Bacterial fatty-acid biosynthesis: a genomics-driven target for antibacterial drug discovery

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In this review we demonstrate how the interplay of genomics, bioinformatics and genomic technologies has enabled an in-depth analysis of the component enzymes of the bacterial fatty-acid biosynthesis pathway as a source of novel antibacterial targets. This evaluation has revealed that many of the enzymes are potentially selective, broad-spectrum antibacterial targets. We also illustrate the suitability of some of these targets for HTS. Furthermore, we discuss how the availability of a robust selectivity assay, mode-of-action assays and numerous crystal structures provide an excellent set of tools with which to initiate integrated programs of research to identify novel antibiotics targeted at these enzymes.

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▼ The majority of our current battery of antibiotics originated from screening campaigns focused on identifying antibacterial entities from natural-product extracts. This approach was effective for several decades, but since the mid-1970s no novel class of broad-spectrum antibiotics has been discovered. Consequently, antibiotic research primarily focused on the fine tuning of existing classes of antibiotics, and incremental improvements in antibacterial potency, spectrum and pharmacokinetic parameters have been introduced to make 'new generation' antibiotics from established classes. In many cases, these new antibiotics are less affected by the established resistance determinants that compromised previous generations. However, there often remains a pool of resistance mechanisms that can further evolve and mutate to eventually compromise the second- and third-generation molecules. This concept is illustrated by the later generation cephalosporins, which were originally unaffected by the ubiquitous TEM-1 and SHV-1 β-lactamases. However, during the past 10 years we have witnessed the widespread emergence of numerous TEM and SHV β-lactamases that possess point mutations in their active sites enabling them to hydrolyze, and thus confer resistance to, these newer generation cephalosporins[1].

Therefore, the majority of currently used antibiotics acts against a limited number of antibacterial targets. Resistance to this current armory of antibacterials is increasing rapidly, challenging our ability to treat infections caused by common bacterial pathogens. Consequently, both the medical community and government organizations recognize the need for novel antibiotics that act via inhibition of novel antibacterial targets.

Bacterial genomics has reshaped antibacterial drug discovery. Advances in DNA sequencing technology are providing the sequences of entire bacterial genomes at an unprecedented rate. This has been demonstrated recently with the generation of high-quality drafts of 15 different bacterial genomes in one month, a rate of >1 genome per 1.5 working days. This effort included sequencing the genome from the 'supergerm', *Enterococcus faecium*, in 1 day. It is estimated that almost 120 genomes will have been completely sequenced within 2–3 years[2]. Access to whole or near-complete gene sets from multiple organisms, in combination with state-of-the-art bioinformatics techniques, greatly facilitates the selection of novel antibacterial targets. First, the genomes of clinically relevant pathogens can be searched for the presence of a specific gene and, therefore,

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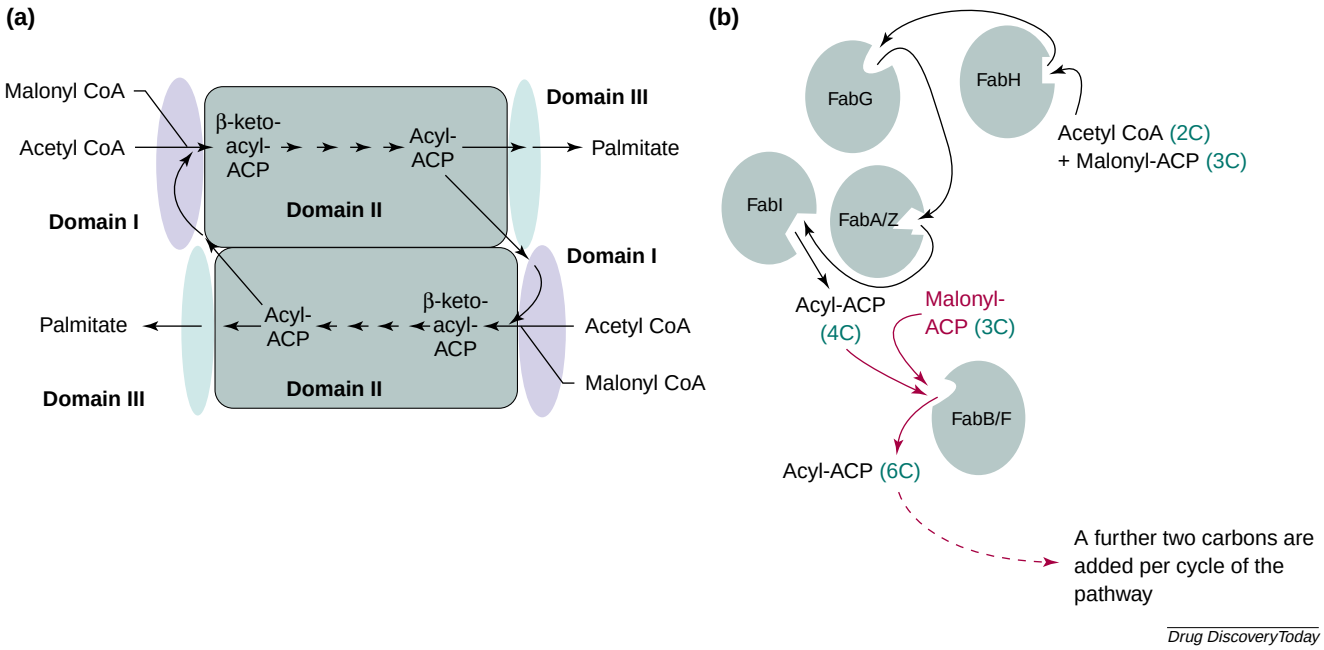


