFabI (2.3 ± 0.4 and 2.9 ± 0.6 nmol/min/mg), whereas the YgaA protein was somewhat less active (0.3 ± 0.02 nmol/min/mg). These data clearly demonstrate that both bsFabI and YgaA have enoyl-ACP reductase activity.

Triclosan Inhibition of the Enoyl-ACP Reductases—We next determined whether bsFabI and YgaA are inhibited by triclosan, a drug known to target ecFabI (11–13) and S. aureus FabI for NADPH (20). Initial rate measurements showed that bsFabI was 8-fold more resistant to triclosan than ecFabI (IC$_{50}$ = 16 and 2 μM, respectively) (Fig. 4). bsFabI contains an Ala at the position equivalent to Gly93 in ecFabI (Fig. 1), and substitutions at this position are known to increase resistance to this inhibitor due to steric interference with drug binding in the substrate pocket (11–13, 28, 29). YgaA also contains an Ala at a position equivalent to Gly93 in ecFabI (Fig. 1) and exhibited the same IC$_{50}$ (16 μM) as bsFabI (Fig. 4).

The key to the effectiveness of triclosan as an antibacterial agent lies in its ability to form a stable ternary complex with the protein and the oxidized cofactor (13). We thus tested whether this complex forms with bsFabI and YgaA proteins. First, we monitored the consumption of the cofactor over an extended time course using drug concentrations that caused 50% inhibition of the initial rate and initiated the reaction with the protein (Fig. 5A). The rate of reaction with bsFabI slowed after several minutes and eventually ceased. This is the behavior of a slow, tight-binding inhibitor (30) and is due to the
time-dependent formation of a stable bsFabI-NAD^+—triclosan ternary complex. This kinetic pattern is the same as observed previously for ecFabI and S. aureus FabI (13, 20). Time-dependent complex formation was not observed with YgaA, although the reaction proceeded at a reduced rate (compared with the uninhibited control) until all of the substrate was exhausted (Fig. 5B). Thus, triclosan behaves as a simple, reversible inhibitor of YgaA. Next, enzyme, drug, and oxidized cofactor were preincubated for 15 min prior to initiating the reaction with substrate. All other factors (enzyme, drug, and substrate concentrations and temperature) were constant. The stable ternary complex formed with the bsFabI enzyme during this preincubation, inactivating the enzyme such that virtually no reaction was observed following addition of substrate (Fig. 5A). With YgaA, the preincubated sample catalyzed the reaction at the same rate as the reaction initiated with enzyme (Fig. 5B), confirming the absence of ternary complex formation. Thus, triclosan inhibited bsFabI by the formation of a stable bsFabI-NAD^+—drug ternary complex, but was a reversible inhibitor of the YgaA enoyl-ACP reductase.

Biological Activities in E. coli—The ability of bsFabI and YgaA to function as enoyl-ACP reductases in vivo was assessed by transforming constitutive expression plasmids into the temperature-sensitive E. coli strain RJH13 (fabI(ts)) (22). Strain RJH13 grows at 30 °C, but cannot grow at 42 °C due to a defective FabI protein (5, 31). Plasmids overexpressing ecFabI, bsFabI, and YgaA all complemented this growth defect, indicating that these genes all encode a functional enoyl-ACP reductase (Table II). The same plasmids were transformed into the wild-type E. coli strain W3110, and the resultant strains were tested for their sensitivity to triclosan (Table II). As expected, strain W3110/pBluescript (empty vector) was sensitive to triclosan. Expression of ecFabI increased resistance to triclosan by ~10-fold, as demonstrated previously (11, 20). Expression of bsFabI conferred a 20-fold increase in resistance on E. coli (Table II), consistent with the in vitro data that bsFabI is more resistant than ecFabI (Fig. 4). In sharp contrast, E. coli cells expressing YgaA grew strongly on plates containing 2000 μg/ml triclosan, a >10,000-fold increase in resistance over wild-type E. coli cells. These data demonstrate the ability of the bsFabI and YgaA proteins to function as enoyl-ACP reductases in
Table I

