Infection with *Mycobacterium tuberculosis* (Mtb) remains a severe global health problem that has prompted an aggressive search for new antibiotic targets and vaccine strategies for this persistent pathogen. Recently, a wide variety of genetic determinants of Mtb pathogenicity have been identified, including several genes involved in the biogenesis of the complex Mtb cell envelope. Among these are the mycolic acid cyclopropane synthases, a family of proteins that modify the major cell envelope lipids of Mtb with a diversity of cyclopropane groups at relatively conserved positions in the meromycolate chain. These lipids form a thick hydrophobic layer in the cell envelope in their covalently linked form and form the envelope structures.

Due to the lack of defined Mtb mutants lacking specific cell envelope structures, understanding of Mtb pathogenesis have come from the creation of defined Mtb mutants that display altered pathogenesis in experimental animals (2). A distinct subset of these mutants have established the pathogenic importance of specific chemical structures in the Mtb cell envelope (3–6). The cell envelope of Mtb is a complex structure that contains many unique lipids and glycolipids including mycolic acids, lipoarabinomannan, trehalose dimycolate, and phthiocerol dimycocerosate (7, 8). These compounds are suspected virulence effectors based on their in vitro activities, but the genetics of their biosynthesis and exact role in pathogenesis has been unclear.

Mycolic acids are very long chain α-alkyl, β-hydroxy fatty acids that are unique to mycobacteria and are greater than 80 carbons in Mtb (9). These lipids form a thick hydrophobic layer in the cell envelope in their covalently linked form and form the lipid groups in Trehalose Dimycolate, an immunomodulatory glycolipid that is noncovalently associated with the Mtb cell envelope (10–12). In pathogenic mycobacteria, but not in non-pathogenic species, mycolic acids are modified with cyclopropyl groups at relatively conserved positions in the meromycolate chain (see Fig. 1 for structures). These cyclopropane residues are added to Mtb mycolic acids by a family of S-adenosylmethionine-dependent methyltransferases that exhibit exquisite substrate specificity for their lipid substrates (9, 13). One such cyclopropane synthase, *pcaA*, was recently shown to be crucial to Mtb persistence and virulence in vivo (5).

Because of its pathogenic importance, we are systematically studying the cyclopropane modification of mycolic acids in Mtb by deleting each putative cyclopropane synthase from *M. tuberculosis* and studying these mutant strains for alterations in cyclopropane content of mycolic acids and alterations in pathogenesis. This approach has been highly informative in elucidating the biosynthetic role of two members of this gene family. *PcaA* was shown to synthesize the proximal cyclopropyl group of the α-mycolate molecule (see Fig. 1) whereas *cmaA2* was shown to be the *trans*-cyclopropane synthase of the oxygenated mycolates (14). Based on these insights, the structural basis for the substrate specificity of these enzymes is being elucidated (15). In this study we report the role of *mmaA2* in mycolic acid modification through the characterization of an Mtb *mmaA2* null mutant. In contrast to its previously assigned function in methoxymycolate modification (16–18), *mmaA2* has an unexpected and non-redundant role in α-mycolate modification and a partially redundant role in methoxymycolate modification.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Media**—Mtb Erdman EF2 is an animal-passaged strain previously described (19). *Mycobacterium smegmatis* mc2155 has been previously described (19). Mtb strains were grown in 7H9 broth or 7H10 agar with 10% oleic acid-albumin-dextrose-catalase supplement (OADC), 0.5% glycerol, 0.05% Tween 80, and where re-
required, hygromycin B (Roche Molecular Biochemicals) (50 μg/ml) and kanamycin (20 μg/ml).

Construction of an mmaA2 Null Mutant by Allelic Exchange and Complementation—The mmaA2 and cmaA1 null mutants were constructed by allelic exchange using hygromycin-marked null alleles designed to delete the entire open reading frame. An mmaA2 null allele was constructed by PCR amplifying the 5’-flanking region of mmaA2 from Mtb genomic DNA using omg 143/144 as primers. This 634-bp PCR product includes the first 15 nucleotides of the mmaA2 open reading frame and includes SpeI and HindIII sites at the 5’ and 3’ ends of the PCR product. The 3’-flanking region of mmaA2 was amplified using omg 145/146 as a 522-bp PCR product that includes the last 14 nucleotides of the mmaA2 open reading frame and includes SpeI and HindIII sites at the 5’ and 3’ ends of the PCR product. Both of these PCR products were cloned, sequenced, and inserted into pmsg 284 flanking the hygromycin resistance gene (14). This final plasmid, pmsg 243, was used to construct a specialized transducing phage as previously described (14, 20). Hygromycin-resistant transductants were screened for allelic exchange by southern blotting. The mmaA2 null mutant (Mt Erdman mmaA2::Hgyg) is mgb 104.

