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Nucleotide Sequence Analysis of a Gene from *Burkholderia* (*Pseudomonas*) *cepacia* Encoding an Outer Membrane Lipoprotein Involved in Multiple Antibiotic Resistance

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Antibiotic-resistant *Burkholderia* (*Pseudomonas*) *cepacia* is an important etiologic agent of nosocomial and cystic fibrosis infections. The primary resistance mechanism which has been reported is decreased outer membrane permeability. We previously reported the cloning and characterization of a chloramphenicol resistance determinant from an isolate of *B. cepacia* from a patient with cystic fibrosis that resulted in decreased drug accumulation. In the present studies we subcloned and sequenced the resistance determinant and identified gene products related to decreased drug accumulation. Additional drug resistances encoded by the determinant include resistances to trimethoprim and ciprofloxacin. Sequence analysis of a 3.4-kb subcloned fragment identified one complete and one partial open reading frame which are homologous with two of three components of a potential antibiotic efflux operon from *Pseudomonas aeruginosa* (*mexA-mexB-oprM*). On the basis of sequence data, outer membrane protein analysis, protein expression systems, and a lipoprotein labelling assay, the complete open reading frame encodes an outer membrane lipoprotein which is homologous with OprM. The partial open reading frame shows homology at the protein level with the C terminus of the protein product of *mexB*. DNA hybridization studies demonstrated homology of an internal *mexA* probe with a larger subcloned fragment from *B. cepacia*. The finding of multiple antibiotic resistance in *B. cepacia* as a result of an antibiotic efflux pump is surprising because it has long been believed that resistance in this organism is caused by impermeability to antibiotics.

Burkholderia (*Pseudomonas*) *cepacia* is an important etiologic agent of nosocomial and cystic fibrosis (CF) infections. Although this organism is rarely pathogenic in healthy hosts, immunodeficient patients (especially those with chronic granulomatous disease) (5, 24, 34) and patients with CF may develop severe and even fatal infections (15, 29). A hallmark of *B. cepacia* infections is the inability to eradicate the organism because of high-level multiple antibiotic resistance (12). The primary resistance mechanism which has been reported is decreased outer membrane permeability (2, 22, 25).

We previously reported the cloning of a chloramphenicol resistance determinant from a CF isolate of *B. cepacia* and determined that decreased antibiotic accumulation was the mechanism of resistance (8). Decreased drug accumulation may result from impermeability or from active efflux. Permeability-mediated resistance in *B. cepacia* has been reported to be caused by changes in lipopolysaccharide (LPS) (10, 22) and pore-forming outer membrane proteins (porins) (2, 25); antibiotic efflux has not been reported for *B. cepacia.*

We subcloned the fragment encoding chloramphenicol resistance and compared the resistance phenotypes encoded by the wild-type and subcloned genes. Nucleotide sequence analysis of the subcloned determinant was performed, and a gene product was identified and characterized.

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MATERIALS AND METHODS

Strains, plasmids, and growth conditions. The *B. cepacia*, *Pseudomonas aeruginosa*, and *Escherichia coli* strains and plasmids used in this investigation are listed in Table 1. The liquid media used in this work were L broth (30) for routine bacterial growth and Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) for MIC determinations. Broth cultures were incubated at 37°C and shaken at 150 cycles per minute. The solid media used were L agar for *P. aeruginosa* and *E. coli* strains and OFPBL (41) for *B. cepacia* strains. Plate cultures were incubated at 37°C. All plasmid-containing strains were grown in the presence of the appropriate antibiotics, i.e., tetracycline (50 μg/ml) for pSP329-based recombinant plasmids and ampicillin (100 μg/ml) for all pUC-based recombinant plasmids. The strains were stored at −70°C in skim milk and inoculated onto fresh agar medium 18 to 40 h prior to use.

MICs. The MICs of chloramphenicol, trimethoprim, and ciprofloxacin were determined for selected *B. cepacia* strains by a broth dilution technique (1).

DNA preparation and analysis. Plasmid DNA was purified from *E. coli* and *B. cepacia* by an alkaline lysis technique followed by phenol-chloroform extraction for small-scale preparations (30) and either ethidium bromide-cesium chloride ultracentrifugation (4) or resin purification (Qiagen, Chatsworth, Calif.) for large-scale preparations. Genomic DNA was isolated from *B. cepacia* and *P. aeruginosa* strains by a modification of the technique of Scordilis et al. (33). Restriction endonucleases were used to digest plasmid and genomic DNAs according to the instructions of the manufacturer (Bethesda Research Laboratories, Bethesda, Md.). DNA was analyzed by 0.7% agarose gel electrophoresis in either Tris-borate or Tris-acetate buffer (30).

