

## IS222, a New Insertion Element Associated with the Genome of *Pseudomonas aeruginosa*

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Received 15 January 1986/Accepted 14 March 1986

**A new insertion element, IS222, was identified to be associated with the DNA of a mutant strain of the converting *Pseudomonas aeruginosa* bacteriophage D3. The insertion sequence was 1,350 base pairs in size and possessed terminal inverted repeats. The nucleotide sequence contained single cleavage sites for *EcoRI* and *PvuI* but none for *BamHI*, *PstI*, *HindIII*, *SmaI*, or *SalI*. By Southern hybridization analysis, no homology was found with genomic DNA from *P. aeruginosa* PAT or *Escherichia coli*. Genomic DNA from the phage host, *P. aeruginosa* PAO, contained two sequences homologous to IS222.**

D3, a temperate bacteriophage belonging to the family *Siphoviridae* (H.-W. Ackermann, personal communication), induces structural changes to the lipopolysaccharides of lysogenized *Pseudomonas aeruginosa* (14). As part of a detailed study on the molecular genetics of this phage and the mechanisms of surface antigen conversion, we prepared a restriction map of D3 DNA (E. Gertman, D. Berry, and A. Kropinski, submitted for publication). In this report we present evidence that a clear plaque-forming mutant of D3, D3c, contains an additional segment of DNA which has the physical properties of an insertion element.

The cleavage pattern generated by digestion of D3<sup>+</sup> (wild type) and D3c DNA with a number of restriction endonucleases is shown in Fig. 1. *BamHI*-B, *HindIII*-B, *SalI*-E, and *SmaI*-B from the mutant are approximately 1,350 (standard deviation, 100) base pairs (bp) larger than the homologous fragment from the wild type. Digestion with *EcoRI* and *PvuI* generated two additional fragments, suggesting that the insert has single sites for these restriction enzymes.

To study further the nature of the insert, D3<sup>+</sup> and D3c DNAs were cut with *BamHI* in the buffer recommended by Maniatis et al. (15) containing bovine serum albumin (20). The fragments were resolved by electrophoresis through 0.5% agarose gels (15), and the *BamHI*-B fragments were recovered by electroelution. These were used to generate the restriction map shown in Fig. 2 and for heteroduplex analysis (Fig. 3). The position of the insert, as determined by measuring 18 heteroduplexes, was 2.65 kilobases (kb) from one of the ends of the *BamHI* fragment, a value which agreed with the data derived by restriction enzyme analysis. The electron micrographs of the heteroduplexes show that the insert displays a classical stem and loop structure. The terminal inverted repeats appear to be 130 bp long. This may represent an overestimate because heteroduplex analysis of Tn3 (11) indicated inverted repeats of 140 bp, while sequence analysis (10) revealed that they were approximately 40 bp. This insertion sequence has received the designation IS222 from E. M. Lederberg (Plasmid Reference Center, Stanford University School of Medicine, Stanford, Calif.).

To determine whether IS222 is present in *P. aeruginosa* genomic or plasmid DNA (17) and its relationship to any insertion element found in *Escherichia coli*, total cellular

