An acyltransferase induced by cold shock in *Escherichia coli*, designated LpxP, incorporates a palmitoleoyl moiety into nascent lipid A in place of the secondary laurate chain normally added by LpxL (HtrB) (Carty, S. M., Sreekumar, K. R., and Raetz, C. R. H. (1999) *J. Biol. Chem.* 274, 9677–9685). To determine whether the palmitoleoyl residue alters the properties of the outer membrane and imparts physiological benefits at low growth temperatures, we constructed a chromosomal insertion mutation in *lpxP*, the structural gene for the transferase. Membranes from the *lpxP* mutant MKV11 grown at 12 °C lacked the cold-induced palmitoleoyltransferase present in membranes of cold-shocked wild type cells but retained normal levels of the constitutive lauroyltransferase encoded by *lpxL*. When examined by mass spectrometry, about two-thirds of the lipid A molecules isolated from wild type *E. coli* grown at 12 °C contained palmitoleate in place of laurate, whereas the lipid A of cold-adapted MKV11 contained only laurate in amounts comparable with those seen in wild type cells grown at 30 °C or above. To probe the integrity of the outer membrane, MKV11 and an isogenic wild type strain were grown at 30 °C or above. To determine whether the palmitoleoyltransferase encoded by *lpxP* confers a selective advantage compared with wild type cells but showed identical resistance when grown at 30 °C. We suggest that the palmitoleoyltransferase may confer a selective advantage upon *E. coli* cells growing at lower temperatures by making the outer membrane a more effective barrier to harmful chemicals.

A unique glycolipid known as lipopolysaccharide is the major component of the outer surface of the outer membranes of Gram-negative bacteria and forms a barrier around the cell (1–3). Compounds with molecular weights of less than 600 penetrate the outer membrane via porins, but larger, potentially harmful agents, including many antibiotics, are excluded (2). Lipid A is the hydrophobic anchor of the lipopolysaccharide molecule (1, 4–6). In wild type *Escherichia coli* grown at 30 °C or above, lipid A consists of a β,1′-6-linked disaccharide of glucosamine that is phosphorylated at the 1- and 4′-positions (Fig. 1) (1, 4–6). *E. coli* lipid A usually contains six acyl chains (1, 4). (R)-3-Hydroxyristoyl groups are located at positions 2, 3, 2′, and 3′ of the glucosamine disaccharide, and two short saturated acyl chains are attached to the (R)-3-hydroxyristoyl groups of the distal unit, forming acyloxyacyl moieties (Fig. 1, “Normal” lipid A) (1, 4–6). Laurate is linked to the (R)-3-hydroxyristoyl residue at position 2′, and myristate is similarly attached at position 3′ (1, 4–6). These so-called “secondary” acyl chains are the same in wild type *E. coli* and *Salmonella*.

Two inner membrane enzymes, LpxL (HtrB) and LpxM (MsbB), which act late in the lipid A pathway (Fig. 1), are responsible for the incorporation of laurate and myristate, respectively (7–9). These enzymes require the presence of a 3-deoxy-o-manno- octulosonic acid (Kdo) disaccharide in their acceptor substrates (Fig. 1) (7–9). *E. coli* mutants lacking LpxM grow at nearly normal rates (10) and contain penta-acylated lipid A species lacking myristate (9, 11). However, cells lacking LpxM are ~10,000-fold less potent in activating human macrophages than wild type *E. coli* (12). Mutants defective in LpxL or in both LpxL and LpxM do not grow above 32 °C on rich medium (10, 13), show increased sensitivity to antibiotics (14), and accumulate tetra-acylated lipid A moieties in their inner membranes at 42 °C concomitant with cessation of growth (11).

