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Amplification of the *bacA* Gene Confers Bacitracin Resistance to *Escherichia coli*

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An *Escherichia coli* genomic library was constructed in order to facilitate selection for genes which confer bacitracin resistance through amplification. One of the plasmids from the library, plasmid pXV62, provided a high level of bacitracin resistance for *E. coli*. Deletion and nucleotide sequence analyses of bacitracin resistance plasmid pXV62 revealed that a single open reading frame, designated the *bacA* gene, was sufficient for antibiotic resistance. The *bacA* gene mapped to approximately 67 min on the *E. coli* chromosome by proximity to a previously mapped locus. The deduced amino acid sequence of the *bacA*-encoded protein suggests an extremely hydrophobic protein of 151 amino acids, approximately 65% of which were nonpolar amino acids. *E. coli* cells containing plasmid pXV62 have increased isoprenol kinase activity. The physical characteristics of the deduced protein and enhanced lipid kinase activity suggest that the *bacA* gene may confer resistance to bacitracin by phosphorylation of undecaprenol.

Bacitracin is a polypeptide antibiotic used clinically in common antimicrobial mixtures. Generally, preparations of bacitracin contain several chemically distinct forms of the compound, with bacitracin A being primarily responsible for antibacterial activity. Although bacitracin may alter membrane permeability or facilitate transport of toxic metals into bacteria, the primary mode of action is thought to be inhibition of peptidoglycan synthesis (24, 25).

The mechanism of inhibition involves the tight binding of bacitracin to a complex of undecaprenyl diphosphate (C_{55}-PP) and a metal cation (23). Normally, C_{55}-PP serves as a membrane-associated carrier for the *N*-acetylmuramyl pentapeptide intermediates for peptidoglycan synthesis and is released at the end of each synthetic cycle (22). Recycling of carrier C_{55}-PP involves its dephosphorylation to C_{55}-P by a phosphatase; then, a new synthetic cycle begins with the acceptance of murine precursors from UDP-acetylmuramyl pentapeptide to C_{55}-P. The model was supported by the observation that accumulation of UDP-acetylmuramyl pentapeptide accompanied the inhibition of bacterial growth with bacitracin (24). Bacitracin apparently inhibits peptidoglycan synthesis by sequestering C_{55}-PP, thus reducing the pool of lipid carrier available for delivering disaccharide pentapeptide to the cell wall.

Despite the relatively widespread use of bacitracin, little is known about bacterial acquisition of resistance to the antibiotic. Fiedler and Rotering (5) reported that mutants of *Escherichia coli* lacking membrane-derived oligosaccharides display reduced sensitivity to bacitracin. This partial resistance occurred in dilute culture medium under conditions of reduced osmolality.

Our present work was initiated with the goal of selecting bacitracin-resistant bacteria which overproduce enzymes for the synthesis of phosphorylated C_{55} lipids. We describe here the selection of plasmids from a genomic library which confer high-level bacitracin resistance upon *E. coli*. The bacitracin resistance gene (*bacA*) on one such plasmid was identified, and the complete nucleotide sequence was determined. Amplification of the *bacA* gene by its inclusion on a multiple-copy-number plasmid is shown to be sufficient for bacitracin resistance and correlates with an increase in membrane-associated isoprenol kinase activity.

MATERIALS AND METHODS

Materials. Bacitracin (>65,000 U/g) and C_{40-60} polyprenol were purchased from Sigma Chemical Co. (St. Louis, Mo.). T-4 DNA ligase and restriction endonucleases were obtained from New England Biolabs (Beverly, Mass.) and GIBCO-BRL (Gaithersburg, Md.). Sequenase (version 2.0) was the product of United States Biochemical Corp. (Cleveland, Ohio), and radionucleotides were obtained from Amersham Co. (Arlington Heights, Ill.). Difco Laboratories (Detroit, Mich.) supplied materials for bacterial growth media. Oligonucleotides were synthesized by the DNA synthesis core facility at the University of Florida Interdisciplinary Center for Biotechnology Research. Other reagents and supplies were purchased from Sigma Chemical Co. and Fisher Scientific (Orlando, Fla.).