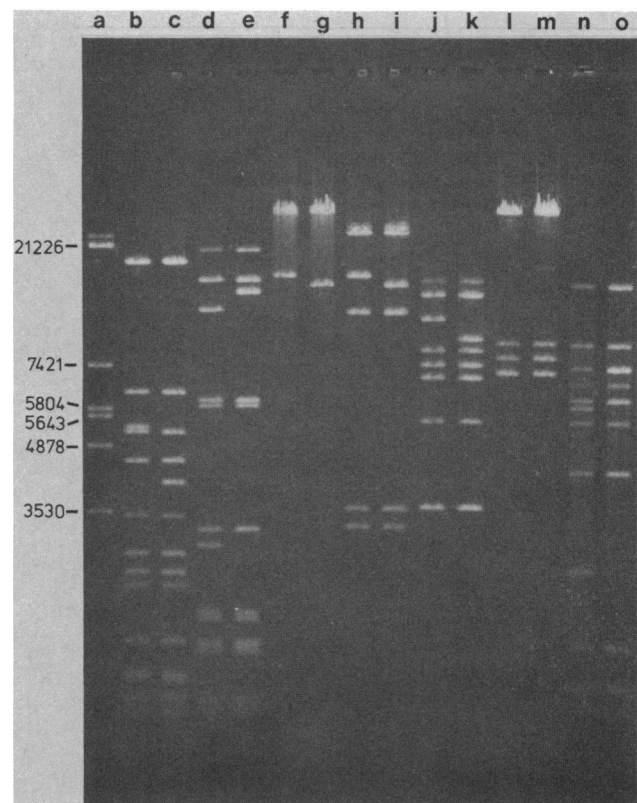


FIG. 1. Comparison of the restriction digestion patterns of phage D3 and D3c DNA. Lane a, lambda DNA digested with *EcoRI*; lanes b and c, D3c and D3 with *SalI*; lanes d and e, D3c and D3 with *EcoRI*; lanes f and g, D3c and D3 with *BamHI*; lanes h and i, D3c and D3 with *SmaI*; lanes j and k, D3c and D3 with *HindIII*; lanes l and m, D3c and D3 with *PstI*; lanes n and o, D3c and D3 with *PvuI*. Following digestion and prior to electrophoresis all samples were heated at 65°C for 10 min. Electrophoresis conditions were as follows: 0.5% agarose (Ultrapure; Bethesda Research Laboratories, Inc., Gaithersburg, Md.); TEB buffer (89 mM Tris hydrochloride, 89 mM boric acid, 2.5 mM EDTA [pH 8.3]); 50 V at room temperature. Numbers to the left of the gels are in base pairs.

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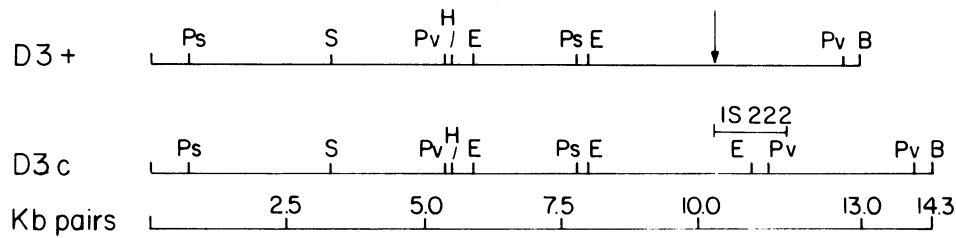


FIG. 2. Restriction map for the *Bam*HI-B fragments from phage D3 and D3c DNA showing the location (arrow) of IS222. The restriction sites are designated as follows: B, *Bam*HI; Ps, *Pst*I; S, *Sma*I; Pv, *Pvu*I; H, *Hind*III; and E, *Eco*RI.

DNA from these organisms was isolated, cut with *Sal*I and *Hind*III, and probed with nick-translated D3 and D3c DNA. No homology was found between D3<sup>+</sup> and genomic DNA from *P. aeruginosa* or *E. coli* (data not shown). In addition, D3c DNA had no homology with DNA isolated from *E. coli*

(Fig. 4, lanes E and J) or from *P. aeruginosa* PAT (Fig. 4, lanes C and H). On the other hand nick-translated D3c DNA did hybridize to 5.0- and 3.45-kb *Sal*I fragments from strain PAO-1 (Fig. 4, lane F) and two of its derivatives, PTO13 (18) (Fig. 4, lane G) and AK1220 (AK1012 carrying FP2 [13]) (lane I). Digestion of DNA from PAO1 and AK1220 with *Hind*III (Fig. 4, lanes A and D) resulted in a single band with homology to IS222, which suggests that the genome may contain two adjacent IS222 sequences separated by a *Sal*I site and bordered by *Hind*III sites. In the case of strain PTO13 the pattern appeared to be different in that two regions of homology (5.6 and 3.0 kb) occurred (Fig. 4, lane B).