Recently, a cold shock-induced enzyme that catalyzes the transfer of a palmitoleoyl chain from palmitoleoyl-acetyl carrier protein to the lipid A precursor Kdo4-lipid IVα was discovered in our laboratory (15). The greater similarity of the palmitoleoyltransferase (LpxP) to the lauroyltransferase (LpxL) than to the myristoyltransferase (LpxM), and the ability of LpxP to use the same Kdo4-lipid IVα substrate as LpxL (Fig. 1), led us to suggest that LpxP acylates the 2′ (R)-3-hydroxyristoyl chain (15). LpxP activity is induced within 15 min following a shift of exponentially growing cells from 37 to 12 °C and peaks after 2 h (15). Appearance of palmitoleoyltransferase activity is correlated with massive accumulation of LpxP mRNA at 12 °C (15).

Modification of bacterial cell membranes in response to cold shock is not unprecedented (16, 17). Following exposure to low
temperatures, some Gram-positive bacteria, like Bacillus subtilis, produce a fatty acid desaturase encoded by des, resulting in production of glycerophospholipids with unsaturated acyl chains in addition to the branched chains normally present in Gram-positive bacteria (17, 18). The fatty acids of E. coli glycerophospholipids contain some unsaturated acyl chains at all growth temperatures (19), but their composition shifts toward greater unsaturation during cold shock because of increased β-ketoacyl-ACP synthase II (FabF) activity (16). Additional acyltransferases that selectively incorporate unsaturated fatty acyl chains into glycerophospholipid are not induced by cold shock in E. coli (16). The constitutive glycerol-3-phosphate acyltransferases of E. coli utilize whatever acyl donors are most abundant in the acyl-ACP and acyl-coenzyme A pools (20–22). LpxP is the first example of a specific acyltransferase that is induced by cold shock (15).

The incorporation of an unsaturated fatty acyl chain into lipid A might alter the physical properties of the outer membrane to benefit the cell in cold environments. We have there-
analyzed by agarose gel electrophoresis, and the 1485-bp fragment containing the kan element was purified from the agarose. pSK57 was digested with NotI, which cuts the lpxP gene at position 417 (15, 25). The kan cassette was then ligated with T4 DNA ligase into pSK57 at this position using the compatible NotI and EcoRI overhangs ending to create plasmid pSK57kan. The lpxP gene was expressed from the T7 promoter of pSK57kan via PCR using primers to the 5′-5′-PGCGGGCCGATATGTTTTCACAATGTC-3′ and 3′-3′-PGCGGGCCGATATGTTTTCACAATGCAAA-3′ ends of lpxP. pMAK705, a vector with a gene for chloramphenicol resistance and a temperature-sensitive origin of replication, was digested with HincII, which yields blunt ends (26). The PCR product containing lpxP-kan was blunt-end ligated into this site, generating the plasmid pSMC9.

Creation of the lpxP-kan Insertion Mutant MKV11—MKV11 (MC1061 lpxP-kan) was constructed according to published procedures (26, 27). pSMC2 was transformed into wild type MC1061 using 100 mM CaCl2 treatment, followed by heat shock (24). The selection for bacterial colonies containing pSMC2 was performed at 30 °C on LB plates containing chloramphenicol. After growth of a single colony to mid log phase in 1 L of medium containing chloramphenicol, cell pellets, and a combination of chloramphenicol and kanamycin at 10 mg/ml, were transformed into MC1061 and plated on LB agar containing chloramphenicol. After three cycles of growth at 30 °C to stationary phase with 100-fold back dilution of a colony containing an integrated plasmid, selection for a recombinant that had retained lpxP-kan on the chromosome and had transferred the intact lpxP gene to the temperature-sensitive covering plasmid was performed in the following manner. Single colonies of bacteria were selected for resistance to chloramphenicol but not 44 °C by replica plating (27). Mutant MKV11, which was identified in this manner, subsequently was found to grow on LB broth in the presence or absence of kanamycin at 30 and 44 °C, indicating that the lpxP covering plasmid could be cured without loss of viability.