Organisms, media, and growth conditions. Bacterial strains and plasmids used in this study are listed in Table 1. Culture medium was either Luria broth (LB) supplemented with glucose (0.2% [wt/vol]) or minimal medium A containing glucose (15). Antibiotics were added to media as appropriate at the following concentrations: ampicillin (100 μg/ml), tetracycline (10 μg/ml), and kanamycin (50 μg/ml). The concentration of bacitracin was 400 U/ml unless otherwise indicated in the text. Liquid cultures were grown with constant mixing in a roller drum or in an orbital shaker at 37°C.

Recombinant DNA techniques. Plasmid DNA was prepared by the method of Garger et al. (6), and smaller quantities were generated by the rapid screen method of Birnboim and Doly (3). Restriction endonuclease digestions and ligations were performed under the conditions recommended by the suppliers. Agarose gel electrophoresis and bacterial transformations were according to standard protocols (18), and

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TABLE 1. List of strains and plasmids

Bacterial strain or plasmid	Genotype or description	Reference or source
E. coli strains		
JM83	*ara* Δ(*lac-proAB*) *rspL* 80*lacZ*ΔM15	J. Messing
1100	*bglR thi-1 rel-1* HfrPO1	17
Plasmids		
pUC19	Ap^r *lacZ*	New England Biolabs
pXV62	Ap^r Bc^r *cca*	This study
pBDC28	Ap^r Bc^r *cca*	This study
pBDC29	Ap^r	This study
pBDC30	Ap^r Bc^r *cca*	This study
pBDC31	Ap^r	This study
pBDC32	Ap^r	This study
pBDC33	Ap^r Bc^r *cca*	This study
pBDC34	Ap^r	This study
pBDC35	Tc^r Bc^r *cca*	This study
pBDC36	Kn^r Bc^r	This study
pPJN1	Ap^r *cca*	This study
pPJN2	Ap^r *cca*	This study
pPJN3	Ap^r Bc^r	This study
pPJN4	Ap^r	This study
pPJN6	Ap^r	This study

Southern blot analysis was as described by Porter et al. (17). Strain TG1 grown in 2XTY medium (15) was employed for production of single-stranded DNA for nucleotide sequence determination. DNA sequencing was by the dideoxynucleotide method with Sequenase under conditions described in the kit.

Preparation and screening of the *E. coli* genomic library. *E. coli* genomic DNA (50 μg) was subjected to partial digestion with *Sau*3AI (8 U for 30 min at 37°C). The DNA was electrophoresed in 1% agarose, the portion of the gel containing fragments of approximately 6.5 to 10.5 kb was excised, and the DNA was electroeluted from the gel. Genomic DNA fragments were ligated to plasmid pUC19 digested with *Bam*HI. *E. coli* JM83 was transformed to ampicillin resistance and screened for the presence of inserted DNA by loss of β-galactosidase activity on plates containing IPTG (isopropyl-β-D-thiogalactopyranoside) (75 μg/ml) and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (40 μg/ml). Individual transformants were selected at random for subsequent screening for bacitracin resistance.

Colonies were lifted with nitrocellulose disks and replica plated onto solid LB medium containing both ampicillin and bacitracin. Colonies of bacitracin-resistant cells were subsequently streaked for purity on LB ampicillin-bacitracin plates. Bacitracin resistance of the transformants was assessed by growth on the bacitracin-containing plates and by determination of growth yield in liquid LB medium containing bacitracin (400 μg/ml). A_{600} was determined in a Beckman DU spectrophotometer.

Plasmid and phage constructions. Plasmids pBDC28 through pBDC34, pPJN1, and pPJN2 were constructed as deletions of the 10.8-kbp bacitracin resistance plasmid pXV62 (Ap^r Bc^r) (Fig. 1). The 10.1-kbp *Bam*HI fragment of plasmid pXV62 was ligated to yield plasmid pBDC28. Plas-