Nucleotide sequence analysis. Double-stranded DNA sequencing was performed by the dideoxy technique of Sanger et al. (32) with some modifications because of the high G+C content of *B. cepacia* (67%): terminal deoxynucleotidyl transferase in the presence of excess nucleotides was used after each termination reaction (18), and the samples were electrophoresed through 7.5% polyacrylamide gels containing 40% formamide. Overlapping sequences from both strands were obtained by using a set of custom-synthesized primers. Nucleotide and translated amino acid sequences were analyzed by using the Blast Network Service at the National Center for Biotechnology Information and the Genetics Computer Group program (University of Wisconsin).

Expression of cloned genes. Both a T7 RNA polymerase-promoter transcription translation technique (36) and an *E. coli* maxicell system (31) were used in an attempt to identify the gene product(s) of the subcloned fragment expressing chloramphenicol resistance. The T7 system used directional cloning of the 4.3-kb *Xba*I-*Hin*dIII fragment (3.4 kb of DNA from K61-3 plus 0.9 kb from the vector, pSP329) from pLIN5 (see Fig. 1) into phagemids encoding the T7 RNA poly-

TABLE 1. Strains and plasmids

Strain or plasmid	Description[a]	Source or reference(s)
Strains		
K61-3	*B. cepacia* CF isolate; Cm^r Tmp^r Cip^r	D. Woods, Calgary, Alberta, Canada
249-2	*B. cepacia* Lys$^-$ Pen^s	3, 9
DH5α	*E. coli endA1 hsdR17 supE44 thi-1* F$^-$ *recA1 gyrA96 relA1* λ$^-$ ϕ80d*lacZ*Δ*M15*	
CSR603	*E. coli recA uvrA*	31
Plasmids		
pSP329	7.0 kb, BHR, mob$^+$, Tet^r	7
pUC18, pUC19	2.7 kb, Amp^r, α-complementation of *lacZ*	Bethesda Research Laboratories
pTZ18R, pTZ19R	2.8-kb pUC-based phagemids, T7 RNA polymerase promoter, Amp^r, α-complementation of *lacZ*	Promega
pGP1-2	7.1-kb T7 RNA polymerase, temperature sensitive, Km^r	36
pLIN1	44.9 kb, BHR, mob$^+$, Km^r, Cm^r, Tmp^r, Cip^r, 21.5-kb *Sal*I insert from K61-3 in pVK102	8
pLIN4	13.3 kb, BHR, mob$^+$, Tet^r, Cm^r, Tmp^r, Cip^r, 6.3-kb *Cla*I insert from pLIN1 in pSP329	This paper
pLIN5	7.0 kb, Amp^r, 4.3-kb *Pvu*II insert from pLIN4 in pUC19	This paper
pLIN6	11.3 kb, BHR, mob$^+$, Tet^r, 4.3-kb *Pvu*II insert from pLIN4 in pSP329	This paper
pPV6	9.2 kb, Amp^r, 4.5-kb *Xho*I-*Hin*dIII insert from *P. aeruginosa* K437 in pAK1900 (encodes *mexA-mexB-oprM*)	26

[a] Abbreviations: Cm^r, chloramphenicol resistant; Tmp^r, trimethoprim resistant; Cip^r, ciprofloxacin resistant; Lys$^-$, lysine auxotroph; Pen^s, penicillin susceptible; BHR, broad host range; Tet^r, tetracycline resistant; mob$^+$, mobilizable; Amp^r, ampicillin resistant; Km^r, kanamycin resistant.

merase promoter (pTZ18R and pTZ19R). These constructs were transformed into DH5α containing pGP1-2, a temperature-sensitive plasmid encoding T7 RNA polymerase, and controlled overexpression was performed (36). The maxicell system (31) with pLIN5 in host strain CSR603 was also used to examine the gene product(s).

DNA hybridization. DNA transfer and hybridization were performed according to the method of Southern (35). Genomic DNAs from *B. cepacia* and *P. aeruginosa* strains were digested with an appropriate restriction endonuclease, electrophoresed through agarose, and transferred to a nylon membrane (Nytran; Schleicher & Schuell, Keene, N.H.). The DNA fragments used as probes were labelled by using the LumiPhos system (Bio-Rad, Richmond, Calif.) and used to detect homologous sequences in the genomic DNA. Detection was performed by autoradiography.

