Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3112–3116, May 1987
Biochemistry

Cloning and DNA sequence of the mercuric- and organomercurial-resistance determinants of plasmid pDU1358

(heavy metal resistance/inducibility/restriction mapping/organomercurial lyase)

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Communicated by I. C. Gunsalus, January 5, 1987

ABSTRACT **The broad-spectrum mercurial-resistance plasmid pDU1358 was analyzed by cloning the resistance determinants and preparing a physical and genetic map of a 45-kilobase (kb) region of the plasmid that contains two separate mercurial-resistance operons that mapped about 20 kb apart. One encoded narrow-spectrum mercurial resistance to Hg^{2+} and a few organomercurials; the other specified broad-spectrum resistance to phenylmercury and additional organomercurials. Each determinant governed mercurial transport functions. Southern DNA·DNA hybridization experiments using gene-specific probes from the plasmid R100 *mer* operon indicated close homology with the R100 determinant. The 2153 base pairs of the promoter-distal part of the broad-spectrum Hg^{2+}-resistance operon of pDU1358 were sequenced. This region included the 3′-terminal part of the *merA* gene, *merD*, unidentified reading frame URF1, and a part of URF2 homologous to previously sequenced determinants of plasmid R100. Between the *merA* and *merD* genes, an open reading frame encoding a 212 amino acid polypeptide was identified as the *merB* gene that determines the enzyme organomercurial lyase that cleaves the C—Hg bond of phenylmercury.**

Resistance to mercurial compounds is widespread in prokaryotes and is usually specified by genes on plasmids or transposons (1–6). Two distinct mercurial-resistance phenotypes have been described: (*i*) resistance to mercuric ions (Hg^{2+}) by enzymatic reduction to Hg^0 and to a limited number of organomercurials by an unknown mechanism (narrow spectrum) and (*ii*) resistance in addition to a wider range of organomercurials including phenylmercuric acetate (PhHgOAc) and thimerosal (broad spectrum). Both narrow- and broad-spectrum determinants specify the enzyme mercuric reductase that converts Hg^{2+} to the less toxic Hg^0, which is then volatilized. Broad-spectrum determinants encode, in addition, the enzyme organomercurial lyase, which cleaves C—Hg bonds to yield Hg^{2+} ions, which are then detoxified by mercuric reductase.

The mercury-resistance determinants also encode transport functions, which carry mercurial compounds across the cell membrane and present the mercurials to the detoxifying enzymes. If the transport functions are expressed in the absence of reductase activity, the cells become hypersensitive to mercurial compounds (7).

The narrow-spectrum *mer* resistance determinants from plasmid R100 (which contains transposon Tn*21* encoding mercury resistance) and transposon Tn*501* have been studied by genetic and DNA sequence analysis (8–13). The *merT* and *merP* gene products are involved in the uptake and transport of Hg^{2+}; *merA* specifies the mercuric reductase enzyme; *merC*, *merD*, and the unidentified reading frames (URF1 and URF2) are of uncertain function. The *merR* gene of R100 is transcribed divergently from *merT*, *merP*, *merC*, *merA*, and *merD* and specifies a regulatory protein that normally represses the transcription of the *mer* operon (14). However, in the presence of subtoxic concentrations of Hg^{2+}, the *merR* product acts as an inducer of the mercurial-resistance phenotype (8). The *merR* product of some broad-spectrum determinants can complement R100 *merR* mutants and regulate the R100 *mer* system (15).

Much less information is available on the genetics of broad-spectrum mercurial-resistance determinants. It seems reasonable that broad-spectrum determinants should be similar in organization to the narrow-spectrum mercurial-resistance operons with an additional gene, *merB*, specifying the organomercurial lyase enzyme. Broad-spectrum mercurial resistance has been located on a 9-kilobase pair (kb) transposon, Tn*3401* (5), whereas on the *Staphylococcus aureus* plasmid pI258, the *merA* and *merB* genes are carried on a 6.4-kb fragment (16), thus indicating close linkage of *merB* to other *mer* genes. However, the *merB* gene of plasmid R831b maps 13.5 kb away from the other *mer* genes (17).

