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Nucleotide sequence and expression of the mercurial-resistance operon from *Staphylococcus aureus* plasmid pI258

(heavy metal resistance/mercuric reductase/organomercurial lyase)

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ABSTRACT **The mercurial-resistance determinant from *Staphylococcus aureus* plasmid pI258 is located on a 6.4-kilobase-pair *Bgl* II fragment. The determinant was cloned into both *Bacillus subtilis* and *Escherichia coli*. Mercury resistance was found only in *B. subtilis*. The 6404-base-pair DNA sequence of the *Bgl* II fragment was determined. The *mer* DNA sequence includes seven open reading frames, two of which have been identified by homology with the *merA* (mercuric reductase) and *merB* (organomercurial lyase) genes from the mercurial-resistance determinants of Gram-negative bacteria. Whereas 40% of the amino acid residues overall were identical between the pI258 *merA* polypeptide product and mercuric reductases from Gram-negative bacteria, the percentage identity in the active-site positions and those thought to be involved in NADPH and FAD contacts was above 90%. The 216 amino acid organomercurial lyase sequence was 39% identical with that from a *Serratia* plasmid, with higher conservation in the middle of the sequences and lower homologies at the amino and carboxyl termini. The remaining five open reading frames in the pI258 *mer* sequence have no significant homologies with the genes from previously sequenced Gram-negative *mer* operons.**

The mercury-resistance determinants from Gram-negative bacteria consist of a regulatory gene (*merR*), an operator/promoter region, and at least three structural genes (1–7). The three structural genes *merT*, *merP*, and *merA* code for a membrane transport protein, a periplasmic Hg^{2+}-binding protein, and the mercuric reductase enzyme subunit, respectively. The reductase functions to detoxify mercury by reducing Hg^{2+} and Hg^{+} ions to elemental Hg^{0} (8). Some plasmid mercurial-resistance determinants also contain an additional gene, *merB*, whose product is the enzyme organomercurial lyase. The lyase cleaves the carbon-mercury bond of organomercurials such as phenylmercuric acetate (9). One product is Hg^{2+}, which is subsequently detoxified by the mercuric reductase. Mercurial-resistance determinants that contain the organomercurial lyase gene are designated broad-spectrum determinants; those without the lyase gene are designated narrow-spectrum determinants (6, 10).

Less is known about mercurial resistance in Gram-positive bacteria. The most thoroughly studied determinant from a Gram-positive bacterium is the broad-spectrum *mer* operon of the *Staphylococcus aureus* plasmid pI258. This *mer* operon was mapped by both deletion mutants (11, 12) and transposon (Tn) insertion mutants (13). These studies established the gene order *merR merA merB*.

Mercury resistance is widely distributed among genera of Gram-negative and -positive bacteria (6, 7, 10). The mercury-resistance determinants from most Gram-negative bacteria are highly homologous (2–4, 6, 14). Conversely, the determinants from Gram-negative sources are less homologous with those from Gram-positive organisms (11, 14).

The DNA of two narrow-spectrum mercurial-resistance determinants from Gram-negative bacteria, those from transposons Tn*21* and Tn*501*, have been sequenced. The sequences were 85% identical at the nucleotide level (2–4). We now have completed the DNA sequence for the broad-spectrum mercury-resistance determinant from plasmid pI258, which was originally isolated from *S. aureus*. Seven large open reading frames (ORFs) were identified and compared with the genes from Tn*21* and Tn*501*. The ORF for the pI258 mercuric reductase was identified. The organomercurial lyase ORF *merB* was identified by comparison with the *merB* lyase gene sequence recently obtained from still another Gram-negative bacterial plasmid (15).‡

MATERIALS AND METHODS

Media and Chemicals. Bacterial cultures for plasmid preparations and *Escherichia coli* strains containing M13 phage were grown in 2XNY broth (16) or on 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) agar (16). Antibiotics and lysozyme were purchased from Sigma. $^{203}Hg(NO_3)_2$ and T4 phage DNA ligase were from New England Nuclear. DNA polymerase I (Klenow fragment) was from United States Biochemical (Cleveland). Restriction endonucleases and BAL-31 exonuclease, from New England Biolabs or International Biotechnologies (New Haven, CT), were used according to the vendors' recommendations.

Mercury-Volatilization Assays. Cells were grown in broth containing tryptone (Difco, 8 g/liter), Casamino acids (Sigma, 2 g/liter), and NaCl (5 g/liter) to the mid-logarithmic phase of growth and harvested by centrifugation, and then the ^{203}Hg-volatilization assays were performed (11).