Outer membrane proteins and lipoproteins. *B. cepacia* cell surface proteins were labelled with sulfono-NHS-biotin (Pierce Chemical Co., Rockford, Ill.) as described by Kocks et al. (16), outer membrane proteins were isolated as previously described (8), and the proteins were electrophoresed on sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred to nitrocellulose. Detection of biotinylated proteins was with horseradish peroxidase-conjugated streptavidin (Pierce) with chemiluminescence staining (ECL; Amersham Life Sciences, Arlington Heights, Ill.).

A modification of the technique of Weinberg et al. (40) was used to evaluate whether relevant proteins were lipoproteins. Briefly, 20 ml of bacterial cells was labelled by growth for 4 h in L broth containing 400 μCi of [^{3}H]palmitate (specific activity, 30 mCi/mmol; Dupont New England Nuclear, Boston, Mass.). The labelled cells were pelleted, resuspended in 100 μl of 1% SDS, boiled for 15 min, and extracted 5 to 10 times with 10 ml of chloroform-methanol (2:1) to remove noncovalently bound [^{3}H]palmitate. SDS-polyacrylamide gel electrophoresis was performed through a 10% gel; after drying of the gel, the lipoproteins (those proteins labelled with ^{3}H) were detected by fluorography.

Nucleotide sequence accession number. The GenBank accession number for *opcM* is U38944.

RESULTS

DNA subcloning. The original cloning of the fragment encoding chloramphenicol resistance from the wild-type CF isolate, K61-3, resulted in plasmid pLIN1, which contained approximately 22 kb of insert DNA from K61-3 in the 23-kb, broad-host-range cosmid vector pVK102 (8). Subcloning utilized the much smaller broad-host-range vector pSP329, which was introduced into strain 249-2 by electroporation (7). Restriction maps of pLIN1, pLIN4 (a pSP329-based intermediary), and pLIN5 (a pUC19-based plasmid containing 3.4 kb of insert DNA from K61-3 plus the 0.9-kb *Acc*I-*Pvu*II fragment of pSP329 [4.3 kb total]) are shown in Fig. 1. A pSP329-based plasmid containing virtually the same 4.3-kb insert as pLIN5 but with *Hin*dIII-*Xba*I sticky ends was constructed because the resistance phenotype is not expressed in *E. coli*. This recombinant plasmid, termed pLIN6, was transformed into strain 249-2 to confirm chloramphenicol resistance and permit MIC determinations.

MICs. The results of MIC determinations for wild-type susceptible and resistant strains as well as strains expressing the cloned and subcloned determinants in broad-host-range vectors are displayed in Table 2. Strain 249-2(pLIN1) has virtually the same resistance profile as the wild-type resistant strain, K61-3. However, the subclone, 249-2(pLIN6), is less resistant to all three antibiotics than both the original clone and the wild-type strain.

Nucleotide sequence analysis. The entire 4.3-kb insert in pLIN5 (3.4 kb from K61-3 and 0.9 kb from pSP329) was sequenced and analyzed (Fig. 2). One complete open reading frame (ORF) was identified on this fragment. Three potential start codons were identified within 11 amino acids of one another. Using the most 5′ of these, the ORF would encompass 1,536 nucleotides and would be predicted to encode a 512-amino-acid polypeptide with a molecular weight of 54,656. The predicted gene product of this ORF has a sequence at its N terminus that is characteristic of a signal sequence (38) as well as a putative lipoprotein signal peptidase cleavage site (14, 39), indicating that this is likely to be a secreted protein. Use of the most 3′ of the three potential start codons would lead to the most hydrophobic signal sequence, and thus this codon would be the most likely start codon for a lipoprotein. The mature protein predicted by this sequence would be 483 amino acids with a molecular weight of 51,627. A hydrophobicity analysis based on the method of Kyte and Doolittle (17) predicts seven potential membrane-spanning domains (data not shown).

TABLE 2. MICs for strains examined in this study

Strain	MIC (μg/ml)		
	Chloramphenicol	Trimethoprim	Ciprofloxacin
K61-3	>200	25	12
249-2	12.5	0.39	0.39
249-2(pLIN1)	>200	12.5	6.25
249-2(pLIN6)	50	3.12	1.56