An identical strategy was employed to construct the cmaA1 null mutant. The PCR primers employed were omg 37/38 and omg 39/40, and the final cmaA1 targeting phage is pmsg 105.

For complementation of the mmaA2 mutant, a wild type copy of mmaA2 under its putative promoter was cloned from the Mtb cosmid MTY20H10. The mma locus was first isolated as a 8864 HindIII fragment. The mmaA2 gene with its putative promoter (16) was subcloned as a 1618-bp Stul/PshAI fragment into pmv306kan, a site-specific integrating vector to create pmsg 256. This single copy complementation plasmid was used to transform mgb 104, and kanamycin-resistant transformants were evaluated for restoration of wild type mycolic acid patterns by radio thin layer chromatography (TLC) of mycolic acids as described below.

Preparation and Analysis of Mycolic Acids—Mycolic acids were labeled in logarithmic phase cultures of Mtb with 50 μCi of [14C]acetate acid (PerkinElmer Life sciences) for 18 h. Mycolic acids were prepared as described previously from whole bacilli (14). For preparation of mycolic acid classes, total mycolic acids prepared from 1 liter of Mtb strains were applied to a 1 mm preparative silica gel TLC plate and developed 7 times with hexanes/ethyl acetate 95:5. Lipids were visualized by rhodamine 6G staining, scraped from the plate, and the silica extracted three times with ethyl ether. Mycolates were then reprecipitated with toluene/acetonitrile before analysis. The purity of each isolated mycolic acid class was evaluated by TLC before structural characterization.

Structural Characterization of Mycolic Acids—For NMR analysis, mycolates were dissolved in Deuterochloroform (Cambridge Isotope Laboratories) and analyzed on a Bruker 500 MHz spectrophotometer. For mass spectrometry, lipids were dissolved in dichloromethane/methanol (1:2) and analyzed by electrospray ionization mass spectrometry on a PE SCIEX API100 mass spectrometer in positive and negative ion mode. Mass spectrometry was performed after regeneration of the free acid from the mycolate methyl ester by saponification. Permanganate cleavage of mmaA2 mutant α-mycolate was performed as previously described (5) on the mycolic acid methyl ester.

RESULTS

Construction of an Mtb mmaA2 Deletion Mutant—We used a genetic approach to understand the biosynthetic role of each member of the mycolic acid methyltransferase gene family. Specifically, we constructed M. tuberculosis null mutants in each methyltransferase and deduced the mycolic acid modification function of each gene through examination of the mutant strains. This approach has been highly informative, defining the biosynthetic role of pcaA as the proximal cyclopropane synthase of the α-mycolate molecule (5) and cmaA2 as the trans-cyclopropane synthase of the oxygenated mycolates (14).

Given this prior data, the biosynthetic origin of the proximal cis-cyclopropyl group of the oxygenated mycolates and the distal cyclopropyl group of the α-mycolates remained unclear (see Fig. 1). To investigate these modifications, we attempted to disrupt mmaA2 by specialized transduction. A targeting construct for mmaA2 was designed to replace the mmaA2 open reading frame with a hygromycin resistance cassette. Although a polar effect on the downstream genes (mmaA3 and mmaA4) is possible, previous experimentation (16) has shown that mmaA3 can be expressed in M. smegmatis from a promoter 3’ of mmaA2. In addition, a polar effect on mmaA3 would be easily detectable by the loss of both methoxymycolates and ketomycolates that would result (6). To disrupt mmaA2, wild type M. tuberculosis Erdman was infected with a specialized transducing phage carrying an mmaA2 knockout construct designed to delete the entire mmaA2 open reading frame. Hygromycin-resistant transductants were screened for allelic exchange at mmaA2 by Southern blotting. All of the hygromycin-resistant transductants contained the mmaA2 disruption (Fig. 2). This Mtb [mmaA2::Hgyg] strain was designated mgb 104 and characterized further.