Bacterial Transformation and DNA Sequencing. Transformation of *E. coli* (16) and *Bacillus subtilis* (17) was done as described. Large-scale isolation of plasmid DNA from *E. coli* (18), *Bacillus* strains (17), or *S. aureus* (12, 19) was performed as described. Replicative forms of M13 and M13 with cloned inserts were prepared by the same procedure used to isolate plasmid DNA (18).

The 6.4-kilobase-pair (kbp) *Bgl* II fragment from plasmid pRAL1 (19) was cloned in both orientations into the *Bgl* II site of M13 bacteriophage mWB2348 (20), and then the hybrid phage DNA (mRAL1 and mRAL2) was introduced into *E. coli* WB373 by transformation (20). For both hybrid phages,

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Abbreviation: ORF, open reading frame.

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‡The sequence reported in this paper is being deposited and the sequence reported in ref. 15 has been deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg, F.R.G.) (accession nos. M15048 and M15049, respectively).

ordered deletions were generated (16, 20). Deletions were sequenced by the dideoxy method as modified (16, 20).

RESULTS

Cloning the Mercury-Resistance Determinant. Digestion of pI258 with restriction endonuclease *Xba* I resulted in four fragments (12), one of which (a 12.5-kbp fragment) contained the mercury-resistance determinant (12, 19). The 12.5-kbp *Xba* I fragment from plasmid pI258 was cloned into the *Xba* I site of pUC12 (ref. 21; the resulting plasmid was called pRAL1 and introduced by transformation into *E. coli* strain JM83; ref. 21). The mercury-resistance determinant from *S. aureus* plasmid pI258 did not confer mercury resistance on *E. coli* JM83(pRAL1) (data not shown). The 12.5-kbp *Xba* I fragment from pRAL1 was cleaved with *Bgl* II and four fragments were produced, with the mercury-resistance determinant located on the 6.4-kbp *Bgl* II fragment (12), which was cloned into cloning vector pBD64 (22) and phage M13. After introduction of the pBD64-derived plasmid (pRAL2) into *B. subtilis* strain BD170 (22) by transformation, mercury-resistant colonies were isolated. Plasmid pRAL2 was obtained from one of these colonies. The presence of the 6.4-kbp *Bgl* II fragment on plasmid pRAL2 was verified by restriction enzyme mapping (data not shown).

Expression of the Mercury-Resistance Determinant. Fig. 1 shows the results of mercury-volatilization experiments with BD170(pRAL2) and control strains. *B. subtilis* BD170-(pRAL2) volatilized mercury 10 times more rapidly when the cells had been preinduced with 2.5 μM Hg^{2+} (Fig. 1*B*). The rate of mercury volatilization by *E. coli* HB101(pDU1003) was greater than that for the mercury-resistant Gram-positive bacteria in this experiment (Fig. 1*A*). All of the mercury-resistant bacteria had an inducible phenotype. *B. subtilis* BD170(pRAL2) was resistant to Hg^{2+}, phenylmercuric acetate and thimerosal, while strain BD170(pBD64) was sensitive to these mercurials (data not shown).

DNA Sequencing. Fig. 2 shows the DNA sequence of the 6404-bp *Bgl* II fragment and the amino acid translations of the major ORFs present on the DNA sequence. Novick *et al.* (12, 13) presented evidence for the direction of transcription in agreement with the orientation deduced from the DNA sequence. Seven ORFs were identified on this strand (Fig. 2). An eighth ORF was identified, on the opposite strand, that if translated from right to left (as shown in Fig. 2) from bp 6266 to 5718 would produce a 182-residue polypeptide.

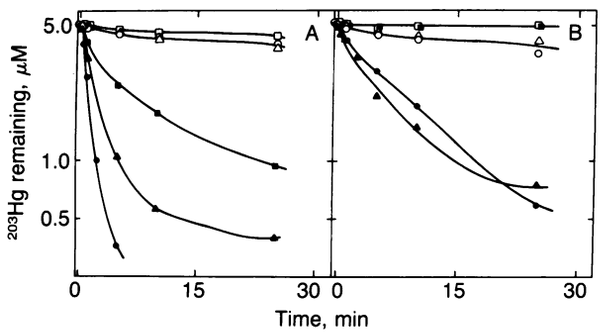

FIG. 1. Volatilization of mercury by intact cells of BD170-(pRAL2) and control strains. Cells were grown in broth to a Klett turbidity reading of 50 (filter 54) and then grown an additional hour in the presence (induced cells, solid symbols) or absence (uninduced, open symbols) of 2.5 μM Hg^{2+}. The cells were harvested by centrifugation and the rate of volatilization of mercury from 5 μM $^{203}Hg^{2+}$ was determined as described (10, 11). (*A*) Circles, *E. coli* HB101(pDU1003) (ref. 1); triangles, *Bacillus* strain I-5 (ref. 23); squares, *Bacillus* strain 473 (ref. 24). (*B*) Circles, *B. subtilis* BD170-(pRAL2); triangles, *S. aureus* RN23(pI258) (ref. 11); squares, *B. subtilis* BD170(pBD64) (ref. 22).

