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ELECTRON-MICROSCOPIC STUDY OF CONJUGATIVE PLASMIDS OF  
SEROLOGICALLY TYPED STRAINS OF *Escherichia coli* AP1

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Plasmid DNAs isolated from cells of *E. coli* AP1 were studied by electron microscopy. Two plasmid DNAs (FBI-1 and FBI-2) with molecular weights of  $35.9 \pm 0.5 \times 10^6$  and  $51.5 \pm 0.6 \times 10^6$  daltons respectively were identified.

KEY WORDS: plasmid DNA; electron microscopy; molecular weight.

Cells of serologically typed strain *Escherichia coli* AP1 contain transmissive F-like conjugation factor, previously described as plasmid FBI [2]. During investigation of the physicochemical properties of the DNA of this plasmid by sedimentation in a neutral glycerol gradient and electrophoresis of its fragments in agarose gel, results were obtained which suggested that a complex consisting of two different plasmids exists in the cells of this strain [1].

TABLE 1. Length of Outlines of Plasmid DNA of *E. coli* AP1 Cells and Their Molecular Weight

No. of specimen	Length of outline of first plasmid	Length of outline of ColEI plasmid DNA, cm	Molecular wt. of first plasmid DNA ( $\times 10^6$ daltons)	No. of specimen	Length of outline of second plasmid DNA, cm	Length of outline of ColEI plasmid DNA, cm	Molecular weight of second plasmid DNA ( $\times 10^6$ daltons)
1	109.0	12.7	36.0	1	162.5	13.0	52.5
2	107.0	13.0	34.6	2	155.0	12.6	51.5
3	114.0	12.8	37.4	3	168.0	13.0	54.2
4	118.0	13.0	38.1	4	162.5	14.0	48.6
5	111.0	12.1	38.5	5	164.0	14.0	49.4
6	95.0	12.1	32.9	6	160.0	12.6	52.5
7	106.0	13.5	33.0				
8	106.0	12.8	34.2				
9	114.0	13.5	35.5				
10	118.0	13.1	37.9				
11	104.0	13.0	34.6				
12	124.0	14.0	37.2				
13	120.0	13.8	36.5				

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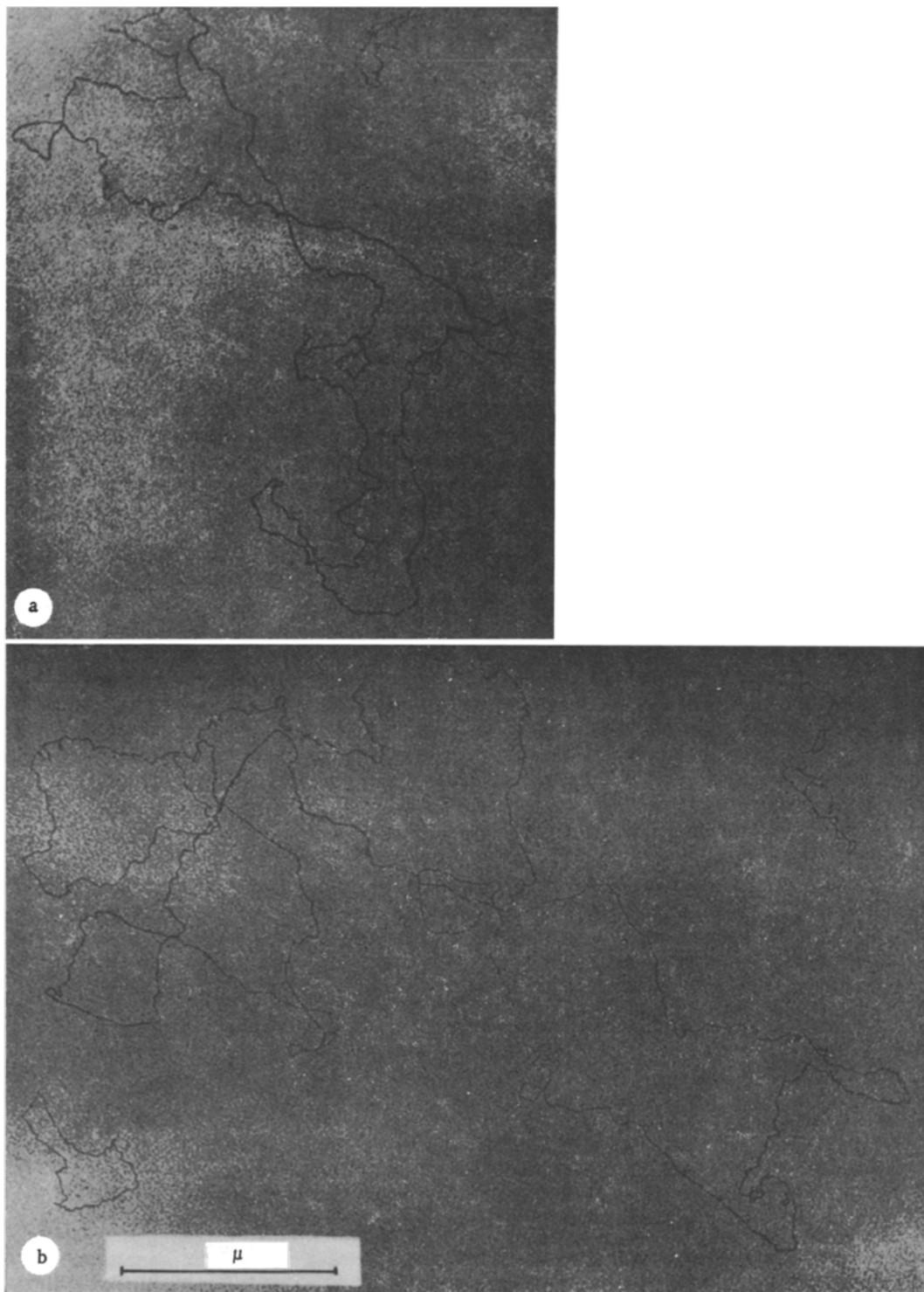


