ISOLATION AND CHARACTERISTICS OF RECOMBINATION-DEFECTIVE MUTANTS OF Escherichia coli

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UDC 576.851.48.095.57

By induction with N-methyl-N'-nitro-N-nitrosoguanidine, rec⁻ mutants were isolated from cultures of Escherichia coli strain P678⁻, a derivative of E. coli K-12. In crossings with standard donors they form recombinants in low frequency.

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Recombination-defective (rec⁻)* mutants of recipient cells of Escherichia coli K-12 have recently been isolated, which cannot form recombinants after crossing with Hfr donor cells because of disturbance of their ability to catalyze one stage of genetic recombination [1, 2, 5, 7]. Since the process of recombination consists of a series of complex physical and chemical events [6], it is assumed that several genes are perhaps concerned in the determination of these events [4, 7]. The possibility likewise is not ruled out that, as a result of mutations, the recipient cells may also lose their power to form contacts with the donor cells or to receive genetic material. For this reason, it is possible in principle to isolate not only various rec⁻ mutants from cultures of the recipient cells, but also mutants of other classes.

This paper describes experiments carried out to isolate rec⁻ mutants from cultures of other strains of E. coli K-12 and also the results of crossing of these mutants with donor cells of intermediate type, carrying F^1-lac^+ sex factors differing in their structure.

EXPERIMENTAL METHOD

The search for rec⁻ mutants was carried out in cultures of recipient strains E. coli P678 $F^-T^-L^-lac^-S^r$ and E. coli J62F^- $Pro^-Try^-His^-lac^-S^r$, treated with N-methyl-N'-nitro-N-nitrosoguanidine. For this purpose,

*The following abbreviations are used in this article: B_1 – thiamine, T – threonine, L – leucine, Pro – proline, Try – tryptophan, His – histidine, lac^+/lac^- – ability/inability to ferment lactose, S^s/S^r – sensitivity/resistance to streptomycin, pho – alkaline phosphatase, pur – purines, Rpho – the gene regulating synthesis of alkaline phosphatase, $T6^r$ – resistance to phage T6.

TABLE 1. Crossing of Intermediate Donors with E.coli P678F^- and with rec⁻ Mutants

Strain	Donor and frequency of transmission of F' factors and chromosomal genes						
	200 PS		1 485		W 1 485		W3747
	$lac^+ S^r$	$T^+ L^+ S^r$	$lac^+ S^r$	$T^+ L^+ S^r$	$lac^+ S^r$	$T^+ L^+ S^r$	$lac^+ S^r$
P678F⁻	100	100	100	100	100	100	100
P678F⁻rec⁻10	55	0,8	100	0,8	100	0,03	100
P678F⁻rec⁻15	100	0,32	100	0,36	1,2	0,3	100
P678F⁻rec⁻48	57	<0,02	74	<0,02	74	<0,04	80
P678F⁻rec⁻75	100	<0,02	100	<0,02	27	<0,04	58

Laboratory of Genetics of Microorganisms, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow (Presented by Academician N. N. Zhukov-Verezhnikov, Academy of Medical Sciences of the USSR.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 7, pp. 100-102, July, 1969. Original article submitted July 5, 1968.

TABLE 2. Crossing of Intermediate Donors with E. coli J62F⁻ and rec⁻ Mutants

Strain	Donor and frequency of transmission of F' factors and chromosomal genes					
	200 PS		1 485		W 1 485	
	lac⁺ Sʳ	Pro⁺ Sʳ	lac⁺ Sʳ	Pro⁺ Sʳ	lac⁺ Sʳ	Pro⁺ Sʳ
J62F⁻	100	100	100	100	100	100
J62F⁻rec⁻308	<0,01	<0,07	70	<0,01	0,002	<0,25

