BEHAVIOR OF F-lac$^+$ FACTOR IN CELLS OF *Escherichia coli* 200 PS AFTER X-RAY IRRADIATION

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The degree of elimination of F-lac$^+$ factor by addition of acridine orange to an irradiated suspension of Escherichia coli 200 PS cells and seeding them after incubation on selective media was determined.

During x-ray irradiation of cells containing the F-lac$^+$ episome, the bond between the bacterial chromosome and F-lac$^+$ factor is broken. As a result of this, the incidence of genetic recombination between F-lac$^+$ factor and bacterial chromosome is reduced.

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F'-factor is known to be formed as a result of genetic recombination between F-factor and chromosome, the sex factor incorporating a certain segment of the chromosome during this process [2]. If the population of cells carrying F'-factor is regarded as a whole, a certain portion of its cells carries F'-factor in the integrated state, and the rest in the autonomous state [5]. Both in Hfr-donors and in some cells with vitamin F', integrated F-factor may pass spontaneously in the autonomous state [5, 7, 8]. The localization of the F-factor can be differentiated by means of acridine orange. Under certain conditions this dye removes autonomous F-factor from cells without eliminating integrated sex factors [4, 8]. In a previous paper we described a state of the F-lac$^+$ factor in which it is bound in a definite manner with bacterial chromosome (but not integrated) and is not susceptible to the action of acridine orange.

The object of the present investigation was to study the behavior of F-lac$^+$ factor in cells during the action of different doses of x-ray irradiation and also to study the possibility of its induction from the integrated or "fixed" state into the autonomous state.

EXPERIMENTAL METHOD

A streptomycin-sensitive strain of Escherichia coli 200 PS lac$^-$, carrying an F-lac$^+$ episome and a streptomycin-resistant strain of E. coli PA678F$^-$, requiring vitamin B_1 (B_1^-), threonine (T^-), and leucine (L^-) for its growth, and not fermenting lactose (lac$^-$) and galactose (gal$^-$), were used in the investigation. Phage f_2 was used as a phage producing specific lysis of the donor cells. A culture of E. coli 200 PS, treated with various doses of x-rays (1000, 5000, 10,000, 15,000, and 20,000 R), was diluted so that each sample contained on the average about 10^3 cells/ml. After incubation of the samples for 18 h with acridine orange in a concentration of 50 μg/ml, they were treated with phage f_2 as described by Adams [1], dilutions were made, and seedings performed on dishes with meat-peptone agar and minimal medium with lactose. Samples not treated with phage were seeded at the same time on these media. By counting the colonies growing on minimal medium with lactose from cells treated and not treated with phase, and also the number of colonies proving fertile when crossed on agar, the proportion of F$^+$lac$^+$ and F$^-$lac$^+$ cells among the lactose-positive bacteria remaining after acridine orange treatment could be determined. Crossing was carried out on minimal medium with glucose and vitamin B_1 and with streptomycin, on which the recipient culture was spread, after which the test lac$^+$ colony was applied as a stroke. Irradiation was given by means of a type RUM-7 apparatus (50 kV, 15 mA, filter Al 0.1 mm).

EXPERIMENTAL RESULTS

Data showing the degree of elimination of F$^+$lac$^+$ factor after x-ray irradiation are given in Table 1. With an increase in dose of irradiation, the proportion of F$^+$lac$^+$ and F$^-$lac$^+$ cells diminished. In control

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TABLE 1. Percentage of Cells of Different Classes after Irradiation with Different Doses of X-rays

Dose of irradiation (in R)	Content of lac^+ cells (in %)	Content of F^-lac^+ cells (in %)	Content of F^+lac^+ cells (in %)
Control without irradiation	63.4	43.3	20.1
1,000	56	36	20
5,000	45	32	13
10,000	28.2	23.6	4.6
15,000	35.2	32.2	3
20,000	37.8	34.2	3.6

experiments in which the original culture was irradiated without addition of the dye, no increase was found in the proportion of cells not fermenting lactose. Cells fermenting lactose but which lost the F-factor were formed as a result of genetic recombination between the F-lac^+ factor and bacterial chromosome, the lactose characteristic remaining on the chromosome, while the F-factor, reverting to the automonous state, was eliminated by acridine orange [3]. This is confirmed by the fact that such clones did not yield recombinants when crossed either on agar or in broth. They were also insensitive to specific phage. The decrease in number of F^+lac^+ cells can be accounted for on the assymption that in some cells F-lac^+ factor was fixed to the chromosome and was not susceptible to the action of acridine orange, while in others it was integrated in the chromosome. X-ray irradiation probably disturbs the bond between the chromosome and F-lac^+ factor. This factor reverts to the autonomous state and is eliminated by acridine orange. Because of disturbance of the bond between F-lac^+ factor and the chromosome occurring during x-ray irradiation, there is less likelihood of recombination between them. As a result, the proportion of F^-lac^+ cells in the population is reduced. The remaining F^+lac^+ cells are those in which F'-factor is integrated into the chromosome. Preliminary analysis of these clones showed that during crossing on agar more than 10 (up to 100) recombinants could be obtained per stroke, whereas the original strain and F^+lac^+ clones from the control, when crossed on agar, yielded from 3 to 6 recombinants per stroke.

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