The first ORF from left to right (Fig. 2) is probably the regulatory gene *merR*. The first ORF runs off the end of the *Bgl* II fragment to the left in Fig. 2, but since the pI258 *mer* operon was inducible as cloned (Fig. 1), it is probable that the initiation site for the *merR* ORF occurs within the *Bgl* II fragment at the position shown in Fig. 2. Possible ribosome binding sites are also indicated in Fig. 2. After the *merR* ORF comes the potential operator/promoter region with dyad symmetries (indicated by arrows in Fig. 2) and an additional set of potential −35 and −10 transcriptional initiation signals, followed by six ORFs (Fig. 2) and a possible transcriptional termination site starting at base 6151.

Analysis of *mer* ORFs. The amino acid sequences of each of the predicted polypeptides were analyzed by the hydropathy method of Kyte and Doolittle (25). ORF3 contained a canonical "leader sequence" (26) with 2 lysines plus an arginine following the N-terminal methionine, and then a series of 19 hydrophobic amino acids (ending with an alanine) prior to the next charged amino acid. ORF4 and ORF5 might encode very hydrophobic polypeptides. The amino acid sequence translated from ORF4 contained several hydrophobic stretches, indicative of four to seven membrane-spanning helices; that of ORF5 contained a central hydrophobic sequence bordered by more hydrophilic polypeptide sequences at both the amino and carboxyl ends.

There is a possibly significant homology between the ORF5 amino acid sequence of pI258 and the amino acid sequence of the MerT membrane transport protein of Tn*21* and Tn*501* of Gram-negative systems. The residues Cys-47, Cys-48, Gly-50, Pro-51, Leu-54, Val-55, Ala-56, Leu-57, Gly-58, and Gly-61 (numbered relative to the initiator methionine) of the ORF5 product correspond to the identical amino acids and spacings at positions 24, 25, 27, 28, 31, 32, 33, 34, 35, and 38 of Tn*21* MerT. Nine of these 10 amino acids are identical in the MerT sequence of Tn*501* as well (2).

The last two ORFs translated left to right (as indicated in Fig. 2) are *merA* (mercuric reductase) and *merB* (organomercurial lyase). The approximate locations of the *merA* and *merB* genes on the 6.4-kbp *Bgl* II fragment had been determined (12, 13). The sequences were compared using the program of Wilbur and Lipman (27), which maximizes the pairing of identical bases (or amino acids). When the DNA sequence of each ORF from the pI258 *mer* operon was compared with the genes from Tn*21* and Tn*501* mercury-resistance operons (2–5), a clearly significant homology was found only for *merA* (data not shown). The *merA* ORF was 44% identical at the DNA level to the *merA* genes from Tn*501* and Tn*21*. Transposons Tn*21* and Tn*501* lack *merB* genes, but the *merB* sequence from a different plasmid from a Gram-negative bacterium became available (15). The DNA sequence of the *merB* ORF of *Serratia* plasmid pDU1358 (15) showed 40% base-pair identities when optimally aligned with the *merB* ORF sequence from pI258 (data not shown).

There were significant homologies between the amino acid sequences of mercuric reductase and organomercurial lyase enzymes from *S. aureus* plasmid pI258 and the comparable amino acid sequences from Tn*21* and pDU1358, respectively (Figs. 3 and 4). The mercuric reductase from pI258 was 40% identical (220/547 residues) to the mercuric reductases from Gram-negative bacteria, and the organomercurial lyase from pI258 was 39% identical (83/212 residues) at the amino acid level to the lyase from pDU1358. The homology between the two organomercurial lyase sequences was strongest in the middle 100 amino acids of the two proteins. Little homology was found between the N- and C-terminal sequences (Fig. 4). Aside from a weak homology between the first ORF, glutathione reductase and lipoamide dehydrogenase, no homology was found between the amino acid sequences of the remaining ORFs of pI258 and the previously identified genes of the plasmids of Gram-negative bacteria.