A polymerase chain reaction using primers specific to the 5′-5′-GCAACCGGCCATATGTTTTCACAATGCAAA-3′ and 3′-3′-GCAAGCTTCTCGAATTACACGAGG-3′ ends of lpxP was performed on genomic DNA obtained from the mutant strain, MKV11, as well as on the excised plasmid pMKV11, isolated from the mutant prior to curing at 44 °C. PCR products were run on a 1% agarose gel, and band sizes were compared with molecular weight standards. A band of ~1 kb, the size of the intact lpxP gene, was produced from the wild type genomic DNA and from the pMKV11 plasmid, verifying that the intact lpxP gene had been excised from the chromosome with the plasmid. However, a band of ~2.4 kb, the size of lpxP-kan, was observed from the genomic DNA obtained from the mutant strain, MKV11, confirming that the lpxP gene was disrupted.

Growth Rate Comparisons— Cultures of wild type and mutant strains were grown at 12 °C starting at an A600 of ~0.03. The A600 was measured every 6–8 h. For prolonged growth rate comparisons, cultures were diluted into fresh medium at regular intervals to maintain cell densities below 0.6.

Preparation of Substrates for Enzymatic Assays—Carrier (Kdo)-4-lipid IVa was prepared by enzymatic synthesis on a 5-mg scale. Acyl-ACP synthase (30). Previous procedures (31) were modified by including an additional purification step (an octyl-Sepharose column) (32) to remove residual non-acetylated ACP, which may be present at higher amounts with unsaturated fatty acids because of the reduced activity of acyl-ACP synthase with palmitoleate as the substrate (33).

Cold Shock Conditions and Preparation of Membranes for Use in Enzyme Assays—Shaking cultures (typically 100 ml) were grown on LB broth at 30 °C to A600 of ~0.4. The cultures were divided into two equal portions. One was incubated further at 30 °C for 2 more hours, and the other was shifted to 12 °C for 2 h. Cells were then harvested by centrifugation at 5000 × g in a Beckman JA4.3 swinging bucket rotor, washed in 10 ml of 30 mM Hepes, pH 7.5, centrifuged a second time, resuspended in 5 ml of Hepes buffer, passed through passage 15 small French pressure cell at 10,000 pounds/square inch. Cell debris was removed by centrifugation at 4000 × g for 10 min, and the resulting cell-free extract was subjected to ultracentrifugation to 100,000 × g for 60 min in a Beckman Ti-70.1 rotor. The supernatant was decanted, and the membranes were homogenized in Hepes buffer. The sample was subjected to a second 60-min ultracentrifugation to remove residual soluble molecules and then resuspended in Hepes buffer to yield final protein concentrations of 1–5 mg/ml, as determined using the BCA assay (34).  

Assay for Palmitoyltransferase Activity—Assay mixtures (15 μl final volume), containing 50 mM Hepes, pH 7.5, 250 mM NaCl, 5 mM MgCl2, 0.1% Triton X-100, 6 μM Kdo-4-2-P-lipid IVa (80,000 ppm/μmol) and 25 μM palmitoyl-ACP, were incubated at 37 °C for 60 min in a Beckman Ti-70.1 rotor. The supernatant was decanted, and the reactions were incubated at 12 °C for 8 h (15), and then 5-μl portions were spotted onto a Silica Gel 60 TLC plate. Substrate and products were resolved by chromatography in the system chloroform, pyridine, 88% formic acid, water (30:70:16:10, v/v). The plate was dried and scanned to a Molecular Dynamics PhosphorImager screen, which was analyzed using ImageQuant version 1.2 software for the Macintosh.

Assay for Lauroyltransferase Activity—Assay mixtures (15 μl final volume) (8), containing 50 mM Hepes, pH 7.5, 50 mM NaCl, 5 mM MgCl2, 0.1% Triton X-100, 6 μM Kdo-4-2-P-lipid IVa (80,000 ppm/μmol) and 12.5 μM lauroyl-ACP, as indicated, were precultured at 30 °C for 10 min. Cell membranes were diluted and added to the reaction mixtures to a final protein concentration of 0.1 mg/ml. The reactions were incubated at 30 °C for 10 min at which point 5-μl portions were spotted onto a Silica Gel 60 TLC plate, which was developed and analyzed as described above for the palmitoyltransferase.