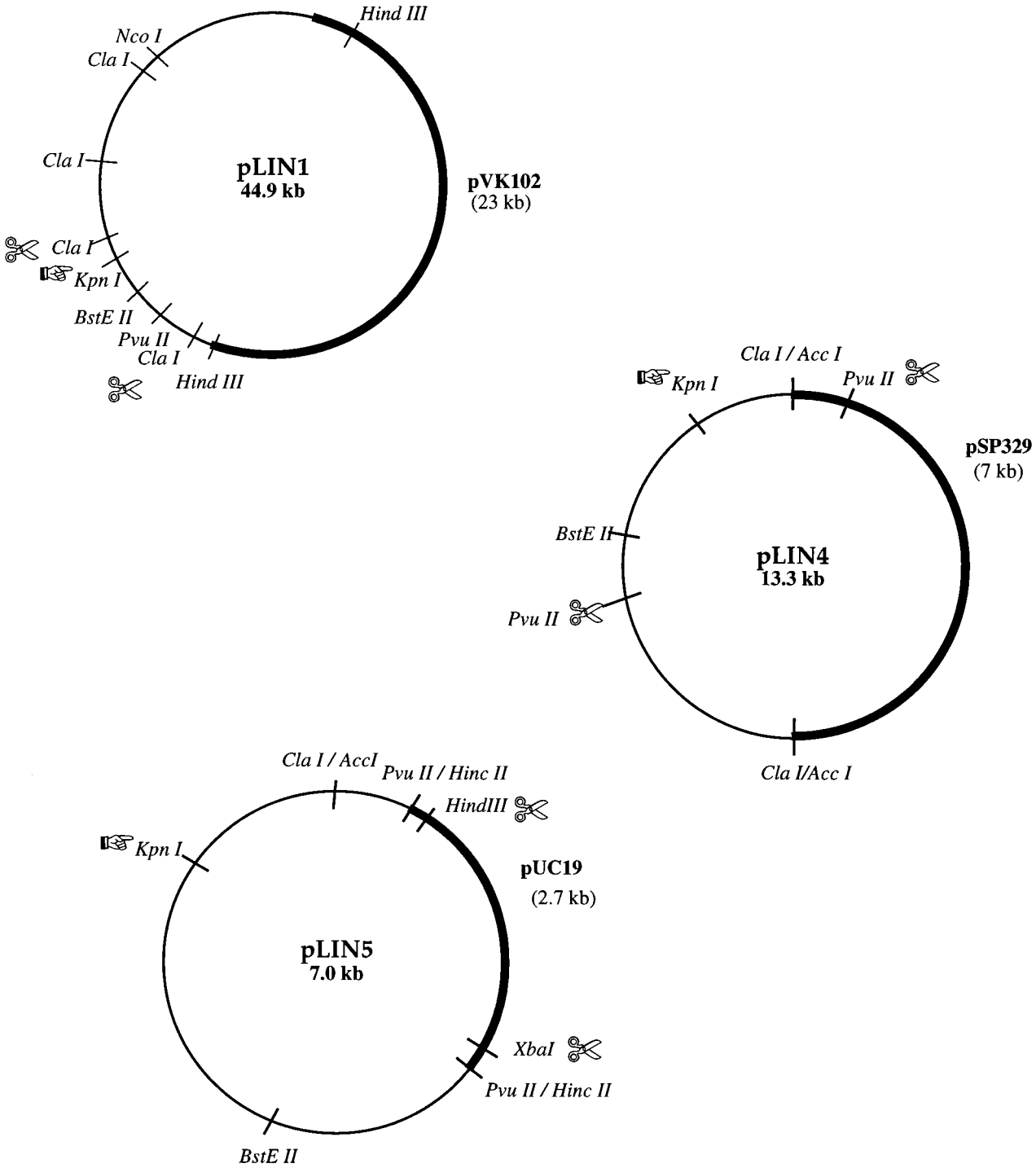

FIG. 1. Restriction maps of selected recombinant plasmids used in this study. pLIN1 contains 21.9 kb of *Sal*I partially digested whole-cell DNA from K61-3 ligated into the *Sal*I site of the cosmid vector pVK102 (8). pLIN4 contains the 6.3-kb *Cla*I fragment of pLIN1 (*Cla*I sites are denoted on the pLIN1 map by scissors) inserted into the *Acc*I site of pSP329. pLIN5 was constructed by digesting pLIN4 with *Pvu*II (denoted on the pLIN4 map by scissors) and ligating the resultant 4.3-kb fragment into the *Hinc*II site of pUC19. The inserted fragment contains the 3.4-kb *Pvu*II-*Cla*I fragment of pLIN4 plus the 0.9-kb *Acc*I-*Pvu*II fragment of pSP329. pLIN6 (not shown) contains the same 4.3-kb *Pvu*II fragment from pLIN4 ligated into the *Hinc*II site of pSP329. The *Kpn*I site (denoted by a hand) was helpful in the subcloning because it was noted that interruption of the *Kpn*I site by either subcloning or ligation into that site resulted in the loss of the resistance phenotype in *B. cepacia*. The vectors used in these constructs are designated by the heavy black lines.

