The Host Specificities of the z20 and CF2004-6 Strains of an *Escherichia coli* O4 Serotype

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SUMMARY

We have discovered different host specificities in the z20 and CF2004-6 strains of a particular *Escherichia coli* O4 serotype. The host specificities are controlled by a chromosomal locus linked to threonine. They are differentiated on the basis of results from plating efficiencies of phage Ø04, conjugation, and generalized transduction. The host specificities of *E. coli* strains K12 and CF2004-6 seem to be similar and distinct from that of z20.

INTRODUCTION

Host-controlled restriction accounts for reduced recombinant frequencies and reduced linkage between markers in matings between strains of *Escherichia coli* K12 in which donor and recipient differ due to mutation at the host specificity locus (Colson, Glover, Symonds & Stacey, 1965; Wood, 1966), or the recipient is lysogenic for phage P1 (Glover, Schell, Symonds & Stacey, 1963; Boice & Luria, 1963; Pittard, 1964; Arber & Morse, 1965). Host-controlled restriction also occurs during inter-strain matings between *E. coli* K12 and B/r (Boyer, 1964), and *E. coli* K12 and B (Arber & Morse, 1965; Copeland & Bryson, 1966), but not during matings between *E. coli* K12 and CF2004-6, a specific serotype of *E. coli* O4 (Zubrzycki & Levinson, 1969).

It was therefore surprising to discover the host specificities of CF2004-6 and z20, which constitute a specific intra-strain fertility system (Siegel & Zubrzycki, 1968; Siegel, 1970), are different. The restriction to be described in this paper affects genetic recombination following conjugation and the plating efficiencies of, and generalized transduction by, phage Ø04.

METHODS

Media. Penassay broth (antibiotic medium no. 3, Difco, Detroit, Michigan, U.S.A.) and trypticase soy agar (Baltimore Biological Laboratories, Cockeysville, Maryland, U.S.A.) were used for the routine cultivation of bacteria. Eosin methylene blue (EMB) agar (Difco) was used to differentiate lactose and non-lactose fermentors. Semisolid agar for plating phage consisted of 0.7% agar in trypticase soy broth (Baltimore Biological Laboratories). The composition of the minimal enriched medium and agar and the concentrations of amino acids and streptomycin (used to counter-select donor strains) were described by Zubrzycki & Levinson (1969).

Bacteria. A recipient, CF2004-6, and a low-frequency donor, z20 (Siegel & Zubrzycki, 1968), are strains isolated from a particular *Escherichia coli* serotype O4:K(undetermined):H5

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Table 1. *Strains of Escherichia coli* used

Strain designation	Strain origin	Pertinent characteristics
CF2004-6	<i>E. coli</i> O4:K(undetermined):H5	prototroph, <i>hsp</i> CF; recipient
CTM-41	CF2004-6	<i>trp</i> , <i>ilv</i> ; used as indicator strain for Ø04
HfrH	K12	<i>hsp</i> K; Hfr
LZ-TM-9	CF2004-6	<i>trp</i> , <i>leu</i> , <i>arg</i> , <i>his</i> , <i>pro</i> , <i>thr</i>
MR19	Z20 × CF2004-6 recombinant*	<i>hsp</i> Z
MR15 and MR25	Thr ⁺ recombinants from Z20 × LZ-TM-9	<i>hsp</i> Z
O4M2	P4X6 × CF2004-6 recombinant†	<i>trp</i> , <i>ilv</i> , <i>hsp</i> CF; Hfr
P4X6	K12	<i>met</i> , <i>hsp</i> K; Hfr
S5 and S10	Thr ⁺ recombinants from HfrH × a <i>thr</i> strain of MR15	<i>hsp</i> K
Z20	<i>E. coli</i> O4:K(undetermined):H5	<i>met</i> , <i>hsp</i> Z; low frequency donor

* Siegel, 1970.

† Zubrzycki & Levinson, 1969.

(simply referred to as *E. coli* O4). Z20 does not behave as a donor with recipient strains of *E. coli* K12, C or B (Siegel & Zubrzycki, 1968; Siegel, 1970). An Hfr strain of CF2004-6, O4M2, was obtained by selection for a terminal marker, lactose, in a mating between P4X6 and a *lac* mutant of CF2004-6 (Zubrzycki & Levinson, 1969). These strains, plus the well-known K12 strains, are listed in Table 1.