Cloning and Expression of the lpxP Gene Behind a T7lac Promoter—The plasmid lpxP structural gene was obtained by PCR using pSK57 (15) as the template. Primers specific for lpxP containing an NdeI restriction site at the 5′ end (5′-GCAACCGGCCATATGTTTTCACAATGCAAA-3′) and an Xhol restriction site at the 3′ end (5′-GCAGTCTCTCGAGTAAATTGTACACGACG-3′) were used. The PCR product was digested with NdeI and Xhol and ligated into the similarly digested vector, pET21a (Novagen). The resulting hybrid plasmid, pMKV2, was transformed into BLR(DE3)pLysS cells. Cell membranes were prepared from selected transformants by sonication, and enzyme activity of the T7lac promoter, which is activated following T7 polymerase expression upon induction with IPTG. Overexpression and optimal enzymatic activity of LpxP was achieved by growing the plasmid-containing cells in LB broth at 37 °C to an A600 of ~0.5, followed by induction with 1 mM IPTG and further incubation at 12 °C for an additional 10 h or at 37 °C for 3 h. Cell membranes from BLR(DE3)pLysS cells containing either pMKV2 or the pET21a+ vector control, grown with and without IPTG, were isolated and washed as above. The palmitoyltransferase assay was performed on these membranes as described above, except that the protein concentration was 0.005–0.01 mg/ml in the assay mixture, and the incubation time was reduced to 5 min at 12 °C, as indicated.

Antibiotic Minimal Inhibitory Concentrations— Cultures were grown from an initial A600 of 0.01 in the presence of a range of antibiotic concentrations for 24 h at 30 °C or for 72 h at 12 °C. The minimal inhibitory concentration was defined as the lowest antibiotic concentration at which no measurable bacterial growth was observed. Experiments were performed in triplicate.

Isolation and Mass Spectrometry of Lipid A Samples—Typically, 200-ml cultures were grown at 12 °C from A600 of ~0.2 to A600 ~0.5. Cells were collected by centrifugation at 5000 × g in a Beckman JA14 rotor at 4 °C and resuspended in 40 ml of phosphate buffered saline (35). Lipid A was isolated from chloroform/methanol-extracted cells by hydrolysis of the residue at 100 °C in 12.5 mM sodium acetate, pH 4.5, and 1% SDS, as described previously (36). Lipid A 1,4′-bisphosphate species were resolved on a 1-ml DEAE-cellulose column equilibrated in water (1:9, v/v), which was eluted stepwise with increasing concentrations of ammonium acetate (36). Spectra were acquired in the negative ion linear mode using a Kratos Analytical (Manchester, UK) matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometer equipped with a 337 nm nitrogen laser, a 20-kV extraction voltage, and time delayed extraction. Each spectrum was the average of 50 shots. The lipid A 1,4′-bisphosphate samples were prepared for MALDI/TOF analysis by depositing 0.3 μl of the sample dissolved in chloroform/methanol (4:1 v/v), followed by 0.3 μl of the matrix, which was a mixture of saturated 6-aza-2-thiothymine in 50% acetonitrile and 10% tribasic ammonium citrate (9.1 v/v). The sample mixtures were allowed to dry at room temperature prior to mass analysis. Hexa-acylated lipid A 1,4′-bisphosphate from wild type E. coli (Sigma) was used as an external standard for calibration.

RESULTS

The Effect of an lpxP Insertion Mutation on E. coli Growth at 12 °C—MKV11 is a mutant of E. coli MC1061 in which a
kanamycin gene cassette is inserted into the lpxP gene (Table I). Because palmitoleoyltransferase activity is not present in MC1061 at 30 °C (15), the insertion mutation should have no effect on viability or growth at 30 °C and above. This was verified by our ability to cure the lpxP mutant of its lpxP covering plasmid at 44 °C. However, given the fact that lpxP is induced by cold shock, it was possible that lpxP might be required for growth below 30 °C. Consequently, the growth of MKV11 at 12 °C was compared with that of MC1061. As shown in Fig. 2, the lpxP::kan strain grows at approximately the same rate as wild type E. coli at low temperatures.