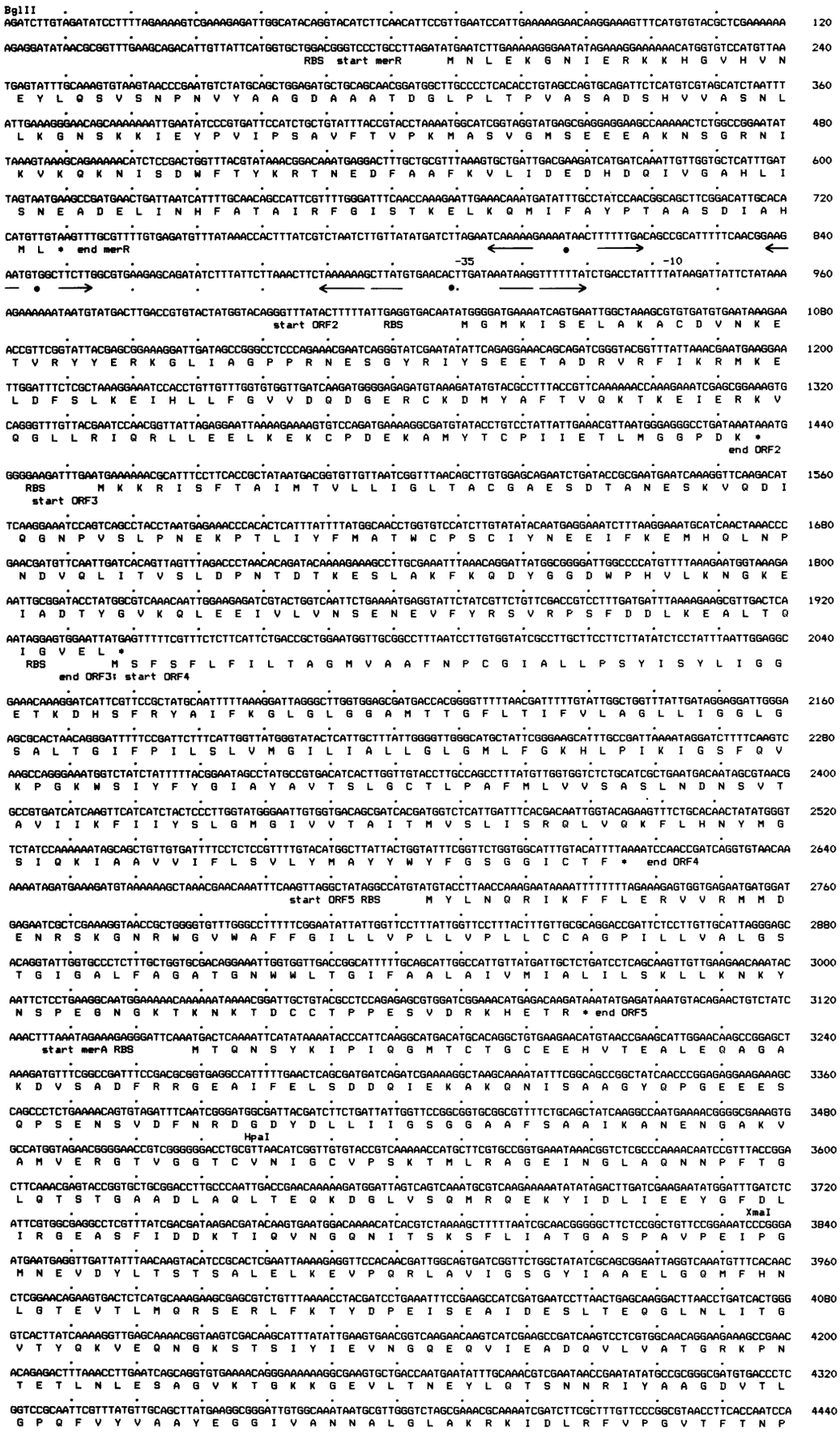

FIG. 2. *(Continued on opposite page.)*

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TCGATCGCCACAGTCGGCTTGACTGAACAACAGGCAAAAGAAAAAGGTTACGATGTCAAAACATCGGTCCTTCCGTTGGACGCTGTACCGCGGGCCTTAGTCAATCACGAAACAACAGGG   4560
 S  I  A  T  V  G  L  T  E  Q  Q  A  K  E  K  G  Y  D  V  K  T  S  V  L  P  L  D  A  V  P  R  A  L  V  N  H  E  T  T  G