MmaA2 Is Required for α-Mycolate Cyclopropanation—To determine the function of mmaA2 in mycolic acid modification, we prepared total mycolic acids from the mmaA2 null mutant and wild type cells and examined these lipids by two-dimensional argmentation TLC. We have used this system previously to assign functions to pcaA and cmaA2 (5, 14). In this system, lipids are separated by polarity in the first dimension and then separated by degree of unsaturation in the second dimension through the use of silver impregnation of the TLC plate. In the absence of a mycolic acid cyclopropane modification, the affected mycolic acids acquire a double bond at the site of the missing cyclopropane ring. These unsaturated mycolic acid derivatives are retarded in their migration in the silver dimension of the TLC plate but maintain the polarity of the parent mycolate.

Wild type Mtb contains three major classes of mycolic acids, α-mycolates, methoxymycolates, and ketomycolates (see Fig. 1 for structures and Fig. 3A for TLC pattern). In the mmaA2 null mutant, the α-mycolate was replaced by a new mycolic acid species that was retarded by silver ions (Fig. 3B, arrow). A small amount of intact α-mycolate is synthesized in the absence of mmaA2. In addition, an unsaturated derivative of the methoxymycolates accumulates in the mmaA2 null mutant (Fig. 3B, arrowhead). To confirm that these mycolic acid derivatives are secondary to the mmaA2 mutation, we transformed the mmaA2 mutant with a complementing single copy plasmid expressing mmaA2 under its putative promoter. Both the α- and methoxymycolic acid defects are attributable to the
mmaA2 mutation as the complemented strain displays wild type mycolic acid patterns (Fig. 3C). These results establish a role for mmaA2 in both α- and methoxymycolate modification. CmaA1 Is Not Required for α-Mycolate Modification—Previous examination of the other members of the mma gene cluster by overexpression in *M. smegmatis* or *M. tuberculosis* had suggested that the mma gene cluster was involved in methoxy-mycolate synthesis (16, 18, 21). Thus, the defective α-mycolate cyclopropanation in the mmaA2 mutant was surprising. The mycolic acid cyclopropane synthetase pcaA cyclopropanates the proximal position of the Mtb α-mycolate (5), and cmaA1 was suspected to cyclopropane the distal position of the α-mycolate based on its activity when overexpressed in *M. smegmatis* (22). To clarify the role of cmaA1 in α-mycolate modification, we constructed a cmaA1 null mutant and examined the mycolic acid phenotype of this strain. The α-mycolate of the cmaA1 null mutant is unaffected by silver impregnation TLC, demonstrating that cmaA1 is not required for α-mycolate modification under the conditions tested (Fig. 3D). These results suggest that cmaA1 may modify lipids other than mycolic acids or that the mycolic acid modification function of cmaA1 is not evident in vitro.

**MmA2 Mutant α-Mycolate Contains a Double Bond and a Cyclopropyl Group**—To specifically define the function of mmaA2 in mycolic acid modification, we purified each mycolic acid class by preparative thin layer chromatography from mmaA2 mutant and wild type cells. These mycolic acid classes were examined first by 500 MHz 1H NMR. The α-mycolate from the mmaA2 mutant contained both cis-cyclopropyl protons and a resonance at 5.33 ppm, consistent with vinyl protons (Fig. 4). The coupling constant of these vinyl protons is 5 Hz, consistent with cis geometry. Vinyl resonances were absent from wild type α-mycolate (Fig. 4). Integration of cyclopropyl proton resonances at −0.33 ppm 1H, and the terminal methyl ester protons at 3.7 ppm 3H revealed a ratio of 1.58:1 (predicted 3:2 or 1.5:1 for dicyclopropanated α-mycolate) in wild type and 2.46:1 (predicted 3:1 in monocy clopropanated mutant α-mycolate). The slight apparent excess of cyclopropyl protons in the mutant α-mycolate likely represents the small amount of intact α-mycolate synthesized in the mutant strain (see Fig. 3). These findings confirm that the mmaA2 mutant α-mycolate is lacking a single cyclopropane group and contains a double bond. To confirm this structure in more detail, we performed two-dimensional 1H COSY NMR of the mmaA2 mutant α-mycolate. This study revealed that the vinyl protons are adjacent to protons at 2.02 ppm, consistent with previously reported resonances of vinyl protons in meromycolate chains (data not shown) (23). These findings are similar to our previous NMR findings when examining the α-mycolate of the pcaA mutant and establish that the mmaA2 mutant α-mycolate lacks either a proximal or distal cyclopropyl group. However these findings do not establish which cyclopropane ring is absent.