Absence of Palmitoleoyltransferase Activity in the lpxP Mutant—Multiple copies of the plasmid pSK57 (15), which contains a 5.6-kb segment of the E. coli genome including the lpxP gene, direct the overproduction of palmitoleoyltransferase activity (15). It was therefore anticipated that a disruption of the lpxP gene would completely eliminate palmitoleoyltransferase activity from the cell. Cultures of MC1061 and MKV11 were grown to early log phase, and a portion of each culture was shifted to 12 °C for 2 h. Membranes were then isolated and assayed for palmitoleoyltransferase activity. As shown in Fig. 3, conversion of the acceptor substrate, (Kdo)2-4P-lipid IVA, to a more hydrophobic species representing (Kdo)2-4P-lipid IVA only occurred in the cold-shocked, wild type cells and was absolutely dependent upon the inclusion of palmitoleoyl-ACP in the reaction system. No residual palmitoleoyltransferase activity from the cell. Cultures of MC1061 and MKV11 were grown to early log phase, and a portion of each culture was shifted to 12 °C for 2 h. Membranes were then isolated and assayed for palmitoleoyltransferase activity. As shown in Fig. 3, conversion of the acceptor substrate, (Kdo)2-4P-lipid IVA, to a more hydrophobic species representing (Kdo)2-4P-lipid IVA only occurred in the cold-shocked, wild type cells and was absolutely dependent upon the inclusion of palmitoleoyl-ACP in the reaction system. No residual palmitoleoyltransferase activity was detected in MKV11. This finding is consistent with the proposal that lpxP is the structural gene for the palmitoleoyltransferase. A small amount of a more rapidly migrating radioactive species (Fig. 3, lane 6) likely arises from the action of the outer membrane enzyme PagP (37), which incorporates a palmitate chain on the hydroxymyristate residue at the 2-position of the proximal unit (Fig. 1), using endogenous glycerophospholipids as donors (38). PagP activity is greatly induced under conditions that activate the PhoP/PhoQ two-component signaling system (37) but not under the growth conditions employed in the present study.

Normal Levels of Lauroyltransferase Activity in Membranes of MKV11—Lauroyltransferase activity (7, 8) was assayed in cell membranes isolated from both MC1061 and MKV11, with and without exposure to cold shock. As shown in Fig. 4, normal amounts of lauroyltransferase activity are present in the membranes of both strains, irrespective of cold shock treatment. The specific activity for the transferase in all membrane preparations is approximately the same (1.04 ± 0.11 nmol/min/mg). In addition to confirming the idea of distinct genes encoding for the lauroyltransferases and palmitoleoyltransferases (Fig. 1), this result also shows that expression of the lauroyltransferase is not affected by cold shock or by the absence of the palmitoleoyltransferase.

