BEHAVIOR OF F-LIKE PLASMIDS IN BACTERIAL CELLS DIFFERING IN THEIR CAPACITY FOR GENETIC RECOMBINATION

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UDC 576.8.095.5

After conjugative transfer of F-like plasmids (FB1, FB1 drd, F'-lac$^+$) into cells of recipient strains of Escherichia coli K-12 with varied recombination capacity the sensitivity of the latter to ultraviolet radiation and the degree of stability of these plasmids during treatment of the bacteria with eliminating agents were determined. The presence of any one of the F-like plasmids studied was shown to have no effect on the sensitivity of the bacterial cells to ultraviolet radiation. On the other hand, the results of a study of spontaneous and induced elimination indicate the existence of considerable differences in the behavior of these plasmids toward eliminating factors. Stability of individual F-like plasmids in cells is presumably largely dependent on genetic differences in the recA region of the host bacteria.

KEY WORDS: bacterial plasmids; elimination; recombination.

Conjugative bacterial plasmids are extrachromosomal genetic elements capable of endowing bacterial cells with various additional properties [1, 13, 15]. Information has also been obtained to show that expression of the functions of the plasmid genes depends to some degree on the genetic properties of the host cell [6, 7, 12].

It was therefore decided to determine experimentally the possible effect of the rec* genotype of Escherichia coli K-12 cells on behavior of the F-like plasmid FB1, previously found in cells of enteropathogenic E. coli of serogroup O6 [5] and of its derepressed mutant FB1 drd, by comparison with the standard F'-lac$^+$ plasmid.

EXPERIMENTAL METHOD

The test objects were strains E. coli K-12 AB1157 rec$^+$ and AB2463 recA13, and also strains of two Rec$^+$ revertants AB2463-4 and AB2463-29, previously isolated from cultures of the recombination-defective mutant AB2463 recA13 [4]. Cells of all these strains carried F-like plasmids FB1, FB1 drd, and F'-lac$^+$ (control). Transfer of these plasmids into the cells of these strains was carried out by crossing donor strains E. coli AP1 (FB1), AP2 (FB1 drd) [5], and 200PS (F'-lac$^+$) with the corresponding recipients. The crosses were carried out by the standard methods [9].

The 18-h broth cultures, washed by centrifugation twice and diluted in phosphate buffer (pH 7.8) to a concentration of $1 \cdot 10^7$ cells/ml were irradiated with ultraviolet light. Samples of 5 ml were placed in dishes and irradiated with an intensity of 1 erg/cm^2/sec. To determine the number of viable cells samples of the irradiated specimens (from the corresponding dilutions) were seeded on nutrient agar (MPA). After incubation of the seedings for 48 h at 37°C the results were read by counting the growing colonies.

*The nomenclature of Demerec et al. [10] is used in this paper and the abbreviations of the symbols for describing the genetic markers are taken from Taylor and Trotter [14].

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 8, pp. 239-241, August, 1977.
Original article submitted January 20, 1977.

TABLE 1. Effect of Ultraviolet Radiation on Viability of E. coli K-12 Cells Containing F-Like Plasmids (in % of control)

Strain	Dose of ultraviolet radiation, erg/cm²					
	2	8	10	24	40	120
AB1157 FB1	—	—	95,0	—	51,59	0,4
AB1157 (control)	—	—	95,0	—	52,94	0,34
AB1157 FB1 drd	—	—	37,0	—	11,4	0,3
AB1157	—	—	37,6	—	12,0	0,4
AB1157 F'-lac+	—	—	60,0	—	23,0	0,58
AB1157	—	—	53,0	—	24,0	0,6
AB2463-4 FB1	—	—	97,0	—	45,0	4,2
AB2463-4 (control)	—	—	69,0	—	30,0	2,0
AB2463-4 FB1 drd	—	—	33,9	—	16,7	0,43
AB2463-4	—	—	52,3	—	12,0	0,56
AB2463-4 F'-lac+	—	—	54,0	—	12,0	0,66
AB2463-4	—	—	63,0	—	10,0	0,43
AB2463-29 FB1	—	—	65,8	—	36,0	1,75
AB2463-29 (control)	—	—	76,0	—	48,6	3,9
AB2463-29 FB1 drd	—	—	48,84	—	22,1	0,5
AB2463-29	—	—	61,0	—	21,7	0,7
AB2463-29 F'-lac+	—	—	69,0	—	8,5	0,7
AB2463-29	—	—	68,0	—	6,5	0,46
AB2463 FB1	20,38	0,32	—	0,005	—	—
AB2463 (control)	18,25	0,51	—	0,004	—	—
AB2463 FB1 drd	23,0	0,54	—	0,004	—	—
AB2463	17,0	0,45	—	0,004	—	—
AB2463 F'-lac+	12,0	0,4	—	0,001	—	—
AB2463	13,0	0,47	—	0,002	—	—