To further define the structural variation in the mmaA2 mutant mycolic acids, we examined each mycolic acid class from wild type and mutant cells by electrospray ionization mass spectroscopy. Wild type α-mycolate contained a series of compounds differing by 28 atomic mass units reflecting chain length variation of two methylene units, as is expected for Mtb mycolic acids (9). The predominant α-mycolate of wild type Mtb Erdman appeared as a sodium adduct in positive ion mode corresponding to a molecular mass of 1151 atomic mass units (Fig. 5). This mass corresponds to a dicyclopropanated α-mycolate of 79 total carbons with a 24-carbon α branch as pictured in Fig. 1 and as reported previously for other strains of Mtb (24, 25). In addition, peaks at 1123 and 1179 reflect smaller and longer chain length variants by two methylene units. Spectra of purified methoxy-and ketomycolates from wild type strains were as reported previously for *M. tuberculosis* (21).

MmA2 mutant α-mycolate contained a similar series of peaks differing by 28 atomic mass units, but were smaller by 14 atomic mass units compared with wild type lipids (Fig. 5). Consequently, the two most abundant α-mycolates in the mmaA2 mutant have molecular masses of 1137 and 1165 atomic mass units with lower abundance isomers at 1132 atomic mass units. These compounds are consistent with a monocy clopropanated, monounsaturated α-mycolate of the same chain lengths as the parent wild type α-mycolate. In addition, *mmaA2* mutant α-mycolate contains a variant with a molecular mass of 1179 atomic mass units, consistent either with some intact α-mycolate of the longest chain length seen in wild type lipids, or a monounsaturated α-mycolate with a methyl branch. The latter is unlikely because NMR of mmaA2 mutant α-mycolate did not reveal a doublet at 0.95–1.05 characteristic of methyl branches in meromycolate chains (16). In addition, the mmaA2 mutant α-mycolate contains a series of peaks of higher molecular weight at 1208, 1236, and 1264 of unclear identity (Fig. 5).

**MmA2 Is a Distal Cyclopropane Synthase of the α-Mycolate**—Based on the assigned function of pcaA in proximal cyclopropane modification of the α-mycolate, the normal α-mycolate in the cmaA1 mutant, and the data presented above, we hypothesized that mmaA2 synthesizes the distal cyclopropane ring of the α-mycolate molecule. To examine this hypothesis, we oxidized the purified α-mycolate from the mmaA2 mutant with potassium permanganate and examined the cleavage products of this reaction by mass spectroscopy. Potassium permanganate oxidizes lipids at the site of double bonds and...
therefore can be used to determine the position of unsaturations in lipid chains. This strategy is similar to that used previously to determine the position of the unsaturation in the M. tuberculosis pcaA mutant/H9251-mycolate (5). Cleavage of the mmaA2 mutant/H9251-mycolate was performed alongside cleavage of the wild type/H9251-mycolate, and both reactions were analyzed by electrospray ionization mass spectroscopy. Cleavage of the mmaA2 mutant/H9251-mycolate produced a base peak of 349 atomic mass units, consistent with the sodium adduct of oxidative cleavage at a distal double bond situated 21 carbons from the end of the meromycolate chain (data not shown). This spacing of the cyclopropyl group/double bond is consistent with the known structure of Mtb/H9251-mycolate as pictured in Fig. 1 (25). This fragment was not detected in wild type/H9251-mycolate cleaved with potassium permanganate. The proximal fragment of cleavage at a distal double bond was detected but of lower abundance than the distal fragment. Neither of the possible fragments from proximal cleavage was detected. This experiment was repeated with similar results. Thus, the mutant α-mycolate in the mmaA2 mutant lacks a distal cyclopropane group, and therefore mmaA2 is necessary for the distal cyclopropane modification of the α-mycolate, an enzymatic function previously attributed to cmaA1 (22).