In the case of *Pseudomonas* spp., only two insertion elements have been reported. One copy of IS22 (16), a 7.3-kb insertion sequence, is found in the *P. aeruginosa* PAO genome and is able to transpose to plasmids such as pKT212. A detailed restriction map has been published (16). IS51 is a 1.3-kb insertion element identified in *Pseudomonas syringae* subsp. *savastanoi* which is cleaved once by *Sal*I and *Bgl*II but not by *Hind*III, *Pst*I, and *Eco*RI (5). Two further elements of approximately the same size as IS222 should be mentioned. The 1,327-bp insert, IS2, has not been identified in *P. aeruginosa* PAO (12) and is not cleaved by *Eco*RI but is cleaved by *Hind*III and *Sma*I (9). IS5 (1,195 bp) (22) shows weak homology, in Southern hybridization analysis, with the *P. aeruginosa* genomic DNA but differs from IS222 on the basis of its smaller size, shorter terminal inverted repeats, and by the fact that it possesses a subterminal *Eco*RI site (8, 21). This transposable element is also present in up to 11 copies in the *E. coli* genome (22). The identification of transposable elements specifically associated with phage or plasmid DNA is not without precedent. A 1.8-kb insertion sequence, IS21 (23), has been found to be associated with plasmid R68.45 but has not been found on the PAO chromosome. Similarly, the 810-bp element IS46 is associated specifically with *IncN* plasmids such as R46 (2).

The phage mutant was received from the laboratory of R. E. W. Hancock (University of British Columbia), in which, as in our laboratory, it was propagated exclusively on strain PAO. While details on the isolation of this mutant have been misplaced, the fact that there is homology between IS222 on D3c and genomic DNA from strain PAO1 suggests that a transposition event must have occurred. It is also interesting that the insert site is 18.2% from one end of the phage genome. The genes regulating lysogeny of enteric

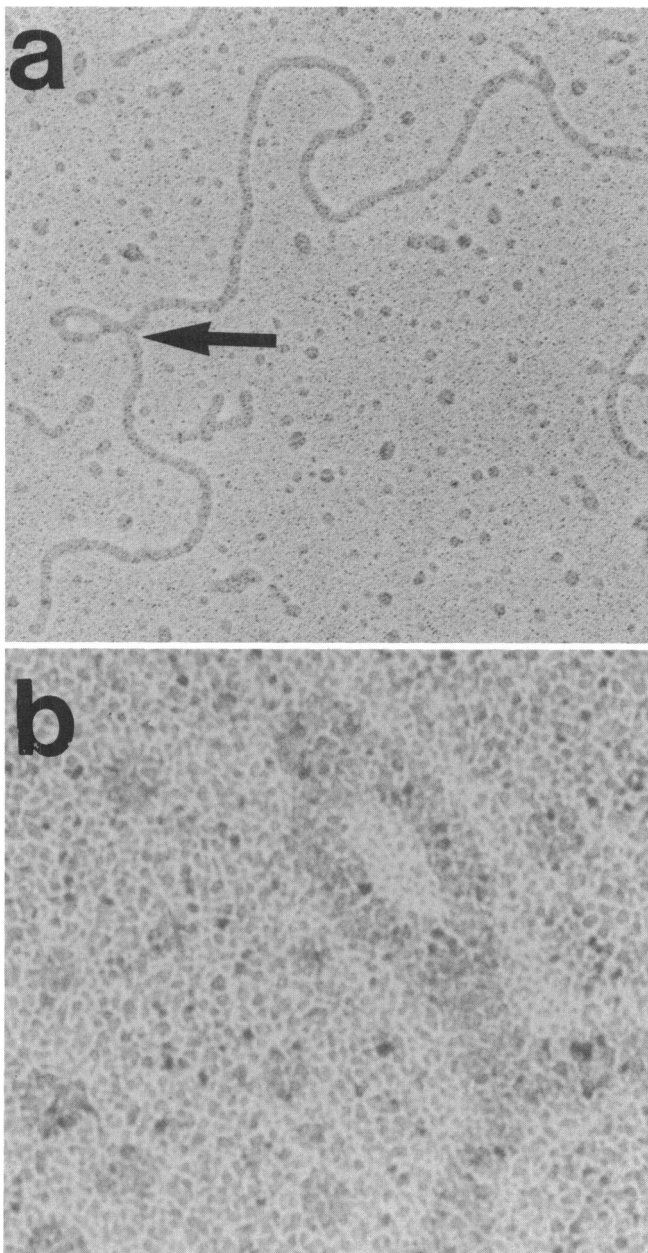


FIG. 3. Heteroduplex analysis of *Bam*HI-B from D3<sup>+</sup> and D3c bacteriophage. *Bam*HI-B were isolated from agarose gels, denatured, and annealed together. The analysis of the heteroduplexes was as described by Davis et al. (7). (a) Electron micrograph of heteroduplex of *Bam*HI-B; the arrow indicates the position of IS222. (b) Higher magnification of IS222 than that shown in panel a showing the hybridized inverted repeats and single-stranded loop.