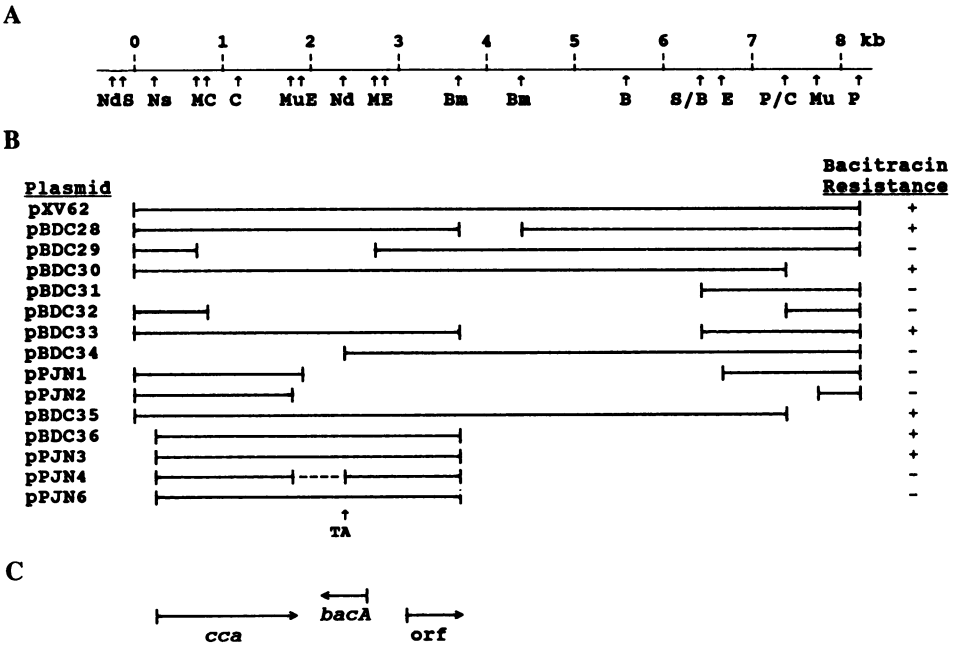

FIG. 1. Restriction map of plasmid pXV62 and deletion mapping of the *bacA* gene. (A) Restriction sites in the genomic insert DNA of plasmid pXV62. Symbols: B, *Bgl*I; Bm, *Bam*HI; C, *Cla*I; E, *Eco*RV; M, *Mlu*I; Mu, *Mun*I; Nd, *Nde*I; Ns, *Nsi*I; P, *Pst*I; S, *Sac*I. (B) Genomic insert DNA in plasmids derived from plasmid pXV62. The bars indicate the genomic DNA present in each plasmid. The dashed line in plasmid pPJN4 shows the replacement of the indicated 600-bp segment with the 6-bp sequence CTGCAG. The arrow labeled TA indicates the position of the 2-bp insert in plasmid pPJN6. The ability of each plasmid to confer bacitracin resistance on *E. coli* is indicated to the right of the bars. Symbols: +, resistance; −, sensitivity. (C) Positions of the *cca* gene (3), the *bacA* gene, and the unidentified partial open reading frame (orf) in plasmid pXV62. The arrows indicate the direction of transcription.

mid pBDC29 was constructed by ligation of the 8.8-kbp *Mlu*I fragment of plasmid pXV62. Plasmid pBDC30 was generated by ligation of the 10.1-kbp *Pst*I fragment of plasmid pXV62. Plasmid pBDC31 was made by ligation of the 4.5-kbp *Sac*I fragment of plasmid pXV62. Plasmid pBDC32 was produced by ligation of the 4.3-kbp *Cla*I fragment of plasmid pXV62. Plasmid pBDC33 was constructed by ligation of the 8.1-kbp fragment generated by digestion of plasmid pXV62 with *Bam*HI and *Bgl*II. Plasmid pBDC34 was made by ligation of the 8.1-kbp *Nde*I fragment of pXV62. Plasmid pPJN1 was made by ligation of the 6.0-kbp *Eco*RV fragment of plasmid pXV62. Plasmid pPJN2 was constructed by ligation of the 4.9-kbp *Mun*I fragment of pXV62.