GTCTATAAACTTGTAGTCAACGCCCAAACCCAGAAATTGATCGGAGCGCACATTGTGAGTGAAAATGCTGGAGATGTGATTTATGCGGCAACGTTAGCGGTTCAATTTGGATTGACCATT   4680
 V  Y  K  L  V  V  N  A  Q  T  Q  K  L  I  G  A  H  I  V  S  E  N  A  G  D  V  I  Y  A  A  T  L  A  V  Q  F  G  L  T  I

GAAGACCTTACGGATAGCTTTGCGCCTTATTTAACGATGGCTGAAGGATTAAAGCTTGCAGCCTTGACGTTTGATAAAGACGTATCGAAATTATCTTGTTGTGCAGGCTAATGGTTCCTT   4800
 E  D  L  T  D  S  F  A  P  Y  L  T  M  A  E  G  L  K  L  A  A  L  T  F  D  K  D  V  S  K  L  S  C  C  A  G  *  end merA
                                                                                              EcoRI
TTGATTCTAATGCTATGTTGCAGGAGGCACTTAAAACAATGAAGTGGAGAAAATATAAGGAGGAATAAGCTTATGAAAAATATTTCAGAATTCTCAGCCCAACTTGATCAAACTTTTGAT   4920
                                                      start merB RBS         M  K  N  I  S  E  F  S  A  Q  L  D  Q  T  F  D

CAAGGGGAAGCCGTCTCTATGGAGTGGTTATTCCGTCCGTTGCTAAAAATGCTGGCGGAGGGCGATCCAGTCCCCGTTGAGGACATCGCGGCGGAGACCGGGAAGCCCGTCGAGGAAGTT   5040
 Q  G  E  A  V  S  M  E  W  L  F  R  P  L  L  K  M  L  A  E  G  D  P  V  P  V  E  D  I  A  A  E  T  G  K  P  V  E  E  V

AAGCAAGTCCTACAGACTCTACCTAGTGTGGAACTTGATGAGCAGGGCCGTGTCGTCGGTTATGGCCTCACACTGTTCCCTACCCCCCATCGCTTCGAGGTTGATGGGAAGCAACTATAT   5160
 K  Q  V  L  Q  T  L  P  S  V  E  L  D  E  Q  G  R  V  V  G  Y  G  L  T  L  F  P  T  P  H  R  F  E  V  D  G  K  Q  L  Y

GCATGGTGCGCCCTTGACACACTTATGTTCCCAGCACTCATCGGCCGGACGGTCCACATCGCTTCGCCTTGTCACGGCACCGGTAAGTCCGTCCGGTTGACGGTGGAACCGGACCGCGTT   5280
 A  W  C  A  L  D  T  L  M  F  P  A  L  I  G  R  T  V  H  I  A  S  P  C  H  G  T  G  K  S  V  R  L  T  V  E  P  D  R  V

GTAAGCGTCGAGCCTTCAACAGCCGTTGTCTCGATTGTTACACCAGATGAAATGGCCTCGGTTCGGTCGGCCTTCTGTAACGACGTTCACTTTTTCAGTTCACCGAGTGCAGCCCAAGAC   5400
 V  S  V  E  P  S  T  A  V  V  S  I  V  T  P  D  E  M  A  S  V  R  S  A  F  C  N  D  V  H  F  F  S  S  P  S  A  A  Q  D

TGGCTTAACCAACACCCTGAGTCGAGCGTTTTGCCCGTTGAAGATGCCTTTGAACTGGGTCGCCATTTGGGAGCGCGTTATGAGGAGTCAGGACCTACTAATGGGTCCTGTTGTAACATT   5520
 W  L  N  Q  H  P  E  S  S  V  L  P  V  E  D  A  F  E  L  G  R  H  L  G  A  R  Y  E  E  S  G  P  T  N  G  S  C  C  N  I

TAATCTTAGGCAATTACGCCTGCGTCAATCTTGGCCTTACGATGATGTCCTGTAACCGAAGCTCTATCTAGTGAAACCAATTTTATGGGAAACCAAATAAATAAAAAGATCAATTGCTTG   5640
 * end merB
                                                                                   end reverse ORF
TGCCTATATCGGGGAGTTAATAAAGCGAGAAAGTGGATTCTTGAATATAGGAGTAAATAAGGTGCCAAAATGGCACCTTATTTAAAATATGTAACGCCAAGGTCTGCCGTATTATATTGC   5760

