INDUCED MUTANTS OF FACTOR F^1-LAC^+ IN Escherichia coli K-12

A. P. Pekhov, A. A. Kusakin, and V. V. Pantyukhova

UDC 576.851.48.095.57

After conversion of sex factor (F) from the integrated state into the autonomous, donor cells of Escherichia coli HfrH become donor type F^+. Sometimes this conversion is accompanied by the formation of a combined structure, consisting of sex factor linked with one or more chromosomal genes. Variants of the autonomous sex factor carrying chromosomal genes are called F^1 factors, and cells possessing this factor are called intermediate donors [6, 8]. A characteristic feature of intermediate donors is that they transfer both factor F^1 and a chromosome segment to F^- recipient cells at the same time.

Since sex factor is constructed from DNA [4, 5, 7], it may be assumed that F^1 factor carries genes controlling several functions (conjugation of cells, transfer of F^1 and the chromosomal segment to F^- cells, antigenic specificity, sensitivity of donor cells to "male" phage, etc.). The identification of these genes is associated with detection of the corresponding mutations of the sex factor.

In the experiments described in this article, mutants of F^1 factor with modified ability to effect their own transfer and the transfer of the chromosomal segment were discovered.

EXPERIMENTAL METHOD

To detect mutants of F^1 factor a prototrophic streptomycin-sensitive (S^s) intermediate donor strain of E. coli 200 Ps/ F^1-lac^+ was used. This strain, whose cells carry the lac_y^- mutation (inability to ferment lactose) on the chromosome and a wild type of factor F^1-lac^+, was obtained from Jacob (France). The recipients were two lactose-negative (lac^-) streptomycin-resistant (S^r) strains of E. coli [1, 2]: C600 F^-, dependent on threonine (thr^-), leucine (leu^-), and thiamine (B_1^-), and J62 F^-, dependent on proline (pro^-), tryptophan (try^-), and histidine (his^-).

Eight-hour broth cultures of E. coli 200 Ps/ F^1-lac^+ were seeded in a volume of 0.1 ml on meat-peptone agar in dishes which were then irradiated with ultraviolet light for 1 min at a distance of 40 cm from a type PRK-4 lamp. The subcultures were incubated at 37° for 24 h, after which the growing colonies (the number of surviving cells was about 1%) were transferred by means of the replica technique to a selective medium, consisting of minimal agar with lactose (1%), streptomycin (250 units/ml), threonine (60 μg/ml), leucine (60 μg/ml), and thiamine (60 μg/ml), covered with a thick suspension of E. coli C600 F^- cells. These subcultures were incubated for 48 h. The results were assessed by growth of the colonies on the replica plates compared with that of the original colonies. Replication of colonies of E. coli 200 Ps/ F^1-lac^+ grown from unirradiated cells on analogous media served as the control.

EXPERIMENTAL RESULTS

In accordance with the technique used, it was assumed that growth of colonies transferred to selective medium is dependent on the activity of the donor cells from which these colonies arise. If the donor ability of the cells remains unchanged after ultraviolet irradiation, these colonies must give growth on the selective medium as a result of crossing with recipient cells. If donor ability is disturbed, growth of the transferred colonies would be impossible.

Using the replica technique, 30,000 colonies grown on meat-peptone agar from irradiated cells were transferred to selective media. As was to be expected, the overwhelming majority of colonies gave growth on the selective medium, and the replica colonies were indistinguishable in size and shape from those in control seedings. Nevertheless, of the total number of colonies investigated, 100 failed to give growth when

Laboratory of Genetics of Microorganisms, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR, V. D. Timakov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 11, pp. 130-133, November, 1967. Original article submitted February 22, 1967.

TABLE 1. Frequency of Appearance of lac^+S^r, $thr^+leu^+S^r$, and pro^+S^r Recombinants

Crosses	Transfer of F^1-lac^+	Transfer of chromosomal genes	
		thr^+ leu^+	pro^+
200 ps — 8 × C600 J62	0	0	0
200 ps — 14 × C600 J62	5×10^{-3}	4×10^{-5}	6×10^{-6}
200 ps — 21 × C600 J62	0	0	0
200 ps — 22 × C600 J62	0	0	0
200 ps — 29 × C600 J62	0	0	0
200 ps — 45 × C600 J62	2×10^{-6}	0	0
200 ps — 65 × C600 J62	0	0	0
200 ps — 74 × C600 J62	$1{,}5\times10^{-4}$	5×10^{-5}	1×10^{-6}
200 ps F^1- lac^+ × C600 J62	$6{,}9\times10^{-2}$	$1{,}1\times10^{-3}$	$1{,}8\times10^{-3}$

transferred to the selective medium. This suggested that these colonies originated from cells carrying a sex factor with modified functions.

