DETECTION AND CHARACTERISTICS OF CONJUGATION FACTORS OF SEROLOGICALLY TYPED STRAINS OF *Escherichia coli*

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Investigation of 100 serologically typed strains of Escherichia coli showed that 25 of them can give rise to genetic recombinants. The donors detected the F, R, or Col conjugation factors and transfer genetic material with a frequency of 10^{-7}-10^{-8} (per donor cell). The F factor of serologically typed strains of E. coli differs considerably from the F factor of E. coli K-12 and it is probably a recessive variant of it.

Genetic analysis of pathogenic bacteria is made more difficult because they have no systems of genetic recombination. Nevertheless, data showing that genetic exchange can take place through conjugation of bacterial cells belonging to individual pathogenic, serologically typed strains of Escherichia coli indicate that the search for conjugation factors in these bacteria could be promising [2, 5, 6].

This paper describes the results of investigations carried out to detect and, if possible, identify conjugation factors in bacteria belonging to several serologically typed strains of E. coli.

EXPERIMENTAL METHOD

Conjugation factors were studied by determining the ability of cells of 100 serologically typed strains of E. coli, of which 29 were reference strains and 71 were of clinical origin, to behave as donors. All these strains belonged to the most widespread serotypes (O6, O25, O26, O55, O86, O111, O124, O128, O20). Their preliminary study showed that all were prototrophs and could utilize lactose (lac^+).* The bacterial cells of 63 strains were sensitive to streptomycin (S^S) whereas reproduction of bacteria of the other strains was not restricted by streptomycin added to the nutrient medium in a concentration of 250 μg/ml (S^r).

The hypothetical donors were crossed with streptomycin-resistant mutants of bacteria of the serologically typed strains O100 (try^-lac^-) and O86 (cys^-lac^-), and also with standard recipient strains E. coli C600 ($F^-thr^-leu^-B_1^-lac^-S^r$ and AB1157 $F^-thr^-leu^-B_1^-pro^-his^-arg^-lac^-S^r$), derivatives of strain E. coli K-12. S^r mutants of serologically typed strains which were sensitive to nalidixic acid (40 μg/ml) were crossed with cells of strain PA-373 $F^-arg^-met^-thr^-leu^-his^-lac^-nal^r$, which also are derivatives of E. coli K-12.

Crossing was carried out by the standard technique [3] and recombinants were selected on appropriate media to which streptomycin (250 μg/ml) or nalidixic acid (100 μg/ml) was added. Sensitivity of the bacteria to "male" and "female" phages was determined by the agar layer method [4]. Ability to produce

*The following abbreviations are used in this paper: lac^+ (lac^-) ability (inability) to ferment lactose, S^S (S^r) sensitivity (resistance) to streptomycin, try^+ (try^-), cys^+ (cys^-), thr^+ (thr^-), leu^+ (leu^-), B_1^+ (B_1^-), pro^+ (pro^-), his^+ (his^-), arg^+ (arg^-), and met^+ (met^-) ability (inability) to synthesize tryptophan, cystein, threonine, leucine, thiamine, proline, histidine, arginine, and methionine, nal^S (nal^r) sensitivity (resistance) to nalidixic acid.

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TABLE 1. Properties of Donor Bacteria of Serologically Typed Streptomycin-Sensitive Strains of E. coli

Strain	Serotype	Frequency of transmission of selective markers to various recipients				Sensitivity to specific "male" phages			
		C600F⁻	AB1157F⁻	O100F⁻	O86F⁻	f_1	f_2	MS2	Qβ
9	O6:K2ac (L):H1	—	—	$<10^{-8}$	—	—	—	—	—
12	O6:K53 (L)	—	—	$<10^{-8}$	10^{-8}	—	—	—	—
13	O6:K54 (L):H10	$0{,}7\cdot10^{-7}$	—	$0{,}2\cdot10^{-7}$	—	—	—	—	—
25	O55	$<10^{-8}$	$1{,}0$—$1{,}2\cdot10^{-8}$	$<10^{-8}$	—	—	—	—	—
27	O55	$<10^{-8}$	—	—	$<10^{-8}$	—	—	—	—
29	O55	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	—	—	—	—
31	O55	$<10^{-8}$	$<10^{-8}$	—	$<10^{-8}$	—	—	—	—
45	O125:B15:H19	$0{,}4\cdot10^{-7}$	—	$<10^{-8}$	—	—	—	—	—
46	O55	$0{,}5\cdot10^{-7}$	—	$<10^{-8}$	—	—	—	—	—
49	O55	$0{,}6\cdot10^{-7}$	—	—	$<10^{-8}$	—	—	—	—

TABLE 2. Properties of Donor Bacteria of Serologically Typed Streptomycin-Sensitive Strains of E. coli