Figure 1. The organization of fatty acid biosynthesis in mammals and bacteria. **(a)** In mammals, fatty acid biosynthesis is performed by a single multifunctional polypeptide complex (existing as a dimer) and the growing acyl chain remains bound until chain elongation is complete (reproduced, with permission, from Ref. 10). **(b)** In bacteria, each of the reactions are performed by discrete proteins, each of which catalyzes a reaction in the pathway; the pathway intermediates are ferried from one specific protein to the next as thioester derivatives of acyl carrier protein (ACP). The figure in brackets (e.g. 2C) denotes the length of the carbon chain.

predictions can be made on the potential spectrum of a given antibacterial target. Furthermore, paralogs within a single organism can also be identified[3]. Second, genomic information from a variety of different organisms enables genes of interest to be rapidly identified and manipulated in organisms other than *Escherichia coli*, such as the key pathogens *Staphylococcus aureus* and *Streptococcus pneumoniae*. For example, advances in gene-knockout technologies now enable the chromosomes of Gram-positive pathogens to be rapidly scanned for genes essential for cell viability, both in the test tube and during infection in the host. Third, it is now possible to make valid predictions on the potential selectivity of a novel bacterial target. Human genome sequences can be searched for the presence of a human equivalent. Where a human ortholog exists, molecular modeling and sophisticated sequence-analysis programs can be used to predict whether selective inhibition of the bacterial enzyme might be possible. Finally, bacterial genomics is making it possible to exploit broadly conserved genes of unknown function via comparative genomic analyses. Typically, these novel targets represent more than 20–30% of each new genome sequenced. In combination, these approaches are empowering an early-phase discovery process for the identification and validation of novel antibacterial targets.

Before the genomics era there was some evidence that particular enzymes in the bacterial fatty-acid biosynthetic pathway held promise as antibacterial targets. However, it has been these genomic-based strategies that have enabled a more informed appreciation of this pathway and other pathways such as peptidoglycan, chorismate[4] and isoprenoid biosynthesis[5] as sources of antibacterial targets. This review will describe the potential for exploiting bacterial fatty-acid biosynthesis and its component enzymes as an antibacterial strategy, and review the currently known inhibitors of these enzymes.

Comparison of human and bacterial fatty-acid biosynthesis

Fatty acid biosynthesis is carried out by the ubiquitous fatty-acid synthase (FAS) system. In the type I system of mammals, including humans, FAS is a single, large polypeptide composed of several distinct domains[6]. In the type II system of bacteria[7], plants[8] and protozoa[9], the FAS components, including the acyl carrier protein (ACP), exist as discrete proteins (Fig. 1). The corresponding activities of the two FAS systems are related in structure and function, but generally lack overall sequence homology. The absolute requirement of type II FAS for bacterial viability, together with its major differences compared with the type I FAS, suggests that

selective compounds, with potential use as broad-spectrum antibacterial drugs, could be obtained. For example, the antituberculosis drug isoniazid can inhibit both FabI and a β-ketoacyl-ACP synthase[11–13], and the antibiotic compound thiolactomycin inhibits all three type II FAS condensing enzymes, but neither of these compounds inhibit type I FAS.