Plasmid pBDC35 was generated by ligation of the 7.4-kbp *Eco*RI-*Pst*I piece of plasmid pXV62 to the 3.5-kbp *Eco*RI-*Pst*I fragment of plasmid pBR322. Plasmids pBDC36 and pPJN3 were produced by ligation of the 3.4-kbp *Nsi*I-*Bam*HI portion of pXV62 to the 4.0-kbp *Pst*I-*Bam*HI and 2.8-kbp *Nsi*I-*Bam*HI fragments of plasmid pACYC177, respectively. Plasmid pPJN4 was generated by ligation of a double-stranded oligonucleotide (AATTCTGCAG and TACTGC AG) to the 4.7-kbp *Mun*I-*Nde*I portion of plasmid pPJN3. Plasmid pPJN6 was made by digestion of plasmid pPJN3 with *Nde*I, blunting the ends by incubation with dATP and dTTP in the presence of DNA polymerase I (Klenow fragment), and subsequent ligation.

Phages M13a36 and M13b36, used for nucleotide sequence determinations, were constructed by ligation of the 3.6-kbp *Bam*HI and *Eco*RI fragments of pXV62 to *Bam*HI- and *Eco*RI-digested phages M13mp18 and M13mp19, respectively.

Cell fractionation. *E. coli* was grown in liquid LB-glucose medium with ampicillin (500 ml). The cells were harvested by centrifugation (10,000 × *g*, 10 min) and washed with TM buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 8.0). The cells were resuspended in TM buffer containing DNase I (10 μg/ml) and disrupted in a French pressure cell (18,000 lb/in^2). The cell homogenate was centrifuged (2,000 × *g*, 5 min) twice prior to use. Membrane fractions were recovered from the resulting low-speed supernatant by centrifugation (110,000 × *g*, 90 min) and resuspended in buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). Protein concentrations were determined by the method of Markwell et al. (14) with bovine serum albumin as the standard.

Isoprenol kinase activity. Isoprenol kinase activity was determined by a modification of the methods of Higashi et al. (9) and of Kalin and Allen (10). Reaction mixtures contained 50 mM Tris-HCl (pH 8.0), 0.2% Triton X-100, 10 mM $MgCl_2$, 10% dimethyl sulfoxide, 1.25 mM NaF, 2.1 mM [γ-^{32}P]ATP (0.37 to 1.4 μCi/μmol), 69 μg of C_{40-60} polyprenol, and cell fraction in a final volume of 0.5 ml. After incubation for 30 min at 37°C, the reaction product was immediately removed by two successive extractions with 1 ml of $CHCl_3$-CH_3OH (2:1 [vol/vol]). The organic phases were pooled and washed with 1 ml of H_2O-CH_3OH (2:1 [vol/vol]). The samples were evaporated to dryness, and radioactivity was determined with ReadyProtein (Beckman Instruments) scintillation fluid. The amount of product formed was expressed as nanomoles of ^{32}P incorporated into $CHCl_3$-extractable material per mg of protein in 30 min.

Nucleotide sequence accession number. The sequence data (see Fig. 3) has been assigned the GenBank accession number L12966.

RESULTS

Identification of bacitracin resistance plasmids. A genomic *E. coli* library was constructed with plasmid pUC19 as a vector in order to isolate genes conferring bacitracin resistance through amplification. *E. coli* was transformed with the library plasmids, and 1,212 transformants were selected at random and screened for growth in the presence of bacitracin (400 U/ml). Eleven of the transformants grew on solid medium containing bacitracin. Inclusion of IPTG in the medium did not appear to alter the growth properties of these strains, suggesting that the *lac* promoter in plasmid pUC19 was not involved in the expression of resistance in these cells. The growth yield of *E. coli* carrying the plasmids in LB medium containing ampicillin and bacitracin was determined as a relative measure of resistance. The growth yield of cells with plasmid pXV62 reached an A_{660} of 1.6, approximately 10-fold higher than that for the strains harboring other plasmids.

Plasmid pXV62 DNA was prepared and transformed into a different *E. coli* host, strain 1100. Resistance to bacitracin was observed in strain 1100 harboring pXV62 grown on both rich LB medium and on minimal medium A. The bacitracin resistance phenotype was clearly shown to be a property conferred on *E. coli* by the presence of plasmid pXV62. It should be noted that individual lots of bacitracin varied with respect to potency over a range of MICs of 100 to 200 U/ml. In every instance, the bacitracin resistance plasmids supported growth of *E. coli* in medium containing at least four times the MIC of antibiotic for strain 1100.