ATTGGAAAAAGAAAACCTTCTGTATAAGGAGATGTTATATGATATTTGGTAAACTGTTCGGCAGTTTGGGCGTAGAAAGCTTTTGCTCCTTGAACATCACGTTTGCCAGTTACAATGTCA   5880

TTTAACAAGTTTAAAGATAAAAAATTCATCGCTTCCTCATGACACTTTGCTGATGCCTCACCTGTAGTTCGATCTAAGTAGACACTTCCATCAAAAGCGGCAATCGCATCATATAAGTGT   6000
                                                                            BamHI
ACGGGCACTTTATAATCGATGGTCTGCTTAAGGAAATCAAGGTGAGGGGTAGGGAAGTTATGAGGGACTGGATCCTTATATACTATCGTGCGTTTCCATGGCCCGCTATTGTACCAAATT   6120

AATCGACTCATTGTTGCTTCCTGGGGATATCCATACTTACTAATTATGGTTTTTGCCCCTTCCAACGGTGGTCCCTGCCAATAGGACAAAATGTGATTTAAATGACTATCCATTGCCATA   6240
                        start reverse ORF
CCTCCTAAACATTTGCCTAAAAGCATTGTATGCCCAATAAATGAATTTTAGGGGTTCTGTTGCAAAGTAAAAAAATATAGCTAACCACTAATTTATCATGTCAGTGTTCGCTTAACTTGC   6360
                                           BglII
TAGCATGATGCTAATTTCGTGGCATGGCGAAAATCCGTAGATCT   6404
```

FIG. 2. DNA sequence of the mercury-resistance determinant contained on the 6404-bp *Bgl* II fragment from pI258. The amino acid translation of the identified ORFs is given in standard one-letter amino acid symbols, and ribosome binding sites (RBS) and stop codons (*) are shown. Dyads of symmetrical sequences in the predicted operator/promoter region are indicated by arrows. Only the anticoding strand is shown.

DISCUSSION

Mercuric reductase belongs to a class of flavin-containing, NAD(P)H-dependent oxidoreductases including glutathione reductase (8, 28, 29) and lipoamide dehydrogenase (30). Comparisons of the amino acid sequences of these enzymes showed that while the pI258 mercuric reductase is 40% identical to both of the mercuric reductases from Gram-negative bacteria (Fig. 3 and data not shown), it is only 30% identical to both glutathione reductase (from human erythrocytes) and lipoamide dehydrogenase (from *E. coli*; data not shown).

The three-dimensional structure of the human glutathione reductase is known (28, 29). The amino acid residues involved in the active site and the binding of NADPH and FAD have been determined (29, 31). If we compare the amino acid sequences of the mercuric reductases from pI258 and Tn*21* (Fig. 3), it is evident that the amino acid matches are not evenly dispersed throughout the sequences but rather are clustered at those positions where previous studies with glutathione reductase (28, 29, 31) have shown that the amino acid residues are involved in the enzymatic activity of this protein (Table 1). There are three pairs of cysteine residues conserved in all three mercuric reductase enzymes: one pair is at the active site (residues 123 and 128 in Fig. 3) and one pair each at the N- and C-termini (residues 15 and 18 and residues 544 and 545, respectively).

The amino acid residues comprising the active site of the pI258 mercuric reductase (residues 118–132, see Fig. 3) are identical to those of Tn*21* and Tn*501* except for the conservative substitutions of a valine for an isoleucine at position 119 and an isoleucine for a valine at position 126 in the pI258 sequence. The active sites for glutathione reductase and lipoamide dehydrogenase (3, 8, 30) are closely related.

Table 1 lists the glutathione reductase amino acid residues involved in making contact with FAD (30, 31), as well as the residues in the equivalent positions in lipoamide dehydrogenase and mercuric reductase. For each comparison, protein sequences were aligned (27). The amino acid sequence of

```
MTQNSYKIPIQGMTCTGCEEHVTEALEQAGAKDVSADFRRGEAIFELSDDQIEKAKQNISAAGYQPGEEESQPSE 75
      : ::::  :  ::  :::                                         ::
   MSTLKITGMTCDSCAVHVKDALEKVPGVQSADVSYAKGSAKLAIEVGTSPDALTAAVAGLGYRATLADAPS 71

NSVDFNRDGDYDLLI----------------IGSGGAAFSAAIKANENGAKVAMVERGTVGGTCVNIGCVPSKTM 134
 :          :                   ::::::: :: :: : :: :   :::: :::::: :::::: :
VSTPGGLLDKMRDLLGRNDKTGSSGALHIAVIGSGGAAMAAALKAVEQGARVTLIERGTIGGTCVNVGCVPSKIM 146

