ISOLATION AND CHARACTERISTICS OF HIGH-FREQUENCY GENETIC DONORS OF A SEROTYPED STRAIN OF *Escherichia coli*

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As a result of crossing cells of donor strain Escherichia coli K-12 (P4X) with serotyped (group 0100) recipient cells E. coli trp⁻lac⁻, nine recombinants possessing sex factor and ability to carry out chromosome transfer with high frequency were isolated. The isolated strains of donor cells carry sex factor in the integrated state and retain their membership of serogroup 0100.

KEY WORDS: Escherichia coli; sex factor; chromosome transfer.

The chief difficulty in the study of the genetic bases of pathogenicity and immunogenicity of bacteria belonging to different species is the absence of crossing systems. Nevertheless, investigations of serotyped pathogenic strains of E. coli isolated from different sources has shown that bacteria of individual strains can carry natural sex factors and are promising material for the search for donor cells among them capable of carrying out genetic transfer with high frequency [1].

Advances in the production of different strains of E. coli K-12 [3] have shown that in the case of serotyped strains of E. coli an effective crossing system can be obtained by introducing the classical sex factor from E. coli K-12 into these bacteria and isolating from the progeny cells carrying sex factor in the integrative state and acting as high-frequency donors of the Hfr type.

The object of this investigation was to introduce sex factor from E. coli K-12 into cells of a serotyped strain of E. coli (serogroup (0100) and to search for high-frequency donors among the recombinants isolated from such a cross.

EXPERIMENTAL METHOD

Donor strain P4X met⁻str^S originating from E. coli K-12 and the recipient serotypes (serogroup 0100) strain E. coli trp⁻lac⁻str^S, isolated in the writer's laboratory by N. I. Shchipkova, were used as the original strains. The 18-h broth cultures of the original strains were diluted 1:20 in nutrient broth (NB) and reincubated to a density of $1 \cdot 10^7$ cells/ml. The donor and recipient cells for crossing were mixed in a tube in the ratio of 1:5, then centrifuged for 10 min and, without pouring off the supernatant, they were incubated for 2 h. The conjugation mixture was then again centrifuged at 4000 rpm for 10 min, washed twice, resuspended in physiological saline up to the initial volume, and samples measuring 0.1 ml were seeded on selected medium (minimal agar with lactose) in dishes. The subcultures were incubated for 48 h at 37°C. Lac$^+$ recombinants appearing on the dishes were purified on Endo's medium, after which their sensitivity to phage f_2 was determined. The donor ability of the phage-sensitive Lac$^+$ recombinants and the direction of the chromosome transfer carried out by them were verified in crosses with E. coli J 62 F⁻pro⁻his⁻trp⁻str^r, C 500 F⁻thr⁻leu⁻thy⁻lac⁻str^r and AB 1157 F⁻thr⁻leu⁻pro⁻arg⁻his⁻lac⁻str^r by

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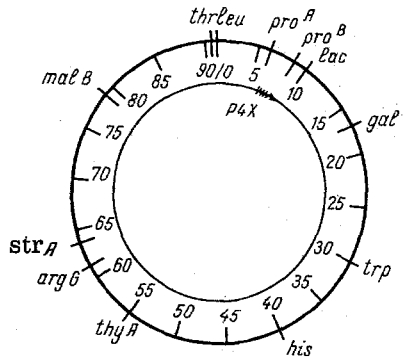

Fig. 1. Chromosome map of E. coli showing genetic markers and the time and direction of their transfer produced by cells of strain Hfr P4X.

TABLE 1. Frequency of Transfer of Genetic Markers from Serotyped Donor Cells to Recipient Cells J62M⁻

No. of donor strain	Marker			
	Pro⁺	His⁺	Trp⁺	Lac⁺
10	$6{,}2 \cdot 10^{-3}$	$1{,}4 \cdot 10^{-6}$	$9 \cdot 10^{-7}$	>0
11	$9{,}6 \cdot 10^{-3}$	$1{,}7 \cdot 10^{-6}$	$2 \cdot 10^{-7}$	>0
13	$4{,}8 \cdot 10^{-3}$	$4{,}5 \cdot 10^{-7}$	$1{,}4 \cdot 10^{-7}$	>0
14	$6 \cdot 10^{-3}$	$8{,}3 \cdot 10^{-8}$	$9{,}5 \cdot 10^{-7}$	>0
19	$6{,}2 \cdot 10^{-4}$	>0	>0	>0
26	$1{,}1 \cdot 10^{-2}$	$9{,}2 \cdot 10^{-7}$	$2{,}2 \cdot 10^{-6}$	>0
30	$4 \cdot 10^{-3}$	$1 \cdot 10^{-6}$	$8{,}3 \cdot 10^{-7}$	>0
37	$3{,}8 \cdot 10^{-3}$	$8{,}6 \cdot 10^{-7}$	$7{,}1 \cdot 10^{-7}$	>0
39	$3{,}6 \cdot 10^{-3}$	$4{,}1 \cdot 10^{-7}$	$4{,}1 \cdot 10^{-7}$	>0