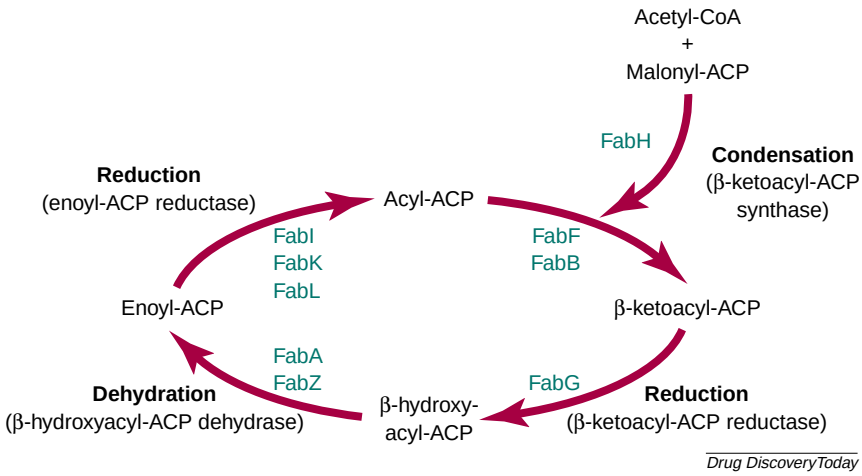


Figure 2. Schematic diagram of the dissociated type 2 bacterial fatty acid biosynthetic pathway. The figure shows a composite view of this pathway and the key enzymes involved. The distribution of particular components varies in a species-specific manner. For example, FabA occurs only in Proteobacteria, whereas FabZ is ubiquitous. Both function interchangeably in elongation cycles up to 10 carbons and both function in the synthesis of saturated fatty acids. FabA is more active in the dehydration of β-hydroxydecanoyl-ACP and also converts a small amount of the product, *trans*-2-decanoyl-ACP, to the *cis* isomer, which is efficiently utilized by FabB, thus diverting the flow of intermediates into unsaturated fatty acid biosynthesis. FabZ is the primary dehydratase involved in further elongation cycles. FabB and FabF both occur in Proteobacteria whereas only a FabF synthase occurs in pathogenic Gram-positive bacteria. In organisms containing both enzymes, FabB has been shown to be essential for unsaturated fatty acid biosynthesis, and FabF is crucial for maintaining membrane fluidity at lower than physiological temperatures. Three different enoyl-ACP reductase enzymes have been discovered in different pathogens, FabI and FabK (Ref. 24) and Fab L (Ref. 15).

Assessing bacterial fatty-acid biosynthesis inhibition: impact of genomics and bioinformatics

Until recently, the majority of research on bacterial fatty-acid biosynthesis has focused almost exclusively on *E. coli*[14] (Fig. 2). Consequently, little was known about the spectrum or characteristics of the component enzymes in different pathogens of clinical interest, or the level of conservation that exists between enzymes from different bacterial species. Access to fully sequenced and assembled bacterial genomes has enabled the identification of many of the component enzymes in *Pseudomonas aeruginosa*, *S. aureus*, *Enterococcus faecalis*, *Haemophilus influenzae* and *S. pneumoniae* (Table 1). For example, data have been published in the past few years on the homologs of *FabH*, *FabI*, *FabG* and *FabD* from *S. aureus*[a,16–19], *FabH* and *FabD* from *S. pneumoniae*[b,20,21] and *FabI* and *FabD* from *P. aeruginosa*[22,23]. Moreover, the identification of these genes has enabled specific pathway enzymes to be cloned, expressed, characterized and progressed to HTS for novel inhibitors.

Access to the fully sequenced genomes of pathogenic organisms facilitates early determination of the potential spectrum of each of the fatty-acid biosynthetic enzymes. Analysis of the enoyl-ACP reductase homologs provides a perfect illustration of the power of genomics and bioinformatics in assessing novel antibacterial targets. Based on work performed on *E. coli* it was believed that FabI was unique in performing the enoyl-ACP reductase reaction in bacteria. However, sequence similarity searches failed to identify a *FabI* homolog in the *S. pneumoniae* genome. Rock and colleagues deduced that *S. pneumoniae* possessed an alternative enoyl-ACP reductase, FabK, which bears no significant homology to FabI. Further bioinformatic analysis found that FabK was present in several other organisms and, in some cases, such as *E. faecalis* and *E. faecium*, both

Table 1. Identification of *Fab* genes in key respiratory and Gram-positive pathogens

Target	*S. aureus*	*S. pneumoniae*	*E. faecalis*	*H. influenzae*
FabG	✓	✓	✓	✓
FabK	×	✓	✓	×
FabI	✓	×	✓	✓
FabA	×	×	×	✓
FabZ	✓	✓	✓	✓
FabB	×	×	×	✓
FabF	✓	✓	✓	×
FabH	✓	✓	✓	✓
FabD	✓	✓	✓	✓

Abbreviations: *S. aureus*, *Staphylococcus aureus*; *S. pneumoniae*, *Streptococcus pneumoniae*; *E. faecalis*, *Enterococcus faecalis*; *H. influenzae*, *Haemophilus influenzae*.
Key: ✓ = homologue present in genome; × = homologue absent from genome.

[a] Payne, D.J. *et al.* Identification and characterization of the *S. aureus* Fab I (enoyl-ACP reductase). *99th American Society of Microbiology General Meeting, Chicago, IL, USA*; Abstract K131

[b] Lin, A.H. *et al.* (1999) The cloning, expression, purification and characterization of the *S. aureus* malonyl co A-Acyl carrier protein transacylase. *99th American Society of Microbiology General Meeting, Chicago, IL USA*; Abstract K119

