SHORT COMMUNICATIONS

Characterization of DNA of Colicinogenic Factor E₁ in a Providence Strain

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Episomes may be characterized by transfer to organisms in which the DNA differs markedly from that of the original strain (Marmur *et al.* 1961). Of the E₁ colicinogenic factors, only the one derived from *Escherichia coli* K-30 has been characterized in this way. In *Proteus mirabilis* this Col E₁ DNA has a buoyant density of 1.710 ± 0.002 g./cm.³ and displays circular forms of three contour lengths with corresponding molecular weights of 4.2, 8.5 and 12.7×10^6 (DeWitt & Helinski, 1965; Bazaral & Helinski, 1968*a*). The higher circular forms in *P. mirabilis* resulted from a possible imbalance in the formation or concentration of enzymes responsible for the duplication of Col E₁ DNA and not from random recombination of monomers (Goebel & Helinski, 1968). In the original *E. coli* strain the Col E₁ DNA is almost exclusively in the monomer form (Bazaral & Helinski, 1968*b*).

Colicin J (CA-62) (Fredericq, 1948) was subsequently classified as an E₁ colicin. Coetzee (1964) transferred this colicinogenic factor to a Providence strain, NCTC9295. This communication reports the nature of this E₁ colicinogenic factor in the Providence host.

The Providence strain carrying Col E₁ (Providence Col E₁⁺) and the non-colicinogenic strain were grown overnight in nutrient broth. DNA was extracted by the phenol technique and fractionated on a MAK column (Roth & Helinski, 1967). Analytical equilibrium centrifugation in a caesium chloride gradient was performed by the method of Meselson, Stahl & Vinograd (1957). *Pseudomonas aeruginosa* ¹⁵N-DNA (density = 1.742 g./cm.³) was used as reference. Preparative equilibrium sedimentation was done according to DeWitt & Helinski (1965). DNA for electron microscopy was taken directly from the MAK column and mixed with 0.4% cytochrome *c* in 0.1 M-ammonium acetate to 2 to 4 µg./ml. DNA was mounted as described by Kleinschmidt & Zahn (1959). Grids were shadowed with 40% palladium-gold and examined in a Philips EM200 electron microscope. Electron micrographs were enlarged and the contour lengths of the DNA molecules measured. Molecular weights were calculated from the contour lengths.

Unfractionated DNA isolated from Providence Col E₁⁺ and the non-colicinogenic strain was examined in CsCl density gradients. Microdensitometer tracings of the DNA profile obtained from both these strains displayed a single DNA component

which banded at 1.700 g./cm.^3 (41 % G + C) which corresponded to the chromosomal DNA of the Providence host. Cesium chloride density-gradient analysis of early DNA fractions from the MAK column revealed an additional DNA species with a density of $1.710 \pm 0.001 \text{ g./cm.}^3$. This DNA was only present in the colicinogenic strain and was assumed to be *Col E*₁ DNA. Alkaline denaturation and neutralization of these MAK fractions resulted in denaturation of the Providence DNA while the *Col* DNA resisted permanent denaturation.

Electron microscopy of the early fractions from the colicinogenic strain revealed open and supercoiled circular DNA molecules (Plate 1). Contour lengths of 65 of these yielded three classes, 2.12 ± 0.06 , 4.25 ± 0.11 and $6.38 \pm 0.16 \mu$, with a predominance of monomer and dimer molecules. Molecular weights of 4.16 , 8.33 and 12.5×10^6 were calculated from the contour lengths.

The *E*₁ colicins share a common receptor site and the colicinogenic factors confer cross-immunity to the colicins. Support for this classification is now provided in that colicinogenic factors isolated from *Escherichia coli* K-30 and paracolon CA-62 are similar in morphology and molecular weight. The demonstration of multiple-length circular *Col E*₁ (CA-62) in the Providence strain agrees with findings for *Col E*₁ (K-30) in *Proteus mirabilis* and may suggest a similar defect in enzymes responsible for *Col E*₁ duplication in these hosts (Goebel & Helinski, 1968).

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EXPLANATION OF PLATE

Electron micrographs of *Col E*₁ DNA isolated from Providence strain 9295 *Col E*₁⁺. (Bar represents 0.5μ).

Fig. 1. Monomeric open circular DNA (2.12μ).

Fig. 2. Supercoiled circular dimer DNA (4.25μ).

Fig. 3. Supercoiled circular trimer DNA (6.38μ).