Fig. 1. Electron micrographs of plasmid DNAs of *E. coli* AP1 (10,000 ×). a) Plasmid DNA (FB1-1) with molecular weight of  $35.9 \pm 0.5 \times 10^6$  daltons; b) plasmid DNA (FB1-2) with molecular weight of  $51.5 \pm 0.6 \times 10^6$  daltons.

To test this hypothesis, in the investigation described below an electron-microscopic study was made of the plasmid DNA of *E. coli* AP1.

#### EXPERIMENTAL METHOD

Plasmid DNA was obtained from cells of *E. coli* AP1 by the method described previously [1].

DNA preparations for electron microscopy were obtained by the micromethod suggested by Lang and Mitani [4]. For this purpose a DNA preparation (0.1-0.2  $\mu\text{g/ml}$  in 0.15 M ammonium acetate buffer) was used with the addition of 1.3  $\mu\text{g/ml}$  cytochrome c and a solution of formaldehyde (in a final concentration of 0.02 M). The DNA-protein film was transferred to grids coated with a carbon support, and then shadowed with tungsten at an angle of  $7^\circ$ , at a distance of 10 cm from the revolving stage (30 rpm). The preparations were examined in the JEM-100B electron microscope (60 kV, objective diaphragm 30  $\mu$ ) with a magnification of 10,000 times. To measure the length of the outline of the plasmid DNA the "internal standard" method [3] was used. A preparation of ColE1 plasmid DNA, whose molecular weight was taken to be  $4.2 \times 10^6$  daltons, was used as the standard specimen. The negatives of plasmid DNA thus obtained were examined on a projector with a magnification of 6.5 times. The length of the outline of each plasmid was measured by means of a curvimeter. On each photograph there were on average up to 4 molecules of DNA ColE1 to 1 molecule of DNA of the plasmid studied.

#### EXPERIMENTAL RESULTS

The results of measurements of the length of the outlines of DNA of ColE1 and the test plasmids, with the molecular weight calculated on this basis, are given in Table 1. The results were subjected to statistical analysis. Electron-micrographs of the test plasmid DNAs are shown in Fig. 1.

The results enable corrections to be made to the previous hypothesis [1] according to which two different plasmids or one plasmid with different forms of its molecules (super-spiral, open, annular, closed, and so on) may be present in a preparation of plasmid DNA from *E. coli* API cells. Electron-microscopic investigation of a plasmid DNA preparation confirms the validity of the first suggestion that two different plasmids exist in the cells of this strain. The mean molecular weight of one plasmid (FB1-1) is  $35.9 \pm 0.5 \times 10^6$  daltons and that of the other (FB1-2) is  $51.5 \pm 0.6 \times 10^6$  daltons.

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