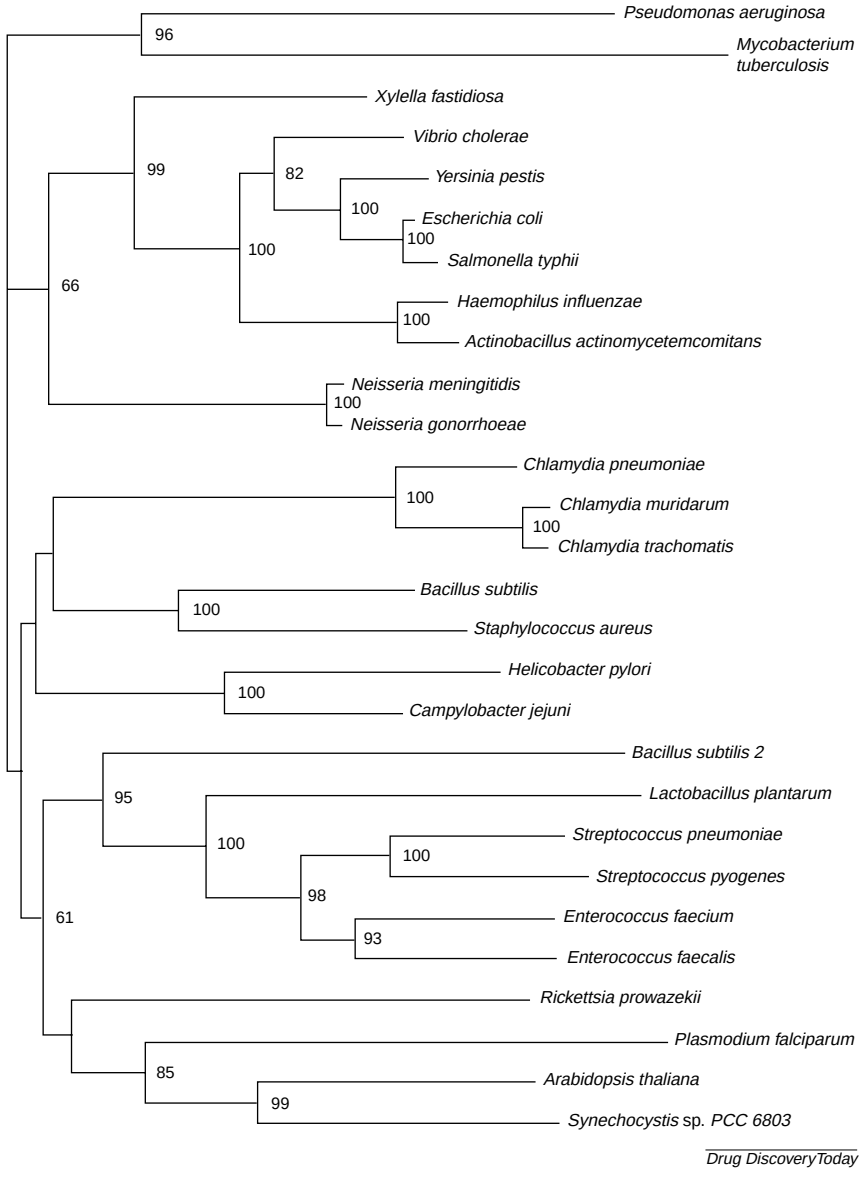


Figure 3. Phylogenetic analysis illustrating the spectrum of FabH. The distance-based FabH phylogeny illustrates the broad range of bacterial species that contain the FabH protein. The numbers in the figure indicate the nodes for which there is a confidence level above 50%. The tree is preferentially rooted with the species *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* to more easily demonstrate the presence of FabH in both Gram-positive and Gram-negative commercially relevant pathogens.

FabK and FabI were present[24] (Table 1). Therefore, progressing a FabI inhibitor might be an effective strategy against specific pathogens such as *Staphylococci* sp., but inhibition of both FabK and FabI would be required to achieve broad-spectrum antibacterial activity. Considering the differences between FabK and FabI this could be challenging. Furthermore, analysis of the *S. aureus*, *S. pneumoniae* and *E. faecalis* genomes illustrates the absence of FabA and FabB in these organisms (Table 1). The above situations exemplify how bioinformatic analysis of a particular antibacterial target can provide spectrum information that could only be obtained via considerable experimentation in the pre-genomics era.

In contrast to FabB, FabA and the enoyl-ACP reductases, other enzymes in the fatty-acid biosynthetic pathway appear conserved among relevant clinical pathogens and thus could be more appropriate choices as broad-spectrum antibiotic targets (Table 1). For example, the phylogenetic analysis of FabH (Fig. 3) demonstrates the conservation of this target in a broad spectrum of bacterial pathogens.

Validation of bacterial fatty-acid biosynthesis as an essential antibacterial target

Confirmation that a target is essential for bacterial survival is an important aspect of the characterization of a novel antibacterial target, and can be achieved by attempting either disruption or complete replacement of the target gene in the organism of choice. Several methods are available for this type of target validation and, with the advent of genomics, it is possible to take a directed, rather than random, approach to defining essential genes. Gene disruption can be achieved by plasmid insertion mutagenesis, whereby an internal fragment of the target gene is cloned into a non-replicative vector containing a selectable marker. The plasmid is introduced into the target organism and transformants are isolated on selective media. Because the vector is unable to replicate in the host, the only way to maintain the selective marker is via homologous recombination with the target gene. Clearly, this will disrupt the gene of interest and, if achieved, appears to demonstrate that the gene is non-essential. Although this is an efficient and rapid method for assessing whether a gene is essential, the results can be misleading. The insertion of a large DNA-sequence into the gene could have effects on the expression of essential genes

downstream of the targeted gene (polar effects), leading to the impression that a non-essential gene is a valid target for antimicrobial therapy. Conversely, this type of experiment leaves the N-terminal portion of the gene intact and, as a result, it is possible to get expression of this part of the protein which, if active, could give the impression that a valid target is non-essential. Allelic replacement mutagenesis, in which the target gene is deleted and replaced with a selectable marker following double-crossover homologous-recombination, is a more effective procedure and has been successfully applied to a comprehensive analysis of the entire set of two-component signal transduction systems from *S. pneumoniae*[25]. The absolute requirement of the fatty acid biosynthetic pathway can be examined in this way, enabling the suitability of each component enzyme to be assessed as a potential antibacterial target.

