APPEARANCE OF THE F'-lac$^+$ FACTOR IN CELLS OF SEROTYPED STRAINS OF Escherichia coli

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During conjugation the sex factor F'-lac$^+$ was transmitted from cells of strain Escherichia coli 200 PS, derived from strain K-12, to lactose-negative cells of E. coli belonging to serological groups O111, O100, O86, and O25. A study of the donor properties of the resulting lactose-positive clonal cultures of E. coli thus obtained showed that they depend on the properties of the bacterial cell systems. The functions of the sex factor were considerably limited in bacteria of serological groups O86 and O111 and were restored when transferred back to cells of the recipient strains of E. coli K-12. In the case of clonal cultures of E. coli belonging to serological groups O25 and O100 this limitation was less marked.

The ability of donor cells of Escherichia coli K-12 to transmit genetic material to recipient cells during conjugation depends on the presence of sex factor in the donors [5, 7].

Since the possibility of transmission of sex factor from E. coli K-12 to bacteria of other strains [4] or of a different species [3] has been demonstrated, experiments were carried out to introduce the replaced sex factor F'-lac$^+$ into cells of serotyped strains of E. coli and then to study their donor properties. The results are described below.

EXPERIMENTAL METHOD

Strains of E. coli of the following serotypes, obtained from Professor I. V. Golubeva (from the collection of the All-Union Escherichia Center at the I. I. Mechnikov Research Institute of Vaccines and Sera) were used: O111:K58(B54), O100:K?:H2, O86:K$^-$:H25, O86A:K61(B7), O55:K59(B5), O26:K60(B6):H46, O25:K19(L):H12. Auxotrophic lactose-negative mutants (lac$^-$),* preserving the O-antigen of the original bacteria, were isolated initially from bacterial populations of these strains after treatment with N-methyl-N-nitro-N-nitrosoguanidine [2]. Later, the F'-lac$^+$ factor of cells of the donor strain E. coli 200 PS, derived from strain K-12, was introduced during conjugation into the lac$^-$ strains. The donor properties of the serotyped bacteria carrying the F'-lac$^+$ factor were studied in crosses with recipient cells of E. coli C600 and E. coli AB1157 and also by determining sensitivity to "male" phages and their relationship to acridine orange. All crosses and treatment of the bacteria with acridine orange were carried out by standard methods [6, 8]. The sensitivity of the isolated donors to specific "male"phages was determined by the agar layer method [1]. The behavior of factor F'-lac$^+$ in recipient strains derived from E. coli K-12 was studied in control experiments.

*The following abbreviations are adopted in this paper: thr – threonine, leu – leucine, try – tryptophan, cys – cysteine, pro – proline, arg – arginine, lac$^+$ (lac$^-$) – ability (inability) to ferment lactose, F – sex factor.

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TABLE 1. Donor Properties of Serotyped Clonal Cultures of E. coli Receiving F'-lac$^+$ Factor from E. coli 200 PS

Donor		Sensitivity of donor's cells to specific "male" phages f_1, f_2, MS2, M2, M12, Qβ, fd	Recipient strains of E. coli K-12	Frequency of transfer of selective markers during conjugation (per donor cell)			
serological group	No. of clonal cultures			lac$^+$	pro$^+$	thr$^+$ leu$^+$	arg$^+$
O100	58	+	C600	2.10^{-3}		5.10^{-4}	
			AB1157	5.10^{-5}	7.10^{-5}	6.10^{-5}	6.10^{-5}
O100	60	+	C600	4.10^{-2}		2.10^{-4}	
			AB1157	8.10^{-3}	2.10^{-4}	2.10^{-5}	6.10^{-5}
O100	95	+	C600	1.10^{-1}		5.10^{-3}	
			AB1157	6.10^{-5}	4.10^{-5}	3.10^{-5}	1.10^{-4}
O25	1	+	C600	1.10^{-2}		1.10^{-4}	
			AB1157	2.10^{-4}	6.10^{-5}	1.10^{-4}	1.10^{-4}
O25	9	+	C600	3.10^{-2}		2.10^{-4}	
			AB1157	1.10^{-4}	2.10^{-5}	5.10^{-5}	9.10^{-5}
O25	14	+	C600	3.10^{-2}		3.10^{-4}	
			AB1157	1.10^{-4}	5.10^{-5}	5.10^{-5}	1.10^{-4}
O86	3	—	C600	3.10^{-3}		3.10^{-5}	
O86	4	—	C600	4.10^{-4}		4.10^{-6}	
O86	31	—	C600	8.10^{-5}		1.10^{-7}	
			AB1157	1.10^{-7}	1.10^{-7}	5.10^{-8}	1.10^{-7}
O111	5	—	C600	1.10^{-8}		3.10^{-8}	
E. coli 200 PS F'-lac$^+$ (K-12)		+	C600	3,7		4.10^{-2}	
			AB1157	1,8	3.10^{-2}	2.10^{-2}	3.10^{-3}

Legend: +(−) sensitivity (resistance) to "male" phages.

