

NOTES

Translocatable Resistance to Mercuric and Phenylmercuric Ions in Soil Bacteria

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Of a sample of 42 gram-negative Hg-resistant bacteria, three (a *Pseudomonas fluorescens*, a *Klebsiella* sp. and a *Citrobacter* sp.) contained translocatable elements conferring resistance to Hg²⁺ (all three) and to Hg²⁺ and phenylmercuric acetate (*P. fluorescens*). The discovery of transposable phenylmercuric acetate resistance extends the range of known resistance "transposons" from heavy metals and antibiotics to organometallic compounds.

Resistance to mercuric ion (Hg²⁺) has been described in a variety of bacterial taxa (5-7), and in at least one report, the prevalence of resistant organisms has been ascribed to environmental pollution (7).

In earlier studies, 97 Hg-resistant bacteria were isolated from soils in or around Christ church in the South Island of New Zealand (W. J. Kelly and D. C. Reanney, unpublished data). These soils had no known history of exposure to mercury in any form. The minimal concentration of Hg²⁺ required to completely inhibit the growth of individual isolates ranged from 10 to 160 µg of Hg²⁺ ml⁻¹. Of the gram-negative members, 35 would accept the broad host range plasmid RP1, which carries the determinants for carbenicillin (Cb), kanamycin (Km), and tetracycline (Tc) resistance (8). Organisms with the RP1 Hg^r phenotype were then crossed with *Escherichia coli* K-12 3272, and transconjugants were selected on Tc to score for acquisition of RP1 and on Hg²⁺ to score for acquisition of mercury resistance. All clones selected on Tc showed resistance to Cb and Km, whereas a variable number showed resistance to Hg²⁺. Clones selected on Hg²⁺ were normally also resistant to Cb, Km, and Tc. Strains with coinherited Hg²⁺, Cb, Km, and Tc resistance were then crossed with a second subline of *E. coli* K-12. Transfer frequencies by using Km selection were in the range of 9.2×10^{-4} to 1.4×10^{-4} per donor cell, whereas with Hg²⁺ selection, the figures lay in the range of 2×10^{-3} to 8×10^{-6} . All clones tested, however, possessed both the RP1

markers and Hg²⁺ resistance, irrespective of the type of selection imposed. Discrepancies between the transfer frequencies on different selective media may reflect differences in the expression of the Km and Hg²⁺ resistance traits. Since Hg²⁺ resistance is an inducible character (11), authentic, Hg²⁺-resistant cells often fail to grow on media with high concentrations of mercuric ion.

In cases in which cotransfer of Hg²⁺ resistance with the RP1 markers was demonstrated, covalent linkage between RP1 DNA and the DNA encoding Hg²⁺ resistance was confirmed by P1 transduction. To determine whether transposition had occurred, we first passed the hybrid RP1 Hg^r plasmids into a *recA* strain of *E. coli* K-12. Transfer-defective mutants of RP1 were then selected from plates flooded with phage PRR1 (which specifically lyses transfer-proficient members of the IncP group to which RP1 belongs [10]). Transfer-defective mutants were then crossed with a different *E. coli* subline containing a mutant of the IncW plasmid R388; this plasmid mutant is not subject to fertility inhibition by RP1 (9). After obtaining a clone containing the markers of both RP1 Hg^r and R388, we performed a second mating with an R⁻ strain and scored for cultures which retained stably linked resistance between the R388 markers (trimethoprim and sulfonamide resistance) and Hg^r in subsequent transfers.

Of the seven RP1-Hg^r hybrids obtained by P1 transduction, three were able to donate the Hg²⁺ resistance phenotype to R388 in the *recA* back-

ground. The original soil donors of the translocatable DNA units were a *Pseudomonas fluorescens*, which was resistant to both Hg^{2+} and to the organomercurial, phenylmercuric acetate (PMA), and a *Klebsiella* sp. and a *Citrobacter* sp., both of which were resistant to Hg^{2+} but not to PMA. The frequency of *recA*-independent movement in all three cases was approximately 1% at 37°C under the experimental conditions used.

Analysis on agarose gels showed significant differences in size between plasmids extracted from clones with and without the PMA^r Hg^r phenotype (Fig. 1D) and between plasmids extracted from clones with and without the Hg^r phenotype (data not shown). Digestion of the hybrid R388 PMA^r Hg^r molecule with the restriction nuclease *Hind*III showed that the en-

zyme cut the hybrid molecule four times, whereas the original R388 plasmid was cut only once. Of the three additional bands obtained from the R388 PMA^r Hg^r DNA, two were obtained from *Hind*III digests of the hybrid form of RP1 PMA^r Hg^r, confirming the presence of identical or similar inserted nucleotide sequences in both plasmids (Fig. 1A and B). An identical result was obtained with DNA extracted from a transconjugant from a second RP1 PMA^r Hg^r × R388 cross, suggesting that the same sequences had "transposed" between the two plasmids. Similar studies have demonstrated the existence of inserted sequences in the two cases of Hg resistance. We estimate the size of the insertion conferring joint PMA-Hg resistance as about 6 megadaltons (Md) and that of the insertions conferring Hg resistance alone as about 5 Md.