Moreover, the antibacterial activity of specific inhibitors of some of the enzymes in the fatty-acid biosynthetic pathway confirms that these components are essential for bacterial viability. Indeed, some of these compounds provided early, and largely ignored, clues to the potential of this area as a target for antibacterial intervention. Cerulenin, thiolactomycin, diazaborines and triclosan (Fig. 4) all possess antibacterial activity mediated via inhibition of one or more of the component enzymes of the pathway.

Diazaborines: inhibitors of FabI

The diazaborine antibacterials have been established for almost 30 years[26]. However, their molecular target was not identified for some time after their discovery. They were initially described as selective inhibitors of lipopolysaccharide biosynthesis[27]. Pretreatment of *E. coli* with diazaborine Sa84.474 made the bacteria less virulent *in vivo* when compared with untreated controls. Subsequently, it was shown that these compounds target the enoyl-ACP reductase, FabI[28]. The molecular mechanism of inhibition of FabI by diazorborines has since been elucidated following the solution of the crystal structure of FabI with a diazorborine complexed in the active site[29]. However, exploitation of these molecules is believed to be restricted by the inherent toxicity associated with this class of compound.

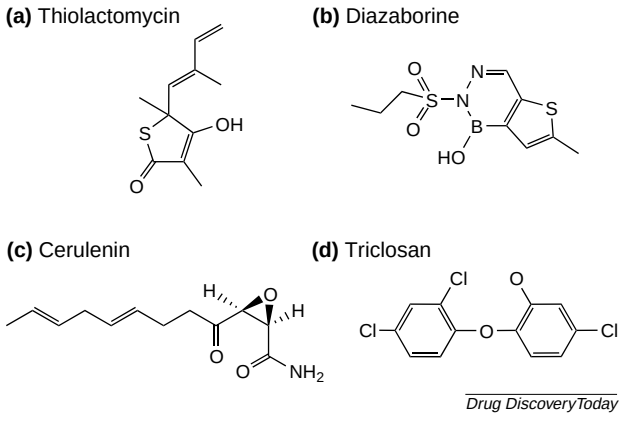


Figure 4. Inhibitors of bacterial fatty acid biosynthetic enzyme: **(a)** thiolactomycin, **(b)** diazaborine, **(c)** cerulenin and **(d)** triclosan.

Cerulenin: inhibitor of FabB and FabF

Cerulenin inhibits the condensation reaction in the pathway that is responsible for the chain-elongation phase of fatty acid biosynthesis. This compound possesses similar potency in this reaction in both mammals and bacteria[30] and, therefore, the poor selectivity and undesirable reactivity of this compound curtailed further exploitation.

Thiolactomycin: inhibitor of FabB, FabF and FabH

Thiolactomycin is a selective inhibitor of the bacterial condensation enzymes[14] (FabB, FabF and FabH), although it is a much less potent inhibitor of FabH. This compound exhibits relatively poor *in vitro* antibacterial activity, and yet, paradoxically, good efficacy in animal infection models was observed[31]. The relatively poor *in vitro* antibacterial activity of thiolactomycin might have prevented further exploition.

Triclosan: inhibitor of FabI

It has recently been shown that the primary antibacterial target of triclosan, and related compounds, is FabI in *E. coli*[32,33]. Identification and characterization of FabI from *S. aureus*[a,18] has confirmed that the antibacterial target of triclosan in this organism is also FabI[c,18]. However, FabK, the enoyl-ACP reductase described above and found in other organisms, is refractory to inhibition by triclosan[24]. Therefore, the mode of action of triclosan against organisms in which FabI is not essential is likely to be via an alternative mechanism, possibly disruption of bacterial membranes.

To date, experimental inhibitors have demonstrated that FabI and the initiating and chain-elongation condensing enzymes (FabH, and FabB and FabF, respectively) are viable antibacterial targets. It is likely that the other pathway components are also attractive antibacterial targets.

The search for novel antibiotics targeted at bacterial fatty-acid biosynthesis

To identify novel and selective inhibitors of these targets it is imperative that HTS assays can be developed to identify

[c] Slater, C. *et al.* (2000) Mode of action of triclosan in *Staphylococcus aureus. American Society of Microbiology, Los Angeles*, CA, *USA*. Abstract A101

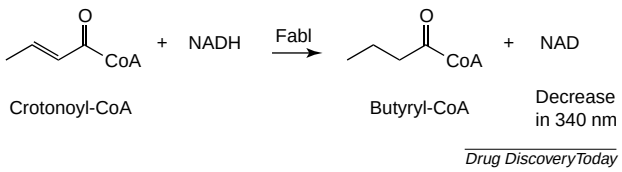


Figure 5. HTS for FabI. FabI catalyzes the NADH-dependent reduction of crotonoyl CoA to butyryl CoA. The conversion of NADH to NAD^+ is followed spectrophotometrically by a decrease in absorbance at 340 nm.

lead compounds, and that a human FAS assay is available to assess their selectivity. Furthermore, structural information on these enzymes is also required to provide tools for the rational exploitation of the novel inhibitors identified by HTS assays. These aspects are described in the next section of this review, using FabI and FabH as examples.