Phage. The generalized transducing phage used in these studies was originally referred to as Ø04-CF (Zubrzycki, Green & Spaulding, 1966), but when modification and restriction of this phage were discovered the hyphenated designation was abbreviated to Ø04 (Siegel, 1970). The phage is specific for isolates of the serotype of *Escherichia coli* O4 used in these studies and will not adsorb to *E. coli* K12.

Notations and abbreviations. The abbreviations for genetic markers conform to the recommendations of Demerec, Adelberg, Clark & Hartman (1966). They are: *arg* (arginine), *his* (histidine), *ilv* (isoleucine), *met* (methionine), *pro* (proline), *thr* (threonine), *trp* (tryptophan), *lac* (lactose), *leu* (leucine), *str* (streptomycin; r for resistant, s for susceptible), *hsp* (host specificity). The notation to designate the bacterial strain on which phage was grown is that recommended by Arber & Linn (1969).

Mating experiments. The smooth strains of *Escherichia coli* do not mate well in broth. Therefore, matings were done on Millipore filters as previously described (Zubrzycki & Levinson, 1969). Linkage between two markers was determined as described by Zubrzycki & Levinson (1969). To get a significant number of recombinants when using the low-frequency donor strain Z20, the donor and recipient must be in contact for a long period of time (Siegel, 1970). This is accomplished simply by mixing equal volumes of young broth cultures containing approximately 1×10^8 donors and 5×10^8 recipients and then spreading 0.2 ml of the mixture on the surface of minimal agar plates. Recombinants were scored after 48 h of incubation at 37 °C.

Transduction experiments. Overnight broth cultures were centrifuged and resuspended in a one-half volume of saline or buffer (minimal enriched medium, minus glucose). One volume of this 2 × concentrated suspension was mixed with one volume of phage diluted in 1 % tryptone broth (Difco) to a titre of 1×10^{10} /ml. The mixture was incubated at 37 °C for 15 min and then 0.1 ml samples were plated in minimal agar using a pour plating technique. Transductants were scored after 48 h of incubation at 37 °C. For transduction, cultures lysogenic for Ø04 were used because the number of transductants is greater than with non-lysogens and over a wide range there is a linear relationship between the number of phage

Table 2. Efficiency of plating of phage Ø04 grown on *Escherichia coli* O4 strains with two different host specificities*

Phage	Host strain			
	CTM-41	MR19	MR15	MR25
Ø04·CF	1·0	0·0013	0·0029	0·0029
Ø04·MR19	0·0012	1·0	0·93	1·05
Ø04·MR15	0·0059	1·04	1·0	0·82
Ø04·MR25	0·0082	1·0	1·2	1·0

* The restriction of Ø04 was not due to differences in adsorption because the kinetics of adsorption were the same in all cases studied. At a bacterial concentration of 3×10^8 /ml about 75 % of phage is adsorbed in 5 min and 94 % in 15 min.

used and the number of transductants (L. Zubrzycki, S. V. Levinson & D. Siegel, unpublished results).

Phage techniques. The techniques for propagating and handling Ø04 were those previously described (Zubrzycki, Green & Spaulding, 1966).

Screening for strains with different host specificities. Bacterial isolates were identified as belonging to one or another host specificity type by determining their ability to restrict Ø04 with different host specificities. To check for modification, plaques of phage which escaped restriction were stabbed with a needle and the phage was suspended in 1 ml of broth. The efficiency of plating (e.o.p.) was determined by comparing the titre on permissive and restricting strains.

RESULTS

Restriction and modification of Ø04

Recombinants from matings between donor z20 and a variety of genetically marked recipient strains of CF2004-6 often yield Ø04 or its tiny plaque-forming variant (Siegel & Zubrzycki, 1968). In a routine check for lysogeny and immunity to Ø04 among recombinants, Siegel (1970) discovered four recombinants on which phage Ø04 would plaque at an e.o.p. of about 10^{-3} . The recombinant chosen for further study, MR19, was found to modify Ø04. The plating efficiencies of stocks of Ø04 grown on CF2004-6 and MR19 (Table 2) show that Ø04·CF is restricted and modified by MR19 to the same extent that Ø04·MR19 is restricted and modified by CTM-41, the routine indicator strain for Ø04 grown on CF2004-6.