In order to identify potential functions of the gene product of the ORF, the GenBank database was searched for sequences exhibiting homology with the predicted gene product. The highest score was demonstrated with the product of *oprK*, a *P. aeruginosa* outer membrane protein which is part of a reported multiple antibiotic efflux operon (27); *oprK* was recently reported (19) to actually be *oprM*, which had been described by Gotoh et al. (13) and Masuda et al. (21). Following

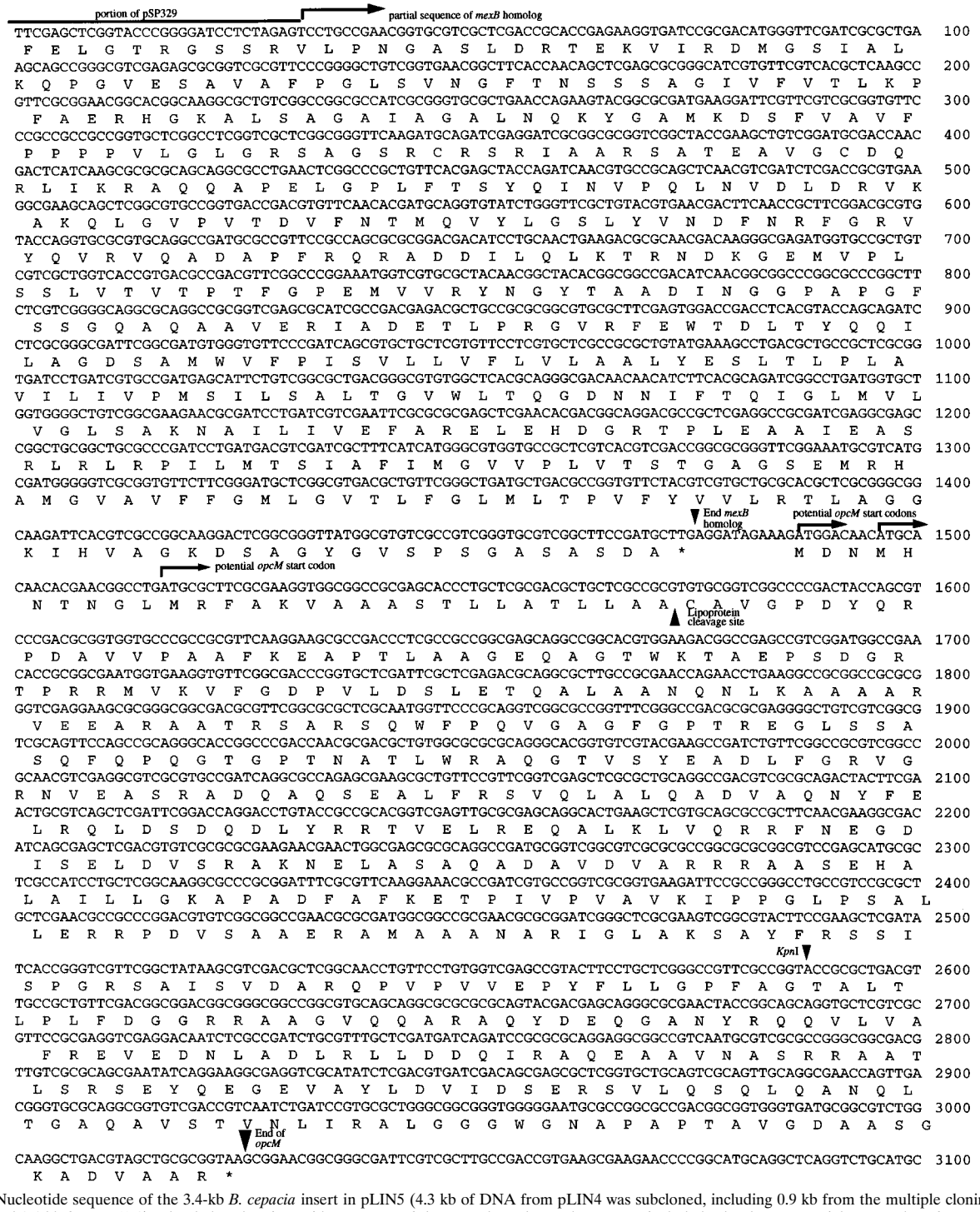

FIG. 2. Nucleotide sequence of the 3.4-kb *B. cepacia* insert in pLIN5 (4.3 kb of DNA from pLIN4 was subcloned, including 0.9 kb from the multiple cloning site of pSP329 and 3.4 kb from K61-3). The deduced amino acid sequences of the *mexB* homolog and *opcM* are included. The three potential start codons for *opcM* are denoted, as are the lipoprotein cleavage site and key restriction endonuclease sites. This sequence has been deposited in the GenBank database.

alignment of homologous sequences, the ORF gene product demonstrated 32% identity and 38% similarity with the product of the *P. aeruginosa* gene. In addition, the predicted mature product of *oprM* has a molecular weight (50,010) similar to that of the ORF gene product and also possesses a signal sequence at its N terminus as well as a putative lipoprotein signal peptidase cleavage site.