Amenability to HTS

Many of the component enzymes are amenable to the development of HTS assays for the discovery of novel inhibitors. Pharmaceutical companies have already started screening some of the component enzymes, as exemplified by the recent report that 200,000 compounds have been screened against *E. coli* FabI; interestingly, this screen identified triclosan as a lead[34].

The availability of the component enzymes from Gram-positive bacterial pathogens has facilitated screening against both FabI and FabH by GlaxoSmithKline. The format for the FabI HTS assay used crotonoyl-CoA as substrate and activity was measured by a decrease in absorbance at 340 nm as a result of oxidation of NADH (Fig. 5). This screen identified several novel leads, two of which are described here.

S. pneumoniae FabH has also been screened. Lack of access to significant quantities of acyl-ACP substrates has previously limited the ability to directly assay many of the component enzymes of fatty acid biosynthesis, although recently this has become less problematic. As large quantities of purified malonyl-ACP were unavailable, *S. pneumoniae* FabH was screened in a coupled assay, in tandem with FabD. Careful characterization of the FabD reaction permitted synthesis of controlled levels of malonyl-ACP that could be utilized by FabH. The use of radiolabeled acetyl-CoA permitted quantitation of FabH activity by monitoring the level of radiolabeled acetoacetyl-ACP production in a filtration assay. The use of this HTS format enabled the discovery of several compound classes that included potent inhibitors of FabH. In addition, a scintillation proximity assay amenable to HTS has also been developed for FabH[35].

The other enzymes in the pathway also appear to be amenable to a HTS format, either as single enzymes, or coupled with another enzyme in the pathway. Finally, it might be possible to configure a 'one pot' HTS assay for all the biosynthetic enzymes in much the same way that has been achieved for the enzymes in the peptidoglycan biosynthesis[36] pathway and this possibility justifies further investigation.

Development of a human selectivity assay

It is essential that the selectivity of HTS leads is evaluated in a timely manner. To facilitate this, human testes FAS1 was cloned, expressed and purified by workers at GlaxoSmithKline, and HTS assays were configured in 96-well microtitre plates[37]. FAS1 is amenable to assaying in several formats. Activity was initially detected by monitoring the incorporation of radiolabelled acetyl-CoA into mature fatty acids; the identity of the latter being confirmed by gas chromatography. However, radioactive filtration-based assays are generally undesirable for a variety of reasons, and so a second continuous spectrophotometric assay was developed. Monitoring the oxidation of NADPH at 340 nm provides a convenient and robust method of determining the selectivity of large numbers of compounds.

Tools for the rational design of novel inhibitors

A wealth of structural data has been generated on *E. coli* FabI, FabF, FabD and FabH. For example, the structure of *E. coli* FabH was recently elucidated[38] and further refined by Qiu and colleagues[39] and these coordinates can be used for the rational design and modification of FabH inhibitors. The physiological form of the enzyme was shown to be dimeric. Three-dimensional structures in the presence and absence of ligands have been refined to 1.46 Å resolution and revealed detailed interactions involved in ligand binding, particularly the mechanism of substrate CoA binding. These structures also provided new insights into the FabH mechanism. At present, no crystal structures of the enzymes from Gram-positive pathogens have been solved, but this situation is likely to change in the near future. Other structures (e.g. FabI and FabF) have been determined with inhibitors complexed in their active site[40–43] and this information will prove invaluable for understanding the mechanism of inhibition by novel inhibitors.

Technologies to track the mechanism of action of novel inhibitors

One of the challenges faced in the discovery and development of novel inhibitors of antibacterial targets is confirming whether the mode of antibacterial action (MOA) correlates with the observed *in vitro* activity against the purified

Table 2. Use of Fab-overexpressing strains to track the mode of antibacterial action

	Minimum inhibitory concentrate μg ml^{-1}		*Staphylococcus aureus* FabI IC_{50} (μM)	Mode of action
	Wildtype	FabI OE		
Tetracycline	0.25	0.25	–	Protein synthesis
Penicillin G	0.008	0.008	–	Cell wall synthesis
Erythromycin	0.25	0.125	–	Protein synthesis
Neomycin	8.0	8.0	–	Protein synthesis
Mupirocin	0.03	0.03	–	tRNA synthetase
Ciprofloxicin	0.125	0.25	–	DNA replication
Triclosan	0.06	2.0	0.98	FabI

target enzyme. Ready access to the gene sequences of the Fab enzymes from different species makes possible the up- or down-regulation of the expression of these enzymes in a tightly controlled manner[44]. If regulation of the levels of a particular gene target results in a selective and concomitant change in antibacterial sensitivity to an inhibitor of the target, this provides corroboration of the mode of antibacterial action. Table 2 illustrates how overexpression of FabI in *S. aureus* gives rise to a selective increase in minimum inhibitory concentration (MIC) of triclosan, demonstrating that FabI is the primary antibacterial target[c,18]. Furthermore, a titratable antisense RNA system has been developed in *S. aureus* that can be used both in MOA studies and to investigate the physiological effect of downregulating *Fab* genes. The use of this technology has been demonstrated with FabI: induction of antisense to the FabI mRNA transcript caused a growth defect or lethal phenotype, depending on induction levels. Downregulation of FabI in this manner also gave rise to a selective increase in sensitivity to triclosan[d]. These systems provide additional validated methodologies for tracking the MOA of novel inhibitors of fatty acid biosynthetic enzymes.