Plasmid pDU1358 is a multiple-antibiotic-resistance IncC incompatability group plasmid (specifying broad-spectrum mercurial resistance) that was originally isolated from a hospital strain of *Serratia marcescens* (18). Here we report a genetic study of mercurial resistance specified by plasmid pDU1358 and show that it carries two *mer* operons (one narrow spectrum and one broad spectrum) that can function independently. We present the DNA sequence of an organomercurial lyase gene from a Gram-negative organism.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. *Escherichia coli* K-12 strains were C600 F^- [*thr-1*, *leuB6*, *lacY1*, *supE44*, λ^- (19)]; DU5111 [Δ(*lac-pro*)XIII *thi rpoB*, Mu (8)]; and WB373 [pOX38 *tra*::Tn*5*-DR2/*hsdR17 supE44*, *thi*, endoI$^-$, phage M13 sensitive (20)]. Plasmid pDU202 is a tetracycline-sensitive variant of plasmid R100-1 (21). Plasmids pBR322 [ampicillin and tetracycline resistance (22)], pUC19 [ampicillin resistance (23)], and pACYC184 [tetracycline and chloramphenicol resistance (24)] served as cloning vectors.

Media and Chemicals. Bacteria were grown in L broth or on L agar (25). Strains carrying M13 were grown in 2XNY broth or on 5-bromo-4-chloro-3-indolyl β-D-galactoside agar (20, 26). Antibiotics and other chemicals were obtained from Sigma or were the best grade available from BDH.

DNA Manipulations. Restriction endonuclease enzymes, T4 ligase, and BAL-31 were purchased from New England Biolabs or Boehringer Mannheim Biochemicals and were

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Abbreviations: PhHgOAc, phenylmercuric acetate; URF, unidentified reading frame; ^{R}, resistance; ^{S}, sensitivity.

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used according to the manufacturer's instructions. DNA polymerase I (Klenow fragment) was purchased from United States Biochemical (Cleveland, OH). Standard molecular cloning, transformation, and electrophoresis techniques (27) were used to clone fragments of pDU1358 into the vector plasmids pBR322, pACYC184, and pUC19.

Susceptibility to Mercurial Compounds. Resistance to mercurial compounds was determined by disk tests and toothpick streaks on Lab Lemco agar (28) as described (8). Inducibility of mercurial-resistance phenotypes was determined by adding subtoxic concentrations of the mercurial compound to exponential-phase L broth cultures 60 min prior to challenge with either Hg^{2+} or PhHgOAc and monitoring growth by the absorbance (600 nm) of the culture.

β-Galactosidase Assays. The production of β-galactosidase from cells containing pDU1161 (8) was measured as described (25).

DNA Sequencing. The chain-termination method (29) as modified by Barnes and Bevan (20) and Misra (26) was used to sequence a 2.1-kb region of the broad-spectrum resistance *mer* operon from pDU1358. The 4.2-kb *Hin*dIII fragment from pHG106 was cloned into M13 mWB2348 (20) and ordered deletions (20) from the primer location were generated using exonuclease BAL-31 (26). Both strands were sequenced.

RESULTS

The Mercurial-Resistance Determinants of pDU1358. A 45-kb region of plasmid pDU1358 was mapped by cloning overlapping restriction nuclease fragments that expressed resistance to antibiotics and mercurial compounds (Fig. 1). Two different fragments of pDU1358 expressed resistance to mercurial compounds when cloned in pBR322 (Fig. 1). One recombinant plasmid (pHG102) expressed a typical narrow-spectrum phenotype, whereas another (pHG103) encoded broad-spectrum mercurial resistance. This broad-spectrum determinant was subcloned on a 4.2-kb *Hin*dIII fragment into pBR322. The resulting plasmid (pHG106) expressed a higher level of resistance to Hg^{2+} and PhHgOAc than did pHG103 (Table 1).

