REMOVAL OF F'-LAC$^+$ FACTOR FROM Escherichia coli K-12 CELLS BY MEANS OF ACRIDINE ORANGE

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Elimination of F'-lac$^+$ factor from cells of Escherichia coli by means of acridine orange is dependent on concentration of the dye and bacteria in the medium. In doses of the dye inhibiting growth of the cell population, removal of the factor does not take place.

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Strains of Escherichia coli carrying F-factor incorporating a chromosome segment (factor F') have been isolated from cultures of E. coli Hfr [2,3,6,8]. Formation of factors F' takes place as a result of genetic recombination between F-factor and bacterial chromosome. Transfer of factor F' from donor to recipient cells takes place at high frequency. By interacting with the homologous part of the chromosome, F'-factor may be incorporated in it [4,9]. Subsequent recombination of F'-factor with chromosome are possible, during which the incorporated segment again remains on the chromosome [5].

Under certain conditions acridine orange has been found to remove autonomous F-factor from cells [7,10]. Since integrated sex factor is insensitive to the dye, treatment of cells carrying F'-factor with it can be used to isolate cell clones of Hfr type.

The object of this investigation was to study the degree of elimination of factor F'-lac$^+$ by the action of acridine orange in various doses on different numbers of cells, and also to determine the frequency of genetic exchange between chromosome and factor F'-lac$^+$ depending on the concentration of this dye. In addition, we investigated the effect of acridine orange on incorporation of factor F'-lac$^+$ into the chromosome of the bacterial cell.

EXPERIMENTAL METHOD

A donor streptomycin-sensitive strain of E. coli 200PS lac$^-$, carrying factor F'-lac$^+$, and a recipient streptomycin-resistant strain E. coli PA678 F$^-$, requiring for its growth vitamin B_1 (B_1^-), threonine (T^-), and leucine (L^-) and not fermenting lactose (lac$^-$), were used in the experiments. RNA containing phage f_2, specifically lysing the donor cells was also used.

Different numbers of cells of strain E. coli 200PS (10^7, 10^6, 10^5, 10^4, and 10^3 cells/ml) were added to test tubes containing meat-peptone broth (pH 7.6), to which acridine orange was added in different concentrations (25, 50, 100, 150, 200, and 300 μg/ml). After incubation at 37° for 18 h, samples were taken from the cultures, treated with phage f_2 by Adams's method [1], diluted, and seeded on plates with meat-peptone agar and minimal medium with lactose. At the same time samples not treated with phage were also seeded. After seeding on minimal medium with lactose, all the cells fermenting lactose were counted. The number of colonies on minimal medium with lactose after treatment with specific phage and the results of crossing on agar were used to determine the proportion of cells loosing and retaining F-factor.

The growing lac$^+$ colonies were crossed on minimal agar with the addition of glucose, streptomycin, and vitamin B_1, for which purpose 0.2 ml of recipient culture was spread over a plate divided into eight sectors, and the test colony was then applied as a stroke to each sector. The results of the seedings were read after incubation for 48 h at 37°.

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TABLE 1. Percentage of Cells Fermenting Lactose as a Function of Number of Cells per ml and Acridine Orange Concentration

Acridine orange concentration in μg/ml	Numbers of cells/ml						
	$1 \cdot 10^7$	$1 \cdot 10^6$	$1 \cdot 10^5$	$1 \cdot 10^4$	$1 \cdot 10^3$		
					lac^+ %	F^+-lac^+ %	F^--lac^+ %
25	94	87	72	67	66	45	21
50	83	80	72	67	64	44	20
100	67	22	7	6	6	1,3	4,7
150	57	12,5	8	4	3,9	1,2	2,6
200	12	1,3	5,4	43,7	50		
300	77	77	98	98	—		

EXPERIMENTAL RESULTS

The results obtained after treating cells of strain 200PS with acridine orange are given in Table 1, showing that with acridine orange concentrations of 25, 50, 100, and 150 μg/ml the percentage of cells fermenting lactose was reduced with a decrease in the cell concentration per milliliter. Characteristically, the degree of elimination of factor F'-lac^+ and its rate at different cell concentrations in the medium varied with each dose. This shows a correlation between the degree of elimination of factor F'-lac^+ and the concentration of acridine orange per cell. The degree of elimination of factor F'-lac^+ also depends on whether the dose of dye used inhibits growth of the cell population or not. For instance, with acridine orange in a concentration of 300 μg/ml no increase took place in the number of cells compared with the number seeded. Probably these doses of acridine orange inhibit replication not only of autonomous F'-factor, but also of bacterial chromosome, and this affects the degree of elimination of F'-lac^+ factor (Table 1). With a cell concentration (10^3 cells/ml) optimal for elimination of F'-lac^+ factor, a great difference was observed in the degree of elimination of the episome when smaller (25, 50 μg/ml) and average (100 and 150 μg/ml) concentrations of dye were used, as shown by the sharp decrease in percentage of both F^+-lac^+ and F^--lac^+ cells. With the lowest concentrations of acridine orange in the medium, gradual accumulation of F^--lac^+ cells formed as a result of genetic interchange between factor F'-lac^+ and chromosome took place. With high (toxic) concentrations of the dye, replication of autonomous F'-factor was sharply inhibited, and the probability of its genetic interchange with bacterial chromosome was diminished.

The decrease in percentage of cells carrying F^+-lac^+ when acridine orange was used in toxic concentration allows these cells to be divided into two classes. One class includes cells in which factor F'-lac^+ is integrated in the chromosome, while the other includes cells in which it is "attached" to the chromosome and likewise is not subject to the action of acridine orange. Probably the doses of dye described above disturb the bond existing between factor F'-lac^+ and bacterial chromosome, so that the factor reverts to the autonomous state and is eliminated. The F'-lac^+ cells thus remaining after treatment with acridine orange in concentrations of 100 and 150 μg/ml belong to the class in which factor F'-lac^+ is integrated in the chromosome. Such cell clones, when crossed on agar, give from 10 to 20 times more recombinants than the original strain.

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