The elements described in this paper display the properties expected of "transposons." We cannot formally exclude the possibility that the original resistance genes were located on small, presumably nonconjugative plasmids which formed cointegrates with RP1 by some form of *recA*-independent recombination. However, repeated gel analyses of DNA extracted from the original *P. fluorescens* strain revealed only one faint plasmid band between the origin and the position of chromosomal DNA. This plasmid was much too big to account for the estimated size differences between RP1 or R388 DNA with and without the inserted module. No plasmids were detected in the original *Klebsiella* and *Citrobacter* sp. As our extraction techniques did not enrich for very large plasmids, we are unable to specify the replicons which are the source of the PMA^r Hg^r and Hg^r genes, but our data clearly favor a transposition model over a cointegration model.

The transposons described in this paper have been assigned the following reference codes: the PMA^r Hg^r transposon, Tn3401, the Hg^r transposon from the *Citrobacter* sp., Tn3402, and the transposon from the *Klebsiella* sp., Tn3403. These three transposons have been compared with each other and with the original Hg^r transposon Tn501 (12) by digesting RP1 containing each of the transposons in turn with a common battery of restriction nucleases (*Eco*RI, *Bam*HI, and *Hind*III). The restriction maps for Tn3402 and Tn3403 were identical. However, the maps for these two transposons differed both from Tn3401 and from Tn501. It appears that the Hg^r phenotype common to all four transposons tested does not result from a single, conserved nucleotide sequence.

Transposable resistance to mercuric ion but not to PMA has been reported (3, 12, 13). The

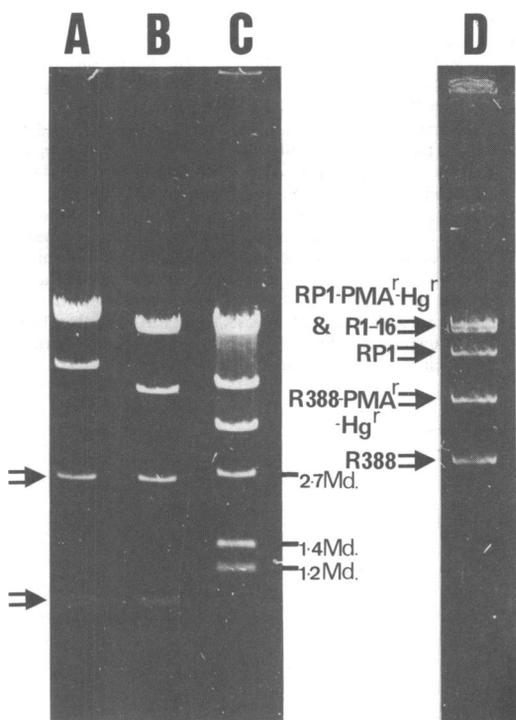


FIG. 1. Agarose gel electrophoresis of intact and endonuclease-digested plasmid DNA. DNA was obtained by cesium chloride-ethidium bromide density gradient centrifugation of DNA precipitated from cleared cell lysates (4). Electrophoresis was for 3 (A, B, and C) and 5 h (D) in Tris-borate-buffered 0.6% agarose. (A) RP1 PMA^r Hg^r digested with *Hind*III; (B) R388 PMA^r Hg^r digested with *Hind*III. Fragments common to both (A) and (B) are marked by arrows. (C) λ DNA digested with *Hind*III; (D) intact R388 (21.5 Md), R388 PMA^r Hg^r (~27.5 Md), RP1 (38 Md), RP1 PMA^r Hg^r (~44 Md), and R1-16 DNA (52 Md).

discovery of a PMA resistance transposon extends the range of known resistance transposons from heavy metals and antibiotics to organo-metallic compounds. Although about 16 clearly different transpositional elements have been described in bacteria (2), most known resistance units have come from organisms of clinical origin. Our data and those of Friello and Chakrabarty (3) suggest that soil may be an important source or reservoir of translocatable elements encoding resistance to heavy metals, as has already been suggested for resistance to antibiotics (1). We cannot explain why transposable PMA^r Hg^r resistance should be readily detectable in soils evidently not subject to direct mercury selection. We note, however, that for many years, mercury was extensively used to extract alluvial gold in various parts of the South Island of New Zealand. The diffusion of Hg²⁺ resistance from bacteria in such highly contaminated areas to those in relatively uncontaminated soils may have been aided by the presence or development of transposable mercury resistance genes.

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