No promoter for the ORF was identified in the sequenced DNA. However, the construct in pSP329, pLIN6, which expressed partial antibiotic resistance in *B. cepacia* was in the proper orientation for the ORF to be transcribed from the *lac* promoter on the vector.

A second, partial potential ORF was identified on the basis of homology data. A 1,323-base DNA segment terminating in

a stop codon was identified upstream of the complete ORF, which continued to the end of the *Pvu*II fragment. A search of GenBank demonstrated a high degree of homology for the product of this sequence with the gene products of *envD* and *acrB* from *E. coli* and, most significantly, with the gene product of *mexB* of *P. aeruginosa*, a component of the multiple antibiotic resistance operon which is upstream from *oprM* (26). Alignment of 443 amino acids demonstrated 41% identity and 56% similarity with *mexB*. The area of greatest similarity was in the C-terminal region of the proteins.

Gene expression. By using an in vivo expression system based on the T7 RNA polymerase-promoter system, it was not possible to express the complete 4.3-kb *Xba*I-*Hin*dIII fragment from pLIN5 (including the 3.4 kb of insert DNA from K61-3 and 0.9 kb of pSP329) in *E. coli* GP1-2. When the fragment was cloned and expressed in pTZ19R, no product was identified. Attempts to express the fragment in pTZ18R resulted in a 20-kDa product which was the result of a spontaneous deletion of the fragment. These findings suggested that the gene product of the 3.4-kb fragment was lethal when expressed in *E. coli* under the control of a strong promoter; this was confirmed by directional cloning in pUC18 and pUC19. All previous subcloning in *E. coli*, including that with pLIN5, had used fragments with complementary ends and thus permitted insertion in either orientation.

An *E. coli* maxicell system was next used in an attempt to identify the gene product(s) of the 3.4-kb fragment. An approximately 55-kDa protein was visualized as the gene product of pLIN5, in addition to the β-lactamase encoded by pUC19 (data not shown). This size is similar to that of the predicted proprotein encoded by the complete ORF.

DNA hybridization studies. The 1.9-kb *Dde*I-*Eco*RI fragment of pLIN5 was used to probe genomic DNA from *B. cepacia* 249-2, K61-3, and antibiotic-susceptible and -resistant clinical isolates, as well as plasmid DNA from pLIN1. Homology was demonstrated with a single band from the genomic DNA from K61-3 and all of the clinical isolates, whether antibiotic resistant or susceptible, but not with genomic DNA from 249-2 (data not shown). 249-2 is a known deletion mutant that is missing 0.8 Mb of DNA from the 2.5-Mb replicon (9). Homologous fragments with three different molecular masses were identified, but there was no correlation between the sizes of the fragments and antibiotic susceptibility or resistance.

The *mexA* probe was constructed by cloning a 1,718-kb *Sma*I-*Hin*dIII fragment from pPV6 (26) and labelling a 621-bp *Pst*I internal fragment of *mexA*; at high stringency (wash temperature, 68°C), homology with K61-3 and pLIN4 was demonstrated (data not shown), suggesting that *B. cepacia* contains homologs of all three genes present in the *P. aeruginosa* operon.

Outer membrane protein profiles. The homology demonstrated between the gene products of the ORF and *oprM* combined with sequence data suggesting that the product of the *B. cepacia* ORF is excreted and has potential transmembrane domains raised the possibility that the *B. cepacia* gene product is an outer membrane protein. For this reason, biotin-labelled outer membrane proteins were prepared for strain 249-2(pLIN4) and for strain 249-2(pLIN4-19), which has the ampicillin resistance gene from pUC19 cloned into the *Kpn*I site of the ORF. The profiles of these two strains were compared (Fig. 3): an approximately 50-kDa outer membrane protein that was absent in the strain with the ORF interrupted was visualized in the strain expressing the ORF. Combined with sequence analysis and the results of experiments with maxicells, this led us to conclude that the gene product is an outer membrane protein. There are many similarities between *oprM*, the *P. aeruginosa* gene identified by Poole et al. (19, 27), and the *B. cepacia* ORF. We have therefore designated the *B. cepacia* gene *opcM* and designated its gene product OpcM, in keeping with the proposed nomenclature for *B. cepacia* outer membrane proteins (6).