Mapping of the bacitracin resistance gene on plasmid pXV62. A series of deletions in the 8.2-kb insert in plasmid pXV62 were engineered to establish the position of the gene conferring bacitracin resistance. A restriction endonuclease map of plasmid pXV62 revealed two or more sites for each of the following enzymes: *Bam*HI, *Cla*I, *Eco*RV, *Mlu*I, *Mun*I, *Nde*I, *Pst*I, and *Sac*I (Fig. 1A). Each of these restriction endonucleases was used to excise a fragment of plasmid pXV62; in addition, another deletion was produced by ligating the compatible ends generated by *Bam*HI and *Bgl*II. The deletion plasmids were transformed into *E. coli* 1100 and tested for growth on medium containing bacitracin (Fig. 1B).

Plasmids pBDC28, pBDC30, and pBDC33 retained bacitracin resistance, indicating that the gene was not associated with the deleted segments. In contrast, plasmids pBDC29, pBDC31, pBDC32, pBDC34, pPJN1, and pPJN2 all failed to confer bacitracin resistance on *E. coli*. Therefore, the deletions in these plasmids must have disrupted the bacitracin resistance gene.

Since both bacitracin and ampicillin were thought to act upon peptidoglycan synthesis, it was conceivable that the observed bacitracin resistance from plasmid pXV62 could have resulted from a synergism between genes carried on the genomic insert DNA and the vector β-lactamase gene. To address this concern, portions of the inserted DNA from plasmid pXV62 were moved into other plasmids which lack ampicillin resistance. The resulting plasmids, pBDC35 (Tc^r) and pBDC36 (Kn^r), both supported growth on medium containing bacitracin (Fig. 1B). Clearly, the β-lactamase gene was not necessary for bacitracin resistance, and the fragment of DNA transferred from plasmid pXV62 was sufficient for antibiotic resistance.

The data from the cloning experiments indicated that the bacitracin resistance gene was located in the 3.4-kbp segment of plasmid pXV62 bounded by the *Nsi*I site and a *Bam*HI site located within the genomic insert (Fig. 1A).