LRAGEINGLAQNNPFTGLQTSTGAADLAQLTEQKDGLVSQMRQEKYIDLIEEYGFDLIRGEASFIDDKTIQVNGQ 209
 ::  :  :    :: :    :                                : :           :       :
IRAAHIAHLRRESPFDGGIAATTPTIQRTALLAQQQARVDELRHAKYEGILEGNPAITVLHGSARFKDNRNLIVQ 221

NITSKSF-------LIATGASPAVPEIPGMNEVDYLTSTSALELKEVPQRLAVIGSGYIAAELGQMFHNLGTEVT 277
              ::::::::::: :::     : ::: ::     : :::::::   : :: : :  ::  ::
LNDGGERVVAFDRCLIATGASPAVPPIPGLKDTPYWTSTEALVSETIPKRLAVIGSSVVALELAQAFARLGAKVT 296

LMQRSERLFKTYDPEISEAIDESLTEQGLNLITGVTYQKVEQNGKSTSIYIEVNGQEQVIEADQVLVATGRKPNT 352
   ::  ::  :: : ::         :              :                  ::  :::::: :::
ILARST-LFFREDPAIGEAVTAAFRMEGIEVREHTQASQVAYINGEGDGEFVLTTAHGELRADKLLVATGRAPNT 370

ETLNLESAGVKTGKKGEVLTNEYLQTSNNRIYAAGDVTLGPQFVYVAAYEGGIVANNALGLAKRKIDLRFVPGVT 427
  : :   ::      :           ::   :::::: :  :::::::: :    : : :            :
RKLALDATGVTLTPQGAIVIDPGMRTSVEHIYAAGDCTDQPQFVYVAAAAGTRAAINMTGGDAALNLTAMPAVV- 444

FTNPSIATVGLTEQQAKEKGYDVKTSVLPLDAVPRALVNHETTGVYKLVVNAQTQKLIGAHIVSENAGDVIYAAT 502
:: :  ::::  :  :   :          : :: ::::: :  : :  ::::        :::    :   ::  :  :
FTDPQVATVGYSEAEAHHDGIKTDSRTLTLDNVPRALANFDTRGFIKLVVEEGSGRLIGVQAVAPEAGELIQTAA 519

LAVQFGLTIEDLTDSFAPYLTMAEGLKLAALTFDKDVSKLSCCAG 547
::     :   : :   ::::: ::::::: :: :::   ::::::
LAIRNRMTVQELADQLFPYLTMVEGLKLAAQTFNKDVKQLSCCAG 564
```

FIG. 3. Alignment of the mercuric reductase amino acid sequences from plasmid pI258 (top lines) and Tn*21* (bottom lines). The sequences were aligned with the program of Wilbur and Lipman (27) using parameters *K*-tuple = 2; window = 20; gap penalty = 5. Standard one-letter symbols for amino acids are used; colons indicate identical residues in both sequences; dashes (indicating gaps) are used to optimize the pairing of the two sequences.

```
MKNISEFSAQLDQTFDQGEAVSMEWLFRPLLKMLAEGDPVPVEDIAAETGKPVEE 55
          :                :  :::  :: : ::       :      : :
   MKLAPYILERLTSVNRTNGTADLLVPLLRELAKGRPVSRTTLAGILDWPAER 52

VKQVLQTLPSVELDEQGRVVGYGLTLFPTPHRFEVDGKQLYAWCALDTLMFPALI 110
:  ::     : : :  :   ::::::  :     :: :   ::::::::: :::::
VAAVLEQATSTEYDKDGNIIGYGLTLRETSYVFEIDDRRLYAWCALDTLIFPALI 107

GRTVHIASPCHGTGKSVRLTVEPDRVVSVEPSTAVVSIVTPDEMASVRSAFCNDV 165
:::     : :  ::  : ::: :      :::     :: : : : : : ::  ::  :
GRTARVSSHCAATGAPVSLTVSPSEIQAVEPAGMAVSLVLPQEAADVRQSFCCHV 162