Sequences of PCR primers used in the construction of the B. subtilis knockout strains

| Primer name | Primer sequences (5’ → 3’) |
|-------------|-----------------------------|
| Cm-Up       | CACAGGAAACACGCTATGACCATGATTTAAACTAACAGACCCCATTAGTCA |
| Cm-Dn       | CATTCAAAATACAGATGGCATTTTATTTTATTTTATTTTTATAATTATTATTC |
| Spe-Up      | CATCCAAATACAGATGGCATTTTATTTTATTTTATTTTTATAATTATTATTC |
| Spe-Dn      | CACAGGAAACACGCTATGACCATGATTTAAACTAACAGACCCCATTAGTCA |
| YgaA-A      | CGTCGGCCCTTTTACAAAG |
| YgaA-B      | TAATCATGGTCACTAGGTGTGCCTGTCCTTCTTGATACGAGTCACAT |
| YgaA-C      | GAAATAAAATAGCTATGCTATTGAGTAAAATGCGGAGGTGATA |
| YgaA-D      | CAGATTCGGCGCTCTC |
| YrpB-A      | CGCCGGCGCATTCCTCAATA |
| YrpB-B      | TAATCATGGTCACTAGGTGTGCCTGTCCTTCTTGATACGAGTCACAT |
| YrpB-C      | GAAATAAAATAGCTATGCTATTGAGTAAAATGCGGAGGTGATA |
| YrpB-D      | GACCGCGATCTTTTACAAAG |
| Yjb-W-A     | AAACCGCGGCTGCAAGT |
| Yjb-W-B     | TAATCATGGTCACTAGGTGTGCCTGTCCTTCTTGATACGAGTCACAT |
| Yjb-W-C     | GAAATAAAATAGCTATGCTATTGAGTAAAATGCGGAGGTGATA |
| Yjb-W-D     | GAAATGTCGGCGAACC |

Table II

Properties of the B. subtilis fabI and fabL (ygaA) genes expressed in E. coli

| Strain          | Clone       | MIC\(\mu g/ml\) | Complements fabI\(ts\)* |
|-----------------|-------------|-----------------|-------------------------|
| W3110/pBluescript | Empty vector | 0.2             | No                      |
| W3110/pFabI     | ecFabI      | 2.0             | Yes                     |
| W3110/pFabI     | bsFabI      | 4.0             | Yes                     |
| W3110/pFabI     | FabL       | >2000           | Yes                     |
| B. subtilis     | None        | 2.0             | NA*                     |

*Triclosan-containing LB (or brain-heart infusion) agar plates were prepared with serial dilutions of triclosan. Cells were plated and incubated at 37 °C. MIC is defined as the concentration of triclosan in the first plate on which there was no growth.

**The plasmids were transformed into E. coli strain RJH13 (fabI) and scored for the ability to support growth at 42 °C.

**Not applicable.

A. Ternary complex formation

\[
\text{FabI} + \text{NADH} \rightarrow \text{FabI-NADH} \rightarrow \text{FabI-NADH}^* \rightarrow \text{FabI-NAD}^* \rightarrow \text{FabI-NAD}^*-\text{triclosan}
\]

B. Reversible inhibition

\[
\text{FabI} + \text{NADPH} \rightarrow \text{FabI-NADPH} \rightarrow \text{FabI-NADPH}^* \rightarrow \text{FabI-NADP}^* \rightarrow \text{FabI-NADP}^*-\text{triclosan}
\]

**Model for mechanism of triclosan inhibition.** A, the FabI family of enoyl-ACP reductases form noncovalent, high-affinity ternary complexes with triclosan and NAD(P)\(^+\)*, preventing the enzyme from participating in the biosynthetic pathway. In this scheme, triclosan functions as a slow-binding inhibitor, and the stable ternary complex takes several minutes to form. B, the FabL enoyl-ACP reductase does not form a high-affinity complex with triclosan and cofactor and is reversibly inhibited by the drug. Thus, the reaction rate is decreased by the presence of triclosan, but is not stopped. Triclosan is an effective antibacterial agent against E. coli and other bacteria that express only FabI, but FabL expression in E. coli is associated with high levels of resistance to the drug.