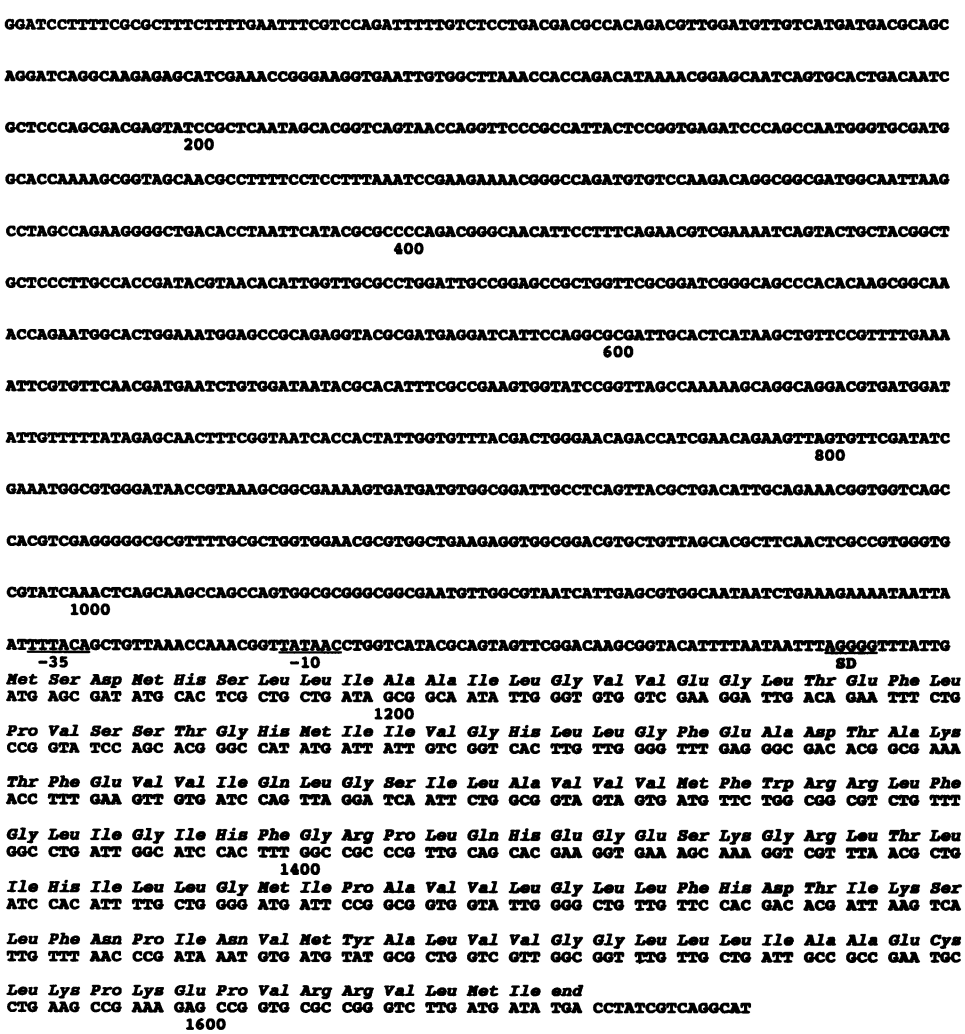

FIG. 2. The nucleotide sequence of the *bacA* gene and the deduced amino acid sequence of its product. The underlined nucleotide segments labeled −35 and −10 indicate the putative promoter for the *bacA* gene. The underlined segment marked SD specifies a possible ribosome binding site.

Nucleotide sequence of the bacitracin resistance gene. A 3.6-kbp segment of plasmid pXV62 containing the bacitracin resistance gene was cloned into bacteriophages M13a36 and M13b36 to facilitate determination of the nucleotide sequence.

Initially, the M13 −40 universal primer was used to determine the 5′ sequences of the 3.6-kbp segment in both phages. A search of GenBank showed that the 250-bp sequence at the 5′ terminus of the 3.6-kbp segment in phage M13b36 was homologous to the 5′ untranslated portion through the initiation codon of the *cca* gene reported by Cudny et al. (4). Restriction endonuclease analysis established the presence of a complete *cca* gene in plasmid pXV62 (Fig. 1C). However, the deletion mapping experiments suggested that the *cca* gene was not responsible for bacitracin resistance. Plasmids pPJN1 and pPJN2 both encoded intact *cca* genes but failed to confer resistance, while the 5′ end of the *cca* gene was deleted in bacitracin resistance-conferring plasmids pBDC36 and pPJN3 (Fig. 1). Since the published nucleotide sequence of the *cca* gene accounted for approximately 2 kbp of the 3.6-kbp segment carrying the bacitracin resistance gene, the sequence of the remaining 1.6 kbp was determined.

Two open reading frames were present in the 1,641-bp nucleotide sequence (Fig. 2). One putative gene (open reading frame) appeared to initiate at the position complementary to nucleotide 611 in the sequence shown and extended past nucleotide 1. However, the deletion mapping experiments were not consistent with this gene specifying antibiotic resistance. The second open reading frame extended from nucleotides 1171 through 1623 (Fig. 1C and 2). The results of the deletion mapping analyses suggested that this gene, designated *bacA*, was responsible for bacitracin resistance.

Disruption of the *bacA* gene. To confirm that the putative *bacA* gene conferred resistance, plasmids pPJN4 and pPJN6 were constructed. Both resulted specifically in disruption of the *bacA* gene carried on bacitracin resistance plasmid pPJN3. Approximately two-thirds of the *bacA* gene was deleted by replacing this portion of the gene with a synthetic oligonucleotide in plasmid pPJN4. Plasmid pPJN6 contains a frameshift mutation engineered by insertion of 2 bp into the *bacA* gene (Fig. 1). Neither plasmid pPJN4 nor pPJN6

supported growth of *E. coli* on bacitracin-containing medium. Importantly, all plasmids which promoted *E. coli* growth on bacitracin-containing medium possessed an intact copy of the *bacA* gene, and plasmids lacking the *bacA* gene uniformly failed to support growth on bacitracin-containing medium.