MmaA2 May Be the Preferred cis-Cyclopropane Synthetase of the Methoxymycolates—The thin layer chromatography analysis of the mmaA2 mutant mycolic acids presented in Fig. 3 shows accumulation of an unsaturated derivative of the methoxymycolates in addition to apparently mature methoxymycolates. These intact methoxymycolates could either represent an intact mixture of cis- and trans-cyclopropanated methoxymycolates, or only trans-cyclopropanated methoxymycolates. To determine the function of mmaA2 in methoxymycolate modification we examined purified methoxymycolates of mutant and wild type strains by NMR. In the absence of mmaA2, intact methoxymycolates with either cis- or trans-cyclopropyl groups are present, showing that mmaA2 is not absolutely required for proper methoxymycolate modification (data not shown). In addition, a resonance at 5.33 ppm in wild type mycolates from the mmaA2 mutant is consistent with a double-bonded methoxymycolate. Thus, methoxymycolates from the mmaA2 mutant

![Fig. 3. MmaA2, but not cmaA1, is required for α and methoxymycolate modification. Each panel is a two-dimensional radio 2D TLC analysis of purified mycolic acids from the indicated strain. The mycolic acids are spotted in the lower left corner of the TLC plate and developed in the first dimension, which lacks silver impregnation. This dimension separates the lipids on the basis of polarity. The plate is then developed in the second dimension, which is impregnated with silver nitrate. This dimension retards lipids with double bonds in relation to saturated or fully cyclopropanated lipids. A, wild type mycolates; B, mmaA2 mutant mycolates. The arrow and arrowhead highlight new mycolic acid species with the polarity of α and methoxymycolates but retarded in the silver dimension. C, mmaA2 mutant complemented with wild type mmaA2. D, cmaA1 deletion mutant.](image)

![Fig. 4. NMR analysis of mmaA2 mutant α-mycolate. One-dimensional 500 MHz 1H NMR analysis of purified α-mycolate from wild type Mtb (A) and the mmaA2 null mutant (B). In the absence of mmaA2, the α-mycolate has acquired a double bond visible at 5.3 ppm (panel B, arrow and inset).](image)

![Fig. 5. Mass spectroscopy of wild type and mmaA2 mutant purified α-mycolate. Peaks represent sodium adducts (+23) of free acid mycolic acids. The top panel is wild type α-mycolate, and the lower panel is mmaA2 mutant α-mycolate. The peaks differing by 28 atomic mass units represent lipid chain length variation of 2 methylene units characteristic of all mycolic acids.](image)
contain both intact and unsaturated methoxymycolates. By comparing the cis- and trans-cyclopropyl resonances from purified methoxymycolates we estimated that wild type methoxymycolates contain a cis/trans ratio of 10:1, whereas mmaA2 mutant methoxymycolates contain a 5:1 ratio. As a control, we performed the same examination of cis/trans ratios in purified ketomycolates from wild type and mutant strains and found these ratios unchanged by the mmaA2 mutation. Thus, in the absence of mmaA2, the relative cis-cyclopropane content of methoxymycolates is reduced by half. These results suggest that in the absence of mmaA2, intact methoxymycolates are synthesized, but the efficiency of cis-methoxymycolate cyclopropanation is reduced, leading to accumulation of a cis double-bonded methoxymycolate derivative and a relative overabundance of trans-cyclopropanated methoxymycolate.

DISCUSSION

The cyclopropane modification of mycolic acids in M. tuberculosis is a unique lipid structure that has been linked to the pathogenesis of this infection. Mtb uses a family of S-adenosylmethionine-dependent methyltransferases to modify the mycolic acids of its cell envelope with a variety of stereochemistries and positions of cyclopropyl group. Given the pathogenic importance of this lipid modification, we are systematically studying the biochemical and pathogenetic function of this gene family through the creation of Mtb null mutants in each synthase. Through this approach, we have defined the pathogenetic role and biochemical function of pcaA as a proximal cis-cyclopropane synthase α-mycolate molecule essential for Mtb virulence in mice (5). In addition, we have defined cmaA2 as the trans-cyclopropane synthase of both the methoxy- and ketomycolates (14). This study establishes the biosynthetic specificity of a third mycic acid cyclopropane synthase, mmaA2.

