SEXUAL HYBRIDIZATION OF A REFERENCE STRAIN OF *Escherichia coli* (SEROTYPE 0111 : B4) WITH STANDARD UNTYPED STRAINS OF *Escherichia coli* F^-

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Previous communications described the positive results of crossing various strains of pathogeneic serotypes of *E. coli* with untyped auxotrophic strains *E. coli* Pa678F$^-$ and 58161F$^+$ [2], and the production of changes in the antigenic structure of *E. coli* as a result of genetic recombination [1]. It should be noted that the typed strains used in these studies were isolated from patients.

TABLE 1. Transfer of Nonselective Signs from Donor to Recipient Cells in Different Crosses

Crosses	Recombinant	Prototrophism	Ability of ferment carbohydrates										Sensitivity to phages								Sensitivity to streptomycin
			Lactose	Glucose	Sucrose	Mannitol	Maltose	Rhamnose	Galactose	Sorbitol	Dulcitol	Arabinose	T1	T2	T3	T4	T5	T6	T7	λ	
O111 :B4F$^+$ × PA678F$^-$	86	+	a	+	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−
	87	+	a	+	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−
O111 : B4F$^+$ × P678F$^-$	76	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−
	88	+	+	+	−	+	+	+	+	−	−	+	+	−	−	−	+	−	−	+	−
	89	+	+	+	−	+	+	+	+	−	−	+	+	−	−	−	+	−	−	+	−
	90	+	a	a	−	+	+	+	+	−	−	+	+	−	−	−	+	−	−	+	−
O111 : B4F$^+$ × C-600F$^-$	7	+	−	+	−	+	g	a	+	+	+	+	+	+	+	+	+	+	+	+	−
	8	+	−	+	−	+	+	a	+	−	−	+	+	+	+	+	+	+	+	+	−
	93	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−
O111:B4F$^+$ W-1F$^-$	1	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	4	+	+	+	ag	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	5	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	6	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	12	+	+	+	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−
	13	+	+	+	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−
	14	+	+	+	−	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−
	16	+	a	+	a	−	+	+	a	+	g	+	+	+	+	+	+	−	+	+	−
	22	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−
	29	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
	52	+	+	+	−	a	ag	ag	+	+	+	+	+	+	+	+	+	+	+	+	−
	54	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−
	56	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−
	64	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−

Legend: a) acid formation; g) gas formation; +) positive reaction, −) negative reaction.

TABLE 2. Serological Properties of Recombinants

Recombinant	Agglutination titers																				
	Living culture										Heated culture										Control
	O111	O55	O86	O125	O126	O18	O127	145	O25	O44	O111	O55	O86	O125	O126	O18	O127	145	O25	O44	
1							—										—				
4						—										200					(+)
7	100	—	100	—		—	200				—	—	—	—		—	—				—
8		400				—	50					—				—	—				—
12	200	50				—	—				400	100				—	—				—
13	100										400										
14	100	—			—	—	—	—			800	400			—	—	—	—			—
16		50				—	—	—				100				100	400	200			++
86	400	—									800	400									—
87	400										1600										—
88	100										800										—
89	200										200										
76	—				—														1600	800	++

Legend: underlined numbers – positive reaction (+++ or ++++); numbers not underlined – reaction + or ++.

TABLE 3. Reaction of Adsorption of Agglutinins by Recombinant No. 87

Serum	Adsorbent	Strain in agglutination reaction	Dilution of exhausted serum						Control
			1:100	1:200	1:400	1:800	1:1 600	1:3 200	
O111:B4	—	O111:B4	++++	++++	+++	+	—	—	—
	O111:B4	O111:B4	++++	++++	++++	++++	++++	+++	—
	O111:B4	№ 87	—	—	—	—	—	—	—
	—	№ 87	—	—	—	—	—	—	—
O111:B4	№ 87	№ 87	++++	+++	++	—	—	—	—
	№ 87	O11 :B4	—	—	—	++	++	—	—
			—	—	—	—	—	—	—
			—	—	—	—	—	—	—

In the present study we investigated the sexual hybridization of a reference strain of E. coli belonging to serotype 0111:B4, with a large number of untyped recipient strains of E. coli.

METHOD

A prototrophic typed reference strain of E. coli (pathogenic serotype 0111:B4), obtained from the International Center in Copenhagen, and standard auxotrophic F^- strains (recipient) of E. coli PA678, P678, C-600, and W-1 were used for crossing.