the standard method. The antigenic properties of the recombinants were determined by the agglutination test on slides and by the line test in tubes with serum against 0100. The behavior of the sex factor of the recombinants towards acridine orange (100 μg/ml) was determined by Hirota's method [2].

EXPERIMENTAL RESULTS

Since the donor cells of strain Hfr P4X carry out chromosome transfer in the order O–pro A–leu–thy A... lac (Fig. 1), selection of Lac⁺ recombinants was based on the assumption that such recombinants must inherit sex factor, for this factor is linked with the lac genetic marker. The formation of recombinants took place with low frequency; on each dish only 4 or 5 recombinant colonies were observed.

After purification on Endo's medium, 112 recombinant colonies were selected for the future work, transferred to NB and, after subculture, each culture was tested for sensitivity to "male" phage f_2. Tests of the sensitivity of these cultures to phage f_2 showed that only nine of them were sensitive (cultures Nos. 10, 11, 13, 14, 19, 26, 30, 37, and 39). All these cultures also proved to be prototrophs and streptomycin-sensitive.

To determine the frequency of chromosome transfer produced by the cells of each of the isolated recombinant strains they were crossed with cells of the recipient strain E. coli F⁻J62, after which the Pro⁺Str^r, His⁺Str^r, Trp⁺Str^r, and Lac⁺Str^r recombinants were selected. The results are shown in Table 1.

As Table 1 shows, the higher frequency of transfer was observed in the case of the Pro⁺ marker. The frequency of transfer of His⁺ and Trp⁺ was lower and that of Lac⁺ was evidently lower still, for no Lac⁺ recombinants were found whatever. Meanwhile cells of strain No. 26 were crossed with AB 1157 F⁻ cells and Pro⁺Str^r, Thr⁺Leu⁺Str^r, Arg⁺Str^r, His⁺Str^r, Trp⁺Str^r, and Lac⁺Str^r recombinants were selected. These crosses yielded the following frequency of transfer of the markers: Pro⁺Str^r $2.4 \cdot 10^{-3}$, Thr⁺Leu⁺Str^r $1.3 \cdot 10^{-3}$, Arg⁺Str^r $7.2 \cdot 10^{-5}$, His⁺Str^r > 0, Trp⁺Str^r > 0, and Lac⁺Str^r > 0. The results indicate that chromosome transfer produced by serotyped donor cells characteristically has a gradient reflected in the following order of transmitted markers: O–Pro–Thr–Leu–Arg–His–Trp–Lac. This gradient corresponds to the gradient of transfer for cells of strain Hfr P4X.

Treatment of cells of Hrf strains with acridine orange does not eliminate sex factor. To prove that the strains of serotyped donors isolated in these experiments belonged to the Hfr type, samples of cells of strain No. 26 were treated with acridine orange in a concentration of 100 μg/ml. The result of acridine orange treatment was determined from the frequency of segregation of Lac⁻ clones in the population of treated Lac⁺ cells seeded on Endo's agar. When no Lac⁻ segregants were found, 10 Lac⁺ colonies developing from cells treated with acridine orange were selected, cultures were grown from them, and each was crossed with E. coli AB 1157, after which Pro⁺Str^r recombinants were selected. The cells of all ten subcultures tested proved to be fertile, for they transferred the Pro⁺-marker at high frequency (of the order of $3 \cdot 10^{-3}$). Acridine orange did not eliminate sex factors from the cells of these treated donor strains.

The results of the experiments with acridine orange thus showed that sex factor in serotyped cells is in the integrated state.

Finally, the study of the antigenic properties of the isolated donor cell strains showed that they still belonged to the serogroup 0100.

It can be concluded from the results of these experiments that the strains of donor cells of E. coli belonging to the serogroup 0100, carrying sex factor from E. coli K-12, thus produced can carry out chromosome transfer with high frequency and with a certain gradient. This property, and also the behavior of their sex factor relative to acridine orange, show that they belong to the Hfr type of donors.

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