Role of mmaA2 in α-Mycolate Modification—Previous examinations of mmaA2 from M. tuberculosis and its homologue from Mycobacterium bovis BCG had been by overexpression of the gene in M. smegmatis, a nonpathogenic mycobacteria that does not produce cyclopropanated mycolic acids (16, 18). These studies revealed that mmaA2 cyclopropanated both the enoxy-mycolate and α-mycolate of M. smegmatis at the proximal position. When mmaA2 was expressed along with the other genes in the mma gene cluster, an intact methoxymycolate was produced. Thus, in M. smegmatis, the activity of mmaA2 was nonspecific, similar to the activity of other cyclopropane synthases in this host (22, 26). As has been shown previously for pcaA and cmaA2, the construction of null mutants in each cyclopropane synthase is a powerful method to deduce the specific biosynthetic function of this gene family. For mmaA2, deletion of the gene from M. tuberculosis revealed an unexpected non-redundant role in the distal cyclopropane modification of the α-mycolate. This function was previously ascribed to cmaA1 (22), a cyclopropane synthase that has no discernible role in mycic acid modification as determined in this study by construction of a cmaA1 null mutant. This surprising role for mmaA2 in distal α-mycolate modification means that mmaA2 is biosynthetically closely linked to pcaA, the proximal cyclopropane synthase of the α-mycolate. Interestingly, the mycic acid phenotypes of the mmaA2 and pcaA mutant differ in ways other than distal versus proximal cyclopropanation. For reasons that are unknown, the pcaA mutant accumulates large amounts of ketomycolates (5), a phenotype that is absent from the mmaA2 mutant (Fig. 3). Although the protein structure of MmaA2 has not yet been solved, comparison of this structure to PcaA may provide further insight into the exquisite catalytic specificity of this gene family (15). Careful examination of the mass spectrum of the mmaA2 mutant α-mycolate revealed that the small amount of intact α-mycolate synthesized in the mutant was only one chain length. This 11-carbon intact α-mycolate is the longest major α-mycolate synthesized in wild type cells, and this result may indicate that some unidentified cyclopropane synthase can modify the distal position in the absence of mmaA2, but that this redundancy is limited to longer chain length lipids.

Role of mmaA2 in Methoxymycolate Modification—The role of mmaA2 in methoxymycolate modification is less clear due to probable redundancy with other cyclopropane synthases. The mmaA2 null mutant accumulates unsaturated derivatives of methoxymycolates but still synthesizes intact methoxymycolates. Characterization of the mmaA2 mutant methoxymycolate by NMR revealed a 2-fold reduction in the relative abundance of cis-cyclopropanated methoxymycolate. This data is most consistent with a role for mmaA2 as the preferred cis-cyclopropane synthase of the methoxymycolates, as had been suggested by its function in M. smegmatis and its genomic organization alongside other putative methoxymycolate biosynthetic genes. However, the production of cis-cyclopropanated methoxymycolate in the mmaA2 mutant demonstrates that another enzyme can perform this function. The most likely candidate for this redundant function is cmaA2, the trans-cyclopropane synthase of the methoxy- and ketomycolates. Although cis-cyclopropanated methoxymycolates are produced in the cmaA2 mutant (14), cmaA2 does have nonspecific cis-cyclopropanating activity in M. smegmatis (26), suggesting that it could serve a cis synthase in the absence of mmaA2.

The new insights into mmaA2 function presented here expand our knowledge of the biosynthesis of the complex M. tuberculosis cell envelope and the biosynthetic specificity of the mycic acid cyclopropane synthases. In addition, the mmaA2 null mutant provides another defined mutant in cell envelope biosynthesis that can be tested in animal models of pathogenesis. It will be particularly interesting to compare the pathogenesis phenotype of the mmaA2 mutant to the pcaA mutant phenotype as these two mutants differ predominantly in the position of the missing cyclopropyl group in the α-mycolate. Characterization of these mutant strains will provide further insight into the relationship between the fine chemical structure of the Mtb cell envelope and specific pathogenesis phenotypes. This information may help validate the mycic acids modification system as an attractive drug target for new antituberculosis drugs and may reveal novel mechanisms by which the host immune system recognizes this fine structure of mycobacterial lipids.

Acknowledgments—We thank Paola Bongiorno, MS, for expert technical assistance and George D. Sukenick, Ph.D., and Sean Cahill, Ph.D., for assistance with NMR studies.