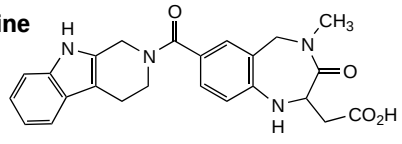
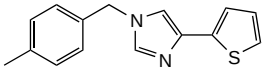
Novel inhibitor-leads of component enzymes

Two of the leads identified from HTS of *S. aureus* FabI at GlaxoSmithKline are shown in Table 3. Both of these compounds inhibited *S. aureus* and *E. coli* FabI. Lead-optimization studies in the imidazole series led to a 16-fold improvement in antibacterial activity and a fivefold improvement in potency of FabI inhibition[45]. The initial benzodiazepine lead possessed no antibacterial activity and exhibited only weak inhibition of FabI. Exploitation of this series yielded a >70-fold increase in the potency of inhibition and MICs of 0.5 μg ml^{-1} have been achieved against *S. aureus*[46]. Moreover, examples from both series exhibit an increase in MIC against strains overexpressing FabI, confirming that their mode of antibacterial action is indeed via inhibition of FabI. These leads demonstrate the potential of screening Fab enzymes to discover new antibiotics for the future.

Conclusions

Access to a broad spectrum of bacterial genomes has played a key role in enabling a thorough assessment of this pathway as a source of novel antibacterial targets. There is already substantial proof that several of these enzymes are well-validated, essential and broad-spectrum antibacterial targets. The development of genomics-based antisense and

Table 3. Hits from HTS of *Staphylococcus aureus FabI*

	HTS lead	IC_{50}(μM)	
		S. aureus FabI	Human fatty acid synthase
Benzodiazapine series		7.8	>100
Imidazole series		2.1	>100

[d] Ji, Y. *et al.* (2001) Validation of drug target by regulated antisense RNA in *Staphylococcus aureus*. *American Society of Microbiology General Meeting: 20–24 May, Orlando, Florida, USA*; Abstract GM01-A-31916

inducible-promoter technologies will provide exquisite tools for the further evaluation of the Fab targets and could provide an insight into which enzyme(s) offer the greatest promise as antibacterial targets. Some of these Fab targets have already been screened and others appear amenable to HTS. The initial success of this area in providing novel antibacterial agents is dependent upon identifying physiologically relevant inhibitors from screening and rational design campaigns. Encompassing all the requisite pharmacological and toxicological features in lead molecules remains a substantial challenge. However, we believe this area has the potential to provide novel, potent and broad-spectrum therapeutic agents and we predict an increase in interest in this pathway by the microbiological and pharmaceutical communities.