There is an *Eco*RI site in the same position in the *merA* genes of Tn*501* and Tn*21* (8, 10, 12, 13) and it appears to be conserved in the narrow- and broad-spectrum mercurial-resistance operons of pDU1358. Subcloning a *Hin*dIII–*Eco*RI fragment from pHG106 and a *Bgl* II–*Eco*RI fragment from pHG102 produced plasmids pHG108 and pHG111, respectively (Fig. 1), both of which conferred hypersensitivity to Hg^{2+} and PhHgOAc. The hypersensitivity phenotype results from expression of transport functions (7) in the absence of reductase activity due to disruption of *merA*. This result allows the *mer* operons to be oriented on the physical plasmid map (Fig. 1).

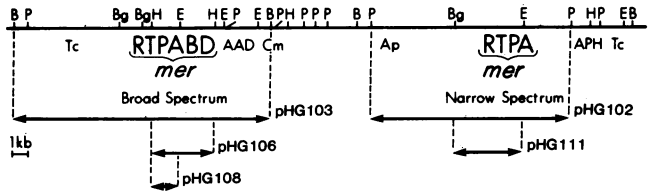

FIG. 1. Restriction nuclease map of plasmid pDU1358 antimicrobial-agent-resistance region as determined by single- and double-restriction enzyme digestion and analysis on agarose gels. Location of the resistance determinants and regions of DNA that have been cloned into vector plasmids are indicated. Restriction nuclease sites: B, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; and P, *Pst* I. AAD, aminoglycoside adenylase; APH, aminoglycoside phosphorylase; Tc, tetracycline resistance; Cm, chloramphenicol resistance; Ap, ampicillin resistance; R, T, P, A, B, and D, the *merR*, *merT*, *merP*, *merA*, *merB*, and *merD* genes. The orientations of the *mer* operons are known from the hypersensitivity of cells with plasmids pHG108 and pHG111 and also from Southern blot analysis (see Fig. 4). The hypersensitivity establishes a transport activity that probably requires *merT* and *merP* functions. We do not know if either operon has a *merC* (10) gene or whether the narrow-spectrum *mer* operon has a *merD* gene.

DNA Sequence. Fig. 2 shows 2153 base pairs (bp) of the promoter-distal part of the pDU1358 broad-spectrum *mer* operon from plasmid pHG106. The major open reading frames are indicated along with predicted amino acid sequences of the polypeptides. The *merA*, *merD*, URF1, and URF2 open reading frames were identified by comparison with the Tn*21* and Tn*501* *mer* sequences (10, 11). In the narrow-spectrum *mer* operons of Tn*21* and Tn*501*, the translational termination codon of the *merA* gene is followed immediately by the ribosome binding site and ATG initiation codon of *merD*. In the pDU1358 broad-spectrum *mer* operon the *merA* gene is followed by a ribosome binding site and an open reading frame of 639 bp corresponding to a 212 amino acid polypeptide (Fig. 2). The predicted N-terminal amino acid sequence differs in only one position from the 46 N-terminal amino acids of the organomercurial lyase protein

Table 1. Resistance levels and complementation between pDU1358 *merR* genes and pDU1161 (*merR*::Tn*5* *merA–lac*)

Plasmid	Inhibition zone,† mm		β-Galactosidase*		
				Induced‡	
	Hg^{2+}	PhHgOAc	Uninduced	With Hg^{2+}	With PhHgOAc
pDU1358 (Hg^R $PhHgOAc^R$)	1	2	4 ± 2	255 ± 56	325 ± 27
None (Hg^S $PhHgOAc^S$)	11	14	30 ± 3	36 ± 2	36 ± 1
pDU1053	8	14	16 ± 1	16 ± 1	14 ± 3
pDU1003	6	14	4 ± 1	103 ± 43	7 ± 1
pHG102	4	14	4 ± 2	139 ± 20	5 ± 2
pHG103	8	5	3 ± 1	112 ± 15	112 ± 17
pHG106	1	0	22 ± 3	21 ± 4	21 ± 3

pDU1358 is the wild-type plasmid (18); pDU1161 contains a *merR*::Tn*5* insertion and a *merA–lac* fusion (8); pDU1053 is a *merR*::Tn*5* insertion mutant of plasmid pDU1003 (8); pDU1003 is the mercurial-resistance-conferring plasmid (8) with the *mer* genes of R100 cloned into pBR322. The structures of the fragments of pDU1358 cloned into pBR322 for plasmids pHG102, pHG103, and pHG106 are shown in Fig. 1. ^{R}, resistance; ^{S}, sensitivity.