The hsp Z locus

The control of modification and restriction observed in MR19 was presumed to be due to a host specificity locus, *hsp Z*, transferred from strain z20. We wanted to incorporate the *hsp Z* locus into a multiply marked recipient CF2004-6 strain to determine whether the locus was located near *thr*, as it is in other *Escherichia coli* strains (Hoekstra & DeHaan, 1963; Boyer, 1964; Colson *et al.* 1965; Wood, 1966). From a mating experiment between z20 and LZ-TM-9, two of twenty-five *Thr*⁺ recombinants tested (MR15 and MR25) clearly restricted and modified Ø04·CF but not Ø04·MR19 (see Table 2).

If the host specificities of *Escherichia coli* K12 and CF2004-6 are similar, as reported by Zubrzycki & Levinson (1969), then replacing the *hsp Z* locus of MR15 or MR25 with the *hsp K* or *hsp CF* locus should restore the host specificity originally demonstrated in strains of CF2004-6.

A *thr* mutant of MR15 was mated with HfrH, P4X6 and o4M2 for varying periods of time up to 15 min and selected for *Thr*⁺ recombinants. The matings were interrupted and limited

Table 3. *Recombinant frequencies for the pro, leu and arg markers after 100 min matings of the Escherichia coli K12 strain P4X6 and derivatives of E. coli O4*

Donor	Recipient	% Recombinants per input donor		
		<i>pro</i>	<i>leu</i>	<i>arg</i>
P4X6 ×	MR15	0.06	0.75	0.29
	LZ-TM-9	0.21	2.0	0.21
	S5	0.41	4.7	0.25

Table 4. *The effects of restriction on the linkage of unselected markers from the crosses in Table 3*

Donor	Recipient	Selected markers ...		Pro ⁺		Leu ⁺		Arg ⁺	
		Unselected ...	...	Leu ⁺	Arg ⁺	Pro ⁺	Arg ⁺	Pro ⁺	Leu ⁺
P4X6 ×	MR15			33.0	2.9	1.7	2.4	0.5	9.1
	LZ-TM-9			50.0	3.4	5.4	3.7	3.2	34.0
	S5			48.0	3.8	4.2	1.6	6.2	29.0

to this short period of time to avoid the transfer of large parts of the donor genome to the recipient. Of the Thr⁺ recombinants tested, 17% (12/70) from the HfrH mating, 16% (12/72) from the o4M2 mating and 37% (19/52) from the P4X6 mating did not restrict Ø04·CF but restricted Ø04·MR19 with an e.o.p. of approximately 10⁻³. To check whether Ø04·MR19 which escaped restriction in the Thr⁺ recombinants was now modified, plaques on eleven arbitrarily chosen recombinants were picked and the phage assayed on MR25 with *hsp Z*. They were now restricted by MR25 with an e.o.p. of approximately 10⁻³.

The *hsp Z* locus is probably to the left of the *thr* locus as depicted on the *Escherichia coli* map of Taylor (1970), since in a separate experiment with P4X6, 100 Leu⁺ recombinants, which did not receive the *thr* marker, still restricted Ø04·CF.

To determine whether the different host specificities of *Escherichia coli* O4 affected genetic recombination during conjugation as they do between other *E. coli* strains, the following crosses were performed. HfrH, P4X6, o4M2, the Hfr donor strain of CF2004-6, were mated with the recipient strains, LZ-TM-9 with *hsp CF*, MR15 and MR25 with *hsp Z* and S5 and S10, two different Thr⁺ recombinants from a P4X6 × MR15 *thr* mating. These latter recipients also received the *hsp K* locus as indicated by an e.o.p. of about one for Ø04·CF. The results from the mating experiments between the three donor strains and the recipients were essentially the same and the results from matings between P4X6, MR15, S5 and LZ-TM-9 are shown in Table 3. The recombinant frequency for the lead marker, *pro* in the case of P4X6, is markedly reduced in matings with MR15 with *hsp Z* when compared with the results using LZ-TM-9 with *hsp CF* and S5 with *hsp K*. The reduction is not as great for the more distal *leu* marker and disappears for the *arg* marker which is even further away from the lead marker. The results in Table 4 show that linkage between *pro*, *leu* and *arg* is also reduced in matings with MR15 when compared with the results using LZ-TM-9 and S5. Figure 1 shows that there was a delay in appearance of the lead marker in matings with MR15.

