lac⁻ MUTANTS OF PATHOGENIC SEROTYPES OF Escherichia coli

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Lactose-negative mutants obtained from cultures of pathogenic serotypes of Escherichia coli by means of ultraviolet irradiation were found to be similar to the original strains in their morphological, cultural, and other biochemical properties. Some mutants acquired antigenic properties different from those of the original strains.

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Isolation of spontaneous and induced lactose-negative (lac⁻) mutants of Escherichia coli has been described by several workers [1-3]. However, the possibility of isolation of lac⁻ mutants from cultures of pathogenic serological types of E. coli has received little study. Nevertheless, such mutants are of great interest for research into the genetics of pathogenic serotypes of E. coli.

The object of the present investigation was to isolate lac⁻ mutants from cultures of various pathogenic serotypes of E. coli and to study their properties.

EXPERIMENTAL METHOD

Experiments to isolate lac⁻ mutants were carried out with pathogenic serotypes O111 : B4 : H2, O55 : B5 : H6, and O26 : B6 : H11 of E. coli, irradiated with ultraviolet rays generated by a BUV-15 lamp. Eight-hour agar cultures were washed off with physiological saline, suspensions containing 1 billion cells/ml were prepared and these were diluted 1:10. The suspension was then poured in volumes of 5 ml into Petri dishes and irradiated for 60-90 sec at a distance of 40 cm from the lamp. During irradiation the dishes with the culture were gently agitated to ensure uniform action of the UV rays on the bacterial cells. With these doses of irradiation, only 0.1% of bacterial cells remained viable. The irradiated culture was transferred in volumes of 0.1 ml to Endo's medium in dishes and spread with a spatula, after which the subcultures were incubated for 24 h. The lactose-negative (white) colonies were picked off and purified by further subcultures on Endo's medium. Samples of unirradiated cultures were investigated in control experiments.

EXPERIMENTAL RESULTS

Lactose-negative mutants were isolated from cultures of all three pathogenic serotypes of E. coli. The mutants were similar to the original strains in their morphological, cultural, and biochemical properties.

The serologic properties of the isolated mutants were studied by the agglutination reaction (on a slide and by the linear method in tubes) with absorbed OB-sera against types O111:B4, O55:B5, and O26:B6.

The original strains were agglutinated (++++) by homologous absorbed OB-type sera on slides, as also were the lactose-negative mutants Nos. 1, 40-49 from pathogenic serotypes O55 : B5 : H6 and O26 : B6 : H11. However, 6 of isolated lactose-negative mutants were not agglutinated by their own sera on slides.

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The serologic properties of the isolated lac$^-$ mutants were next studied by the linear agglutination reaction in tubes with homologous sera, which were agglutinated on slides (++++). These experiments showed that some of them completely retained the antigenic properties of the original strains, while the others had lost them.

The nutritional requirements, resistance to coliphages, and relationship to streptomycin of the isolated lac$^-$ mutants were identical with those of the original strains.

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