TABLE 2. Results of Treatment of Bacteria of Donor Clonal Cultures O100F lac$^+$ and O25F lac$^+$ with Acridine Orange (AO)

Donor		Addition of AO (in μg/ml medium)	Percentage of lac$^-$ bacteria in treated population	Sensitivity of clones of E. coli isolated after AO-treatment to "male" phage MS2			
serological group	No. of clonal cultures			total number studied		number sensitive to MS2	
				lac$^+$	lac$^-$	lac$^+$	lac$^-$
0100	58	0 (Control)	<0,1	50	—	35	—
0100	58	75	<0,1	50	—	0	—
0100	60	0	2,4	50	50	50	7
0100	60	75	97,9	50	50	12	0
0100	95	0	<0,1	50	—	50	—
0100	95	75	<0,1	50	—	2	—
025	1	0	16	20	20	20	2
025	1	75	90	20	20	6	0
025	9	0	3	10	10	10	1
025	9	75	94	10	10	4	0
025	14	0	10	20	20	20	3
025	14	75	89	20	20	7	0
E. coli 200 PS		0	5,5	50	20	50	6
F'-lac+ (K-12)		75	91,6	50	50	16	0

EXPERIMENTAL RESULTS

In crosses between E. coli K-12/F'-lac$^+$ with try$^-$lac$^-$ E. coli cells of serological group O100 the frequency of appearance of O100 lac$^+$-merodiploids was 4×10^{-1} per donor cell, whereas in crosses in which lac$^-$, cys$^-$lac$^-$, and lac$^-$ cells belonging to serological groups O25, O86, and O111 were used as recipients the frequency of appearance of lac$^+$ merodiploids was 1×10^{-2}, 5×10^{-3}, and 4×10^{-4}, respectively. In crosses between E. coli K-12/F'-lac$^+$ with lactose-negative mutants of E. coli cells belonging to serological groups O86A, O55, and O26 transmission of the replaced sex factor could not be demonstrated.

Isolated lactose-positive colonies were selected from the three crosses in which transfer of the sex factor was detected, clonal cultures were obtained from them and, after determination of their sensitivity to "male" phages, their ability to perform episomal and chromosomal transfer was studied. The results obtained by a study of these properties in the isolated cultures, described conventionally as O100F lac$^+$, O25F lac$^+$, O86F lac$^+$, and O111F lac$^+$, are given in Table 1. Clearly bacteria of serological groups O100 and O86 receiving the F'-lac$^+$ factor were similar to cells of strain 200 PS/F'-lac$^+$ in their sensitivity to specific DNA- and RNA-containing "male" phages but they were less able to perform episomal and chromosomal transfer during conjugation with recipient strains E. coli C600 and AB1157. In the case of clonal strains O86F lac$^+$ and, in particular, O111F lac$^+$ the bacteria were resistant to male phages, and the frequency of transfer of chromosomal markers was low.

The results of determination of the eliminating activity of acridine orange on sex factors of the serotyped bacteria are given in Table 2. They show that the sex factor linked with the lac^+ gene in cells of clonal cultures Nos. 1, 9, and 14 of serological group O25 and of clonal culture No. 60 of serological group O100 is in an autonomous state; i.e., it behaves like the F'-lac^+ factor. The character of its replication in these bacteria in the presence of acridine orange is similar to that in the case of cells of E. coli 200 PS. The sex factor of bacteria of clonal cultures Nos. 58 and 95 of serological group O100 in the presence of acridine orange behaves like the F-factor of the donor strains of E. coli K-12 type F^+.

Further experiments showed that after transfer of the F'-lac^+ factor from bacteria of serological groups O86 and O111 to cells of recipient strains E. coli C600 and AB1157, the merodiploids formed became sensitive to "male" phages. The results demonstrate the similarity and differences in the behavior of the sex factor F'-lac^+ depending on the character of the cell systems in which it replicates. A considerable limitation of the functions of the F'-lac^+ factor of E. coli K-12 was observed in the cells of serological groups O86 and O111, whereas this limitation was less marked in cells O100 and O25.

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