*Units are according to Miller (25). The numbers are the means ± SD of two determinations each from two independent experiments ($n = 4$).

†Inhibition zone diameters (minus 6-mm disk diameter) with 37 nmol of Hg^{2+} or 30 nmol of PhHgOAc (8) for strain C600 carrying only the one indicated plasmid.

‡Induction was with 1 μM Hg^{2+} or 0.5 μM PhHgOAc.

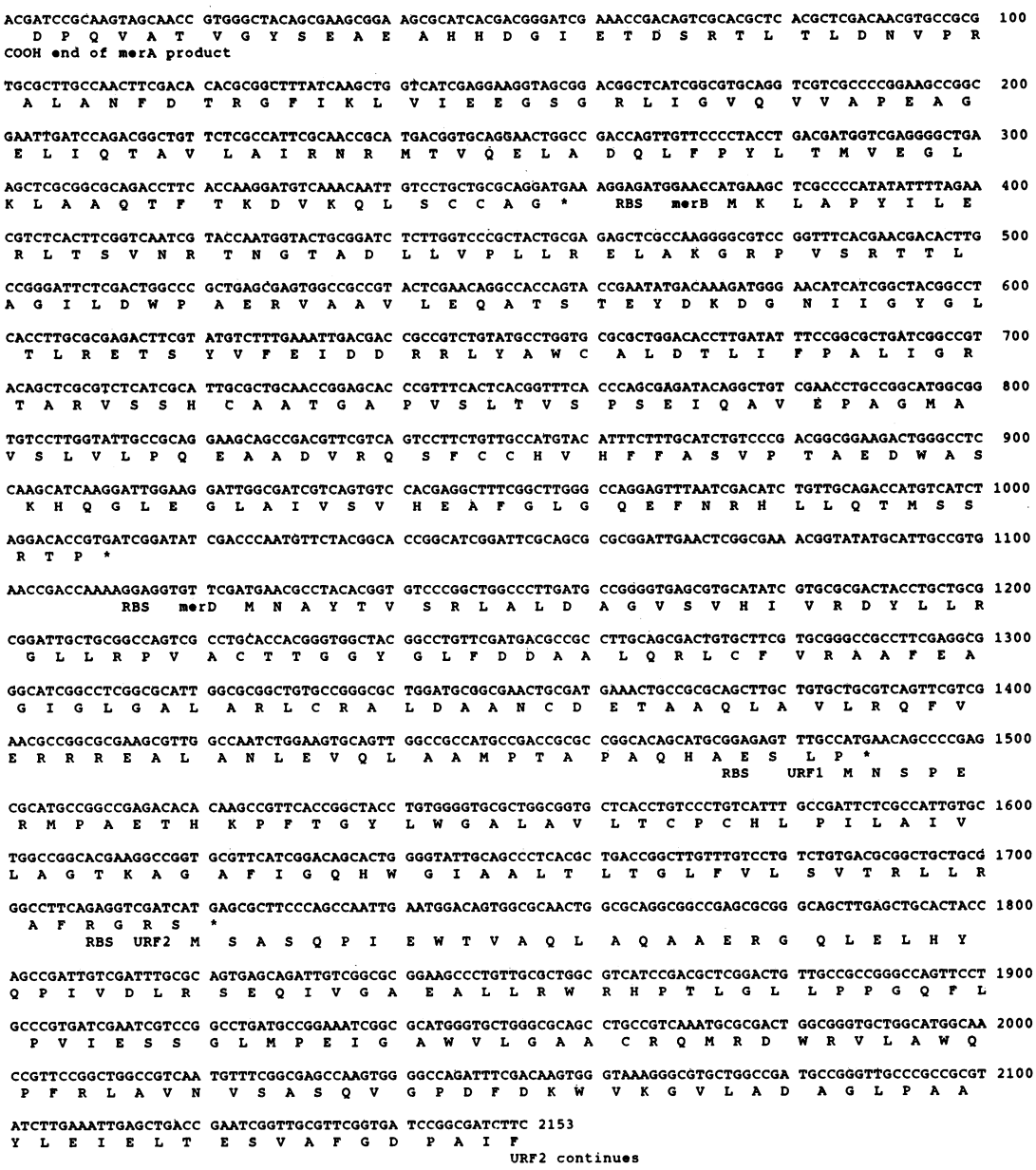

FIG. 2. DNA sequence of 2153 bp of the plasmid pDU1358 broad-spectrum *mer* operon, including the *merB* region, from plasmid pHG106. Only the strand equivalent to the mRNA (anticoding strand) is shown. Ribosome binding sites (RBS) and predicted polypeptide sequences are shown; * represents stop codons. The *merA*, *merD*, URF1, and URF2 sequences were identified by their >80% identity with those of R100 (9–11) at the amino acid level (data not shown).

from plasmid R831b (30). There is also significant homology (39% identical amino acids) between the predicted amino acid sequence of the newly sequenced *S. aureus* organomercurial lyase (33) and the amino acid sequence of the pDU1358 enzyme (Fig. 3). A BAL-31 deletion mutant (pHG114), which consisted of pHG106 with DNA deleted from the promoter-distal *Hin*dIII site to position 425 in Fig. 2, lost PhHgOAc resistance but retained Hg^{2+} resistance. A similar mutant (pHG115) that was deleted only up to position 1291 (from the same *Hin*dIII site) retained full broad-spectrum resistance to Hg^{2+} and PhHgOAc, confirming that the open reading frame between *merA* and *merD* is the *merB* gene and also indicating that *merD*, URF1, and URF2 are not essential for expression of Hg^{2+} and PhHgOAc resistances.

