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Characterization of Deoxyribonucleic Acid from *Yersinia pestis* by Ethidium Bromide-Cesium Chloride Density Gradient Centrifugation

ROSELYN V. LITTLE AND ROBERT R. BRUBAKER

Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48823

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No plasmids were detected in cells of *Yersinia pestis* which were bacteriocinogenic and positive for three additional determinants of virulence known to be lost at high frequency.

The five established determinants of virulence in *Yersinia pestis*, the causative agent of bubonic plague, consist of the abilities to produce V and W antigens (*vwa*$^+$), elaborate envelope or fraction 1 antigen (*fra*$^+$), synthesize a surface structure which permits absorption of certain dyes and pigments (*pgm*$^+$), express bacteriocin (pesticin I) activity (*pst*$^+$), and produce endogenous purines (3). The mutation to *pst*$^-$ occurs at low frequency (5), but this gene, in view of the nature of its product, is assumed to exist on an extra-chromosomal replicon (4). In contrast, mutations to *vwa*$^-$ and *pgm*$^-$ occur at the high rates of 10^{-4} (6) and 10^{-5} (2), respectively, and that to *fra*$^-$ may also occur at high frequency (7). The independent loss of these four genes is evidently irreversible in contrast to loss of genes responsible for purine biosynthesis; the latter are presumably located on the chromosome proper. To determine if *vwa*, *fra*, *pgm*, or *pst* normally exists on plasmids, an analysis of deoxyribonucleic acid (DNA) of *Y. pestis* was undertaken by ethidium bromide-cesium chloride density gradient centrifugation (1).

DNA of *Y. pestis* strain K25 (*vwa*$^+$, *fra*$^+$, *pgm*$^+$, *pst*$^+$, *gua A*$^-$) was labeled by cultivation of cells at 26 and 37 C in 20 ml of heart infusion-broth containing added 0.001 M guanosine, 0.001 M adenosine, and ^{3}H-thymidine (5 μCi/ml). The organisms were harvested by centrifugation in the late logarithmic growth phase and washed in 0.05 M tris(hydroxymethyl)aminomethane-chloride, 0.05 M NaCl, 0.005 M ethylenediaminetetraacetate buffer (TES), *p*H 8.0. Lysates were prepared by the method of Bazaral and Helinski (1) with the following two modifications. First, a 10% solution of the detergent Brig-58 was substituted for sarkosyl and, second, partial lysates were alternately frozen (in nitrocellulose tubes) in an ethanol-dry ice bath and then thawed in a 37 C water bath; this procedure was repeated at least eight times.

A 3.0-ml sample of the resulting complete lysate was gently mixed in a vial containing 6 g of anhydrous CsCl, 0.5 ml of a 0.5% solution of ethidium bromide, and 2.7 ml of TES. This preparation was transferred to a polyallomer centrifuge tube, overlayered with light mineral oil, and centrifuged for 36 hr at 117,000 $\times$ *g* in a Ti50 fixed-angle rotor at 15 C in a Beckman model L preparative ultracentrifuge. After centrifugation, fractions (15 drops) were collected, and 20-μliter portions of each fraction were spotted on discs of Whatman no. 1 filter paper. The discs were dried, washed in cold 5% trichloroacetic acid, rinsed in 95% ethanol, and dried. Radioactivity was then

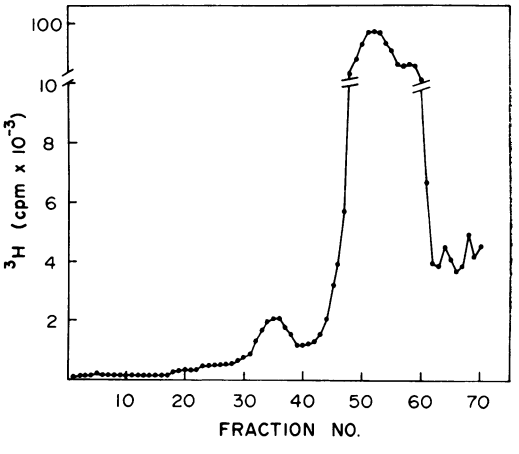

FIG. 1. *Ethidium bromide-cesium chloride density profile of DNA from E. coli JC411 (Col E$_1$).*

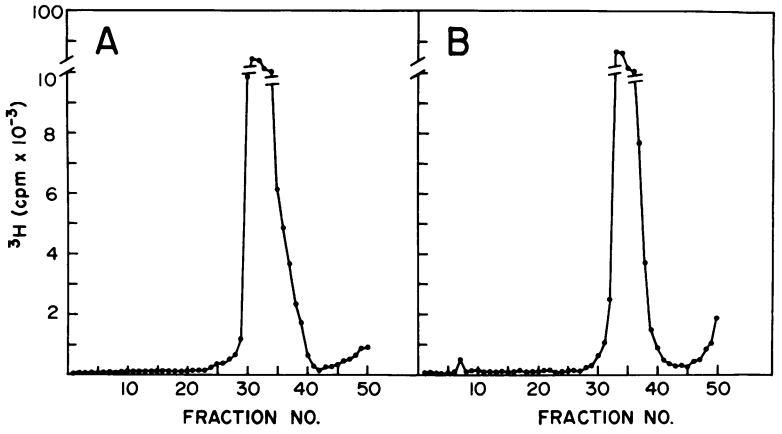

FIG. 2. *Ethidium bromide-cesium chloride density profile of DNA from Y. pestis strain K25 cultivated at 26 C (A), and 37 C (B).*

determined in a Packard Tricarb scintillation counter with toluene base containing 0.4% 2,5-diphenyloxazole (PPO) and 0.005% 1,4-di-2-(5-phenyloxazolyl)-benzene (dimethyl POPOP).

The validity of this procedure is shown in Fig. 1 in which a plasmid, known to regulate the expression of colicin E_1, was separated from chromosomal DNA of *Escherichia coli* strain JC411 (Col E_1). In contrast, only a single peak of chromosomal DNA was obtained from lysates of *Y. pestis* (Fig. 2). Accordingly, under the cultural conditions that were employed it seems possible that *vwa*, *fra*, *pgm*, and *pst* were located on the chromosome proper or could not be detected by the methods employed. These results do not, of course, eliminate the possibility that these four genes were located on integrated episomes in the majority of the population during cultivation by the procedure described.

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