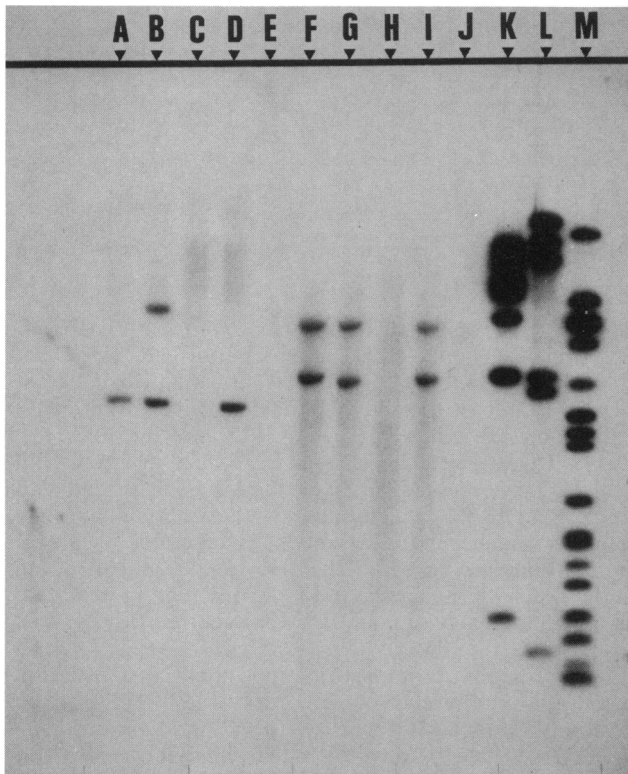


FIG. 4. Southern blot analysis for sequences homologous to IS222 in *P. aeruginosa* PAO and *E. coli* genomic DNAs. DNA was isolated from the *Pseudomonas* strains by sodium dodecyl sulfate lysis of lysozyme-EDTA-induced spheroplasts by a modification of the procedures of Chesney et al. (3) and Coleman et al. (4). DNA from *E. coli* was obtained commercially from P-L Biochemicals, Inc., Milwaukee, Wis. Bacterial DNA (10 to 18  $\mu$ g) was digested with 40 U of *Hind*III and 40 U of *Sall* for 5 h at 37°C. This material was concentrated by ethanol precipitation prior to gel analysis. Phage D3c DNA was also digested with these enzymes and with *Sma*I. Chromosomal DNA (10  $\mu$ g) and 10 ng of D3c DNA were electrophoresed through a 0.7% agarose gel. Following electrophoresis the DNA was denatured and transferred to Gene Screen Plus (New England Nuclear Corp., Boston, Mass.) under the conditions specified in the product information. This was probed with <sup>32</sup>P-labeled D3c DNA prepared by nick translation with the Nick Translation Reagent Kit (Bethesda Research Laboratories) and [ $\alpha$ -<sup>32</sup>P]dCTP(3,200 Ci/mmol; New England Nuclear). The following DNAs were analyzed with the indicated restriction endonucleases: D3c DNA (lanes K, *Hind*III; L, *Sma*I; M, *Sall*); PAO1 chromosomal DNA (lanes A, *Hind*III; F, *Sall*); PTO13 (lanes B, *Hind*III; G, *Sall*); PAT (lanes C, *Hind*III; H, *Sall*); AK1012(FP2) (lanes D, *Hind*III; I, *Sall*); *E. coli* DNA was digested with *Hind*III (lane E) and *Sall* (lane J).

temperate phages such as lambda (6),  $\phi$ 80 (19), and P2 (1) are found clustered at sites approximately 25% from the right end of the molecules. While we were able to clone fragments of D3<sup>+</sup> DNA which gave rise to the convertant phenotype, no such clones were isolated from D3c, suggesting that IS222 may be inserted within the structural genes for conversion. We have recently cloned a fragment of D3c DNA carrying IS222 (D. Berry, A. Kropinski, and E. Gertman, unpublished data).

This study was supported by grants from the Medical Research Council of Canada (to A.M.K.) and the Natural Sciences and Engineering Research Council of Canada (to A.M.K. and B.N.W.).

Strains PAO1 and PTO13 were kindly provided by B. W. Holloway.

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