HFFSSPSAAQDWLNQHPESSVLPVEDAFELGRHLGARYEESGPTNGSCCNI 216
::: :    : ::    :        :        :                : :
HFFASVPTAEDWASKHQGLEGLAIVSVHEAFGLGQEFNRHLLQTMSSRTP  212
```

FIG. 4. Alignment of the organomercurial lyase amino acid sequences from plasmid pI258 (top lines) with that from pDU1358 (bottom lines) (see Fig. 3).

Table 1. Comparison of mercuric reductase, glutathione reductase, and lipoamide dehydrogenase amino acids that may make contact with FAD or NAD(P)H

Glutathione reductase*	Lipoamide dehydrogenase†	Mercuric reductase		
		pI258	Tn*21*‡	Tn*501*‡
	FAD contacts			
I 26	L 11	I 91	I 103	I 104
G 27	G 12	G 92	G 104	G 105
G 28	A 13	S 93	S 105	S 106
G 29	G 14	G 94	G 106	G 107
S 30	P 15	G 95	G 107	G 108
G 31	A 16	A 96	A 108	A 109
G 32	G 17	A 97	A 109	A 110
V 49	V 34	V 114	I 126	I 127
E 50	E 35	E 115	E 127	E 128
S 51	R 36	R 116	R 128	R 129
T 57	V 43	T 122	T 134	T 135
C 58	C 44	C 123	C 135	C 136
C 63	C 49	C 128	C 140	C 141
K 66	S 52	S 131	S 143	S 144
K 67	K 53	K 132	K 144	K 145
H 129	L 115	E 195	S 209	E 210
A 130	G 116	A 196	A 210	A 211
A 155	A 143	A 219	A 238	A 239
T 156	A 144	T 220	T 239	T 240
Y 197	I 184	Y 260	V 279	V 280
E 201	E 188	E 264	E 283	E 284
R 291	R 275	R 348	R 366	R 363
V 329	I 310	A 386	A 404	A 401
D 331	D 312	D 388	D 406	D 403
T 339	A 320	V 396	V 414	V 411
F 372	Y 351	F 428	F 445	F 442
H 467	H 444	Y 521	Y 538	Y 535
E 472	E 449	E 526	E 543	E 540
	NAD(P)H contacts			
G 194	G 181	G 257	G 276	G 277
A 195	G 182	S 258	S 277	S 278
G 196	G 183	G 259	S 278	S 279
Y 197	I 184	Y 260	V 279	V 280
I 198	I 185	I 261	V 280	V 281
E 201	E 188	E 264	E 283	E 284
I 217	E 204	Q 280	A 299	A 300
R 218	M 205	R 281	R 300	R 301
H 219	F 206	S 282	S 301	N 302
R 224	P 211	K 287	R 306	R 307
L 285	V 269	V 342	L 360	L 357
L 286	L 270	L 343	L 361	L 358
W 287	V 271	V 344	V 362	V 359
A 288	A 272	A 345	A 363	A 360
I 289	I 273	T 346	T 364	T 361
G 290	G 274	G 347	G 365	G 362
R 291	R 275	R 348	R 366	R 363
D 331	D 312	D 388	D 406	D 403
V 370	I 349	V 426	V 443	V 440

Amino acid residues (standard one-letter symbols) and their position numbers are given.
*Human glutathione reductase sequence (28, 29, 31).
†*E. coli* lipoamide dehydrogenase sequence (30).
‡From ref. 3.

glutathione reductase is overall only about 25–27% identical to each of the three mercuric reductase sequences. However, a comparison of the ($3 \times 28 = 84$) mercuric reductase amino acid residues listed in Table 1 to the glutathione reductase FAD contact positions shows that 60% (50/84) of the amino acid residues are identical. For the three mercuric reductases, the residues listed in Table 1 are identical in 89% (25/28) of the positions as compared with only 40% for the entire amino acid sequences of the pI258 and Tn*21* proteins. For glutathione reductase vs. pI258 mercuric reductase, 64% (18/28) of the positions shown are identical, compared with the 26% match for the overall sequence. Similarly, the lipoamide dehydrogenase sequence and the mercuric reductase sequences were identical in 51% (43/84) of the possible matches in Table 1, much higher than the 26% overall identity. A similar analysis of glutathione reductase residues involved with binding of NADPH (28–30) and the comparable positions in lipoamide dehydrogenase and mercuric reductase (Table 1) showed similar higher frequencies of identities than did the overall sequences.

When the amino acid sequences from the pI258 ORFs were compared with the National Biomedical Research Foundation data base of amino acid sequences§, only the pI258 *merA* sequence had a strong significant homology with sequences from the data base. The pI258 mercuric reductase was related to the mercuric reductase from Tn*501* and plasmid R100 and to glutathione reductase and lipoamide dehydrogenase.

§Protein Identification Resource (1987) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 12.0.

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