Southern Blot Analyses. Southern blot DNA·DNA hybridization (32) experiments were done using ^{32}P-labeled probes that included (*i*) most of the *merR* gene, (*ii*) most of the *merT* and *merP* genes, or (*iii*) the 5′ end of the *merA* gene from plasmid R100 [in the form of restriction nuclease fragments from mutant plasmid pDU1205 (14)]. These probes were blotted against pDU1205 DNA, where all three probes hybridized, as expected (Fig. 4). The *merT–merP* and the *merA* also hybridized with fragments from plasmids pHG102 and pHG106 (Fig. 4 *B* and *C*), demonstrating a close relationship between the R100 and pDU1358 sequences in these genes. However, the R100 *merR* probe hybridized with DNA of plasmid pHG102 (with the narrow-spectrum *mer* operon of pDU1358) but did not hybridize with DNA from plasmids pHG103 and pHG106 (with the broad-spectrum *mer* operon of pDU1358; Fig. 4*A*). This result shows a surprising lack of close sequence relationship between the two *merR* genes of plasmid pDU1358.

Inducibility of the pDU1358 *mer* Operons. The regulation of the narrow- and broad-spectrum mercurial-resistance operons was studied by (*i*) liquid culture growth experiments of strains with pDU1358 and (*ii*) complementation tests with

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MKLAPYILERLTSVNRTNGTADLLVPLLRELAKGRPVSRTTLAGILDWPAERVAAVL 57
:                :  :::  :: : ::     :    : : :  ::
MKNISEFSAQLDQTFDQGEAVSMEWLFRPLLKMLAEGDPVPVEDIAAETGKPVEEVKQVL 60

EQATSTEYDKDGNIIGYGLTLRETSYVFEIDDRRLYAWCALDTLIFPALIGRTARVSSHC 117
  : : :  :::::: :   :: :  :::::::::: ::::::::    : :
QTLPSVELDEQGRVVGYGLTLFPTPHRFEVDGKQLYAWCALDTLMFPALIGRTVHIASPC 120