Location of the *bacA* gene in the chromosome. Since the *bacA* gene was adjacent to the *cca* gene in plasmid pXV62 and the *cca* gene has been mapped to 67 min on the *E. coli* genetic linkage map (2), the location of the *bacA* gene in the *E. coli* chromosome could be readily determined by establishing that both genes were in fact adjacent in the chromosome. Southern analyses were performed to confirm that the two genes were indeed closely linked in *E. coli* 1100 (data not shown).

Isoprenol kinase activity. The deduced amino acid content and primary sequence of the *bacA* gene product suggested characteristics similar to those of other reported bacterial lipid kinases (see below). Given these similarities and the fact that increased availability of C_{55}-P could account for bacitracin resistance, the level of isoprenol kinase activity was investigated in cells with normal and amplified levels of the *bacA* gene.

Cell fractions were prepared from *E. coli* 1100 and from strain 1100 harboring plasmid pXV62. Isoprenol kinase activity was determined by measuring the incorporation of radioactivity from [γ-^{32}P]ATP into lipid with a mixture of exogenous isoprenols (C_{40-60}) as substrate. The kinase activities (in nanomoles per milligram of protein) were as follows: strain 1100, 0.86 (homogenate) and 1.6 (membrane fraction), and strain 1100 with plasmid pXV62, 2.6 (homogenate) and 7.0 (membrane fraction). For experiments with strain 1100 carrying plasmid pXV62, cells were grown in 200 to 300 U of bacitracin per ml. The whole-cell homogenate of the cells with pXV62 had higher isoprenol kinase activity than the homogenate from the parental strain. Furthermore, kinase activity in the membrane fraction from cells with plasmid pXV62 was enriched to a level more than fourfold higher than the level found in membranes from the parental strain. It should be noted that this increase in isoprenol kinase activity was reproducible through several independent membrane preparations.

Increases in kinase activity of over twofold were also observed between homogenates and membranes of the parental strain and *E. coli* carrying plasmid pXV62 when kinase activity was measured in the absence of exogenous substrate (data not shown). However, activity was not markedly increased by addition of exogenous isoprenol. This suggested that the enzyme was utilizing a substantial pool of endogenous membrane-associated substrate. These observations are not necessarily inconsistent with the hypothesis that *bacA* encodes undecaprenol kinase. Early studies of the enzyme by Higashi et al. (9) showed a large endogenous isoprenol pool in the membranes of *Staphylococcus aureus* and no exogenous polyprenol stimulation of the enzyme in membranes of *Micrococcus lysodeikticus*.

Although suggestive, the evidence was not sufficient to conclude that the *bacA* gene encodes undecaprenol kinase. Increased C_{55}-PP synthesis could have accounted for bacitracin resistance. However, levels of C_{55}-PP synthetase activity in cell homogenates from cells carrying plasmid pXV62 were not significantly higher than in homogenates derived from the parental strain (data not shown) (1). Moreover, lipid kinases are known to be promiscuous with respect to substrate specificity, and so the activity may reflect the expression of an enzyme which does not normally function in isoprenoid metabolism (20, 21).

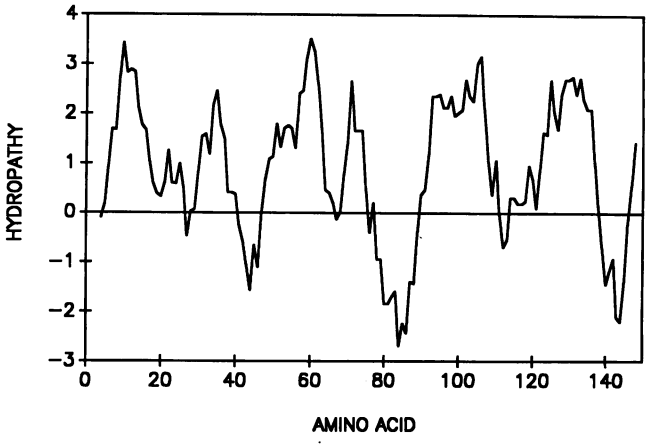

FIG. 3. Hydropathy plot of the deduced *bacA*-encoded protein. Hydropathy was calculated according to the algorithm of Kyte and Doolittle (11) with a window of seven amino acids.