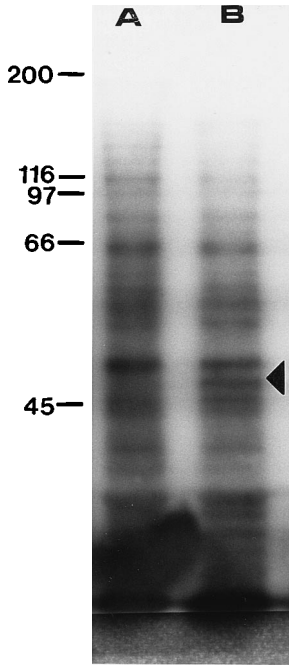

FIG. 3. Sulfono-NHS-biotin-labelled outer membrane proteins of 249-2(pLIN4-19) (lane A), which has the ampicillin resistance gene from pUC19 cloned into the *Kpn*I site of the ORF, and of 249-2(pLIN4) (lane B). An approximately 50-kDa outer membrane protein (arrowhead) that is not seen in the strain with the interrupted gene, 249-2(pLIN4-19), is identified in 249-2(pLIN4). Molecular size standards (in kilodaltons) are shown on the left.

Identification of lipoproteins. [^{3}H]palmitate labelling was used to identify lipoproteins in strains which contain or do not contain *opcM* (Fig. 4). An approximately 51-kDa ^{3}H-labelled protein was identified in 249-2(pLIN1) and 249-2(pLIN4), strains expressing OpcM, suggesting that OpcM is a lipoprotein.

DISCUSSION

We have identified and subcloned from *B. cepacia* a fragment encoding resistance to chloramphenicol, trimethoprim, and ciprofloxacin. Because of the intrinsic resistance of *B. cepacia* to the aminoglycosides (22) and the readily inducible resistance to β-lactams (2, 28), these three agents are frequently used to treat *B. cepacia* infections. Thus, the multiple resistance phenotype described here is clinically significant.

Previous studies of antibiotic resistance in *B. cepacia* have identified diminished outer membrane permeability as the primary mechanism of resistance (2, 22, 25). Porin-mediated impermeability has been proposed for hydrophilic compounds such as β-lactam antibiotics, and the lack of a cation-binding site could explain intrinsic resistance to polycations such as aminoglycosides and polymyxin. Parr et al. (25) identified diminished porin-mediated diffusion of β-lactams because of small porin channel size, as well as small single-channel conductance. In both antibiotic-resistant mutants and CF clinical isolates, Aronoff (2) observed decreased expression of two major outer membrane proteins identified as porins. The in-

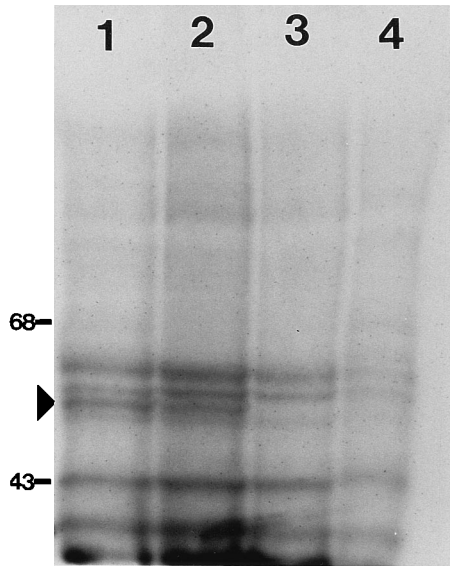

FIG. 4. Lipoproteins isolated from *B. cepacia* 249-2 and its *opcM*-expressing derivatives. Log-phase cells were pulse-labelled with [^{3}H]palmitate, and the lipoproteins were extracted from cell pellets. After SDS-polyacrylamide gel electrophoresis, the gels were dried and subjected to fluorography. Lane 1, 249-2(pLIN1); lane 2, 249-2(pLIN4); lane 3, 249-2(pLIN6); lane 4, 249-2(pSP329). An approximately 50-kDa lipoprotein (arrowhead) that is not seen in 249-2(pLIN6) and 249-2(pSP329) is demonstrated in 249-2(pLIN1) and 249-2(pLIN4). Molecular size standards (in kilodaltons) are shown on the left.

trinsic aminoglycoside and polymyxin resistance which is a hallmark of *B. cepacia* has been related to inaccessible LPS binding sites in the *B. cepacia* outer membrane (10, 22). Our previous research also suggested permeability changes as the mechanism of resistance to chloramphenicol (8).