AATGAPVSLTVSPSEIQAVEPAGMAVSLVLPQEAADVRQSFCCHVHFFASVPTAEDWASK 177
  ::  : ::: :    :::   :: : : : :: ::  :::: :  : ::
HGTGKSVRLTVEPDRVVSVEPSTAVVSIVTPDEMASVRSAFCNDVHFFSSPSAAQDWLNQ 180

HQGLEGLAIVSVHEAFGLGQEFNRHLLQTMSSRTP 212
:   :    :          :  :
HPESSVLPVEDAFELGRHLGARYEESGPTNGSCCNI 216
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FIG. 3. Alignment of predicted organomercurial lyase amino acid sequence from pDU1358 with that from *S. aureus* plasmid pI258 (33). The alignment was run using the Wilbur and Lipman (31) program with parameters K-tuple = 2; window size = 20; and gap penalty = 5. A colon indicates identity.

pDU1161, which is a plasmid R100 derivative lacking *merR* function and containing a transcriptional *merA–lac* fusion (Table 1). The growth experiments showed that cells containing pHG102 could be induced to express Hg^{2+} resistance with subtoxic concentrations of Hg^{2+} but not with PhHgOAc (Fig. 5*A*). Similar results to those with pHG102 were obtained with $merR^+$ derivatives of narrow-spectrum resistance plasmid R100 (data not shown). Cells containing pHG103 (Hg^R $PhHgOAc^R$) were induced with Hg^{2+} (Fig. 5*B*) and PhHgOAc (Fig. 5*C*) and expressed broad-spectrum mercurial resistance, although Hg^{2+} acted as a relatively poor inducer of PhHgOAc resistance (Fig. 5*B*). There was no difference in resistance levels between uninduced and induced pHG106-carrying cells after challenge (data not shown), indicating constitutive expression of the *mer* genes on this plasmid. This is probably due to the loss of the *merR* gene during subcloning.

Complementation Tests. The ability of the recombinant plasmids carrying the pDU1358 narrow- and broad-spectrum resistance operons to complement a *merR* mutant derived from plasmid R100 was tested using a transcriptional *lac* fusion to *merA* to monitor expression of the R100 *mer* operon. The presence of pHG102 repressed β-galactosidase expression by pDU1161 in the absence of induction by 85% (Table 1). Expression was elevated substantially following induction with a subtoxic concentration of Hg^{2+}. This indicates that the *merR* gene product of the narrow-spectrum pDU1358 *mer* operon is similar in function to that of R100. Only Hg^{2+} and not PhHgOAc acted as an inducer. The pDU1358 broad-spectrum *mer* operon

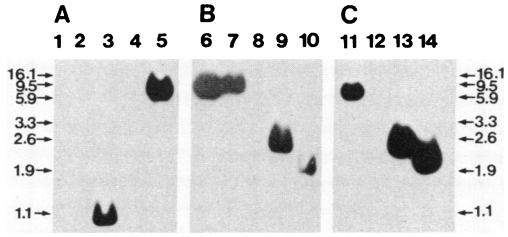

FIG. 4. Southern blot DNA·DNA hybridization with *merR*-, *merT–merP*-, and *merA*-specific probes from plasmid R100. (*A*) *merR* probe [375-bp *Dde* I–*Bam*HI fragment from plasmid pDU1205 (14)]. (*B*) *merT–merP* probe (574-bp *Nae* I fragment from pDU1205). (*C*) *merA* probe (844-bp *Sfi* I–*Eco*RI fragment from pDU1205). Hybridization against DNA digested with restriction endonucleases, electrophoresed on a 1% agarose gel, and transferred to nitrocellulose (32). Lanes 1, 8, and 12, *Eco*RI-digested pBR322; lanes 2, 10, and 14, pHG106 digested with *Hin*dIII and *Eco*RI; lanes 3, 9, and 13, pDU1205 digested with *Bam*HI, *Pst* I, and *Eco*RI; lane 4, pHG103 digested with *Hin*dIII; lanes 5, 7, and 11, pHG102 digested with *Bgl* II and *Pst* I; lane 6, pDU1205 digested with *Eco*RI. Size markers (in kb) are from positions of λ phage DNA digested with *Eco*RI and *Bam*HI on the eithidium bromide-stained agarose gels (which are not shown).