The initial typed strain fermented lactose, glucose, sucrose, maltose, rhamnose, galactose, arabinose, mannitol, and sorbitol, but not dulcitol, and was resistant to phages of the T group and phage λ and sensitive to streptomycin. Strains PA678, P678, and C-600 do not ferment lactose and sucrose (W-1 ferments lactose), but all the recipient strains ferment glucose, rhamnose, arabinose, and sorbitol. In addition, strains C-600 and W-1 ferment mannitol and galactose, while PA678 and P678 do not have this property; strains P678, C-600, and W-1 ferment maltose and dulcitol. Strains PA678, P678, and C-600 are resistant to phages T_1, T_5, and λ, and strains PA678 and P678 to phage T_6. Phage sensitivity was observed in all other cases.

Crossing was carried out by Lederberg's method [3]: 16-18 h broth cultures (of typed and untyped strains) were seeded in doses of 0.5 ml into a tube containing 6 ml of meat-peptone broth, which was incubated for 20 h at 37°. The mixed culture was centrifuged, the residue was suspended in 0.5 ml of physiological saline, and one

drop of the suspension was placed on the surface of a selective medium in dishes, and spread out. The selective medium consisted of minimal agar medium with 1% glucose and streptomycin in a dose of 150 mg/ml. The control consisted of seeding of the original cultures on the same medium. The seedings were incubated for 5 days at 37°. If colonies of mixed cultures grew on the selective medium but no growth took place on the control plates, these colonies were identified as recombinants.

The fermentation properties, relationship to phages of the T group and to phage λ, and also the serological properties of the isolated recombinants with typed adsorbed OB sera in the agglutination reaction, were investigated.

RESULTS

The results of crossing between the typed and untyped strains showed that in all combinations the crosses were fertile. Accordingly the tested typed strain E. coli 0111:B4 could be classed as an F^+ strain (possessing fertility factor).

During sexual hybridization the ability to ferment several sugars was transmitted from the donor strain to the recombinants (Table 1). However, this ability was not transmitted to some of the recombinants and they acquired a new property: they did not ferment certain sugars which were fermented by the initial strains.

This may be seen from the example of recombinant No. 89, which did not ferment arabinose, and hybrid No. 90, which did not ferment sorbitol, although the parent strains fermented these carbohydrates.

Analysis of the relationship between recombinants and phages showed that resistance to individual phages was transmitted from the donor to some hybrids, whereas others did not acquire this property but, on the contrary, were sensitive to these phages. For example, recombinants Nos. 88, 89, and 90 were sensitive to phages T_1, T_5, and λ, while hybrid No. 12 was sensitive to phage T_6, although the parent strains of these recombinants were not lysed by these phages.

The serological properties of 23 recombinants were investigated by means of the agglutination reaction on glass slides, the linear agglutination reaction in tubes, and Castellani's reaction with a series of adsorbed typed OB coli sera against types 0111:B4, 055:B5, 025:B6, 020:B145, 086:B7, 0119:B14, 0128:B12, 0127:B8, $018_{a,c}$:B21, 0125:B15, 025:L77, and 044:L78.

The "smooth" or "rough" state of the cultures were determined by the boiling test. Analysis of the serological properties of the recombinants (Table 2) showed that during sexual hybridization of the pathogenic typed strain with the untyped isolated recombinants the antigenic properties of the donor were obtained, but those recombinants which had not acquired these properties possessed new serological properties belonging to different serotypes. For example, in recombinant No. 76 from the cross 0111:B4 F^+ × Pa678 F^-, antigens of serotypes 025:L77 and 044:L78 were found.

To confirm the presence of donor antigen 0111 in individual recombinants, the reaction of adsorption of agglutinins were carried out by Castellani's method. Recombinant No. 87 was used as adsorbent, for it was agglutinated in high titers by 0111 antiserum in the living and heated states (Table 3).

A noteworthy feature in recombinant No. 76 and certain other hybrids was the relationship between the acquisition of sensitivity to various phages and the change in antigenic properties—the appearance of a new antigenic component, both of which evidently appeared at the same time. These findings are in agreement with the results obtained previously during the investigation of typed strains, isolated from patients, for fertility [1, 2].

Hence, during genetic recombination, some of the hybrids obtain their biochemical and serological properties and phage resistance from the donor, while others do not acquire these properties but possess new ones, so that they differ from the parent strains.

LITERATURE CITED

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