Mass Spectrometry of Lipid A from Wild type E. coli and MKV11 Grown at 12 °C—To demonstrate the importance of the palmitoleoyltransferase during cold shock in living cells, lipid A 1,4'-bisphosphate species were isolated from MC1061 and MKV11 grown continuously at 12 °C to mid-log phase (A600 = 0.5). The hexa-acylated lipid A 1,4'-bisphosphates, purified by chromatography on DEAE-cellulose (36), were analyzed by MALDI/TOF mass spectrometry in the negative mode. Major peaks for lipid A from wild type cells were observed at m/z 1851.5 and 1797.4 (Fig. 5). The larger peak at m/z 1851.5, representing about two-thirds of the total, is attributed to [M - H]+ of a cold-adapted lipid A molecule containing a palmitoleate residue in place of laurate (Fig. 1). The smaller peak at m/z 1797.4 represents the [M - H]+ ion derived from “normal” E. coli lipid A (Fig. 1), which contains laurate in acyloxyacyl linkage at position-2'. The presence of the latter species in cells at 12 °C is consistent with the in vitro assay data (Fig. 4), demonstrating that the lauroyltransferase activity is intact in membranes isolated from cold-shocked cells. Additional minor peaks at m/z 1769.4 and 1824.0 (Fig. 5) likely represent lipid A molecules lacking two methylene units, when compared with the two major species; these probably arise from the slow incorporation of shorter acyl chains into the acceptor (Kdo)2-lipid IVα by the late acyltransferases (7-9).
Lipid A was also isolated from cold-shocked MKV11 to verify that the palmitoleoyltransferase was not functional in vivo and to ensure that no other modifications of the lipid A molecule were occurring in its absence. The mass spectrometry in the negative mode (Fig. 6) shows only a single major peak at m/z 1797.4, which is indistinguishable from the signal obtained with lipid A of wild type cells grown at 30 °C or above (Fig. 1). In the absence of the palmitoleoyltransferase, the lauroyltransferase is fully functional at 12 °C, indicating that the two enzymes must compete for the precursor (Kdo₂-4°-32P-lipid IV₈) in wild type cells at 12 °C (Figs. 1 and 5). The absence of other major lipid A species also confirms that no alternative modifications of the lipid A molecule in membranes of strain BLR(DE3)pLysS cells containing the lpxP gene are required for protection against harmful agents in the environment.

Massive Overproduction of Palmitoleoyltransferase in Cells Expressing lpxP Behind a T7lac Promoter—The lpxP gene was cloned into a T7 polymerase expression vector, pET21a⁺, to confirm that overexpression of the LpxP protein from a non-native promoter results in increased in vitro palmitoleoyltransferase activity. As shown in Table II, massive overproduction of palmitoleoyltransferase activity is observed only in membranes of strain BLR(DE3)pLysS cells containing the lpxP gene behind the T7lac promoter on pMKV2. The membranes from cells induced with IPTG at 12 °C have a specific activity of 44.3 × 10³ pmol/min/mg, nearly 6000-fold greater than wild type, cold-shocked cells (Table II). This establishes that LpxP is necessary and sufficient for the conversion of (Kdo₂-4°-32P-lipid IV₈ to Kdo₂-4°-32P-palmitoleoyl-lipid IV₈ occurring only in the presence of palmitoleoyl-ACP. The laurayltransferase activity was not affected by the overproduction of LpxP.

Antibiotic Hypersensitivity of MKV11 during Cold Shock—As lpxP gene expression is not required for growth in liquid medium (Fig. 2) or on agar plates at 12 °C, the effect of lpxP inactivation on antibiotic resistance was determined at cold temperatures. Five antibiotics from different major structural classes were tested (Table III). Erythromycin had no effect on either strain at both temperatures at concentrations below 500 μg/ml. However, the minimal inhibitory concentrations for rifampicin and vancomycin are 10-fold less for the mutant than for the wild type at 12 °C (Table III). Bacitracin and fusidic acid also inhibited the growth of the mutant at lower concentrations than wild type during cold shock. Therefore, it is necessary for E. coli to incorporate an unsaturated fatty acid into its lipid A at low temperatures to maintain an optimal barrier for protection against harmful agents in the environment.

DISCUSSION

We have constructed a mutant of E. coli (MKV11) containing a kanamycin resistance cassette insertion in the structural gene of the palmitoleoyltransferase, lpxP, involved in the biosynthesis of cold-adapted lipid A (15). In contrast to its parental strain, MC1061, membranes isolated from this mutant do not convert the lipid substrate for LpxP, Kdo₂-lipid IV₈ to Kdo₂-(palmitoleoyl)-lipid IV₈ (Figs. 1 and 3) and cannot incorporate any palmitoleoyl into its lipid A in vivo during cold shock.
The absence of the lpxP gene product does not confer any obvious growth phenotype in nutrient broth at low temperatures (Fig. 2). Furthermore, the inner and outer membranes of wild type and mutant cells do not appear to differ appreciably in their protein or lipopolysaccharide core composition, as judged by gel electrophoresis (data not shown). The ratio of glycerophospholipids to lipid A (normally about 10:1) is also not affected (data not shown). The absence of a strong phenotype associated with lpxP inactivation is a little surprising, considering that two-thirds of the lipid A species isolated from wild type E. coli cultured under cold shock conditions contain a palmitoleoyl moiety (Fig. 5). It is possible that the up-regulation of unsaturated fatty acid biosynthesis and the increased incorporation of these fatty acids into the glycerophospholipids (19) during growth at low temperatures provide sufficient fluidity to maintain cell viability.