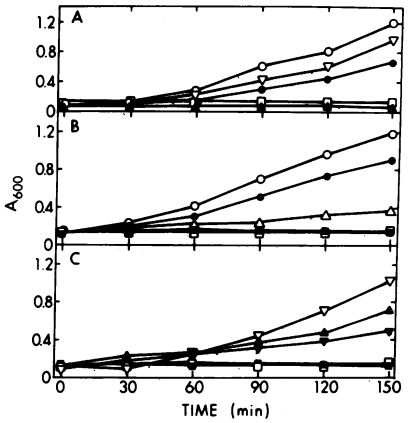

FIG. 5. Growth inhibition experiments with *E. coli* C600 containing recombinant plasmids. Strain C600 (pHG102) (*A*) or C600 (pHG103) (*B* and *C*) cultures were grown in L broth at 37°C. Samples were taken at 30-min intervals and the absorbance at 600 nm was measured. Cultures were induced either with 2.5 μM Hg^{2+} or with 1 μM or 2.5 μM PhHgOAc or were uninduced. After 60 min of induction, a challenging concentration (25 μM) of Hg^{2+} or PhHgOAc was added. A lower inducing concentration of PhHgOAc (1 μM) was used for cells containing plasmid pHG102 as 2.5 μM PhHgOAc inhibited growth. ○, Induced with Hg^{2+}; ●, induced with Hg^{2+}, challenged with Hg^{2+}; △, induced with Hg^{2+}, challenged with PhHgOAc; □, challenged with Hg^{2+}; ▽, induced with PhHgOAc; ▲, induced with PhHgOAc, challenged with Hg^{2+}; ▼, induced with PhHgOAc, challenged with PhHgOAc; ■, challenged with PhHgOAc.

on pHG103 also carries a *merR* regulatory element that can complement pDU1161. In this case induction was achieved with Hg^{2+} and PhHgOAc (Table 1), implying that the broad-spectrum *merR*-encoded protein interacted with Hg^{2+} and PhHgOAc. This complementation of R100 *merR* function by plasmid pHG103 contrasts with the absence of Southern blot hybridization between the R100 *merR* gene probe and plasmid pHG103 (Fig. 4*A*, lane 4).

The pDU1358 broad-spectrum *mer* operon on pHG106 neither repressed (in the absence of inducer) nor elevated (in the presence of inducer) the expression of β-galactosidase (Table 1). This is consistent with the finding that pHG106 expressed Hg^{2+} and PhHgOAc resistances constitutively and presumably lacks *merR* function.

DISCUSSION

We report here the analysis of the broad-spectrum mercurial-resistance plasmid pDU1358 and the existence of two separate *mer* operons, one narrow spectrum and the other broad spectrum. These were cloned separately from pDU1358 and both determinants expressed inducible Hg^{2+} resistance, demonstrating that both encoded all of the proteins necessary for resistance. The hypersensitivity-conferring subclones pHG108 and pHG111 confirmed that each operon had functional mercurial transport genes. Induction experiments and complementation tests showed that pHG103 specified a *merR* function (Fig. 5) that could regulate the R100 *mer* operon (Table 1). These findings are consistent with published data (17) on the broad-spectrum determinant of plasmid R831b. Based on insertional inactivation, a *merB* gene was mapped 13.5 kb from a typical narrow-spectrum *mer* operon. However, it was presumed that the distal *merB* gene was independent of the other *mer* genes (17). Recent unpublished DNA sequencing data suggested that *merB* of R831 was linked to a (probably) nonfunctional *merA* gene (4). Perhaps the R831b *mer* region originally carried two complete *mer* operons like pDU1358 but has suffered DNA rearrangements rendering some genes of the broad-spectrum *mer* operon nonfunctional. A broad-spectrum *mer* determinant

alone would confer the same resistance phenotype as broad- and narrow-spectrum *mer* determinants together. It seems reasonable to suppose that pDU1358 evolved by the stepwise acquisition of resistance determinants to form a composite multiresistance plasmid. This hypothesis is supported by the finding that pDU1358 carries two tetracycline-resistance determinants and two different aminoglycoside-resistance determinants.

