DNA Sequence Analysis of the Organomercurial-Resistance Determinants from *Pseudomonas* K-62 Plasmid pMR26

MASAKO KIYONO, TOMOKO OUMRA, MANABU INUZUKA, HIROYUKI FUJIMORI and HIDEMITSU PAN-HOU

Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-01, Japan and Department of Biochemistry, Fukui Medical School, 23 Shimoazuki, Matsuoka, Fukui 910-11, Japan

**Introduction**

A recombinant plasmid pMRA17, which was constructed by inserting the 6.6-kb SacI fragment of the 26-kb plasmid designated pMR26, from *Pseudomonas* K-62 into the SacI site of Bluescript II, inducibly encoded the typical broad-spectrum mercurial resistance basis on the degradation of organomercurials and reduction of the resultant Hg$^{2+}$ to Hg$^0$. In this paper, we present the DNA sequence of the region required for broad-spectrum mercury resistance in pMRA17.

**Methods**

Standard molecular cloning, transformation and gel electrophoresis techniques were used. The diodeoxy chain-termination DNA sequencing method of Sanger *et al.* was used to sequence both strands of the 5.5 kb BglII-SacI region of the mercury resistance operon from plasmid pMRA17. Overlapping ordered sequencing deletions from the primer location were generated by digestion with appropriate restriction enzymes and exonuclease III.

**Results**

Six open reading frames (ORFs) were found on the DNA sequence of a 5504-bp BglII-SacI fragment encompassing the broad spectrum mercurial resistance operon of pMRA17. Five of these were identified as merR, merT, merP, merA, and merB by comparison with the DNA sequence of the mer operons from Tn501, Tn21, pDU1358, and pJ258. The remaining ORF in the operon has no significant homology with the genes from previously sequenced mer operon. The regulatory region before the major transcription initiation site containing potential $-35$ and $-10$ sequences and dyad symmetrical sequences, which may be the merR binding sites for transcriptional regulation is also found in pMRA17 mer operon. Deletion analysis of the mer operon showed the organization of the mer genes on the operon is in the order of merR, operator/promoter, merT, merP, merA, URF, and merB.

**Discussion**

The pMRA17 merR, merT, merP possessed a relatively high degree of similarity whereas the merA and merB showed somewhat less identity in both DNA and amino acid sequences with those from previously published mer genes. Most of the characteristic and functional sequences (or residues) in the published mer polypeptides were conserved in the respective protein encoded by mer gene from pMRA17. The pMRA17 sequence shown is more similar to that of Tn21 (originally from a *Shigella* sp.) than to that of Tn501 (originally from a *Pseudomonas* sp.) and pDU1358 (originally from a *Serratia* sp.). The origin of the pMRA17 (constructed from *Pseudomonas* K-62) mercurial resistance genes is indeed interesting but at present we do not have enough information to warrant further discussion.

**References**