To determine the phenotype, each of the 100 colonies was taken from the original dishes and suspended in 1 ml meat-peptone broth; three media were seeded at the same time with the suspension: minimal agar with lactose, minimal agar with lactose and streptomycin, and Endo's medium. The results of these subcultures showed that all the colonies were phenotypically indistinguishable from colonies growing after seeding bacteria of the original strain. Since colonies of lac^- segregants also developed on Endo's medium along with the lac^+ colonies, for subsequent experiments to confirm the results obtained only lac^+ colonies were chosen.

In the next series of experiments the donor ability of the 100 reisolated lactose-positive clone cultures was tested by crossing each of them with cultures of recipient strains in a liquid medium. The transferability of factor F^1-lac^+ and also of chromosomal genes thr^+, leu^+, and pro^+ was determined. Eight-hour clone cultures of lac^+ cells were mixed with 8-hour cultures of E. coli C600 F^- and J62 F^- in the ratio 1:10. After incubation for 2 h, the mixture of 200Ps × C600 was diluted and seeded on media permitting selection of lac^+S^r and $thr^+leu^+S^r$ recombinants and the 200Ps × J62 mixture was seeded on media selecting pro^+S^r recombinants.

The results of crossing the clone cultures with cultures of recipient cells in meat-peptone broth differed essentially from the results of the analogous crosses performed previously on agar. Of the 100 cultures, the cells of 92 cultures crossed with cells of the recipient strains. The frequency of transfer of the sex factor and of chromosomal genes in these crosses was the same when compared with the frequency of transfer in control crosses. Consequently, further cultivation facilitated restoration of the donor ability of the cells of many of the clone cultures isolated immediately after ultraviolet irradiation of the original specimens. So far as the other eight clone cultures isolated from irradiated cells are concerned, the results obtained by crossing them with cultures of recipient strains demonstrate a permanent change in activity of the sex factor (see Table 1).

The results given in Table 1 show that cells of clone cultures 200 Ps—14, 200 Ps—45, and 200 Ps—74 are much less able to transfer factor F^1-lac^+ and chromosomal genes than cells of the original strain. The order of transfer of chromosomal genes is preserved. Meanwhile cells of the other five clonal cultures lost their ability to transfer genes. Since these cells were lac^+, the possible assumption that they

had lost sex factor is ruled out. The loss of donor ability by the cells in this particular case is associated with mutation of the sex factor.

In the final series of experiments cells of clonal cultures with modified donor ability were tested for sensitivity to phage f2, causing lysis specifically of Hfr, F^+, or F^1 cells only. All the cultures isolated were found to be sensitive to this phage.

This investigation thus showed that clonal cultures of E. coli K-12 cells carrying mutant factor F^1-lac^+ can be isolated.

Mutations of factor F^1 have been described previously by Cuzin [3], who isolated clonal cultures of E. coli R^1-lac^+, whose cells transferred factor F^1 and individual chromosomal genes with very low frequency when crossed with F^- cells. Since the isolated cultures were resistant to "male" phage, their nature was explained by mutations of factor F^1 manifested phenotypically as a change in the surface structures of the donor cells, preventing their conjugation with F^- cells. In the present case the defective character of the mutants discovered may be attributed to injuries of a different order, for the cells of the isolated clonal cultures remained sensitive to "male" phage. This means that conjugation and the establishment of effective contacts between donor cells carrying mutant sex factor and F^- cells are undisturbed. Consequently, the isolated clones of cells possess the factor F^1-lac^+, which carries mutations damaging transfer of the factor F^1-lac^+ itself and its ability to mobilize the chromosomal segment for transfer, rather than conjugation of the crossed cells. In other words, the results of these experiments mean that sex factor genes are functionally heterogeneous, and their functions exhibit specialization.

LITERATURE CITED

1. R. K. Appleyard, Genetics, 39, 440 (1954).
2. R. S. Clowes and D. Rowley, J. Gen. Microbiol., 11, 250 (1954).
3. F. Cuzin, C. R. Acad. Sci., Paris , 255, 1149 (1962).
4. P. Driskell and E. Adelberg, Bact. Proc.,186 (1961).
5. S. Falkow and R. Citarella, J. Molec. Biol., 12, 138 (1965).
6. F. Jacob and E. L. Wollman, Sexuality and the Genetics of Bacteria, New York (1961).
7. R. Lavalle and F. Jacob, C. R. Acad. Sci., Paris , 252, 1878 (1961).
8. P. Sneath, Brit. Med. Bull., 18, 41 (1962).