DISCUSSION

This work describes the cloning, complete nucleotide sequence, and genomic location of the *bacA* gene from *E. coli*. Amplification of the *bacA* gene by expression from a multiple-copy-number plasmid in *E. coli* provides resistance to at least 400 U of bacitracin per ml. The resistance phenotype correlates with an increase in membrane-associated isoprenol kinase activity.

The *bacA* gene has an open reading frame of 453 bp. The likely promoter, with near-consensus −35 and −10 sequences, is located approximately 60 bp 5′ with respect to the putative initiation codon (Fig. 2). A purine-rich segment suitable for specification of a Shine-Dalgarno ribosome binding site is centered 10 bases ahead of the initiation codon.

The deduced amino acid sequence of the *bacA*-encoded protein suggests an extremely hydrophobic protein of 151 amino acids (Fig. 2). Approximately 65% of the protein consists of nonpolar amino acids, with three aliphatic amino acids, leucine, isoleucine, and valine, accounting for 37% of the protein. Hydropathy analysis of the *bacA*-encoded protein appears consistent with a membrane-associated protein with at least four, and perhaps as many as six, membrane-spanning regions (Fig. 3).

An enzyme that is involved in C_{55}-PP metabolism and that has characteristics similar to those of the *bacA*-encoded protein has been described. C_{55}-isoprenoid alcohol phosphokinase (undecaprenol kinase) from *S. aureus* is of comparable size and has a high hydrophobic amino acid content (19). It seems plausible that the *bacA* gene may encode a similar enzyme in *E. coli*. This speculation is also supported by the knowledge that bacitracin inhibits C_{55}-PP phosphatase, resulting in an accumulation of C_{55}-PP units. The consequence of this is a decrease in C_{55}-P, which is required for initiation of the lipid cycle of peptidoglycan synthesis. Since C_{55}-isoprenoid alcohol phosphokinase catalyzes the ATP-dependent phosphorylation of undecaprenol to C_{55}-P, the presence of adequate undercaprenol and higher-than-parental levels of kinase could account for sufficient C_{55}-P to overcome the inhibition effects of bacitracin on peptidoglycan synthesis. The hypothesis that the *bacA* gene may

encode a lipid kinase is also supported by the observation that another *E. coli* enzyme involved in lipid phosphorylation, diacylglycerol kinase, is a small hydrophobic protein (12, 13). However, purified *bacA* gene product has not yet been definitively shown to have undecaprenol kinase activity.

Like bacitracin, colicin M inhibits peptidoglycan and lipopolysaccharide O-antigen biosynthesis by interfering with lipid carrier recycling (7, 8). However, the authors concluded that colicin M action appeared to involve prevention of the regeneration of undecaprenyl phosphate by a mechanism different from that of bacitracin. It is also noteworthy that a low-molecular-weight protein, the *cmi* gene product, which renders *E. coli* resistant to colicin M, has been identified (16). The *cmi*-encoded protein does not provide bacitracin resistance and bears no homology to the *bacA* gene product.

The fact that amplification of a single small gene, such as the *bacA* gene, provides a very significant increase in *E. coli* resistance to bacitracin suggests that this is a plausible mechanism for resistance in the clinical setting. The level of resistance apparently does not correlate with the extent of amplification. Most of the plasmids reported here, including plasmid pXV62, are derived from the high-copy-number plasmid pUC19. However, plasmids pBDC36 and pPJN3 are derived from the relatively low-copy-number plasmid pA CYC177, and these plasmids confer resistance at a level comparable to that of the other *bacA* gene plasmids. This observation suggests that only a low level of amplification of the *bacA* gene would be sufficient for clinically important antibiotic resistance. A test of this hypothesis awaits measurement of the minimum level of *bacA* gene amplification required for bacitracin resistance and a screen of clinically derived bacitracin-resistant specimens for amplification of the *bacA* gene.

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