REFERENCES

1. Dye, C., Scheele, S., Dolin, P., Pathania, V., and Raviglione, M. C. (1999) J. Am. Med. Assoc. 282, 677–684.
2. Glickman, M. S., and Jacobs, W. R., Jr. (2001) Cell 104, 477–485.
3. Camacho, L. R., Ensergueix, D., Perez, E., Gicquel, B., and Guilhot, C. (1999) Mol. Microbiol. 34, 257–267.
4. Cox, J. S., Chen, B., McNeil, M., and Jacobs, W. R., Jr. (1999) Nature 402, 79–83.
5. Glickman, M. S., Cox, J. S., and Jacobs, W. R., Jr. (2000) Mol. Cell 5, 717–727.
6. Dubnau, E., Chan, J., Raymond, C., Mohan, V. P., Lanceille, M. A., Yu, K., Quemard, A., Smith, I., and Daffe, M. (2000) Mol. Microbiol. 36, 630–637.
7. Brennan, P. J., and Nikaido, H. (1996) Annu. Rev. Biochem. 64, 29–63.
8. Daffe, M., and Draper, P. (1998) Adv. Microb. Physiol. 39, 131–203.
9. Barry, C. R., Lee, E. R., Mdluli, K., Sampson, A. E., Schroeder, B. G., Slavysen, R. A., and Yuan, Y. (1998) Prog. Lipid Res. 37, 143–179.
10. Peres, R. L., Roman, J., Rosen, S., Little, C., Oben, M., Indriago, J., Hunter, R. L., and Actor, J. K. (2000) J. Interferon Cytokine Res. 20, 795–804.
11. Oswald, I. P., Dozios, C. M., Fournout, S., Petit, J. F., and Lemaire, G. (1999) Eur. Cytokine Netw. 10, 533–542.
12. Oswald, I. P., Dozios, C. M., Petit, J. F., and Lemaire, G. (1997) Infect. Immun. 65, 1364–1369.
13. Schroeder, B. G., and Barry, C. E., III (2001) Bioorg. Chem. 29, 164–177.
mma2 of *M. tuberculosis*

14. Glickman, M. S., Cahill, S. M., and Jacobs, W. R., Jr. (2001) *J. Biol. Chem.* 276, 2228–2233
15. Huang, C. C., Smith, C. V., Glickman, M., Jacobs, W. R., Jr., and Sacchettini, J. C. (2001) *J. Biol. Chem.* 277, 13106–13114
16. Yuan, Y., and Barry, C. E., III (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 12828–12833
17. Dubnau, E., Marrakchi, H., Smith, I., Daffe, M., and Quemard, A. (1998) *Mol. Microbiol.* 29, 1526–1528
18. Dubnau, E., Laneille, M. A., Soares, S., Benichou, A., Var, T., Prome, D., Prome, J. C., Daffe, M., and Quemard, A. (1997) *Mol. Microbiol.* 23, 315–322
19. Snapper, S. B., Melton, R. E., Mustafa, S., Kieser, T., and Jacobs, W. R., Jr. (1990) *Mol. Microbiol.* 4, 1911–1919
20. Bardarov, S., Bardarov, S., Jr., Pavelka, M. S., Jr., Sambandamurthy, V., Larsen, M., Tufariello, J., Chan, J., Hatfull, G., and Jacobs, W. R., Jr. (2002) *Microbiol.* 148, 3007–3017
21. Yuan, Y., Crane, D. C., Musser, J. M., Sreevatsan, S., and Barry, C. E., III (1997) *J. Biol. Chem.* 272, 10041–10049
22. Yuan, Y., Lee, R. E., Besra, G. S., Belisle, J. T., and Barry, C. E., III (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 6630–6634
23. Yuan, Y., Zhou, Y., Crane, D. D., and Barry, C. E., III (1998) *Mol. Microbiol.* 29, 1449–1458
24. Watanabe, M., Anyagi, Y., Mitome, H., Fujita, T., Naoki, H., Ridell, M., and Minnikin, D. E. (2002) *Microbiol.* 148, 1881–1902
25. Watanabe, M., Anyagi, Y., Ridell, M., and Minnikin, D. E. (2001) *Microbiol.* 147, 1825–1837
26. George, K. M., Yuan, Y., Sherman, D. R., and Barry, C. E., III (1995) *J. Biol. Chem.* 270, 27292–27298