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References

1 Jacoby, G.A. and Medeiros, A.A. (1991) More extended spectrum β-lactamases. *Antimicrob. Agents Chemother.* 35, 1697–1740
2 Amber, D. (2000) DOE's microbial genomics program focuses on high-throughput draft sequencing. *The Scientist* 14, 1
3 Brown, J.R. and Warren, P.V. (1998) Antibiotic discovery: is it all in the genes? *Drug Discov. Today* 3, 564–566
4 Payne, D.J. *et al.* (2000) The impact of genomics on novel antibacterial targets. *Curr. Op. Drug Discov. Develop.* 3, 177–190
5 Wilding, E.I. *et al.* (2000) Identification, evolution and essentiality of the mevalonate pathway for isopretentyl diphosphate biosynthesis in Gram-positive cocci. *J. Bacteriol.* 182, 4319–4327
6 Chirala, S.S. *et al.* (1997) Animal fatty acid synthase: functional mapping and cloning and expression of the domain I constituent activities. *Proc. Natl. Acad. Sci. U. S. A.* 94, 5588–5593
7 Tsay, J.T. *et al.* (1992) Isolation and characterization of the β-ketoacyl-acyl carrier protein synthase III gene (FabH) from *Escherichia coli* K-12. *J. Biol. Chem.* 267, 6807–6814
8 Clough, R.C. *et al.* (1992) Purification and characterization of 3-ketoacyl-acyl carrier protein synthase III from spinach. A condensing enzyme utilizing acetyl-coenzyme A to initiate fatty acid synthesis. *J. Biol. Chem.* 267, 20992–20998
9 Waller, R.F. *et al.* (1998) Nuclear-encoded proteins target to the plastid in *Toxoplasma gondii* and *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U. S. A.* 95, 12352–12357
10 Wakil, S.J. (1989). Fatty acid synthase, a proficient multifunctional enzyme. *Biochemistry* 28, 4523–4530
11 Banerjee, A. *et al.* (1994) *InhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science* 263, 227–230
12 Rozwarski, D.A. *et al.* (1998) Modification of the NADH of the isoniazid target (*InhA*) from *Mycobacterium tuberculosis*. *Science* 279, 98–102
13 Mdluli, K. *et al.* (1998) Inhibition of a *Mycobacterium tuberculosis* β-ketoacyl ACP synthase by isoniazid. *Science* 280, 1607–1610
14 Magnuson, K. *et al.* (1993) Regulation of fatty acid biosynthesis in *Escherichia coli*. *Microbiol. Rev.* 57, 522–542
15 Heath, R.J. *et al.* (2000) The enoyl-[acyl-carrier-protein] reductases FabI and FabL from *Bacillus subtilis*. *J. Biol. Chem.* 275, 40128–40133
16 Debouck, C. *et al.* (1999) *Staphylococcus aureus* FabH. EP916730A2
17 Huang, J. and McDevitt, D. (1998) *Staphylococcus aureus* FabG. US6110704
18 Heath, R.J. *et al.* (2000) Inhibition of the *Staphylococcus aureus* NADPH-dependent enoyl-acyl carrier protein reductase by triclosan and hexachlorophene. *J. Biol. Chem.* 275, 4654–4659
19 Gentry, D.R. *et al.* (1998) *Staphylococcus aureus* malonyl-CoA:ACP transacylase (Fab D). EP843016-A2
20 Gentry, D.R. *et al.* (1998) *Streptococcus pneumoniae* FabH. WO9817779-A1
21 Gentry, D.R. (1998) *Streptococcus pneumoniae* FabD. WO9822133-A1
22 Kutchma, A.J. *et al.* (1999) Characterization of a *Pseudomonas aeruginosa* fatty acid biosynthetic gene cluster; purification of acyl carrier protein (ACP) and malonyl-coenzyme A: ACP transacylase (Fab D). *J. Bacteriol.* 181, 5498–5504
23 Hoang, T.T. and Schweizer, H.P. (1999) Characterization of *Pseudomonas aeruginosa* enoyl-acyl carrier protein reductase (FabI): a target for the antimicrobial triclosan and its role in acylated homoserine lactone synthesis. *J. Bacteriol.* 181, 5489–5497
24 Heath, R.J. and Rock, C.O. (2000) A triclosan-resistant bacterial enzyme. *Nature* 406, 145–146
25 Throup, J.P. *et al.* (2000) A genomic analysis of two-component signal transduction in *Streptococcus pneumoniae*. *Mol. Microbiol.* 35, 566–576
26 Gronowitz, S. *et al.* (1971) Antibacterial borzaro derivatives. *Acta Pharm. Suecica* 8, 377–390
27 Lam, C. *et al.* (1987) Effect of diazaborine derivative (Sa 84.474) on the virulence of *Escherichia coli*. *J. Antimicrob. Chemother.* 20, 37–45
28 Turnowsky, F. *et al.* (1989) *EnvM* genes of *Salmonella typhimurium* and *Escherichia coli*. *J. Bacteriol.* 171, 6555–6565
29 Baldock, C. *et al.* (1996) A mechanism of drug action revealed by structural studies of enoyl reductase. *Science* 274, 2107–2110
30 Omura, S. (1981) Cerulenin. *Methods Enzymol.* 72, 520–532
31 Miyakawa, S. *et al.* (1982) Thiolactomycin, a new antibiotic IV. Biological properties and chemotherapeutic activity in mice. *J. Antibiot.* 35, 411–419
32 McMurry, L.M. *et al.* (1998) Triclosan targets lipid synthesis [letter]. *Nature* 394, 531–532
33 Heath, R.J. *et al.* (1998) Broad spectrum antimicrobial biocides target the FabI component of fatty acid synthesis. *J. Biol. Chem.* 273, 30316–30320
34 Ward, W.H.J. *et al.* (1999) Kinetic and structural characteristics of the inhibition of enoyl(acyl carrier protein) reductase by triclosan. *Biochemistry* 38, 12514–12525
35 He, X. *et al.* (2000) Development of a scintillation proximity assay for β-ketoacyl carrier protein Synthase III. *Anal. Biochem.* 282, 107–114
36 Wong, K. *et al.* (1998) Engineering a cell-free murein biosynthetic pathway: Combinatorial enzymology in drug discovery. *J. Am. Chem. Soc.* 120, 13527–13528
37 Bergsma, D.J. *et al.* (2000) Human FAS. WO051430A1
38 Qiu, X. *et al.* (1999) Crystal structure of β-ketoacyl-acyl carrier protein synthase III. A key condensing enzyme in bacterial fatty acid biosynthesis. *J. Biol. Chem.* 274, 36465–36471
39 Qiu, X. *et al.* (2001) Refined structures of β-ketoacyl-acyl carrier protein synthase III. *J. Mol. Biol.* 307, 341–356
40 Qiu, X. *et al.* (1999) Molecular basis of triclosan activity involves a flipping loop in the active site. *Protein Sci.* 8, 2529–2532
41 Heath, R.J. (1999) Mechanism of triclosan inhibition of bacterial fatty acid synthesis. *J. Biol. Chem.* 274, 11110–11114
42 Levy, C.W. *et al.* Molecular basis of triclosan activity [letter]. *Nature* 398, 383–384
43 Moche, M. *et al.* (1999) Structure of the complex between the antibiotic cerulenin and its target, β-ketoacyl-acyl carrier protein synthase. *J. Biol. Chem.* 274, 6031–6034
44 Zhang, L. *et al.* (2000) Regulated gene expression in *Staphylococcus aureus* for identifying conditional lethal phenotypes and antibiotic mode of action. *Gene* 255, 297–305
45 Heerding, D. and Newlander, K.A. (2000) Antibacterial compounds. WO00071120
46 Miller, W.H. *et al.* (2000) FabI Inhibitors. WO00063187