Interestingly, however, the integrity of the mutant outer membrane is altered during cold shock, as determined by antibiotic resistance studies. A 10-fold greater sensitivity to rifampicin and tetracycline verifies that incorporation of an unsaturated fatty acid during cold shock, although not required for cell survival, is essential for the maintenance of an effective outer membrane barrier. Although we favor the view that the outer membrane is leaky when the palmitoleoyl moiety is not incorporated into lipid A, we cannot entirely exclude the possibility that export of these antibiotics is compromised.

The activity of the lauroyltransferase (LpxL) (8) is not affected in mutant cells grown at both 12 and 30 °C, confirming that the lauroyltransferase and palmitoleoyltransferase are distinct enzymes (Fig. 1). LpxL activity does not decrease in wild type cells grown at 30 °C or above (Fig. 1). The minor peaks at m/z 1824.0 and 1769.4 are consistent with structural variants lacking two methylene units, when compared with the major lipid A species at m/z 1851.5 and m/z 1797.4, respectively. These variants may arise because of the lack of absolute chain length specificity of LpxM (9). Lipid A isolated from wild type cells grown at 30 °C or above contains only the species with m/z 1797.4 (data not shown).

The activity of the lauroyltransferase (LpxL) (8) is not affected in mutant cells grown at both 12 and 30 °C, confirming that the lauroyltransferase and palmitoleoyltransferase are distinct enzymes (Fig. 1). LpxL activity does not decrease in wild type cells upon cold shock indicating that there must be competition between LpxL and LpxP activity during growth at low temperatures. The composition of the acyl-ACP pool clearly must influence the ability of these enzymes to catalyze their respective acylation reactions at 12 °C. The increase in activity of β-ketoacyl-ACP synthase II at cold temperatures (a direct effect on the enzyme) (16) may result indirectly in the diminished availability of lauroyl-ACP relative to palmitoleoyl-ACP. In vitro, the specific activity of LpxP is at least an order of magnitude lower than that of LpxL (15), but to date, the opti-
**TABLE III**

Hypersensitivity of MKV11 to certain antibiotics at 12 °C

| Antibiotic | MIC<sup>a</sup> (µg/ml) |
|------------|-------------------------|
|            | 30 °C<sup>b</sup> | 12 °C<sup>b</sup> | 30 °C<sup>b</sup> | 12 °C<sup>b</sup> |
| Rifampicin  | 823           | 10            | 10          | 10          |
| Erythromycin| 734           | 500           | 500         | 500         |
| Vancomycin  | 1449          | 500           | 500         | 500         |
| Bacitracin   | 1421          | >500          | >500        | 500         |
| Fusidic acid | 517           | >1000         | >1000       | 500         |

<sup>a</sup> MIC, minimal inhibitory concentration.

<sup>b</sup> Growth temperatures are shown at 30 and 12 °C.

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<sup>2</sup>M. K. Vorachek-Warren and C. R. H. Raetz, unpublished observations.
An *Escherichia coli* Mutant Lacking the Cold Shock-induced Palmitoleoyltransferase of Lipid A Biosynthesis: ABSENCE OF UNSATURATED ACYL CHAINS AND ANTIBIOTIC HYPERSENSITIVITY AT 12 °C

Mara K. Vorachek-Warren, Sherry M. Carty, Shanhua Lin, Robert J. Cotter and Christian R. H. Raetz

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