The studies presented here suggest that resistance to chloramphenicol, trimethoprim, and ciprofloxacin is the result of active drug efflux. Nucleotide sequence analysis has identified significant homology between the subcloned fragment and a portion of a multiple antibiotic resistance efflux operon (*mexA-mexB-oprM*) from *P. aeruginosa* (26, 27).

The predicted gene product of *opcM* is an outer membrane protein which has homology with a *P. aeruginosa* outer membrane protein encoded by *oprM*. OprM is a 50-kDa mature protein which results from cleavage of a signal sequence and which is also a putative lipoprotein on the basis of the presence of a lipoprotein signal peptidase cleavage site (19, 27). N-terminal amino acid sequencing of OprM was unsuccessful, again suggesting that it is a lipoprotein. OpcM is also an approximately 50-kDa outer membrane lipoprotein which results from cleavage of a proprotein. Three potential start codons have been identified for *opcM*, and possible ribosomal binding sites can be identified for each of them. Because the most 3′ of the possible start codons would result in the most hydrophobic signal sequence, the third methionine is the most likely start codon.

Homology with a second protein encoded by the *mexA-mexB-oprM* operon has been deduced for the gene product of a second, partial potential ORF within the 3.4-kb subcloned fragment. This coding sequence is 5′ of *opcM* and is homologous with the product of *mexB*, which is 5′ of *oprM*. DNA hybridization experiments demonstrate homology of the larger, 6.3-kb subcloned DNA fragment in pLIN4 with the *P. aeruginosa mexA* gene. Taken together, these results would fit with the prediction that *B. cepacia* contains homologs of the *P. aeruginosa* multiple antibiotic resistance operon *mexA-mexB-oprM* (26, 27).

The mechanism of multiple antibiotic resistance encoded by *mexA-mexB-oprM* is predicted to be an antibiotic efflux pump (27). This efflux pump appears to be a member of the RND family of membrane transporters, which includes the products of *czcA* and *acrB* (11, 20). A model has been proposed which hypothesizes that the *mexB* product is an inner membrane pump, the *oprM* product is an outer membrane channel-forming protein, and the *mexA* product is a protein that spans the periplasmic space between the *mexB* and *oprM* products (23). This model suggests that all three gene products encoded by the operon are required for resistance to chloramphenicol, ciprofloxacin, and tetracycline. This may not be true for *B. cepacia*. A direct comparison of the two organisms is made difficult by the fact that Poole et al. (26, 27) characterized the *P. aeruginosa* system by using insertional mutagenesis to knock out each of the components. In contrast, we cloned what we hypothesize to be the entire operon into a *B. cepacia* deletion mutant, 249-2, which lacks the sequence. In addition, we subcloned a smaller fragment encoding only the outer membrane protein, OpcM, into 249-2. The antibiotic susceptibilities determined for the resultant strains suggest that the product of *opcM*, by itself, is sufficient to confer partial resistance to chloramphenicol, trimethoprim, and ciprofloxacin or that it forms a channel that can be shared by other efflux systems. DNA hybridization demonstrated the presence of sequences homologous with *opcM* in both antibiotic-susceptible and -resistant CF isolates. If the entire operon was present in susceptible strains, this would imply a regulated gene. Alternatively, other homologs of this family of transporters may be present in *B. cepacia*. Poole et al. (27) found homology of *oprM* with a *B. cepacia* fusaric acid resistance gene, *fusA* (37). However, our sequence analysis did not identify significant homology between *opcM* and *fusA*.

An additional difference between the multiple antibiotic resistance described for *P. aeruginosa* and that which we have characterized for *B. cepacia* is the iron regulation of the *P. aeruginosa* system. Because Poole et al. (26, 27) were working with the gene cluster in the wild-type resistant strain and we have cloned it into an antibiotic-susceptible deletion mutant (9), direct comparison of regulation is not possible at this time. However, the genes encoding multiple antibiotic resistance in *B. cepacia* appear to be related to the efflux-mediated multiple antibiotic resistance operon in *P. aeruginosa*. Further subcloning and sequence analysis of pLIN1 will be critical in determining whether a complete *mexA-mexB-oprM* homolog is present in *B. cepacia*. Additional studies using mutagenesis of the wild-type resistant strain to inactivate the resistance determinants will contribute to our ability to directly compare the regulations and functions of the systems in *B. cepacia* and *P. aeruginosa.*

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