To determine the frequency of spontaneous elimination of the plasmids, seedings were made of 18-h broth cultures of the corresponding bacteria on MPA plates to obtain isolated colonies. The clones of bacteria subsequently grown (50 in each case) were studied for sensitivity to donor-specific phage MS 2. The frequency of elimination of plasmids induced by ultraviolet radiation and by ethidium bromide was determined in the same way. The bacteria were treated with ethidium bromide by the standard method [8]. The sensitivity of the cells carrying plasmids FB1 drd and F'-lac+ to phage MS 2 was determined by the agar layer method [11]. The sensitivity of bacteria containing repressed F-like plasmid FB1 to phage MS 2 was determined by measuring the increase in titer of this phage. For this purpose phage-bacteria mixtures were prepared and incubated for 18 h at 37°C. The number of phage particles in specimens from these mixtures was determined by seeding from appropriate dilutions in 0.6% MPA containing cells of the indicator strain Jc158Hfr. In all cases the results were read after incubation of the seedings for 18 h.

EXPERIMENTAL RESULTS

As already stated, by conjugation of the corresponding bacteria the plasmids FB1, FB1 drd, and F'-lac+ were introduced into cells of strains E. coli K-12 AB1157 rec+, AB2463 recA13, AB2463-4 Rec+, AB2463-29 Rec+. The cells of these strains differed in their ability to carry out recombination and repair of injuries to DNA [2, 3]. To determine the possible effect of the plasmids studied on the viability of the carrier bacteria and also to determine the frequency of their elimination the cultures were irradiated with ultraviolet light in phosphate buffer (pH 7.8) in doses of 10, 40, and 120 erg/cm² (in the case of recombination-defective mutant AB2463 recA13). As the results in Table 1 show, the presence of any one of the three F-like plasmids in the E. coli K-12 cells had no effect on their sensitivity to the lethal action of ultraviolet radiation irrespective of the genetic features of the strains.

On the other hand the results of the experiments to determine the stability of these plasmids in cells of E. coli strains K-12 AB1157 rec+, AB2463 recA, AB2463-4 Rec+, and AB2463-29 Rec+ (the cells of the Rec+ revertants carry back mutations and suppressor mutations respectively [3]) indicate the existence of definite differences in the behavior of plasmids FB1 and FB1 drd compared with F'-lac+ (Table 2). As Table 2 shows, spontaneous elimination of plasmid FB1 and of its derepressed variant FB1 drd could not be detected. These plasmids also persisted in the cells after exposure to ultraviolet radiation (in various doses) and also to ethidium bromide in high concentration (400 μg/ml). Plasmid F'-lac+ was characterized by a high frequency of both spontaneous and induced elimination. The results (Table 2) also indicate that the frequency of elimination of plasmid F'-lac+ depends on the recombination capacity of the host cells. If the bacterial cells had normal capacity for genetic recombination, total (100%) elimination of the F'-lac+ plasmid occurred after exposure to ultraviolet radiation in the dose of 40 erg/cm². On the other hand, the dose of irradiation of cells of the recombination-defective strain AB2463 recA giving the same effect on their viability (Table 1) gave rise to virtually no

TABLE 2. Eliminating Effect of Ultraviolet Radiation and Ethidium Bromide Relative to F-Like Plasmids in E. coli K-12 Cells

Strain	Plasmids	Spontaneous elimination, %	Percentage of cells losing plasmid after different doses of ultraviolet radiation, erg/cm²						Eliminating effect of ethidium bromide, %
			2	8	10	24	40	120	
AB1157 rec+	FB1	<2	—	—	<2	—	<2	<2	<2
	FB1 drd	<2	—	—	<2	—	<2	<2	<2
	F′-lac+	47	—	—	47	—	100	100	50
AB2463 rec−	FB1	<2	<2	<2	—	<2	—	—	<2
	FB1 drd	<2	<2	<2	—	<2	—	—	<2
	F′-lac+	<1	<1	<1	—	25	—	—	3
AB2463-4 Rec+	FB1	<2	—	—	<2	—	<2	<2	<2
	FB1 drd	<2	—	—	<2	—	<2	<2	<2
	F′-lac+	34	—	—	47	—	100	100	38.5
AB2463-29 Rec+	FB1	<2	—	—	<2	—	<2	<2	<2
	FB1 drd	<2	—	—	<2	—	<2	<2	<2
	F′-lac+	35	—	—	36	—	100	100	39

eliminating action on this plasmid. A similar effect was found when the eliminating activity of ethidium bromide was studied (Table 2).

The results are evidence of considerable differences in the behavior of individual F-like plasmids in cells of E. coli K-12 when treated with eliminating agents. The stability of the individual F-like plasmids may also be presumed to depend largely on genetic differences in the recA region of the host bacterial cells. Whereas the stability of plasmid F′-lac⁺ depended on the genetic properties of the host cell, the F-like plasmids FB1 and FB1 drd showed similar behavior irrespective of the recombination capacity of the bacteria studied.

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