![Model for mechanism of triclosan inhibition](image)

**FIG. 6.** Model for mechanism of triclosan inhibition. A, the FabI family of enoyl-ACP reductases form noncovalent, high-affinity ternary complexes with triclosan and NAD(P)\(^+\)*, preventing the enzyme from participating in the biosynthetic pathway. In this scheme, triclosan functions as a slow-binding inhibitor, and the stable ternary complex takes several minutes to form. B, the FabL enoyl-ACP reductase does not form a high-affinity complex with triclosan and cofactor and is reversibly inhibited by the drug. Thus, the reaction rate is decreased by the presence of triclosan, but is not stopped. Triclosan is an effective antibacterial agent against E. coli and other bacteria that express only FabI, but FabL expression in E. coli is associated with high levels of resistance to the drug.

![Model for mechanism of triclosan inhibition](image)

**Table III**

Growth phenotypes and triclosan sensitivity of knockout strains of B. subtilis

| Gene(s) deleted a | Growth phenotype | Triclosan MIC \(\mu g/ml\) |
|-------------------|------------------|---------------------------|
| fabI              | Wild-type        | 1.5                       |
| yrpB              | Wild-type        | 1.5                       |
| ygaA (fabL)       | Small colonies   | 0.006                     |
| fabI, yrpB        | Wild-type        | 1.5                       |
| fabI, ygaA        | Not obtained b   | Not obtained              |
| yrpB, ygaA        | Small colonies   | 0.006                     |
| Wild-type         | Wild-type        | 1.5                       |

a Gene deletions were accomplished, and the resulting strains were examined for growth and triclosan sensitivity as described under "Experimental Procedures." b This double knockout strain could not be constructed.

**Function of FabI and FabL (YgaA) in B. subtilis—The physiological function of the two enoyl-ACP reductases in B. subtilis was examined by knocking out the genes and determining the effect of the gene disruptions on cell growth and triclosan sensitivity. Neither the fabI nor ygaA gene was essential (Table III). We were unable to generate a fabI ygaA double knockout, suggesting that the two genes serve redundant functions (Table III). We next examined the sensitivity of the strains to triclosan (Table III). The fabI knockout strain had the same MIC for triclosan as the wild-type strain. However, the ygaA knockout strain exhibited a 250-fold decrease in the triclosan MIC. These data demonstrate that the expression of FabL confers resistance to E. coli cells expressing YgaA.**

**Enoyl-ACP Reductases FabI and FabL from B. subtilis**

The characterization of YgaA defines a distinct class of enoyl reductases that are usually classified within the short-chain alcohol reductase/dehydrogenase family. Analysis of the public data bases with the YgaA protein returned the highest scoring

3 R. J. Heath and C. O. Rock, unpublished observations.
match (38% identical) to a *Streptomyces coelicolor* protein termed CheA (GenBank™/EBI Data Bank accession number AAC44655) that has enoyl-CoA reductase activity (33). Similar matches were uncovered in *Camyloobacter jejuni* (40% identical; annotated as putative oxidoreductase; GenBank™/EBI Data Bank accession number CAB75072) and *Helicobacter pylori* (39% identical; annotated as a 7-a-hydroxysteroid dehydrogenase; GenBank™/EBI Data Bank accession number F64646). Each of these predicted proteins possesses the Tyr-Xaa-Lys spacing rather than the Ser-Tyr-Lys catalytic triad of short-chain alcohol reductases/dehydrogenases. The *S. coelicolor* cheA gene is in an operon encoding enzymes involved in the synthesis of the polyketide antibiotic ansatrienin and functions as an enoyl-CoA reductase in this pathway (33). Genes related to the *S. collinus* ansatrienin biosynthetic genes are not found in *B. subtilis*, which does not make this antibiotic (33); and the region on the *B. subtilis* chromosome where ygaA resides does not appear to contain genes related to polyketide synthases. Thus, the YgaA protein appears to be an enoyl reductase different from CheA. YgaA is active with both NADH and NADPH and reduces all of the intermediates in the pathway and was consistent with the existence of a second triclosan target in this Gram-positive organism (32). These experiments indicate that bacteria that express FabL would be refractory to inhibitors that are specifically tailored to FabI, although the similarity between these two proteins (Fig. 1) suggests the potential for the discovery of compounds that are effective against both enzymes.

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