We pursued our studies on differentiating the host specificities of *Escherichia coli* K12 and CF2004-6 from those of Z20 using transduction experiments. As shown in Table 5, the relative transduction frequencies by phage Ø04·MR19 into strain LZ-TM-9(Ø04) with *hsp CF* and strain S5(Ø04) with *hsp K* were lower than into strain MR15(Ø04) with *hsp Z*.

These results, together with the plating efficiencies of Ø04 and the conjugation experiments,

Fig. 1. Interrupted mating experiment between p4x6 and s5 (●), LZ-TM-9 (■) and MR15 (▲), for the early marker *pro*.

Table 5. Transduction frequencies for the markers listed relative to those by Ø04·CF into LZ-TM-9 (Ø04)* which was assigned a value of one

Phage	Recipient	<i>trp</i>	<i>leu</i>	<i>arg</i>	<i>his</i>	<i>pro</i>
Ø04·CF	} × LZ-TM-9 (Ø04) {	1·0	1·0	1·0	1·0	1·0
Ø04·MR19		0·26	0·5	0·5	0·13	0·1
Ø04·CF	} × S5 (Ø04) {	0·5	0·77	0·69	0·58	1·3
Ø04·MR19		0·041	0·15	0·23	0·064	0·017
Ø04·CF	} × MR15 (Ø04) {	0·054	0·031	0·056	<0·01	0·02
Ø04·MR19		1·2	1·6	2·1	1·4	0·12

* Recipients used were lysogenic derivatives (see Methods).

are consistent with our conclusions that the *hsp Z* and *hsp CF* loci are closely linked to *thr* and are allelic to *hsp K*. But the host specificities of *hsp CF* and *hsp K* are similar and distinct from that determined by *hsp Z*.

DISCUSSION

CF2004-6, a recipient, and z20, a low-frequency donor, are strains isolated from a particular *Escherichia coli* O4 serotype. They constitute therefore an intra-strain fertility system (Siegel & Zubrzycki, 1968; Siegel, 1970).

Discovery of host specificity differences in *Escherichia coli* O4 had to await the chance observation that some recombinants of a z20 and CF2004-6 mating restricted Ø04. We could not have observed this restriction merely by comparing plating efficiencies of Ø04 on z20 and CF2004-6 because z20 is lysogenic for a co-immune small plaque-forming variant of Ø04 (Siegel, 1970). The recombinants used in the studies reported here are not lysogenic for Ø04 or its small plaque-forming variant. This eliminates the possibility that the small plaque-forming variant of Ø04 controls the restriction that was observed.

One of the more dramatic results accompanying restriction is the degradation of ³²P-labelled phage DNA (Arber, 1965). In preliminary work we made repeated attempts to

detect degradation of ^{32}P -labelled $\phi\text{O}4$ DNA during restriction but could not (Siegel, 1970). It may be that our techniques were not sensitive enough to determine the partial degradation of phage DNA that often accompanies restriction (Fukasawa, 1964; Uetake, Toyama & Hagiwara, 1964; Pizer, Smith, Miovic & Pylkas, 1968). We became convinced, however, that we were observing host-controlled restriction and modification controlled by the different host specificities of the CF2004-6 and Z20 strains of *Escherichia coli* O4 because: (i) phage $\phi\text{O}4$ which escapes restriction by a second host (be it Z20 or CF2004-6) is modified and, in turn, restricted by the first host; (ii) restriction affects conjugation and transduction; and (iii) the host specificity loci of CF2004-6 and Z20 are closely linked to the *thr* locus.

These results therefore indicate that members of an intra-strain fertility system may have different host specificities but as Zubrzycki & Levinson (1969) have shown, the host specificities of an inter-strain fertility system such as that between *Escherichia coli* K12 and the CF2004-6 strain of *E. coli* O4 can be similar.

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