The constitutive expression of mercuric resistance conferred by plasmid pHG106 [and by the same 4.2-kb *Hin*dIII fragment cloned into pBR322 in the opposite orientation (data not shown)] raises an interesting possibility. Either the *mer* operon is being transcribed constitutively in both orientations from pBR322 vector promoters or this fragment with its inactivated *merR* gene function (Table 1) functions constitutively from the *mer* operon promoter. There are problems with both alternatives: interruption of pBR322 DNA at the *Hin*dIII site inactivates the major tetracycline-resistance promoter P2, and therefore we would expect transcription from the remaining ampicillin-resistance promoter P1 (34) in one orientation but not in the other. The *merR* product of plasmid R100 is needed for positive transcriptional control (8, 14) and *merR* mutants of R100 are mercury sensitive and not constitutively resistant. It is possible that the pDU1358 broad-spectrum mercurial-resistance operon *merR* is sufficiently different from that of R100 that it functions negatively and not positively. Further fusion and DNA sequencing studies will clarify this matter.

The predicted amino acid sequence of the *merB* polypeptide is almost identical with the N-terminal amino acid sequence directly determined for the organomercurial lyase specified by plasmid R831b (30). The protein also has considerable homology with the central 100 amino acids of the organomercurial lyase from *S. aureus* plasmid pI258 (33) and a deletion affecting this sequence eliminated expression of PhHgOAc resistance. The lyase protein of plasmid pDU1358 does not have an N-terminal sequence related to Gram-negative leader sequences (Fig. 3), suggesting that it is a cytoplasmic protein. The G+C content of the lyase gene is 56 mol%, significantly lower than the average G+C content of the *mer* genes of Tn*21* (65 mol%) and Tn*501* (64 mol%). Possibly *merB* has a different origin from the other *mer* operon genes, and broad-spectrum operons may have evolved by the insertion of DNA carrying the *merB* gene into a narrow-spectrum *mer* operon.

The plasmid pDU1358 and R100 *mer* operons and their polypeptide products show remarkable sequence conservation. The C-terminal 20% of the *merA* protein of pDU1358 is 96% identical with the corresponding sequence of the R100 *merA* polypeptide. A similar level of identical amino acids was found between Tn*21* and Tn*501* *mer* polypeptides (9–11). This suggests a common origin for all three *mer* determinants with the exception of the *merB* gene. Particularly interesting is the conservation (now found in three different mercurial-resistance determinants) of *merD*, URF1, and URF2, which appear to have no essential role in either narrow- or broad-spectrum mercurial resistance.

Fig. 3 shows that the organomercurial lyase polypeptides from plasmid pDU1358 and from *S. aureus* plasmid pI258 (33) share 40% identical positions overall. There are areas of higher homology: amino acids 92–110 of the pDU138 polypeptide have 18/19 positions identical with the corresponding amino acids of the pI258 organomercurial lyase polypeptide. This region contains two conserved cysteine residues that may be of importance in binding mercurial compounds. There is another conserved cysteine (position 159) followed on pDU1358 by a second cysteine (position 160). It is likely that regions of close homology between these organomercurial lyase polypeptides are functionally important and represent the active site of the enzyme.

Brown (35) proposed a model for Hg^{2+} detoxification that involves the initial sequestering of Hg^{2+} ions as a dithiol adduct of the *merP* product in the periplasmic space. The Hg^{2+} is then transferred across the cytoplasmic membrane by paired cysteines in the transmembrane *merT*-encoded protein to the N-terminal cysteines of mercuric reductase. Evidence presented here on the mutants conferring hypersensitivity (pHG108 and pHG111) suggests that PhHgOAc may also be transported by the *merT* and *merP* products. The hypersensitivity of R100 *merA* mutants also extended to PhHgOAc (7).

Annette Hearne helped with the graphics. The work in Dublin was supported by a grant from the Irish Medical Research Council and that in St. Louis was supported by National Institutes of Health Grant AI15672.

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