Strain	Serotype	Sensitivity to antibiotics in minimal inhibitory concentrations (μg/ml)							Frequency of appearance of met^+nal^r recomb. in crosses with E. coli PA-373	Frequency of transmission of R-factor	Sensitivity to specific "male" phages			
		Str	Tc	Ne	Km	Lm	Pn (in units/ml)	Pl			f_1	f_2	MS2	Qβ
30	O124	500	>100	10	5	>100	>1000	1,0	$1{,}6\cdot10^{-8}$	$0{,}3\cdot10^{-4}$	—	—	—	—
38	O111	>1000	2,5	10	10	>100	>1000	0,1	$1{,}7\cdot10^{-8}$	$0{,}5\cdot10^{-4}$	—	—	—	—
54	O55	>1000	>50	>100	>25	>50	>1000	>10	$<10^{-8}$	$4{,}4\cdot10^{-2}$	—	—	—	—
75	O25	>1000	>50	>25	>25	>50	>50	>10	$<10^{-8}$	$5{,}0\cdot10^{-2}$	—	—	—	—
86	O26	500	>50	10	25	>50	>100	10	$<10^{-8}$	$4{,}0\cdot10^{-2}$	—	—	—	—

Legend: Str) streptomycin; Tc) tetracycline; Ne) neomycin; Km) kanamycin; Ch) chloramphenicol; Pn) penicillin; Pl) polymixin.

colicins was determined by the stab method [1], sensitivity to antibiotics was tested by the serial dilution method in nutrient broth, and hemolytic activity was studied in 4% blood agar.

EXPERIMENTAL RESULTS

The results of crossing bacterial cells of 63 S^S strains of the various E. coli serotypes with auxotrophic S^r mutants of E. coli K-12 and auxotrophic mutants of serotypes O100 and O86 showed that the cells of 16 strains have donor ability, i.e., ability to transmit genetic material and to give rise to genetic recombinants: $thr^+leu^+S^r$, pro^+S^r, his^+S^r, arg^+S^r, try^+S^r, and cys^+S^r. So far as S^r mutants of the typed bacteria are concerned, fertility was discovered in nine of the 37 strains during selection of met^+nal^r recombinants in crosses with E. coli PA-373.

While these donors were able to transfer genetic material, they did so at a frequency below that of the transfer usually carried out by type F^+ donor cells. As Table 1 shows, the frequency of appearance of recombinants in crosses of serologically typed donors with untyped and typed recipients by conjugation for 2 h was of the order of 10^{-7}-10^{-8} (per donor cell).

To obtain further data on the properties of the donor bacteria of all fertile streptomycin-sensitive strains discovered, their sensitivity was determined to the specific "female" phage II and to the "male" phages f_1, f_2, MS2, and Qβ. These experiments (Table 1) showed that the bacteria investigated are resistant not only to "female" phage, but also to all "male" phages. Nevertheless, to confirm the results concerning the presence of conjugation factor in the donor cells experiments were carried out to determine the transmissiveness of this factor. Analysis of recombinants obtained by crossing bacteria of three donor S^S strains with the corresponding recipients showed that the conjugation factor of the donors discovered is transmissive, and that transmissiveness was exhibited by 57, 30, and 20% of the total number of recombinants tested (respectively). On this basis the conjugation factor was identified as factor F.

Besides identifying the F factor in bacterial cells of the serologically typed strains, they were also investigated for the presence of colicinogenic factors. Tests on the donor strains for ability to produce colicins revealed this property in six cases. Sample analysis of recombinants obtained by crossing three donor strains with the corresponding recipients showed that the frequency of transmission of colicinogenicity was 10, 60, and 10%, respectively. These results suggest that donor cells of six serologically typed strains carry colicinogenic factors which, in their properties resemble type I Col-factor.

These results suggest that the cells of 10 streptomycin-sensitive serologically typed donor strains of bacteria carry a conjugation factor similar to factor F whereas the bacteria of the remaining six strains carry colicinogenic factor. No R-factors were discovered in this group of donors.

In further experiments the donor cells of 10 typed streptomycin-sensitive strains were crossed as recipients with donor cells of E. coli Hfr $Hthr^{-}lac^{-}S^{r}$. The results of these experiments showed that cells of five of the 10 donor strains can act as recipients, because $thr^{+}S^{r}$ recombinants appeared in the crosses. In parallel experiments in which cells of serologically typed strains without donor ability were used as recipients, analogous recombinants also were found. In conjunction with evidence of the resistance of donor cells to "male" phages, these results show that the identified F factor was heterologous relative to the F factor present in cells of individual strains of E. coli K-12.

In the next series of experiments the nature of the conjugation factor was studied in donor cells resistant to streptomycin. For this purpose, the sensitivity of bacteria of nine donor strains to another six antibiotics (tetracycline, chloramphenicol, kanamycin, neomycin, penicillin, polymixin M) was determined to begin with. They were found to be resistant to most of these antibiotics. In experiments to study the transmission of markers of antibiotic resistance of the test donors to sensitive recipients, the determinants of drug resistance were found to be transmitted linked with the conjugation factor, and in a considerably higher frequency than determinants with a chromosomal localization. As Table 2 shows, the frequency of transmission of resistance to, for example, chloramphenicol was 10^{-2}-10^{-4} (per donor cell). Consequently, the conjugation factors of the bacteria of these nine serologically typed strains were similar to the typical R-factor.

In conclusion, donor cells of all 25 serologically typed donor strains were tested for hemolytic activity. It was found in one case, but in the next experiments the transmissive character of the hemolytic activity could not be shown.

It can be concluded from the results of these observations that bacteria of serologically typed strains of E. coli can carry various conjugation factors. So far as F factor in the case of serologically typed strains of E. coli is concerned it differs considerably from the F-factor of E. coli K-12 and, in some cases, it is probably one of its repressed variants.

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