nitrosoguanidine was added to 6-8 hour broth cultures in a concentration of 50 μg/ml and the cultures were incubated at 37° for 1 h with aeration. Cells treated with the mutagen were then washed twice by centrifugation and the suspensions were seeded on meat-peptone and minimal agar, with the essential additives, or in meat-peptone broth, from which samples were taken after incubation at 37° for 18-20 h, and also seeded on meat-peptone and minimal agar. The growing colonies were transferred by the replica technique to minimal agar media containing additives permitting selection of $T^+L^+S^r$ and Pro^+S^r recombinants, covered with a thick suspension of donor cells E. coli HfrH $B_1^-S^r$ (order of transfer: O–T–L–Pro–Try–His). Colonies whose impressions did not give growth on the selective media were removed from the original dishes and seeded in meat-peptone broth, and after verification of the genotype, the cultures were studied in crossings with the same donor by standard methods [3]. In control experiments the same method was used to analyze colonies grown from cells not treated with the mutagen. The effectiveness of this last procedure was checked by parallel detection of additional nutrient requirements in the cultures. Crossing of the isolated rec⁻ mutants with cells possessing sex factor F^1 was also carried out by standard methods [3].

EXPERIMENTAL RESULTS

In the course of the experiments 3100 colonies of E. coli P678F⁻ and 1000 colonies of E. coli J62F⁻, grown from cells treated with nitrosoguanidine, were transferred to selective media.

Analysis of growth of impressions of these colonies showed that in the first case no growth was given by impressions of 190 colonies, and in the second case by impressions of 32 colonies. However, in crossings carried out by standard methods, by mixing recipient cells isolated from single colonies of clone cultures with HfrH cells in meat-peptone broth, a low frequency of recombination was found only in experiments with four clone cultures of E. coli P 678F⁻ and one clone culture of E. coli J62F⁻. It will be noted that in the experiments to check the mutagenic activity of nitrosoguanidine, 30 of the 200 colonies investigated showed the presence of additional nutrient requirements.

After establishment of the identity of the isolated cultures with the original, they were preliminarily defined as rec⁻ cultures and designated P678F⁻ rec⁻10, P678F⁻rec⁻15, P678F⁻rec⁻48, P678F⁻rec⁻75, and J62F⁻rec⁻308. This conclusion was based on the following consideration. The ability of cells of the isolated cultures to act as recipients was expressed as a percentage of the appearance of recombinants in their crossings with HfrH cells relative to the frequency of appearance of analogous recombinants in crossings in which cells of the original strains (of wild type) were the recipients. In HfrH × P678F⁻rec⁻ crossings the frequency of appearance of $T^+L^+S^r$ recombinants, from the results of a series of experiments, was between 0.04 and 0.2% of the frequency of appearance of these recombinants in the control crossings (HfrH × P678F⁻). In HfrH × J62F⁻rec⁻308 crossings the frequency of appearance of Pro^+S^r recombinants was 0.11-2.8% of the frequency of appearance of the analogous recombinants selected in the control crossings (HfrH × J62F⁻).

To confirm the nature of the rec⁻ mutants detected, their ability to act as recipients was established in crossings with E. coli 200PS, 1485, W1485, and W3747, the cells of which carrying sex factors F^1-lac⁺, F^1-lac⁺, F^1-lac⁺, F^1-gal⁺, and F13-lac⁺pho Rpho $T6^r$ respectively. Partial diploids lac^+S^r and recombinants $T^+L^+S^r$ and Pro^+S^r were selected and their frequency of appearance determined as a percentage of the frequency of genetic transfer in control crossings in which the recipients were E. coli P678F⁻ and J62F⁻. The results obtained are given in Tables 1 and 2.

As Table 1 shows, the frequency of appearance of $T^+L^+S^r$ recombinants compared with the control was just as low as in the preceding crossings, where HfrH cells were the donors. Meanwhile the frequency of appearance of lac^-S^r diploids in nearly all cases was 55-100% of the frequency of appearance of these

diploids in the control crossings. The only exception was mutant P678F⁻rec⁻15, which received one of the sex factors at a lower frequency. The isolated rec⁻ mutants did not lose their ability to receive F^1 sex factors of different types. However, replication of one of them under these conditions was apparently suppressed. So far as the results given in Table 2 are concerned, the high frequency of formation of lac^+S^r diploids in crossing 1485 × J62F⁻rec⁻308 only, in the absence of Pro^+S^r recombinants, still does not constitute a basis for acceptance of the recombination nature of this mutant, for in other crossings the lac^+S^r diploids were absent.

The results of these experiments thus confirm the conclusion that the isolated cultures were rec⁻ mutants. They also demonstrate that the rec⁻ mutation has some influence on replication of the various F^1 factors in cells carrying this mutation. The nature of this phenomenon requires further study.

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