Genet. Res., Camb. (1970), **16**, *pp.* 215–224
Printed in Great Britain

The inhibitory action of transfer factors on lysis of *Escherichia coli* K12 by phages $\mu 2$ and $\phi 2$

By J. S. PITTON* and E. S. ANDERSON

Enteric Reference Laboratory, Public Health Laboratory Service, Colindale Avenue, London N.W. 9

(*Received* 29 *May* 1970)

SUMMARY

A selection of *fi*$^+$ resistance factors and transfer factors, when introduced into K12F$^+$, showed a range of inhibitory activity of lysis by the male-specific phage μ 2. This range can be used to subdivide the *fi*$^+$ factors into *fi*$^{+1}$, *fi*$^{+2}$, *fi*$^{+3}$ and *fi*$^{+4}$ classes, according to the degree of inhibition of μ 2 lysis. To this subdivision can be added restriction of the 'female-specific' phage ϕ 2.

Introduction of all the *fi*$^+$ factors tested into K12HfrH totally inhibited lysis by μ 2 in spot tests, but with two *fi*$^{+1}$ and one *fi*$^{+2}$ factors visible lysis was obtained in agar-layer tests. These three factors caused least inhibition of transfer of *pro* by HfrH. It can be assumed that both tests reflect lower inhibition of sex fimbrial formation by these *fi*$^+$ factors than by the remainder.

The *fi*$^-$ factors, when introduced into K12, can be subdivided on the basis of restrictive effects on phage ϕ 2.

These effects can be added to phage restriction in the salmonellae for the purposes of further classification of the transfer factors and R-factors.

1. INTRODUCTION

Transfer factors responsible for the mobility of determinants for resistance to antibiotics and other characters can be crudely divided into two main groups, in relation to their ability to inhibit the fertility of strains of *Escherichia coli* K12 carrying the F factor (Watanabe & Fukasawa, 1962; Watanabe, Fukasawa & Takano, 1962; Watanabe *et al.* 1964). The factors which repress conjugation were called fertility inhibition + (= *fi*$^+$), and those which do not repress it, *fi*$^-$. It was found that the loss of F fertility caused by *fi*$^+$ factors was accompanied by loss of sensitivity to male-specific phages, while *fi*$^-$ factors did not affect this sensitivity.

The degree of inhibition of lysis by male-specific phages resulting from carriage of transfer factors can be determined by two simple methods. The spotting of phage on to surface cultures (spot test) provides a screening technique which gives rapid results. However, the sensitivity of the spot test is limited. The determination of the efficiency of plating (EOP) by the agar-layer technique gives results

* Present address: University Institute of Medical Microbiology, University of Geneva, Geneva, Switzerland.

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press

which enable the inhibitory effect of different factors to be compared on a quantitative basis. Moreover, plaque morphology is more easily observed with agar-layer than with spot tests.

In this article we shall discuss the effect of various *fi*+ factors, in different strains of *E. coli* K12, on multiplication of the male-specific phage μ 2 (Dettori, Maccacaro & Piccinin, 1961) and of the so-called 'female-specific' phage ϕ 2 (Cuzin, 1965).

2. MATERIALS AND METHODS

(i) *Media*

Liquid medium for the growth of cultures contained 20 g of Bacto dehydrated nutrient broth (Difco Laboratories) and 8·5 g of NaCl/l. Solid medium was prepared by adding 13 g of New Zealand powdered agar per litre of this medium. Semi-solid agar for agar-layer tests was prepared by adding powdered agar in a concentration of 5 g/l. of nutrient broth.

(ii) *Transfer factors*

The transfer factors studied were all isolated from wild strains of *Salmonella typhimurium* or *S. paratyphi* B carrying various resistance determinants. One

Table 1. *Origin of transfer factors and R-factors* (*donor strains*)

ERL no.	Phage type	Resistance of wild parental strain	Designation of R-factors or transfer factors used in present experiments	*fi* character
		Salmonella serotype: *S. typhimurium*		
RT1	29	ASTSuFu	Δ	*fi*−
1R683	29	SSuTFu	683T	*fi*−
4R256	29	CKSSuTFu	256T	*fi*−
3R129	6	T	129	*fi*+
3R130	6	T	130	*fi*+
RT780	6	T	780	*fi*+
3R118	29	T	118	*fi*+
3R125	6	T	125	*fi*+
1R726	29	Sensitive	x_5*	*fi*+
4R256	29	CKSSuTFu	256C	*fi*+
		Salmonella serotype: *S. paratyphi* B		
7R334†	3a var. 4	ACKSSu	334	*fi*+

Resistances: A = ampicillin, S = streptomycin, C = chloramphenicol, T = tetracycline, K = neomycin-kanamycin, Su = sulphonamides, Fu = furazolidone.

* The transfer factor x_5 came from a wild drug-sensitive strain. It was associated with a determinant for ampicillin resistance by the triparental cross of Anderson (1965*a*, *b*).

† The resistance of the strain of *S. paratyphi* B was shown to be transferable in 1964 (E. S. Anderson & N. Datta, unpublished). The R factor, which corresponds to ERL factor 334, was designated RI by Meynell & Datta (1966).

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press

strain, 1R726, carried a transfer factor but no resistance determinant. The characters of these strains are summarized in Table 1.

Using the techniques of Anderson & Lewis (1965*a*, *b*) these factors, with or without their resistance determinants, were transferred into four different strains of *E. coli* K12: *E. coli* K12F$^+$met$^-$ = K12F$^+$; *E. coli* K12F$^-$ prototrophic = K12F$^-$; *E. coli* K12F$^+$defmet$^-$ = K12F$^+$def (this strain carries a defective F factor (Hayes, 1953)); *E. coli* K12HfrH = HfrH.

(iii) *Phages*

The phages were the male-specific phage μ 2 (Dettori *et al.* 1961), of which the titre was about 10^{12} plaque-forming units (pfu)/ml, and the 'female-specific' phage ϕ 2 (Cuzin, 1965), with a titre of about 10^9 pfu/ml.

(iv) *Determination of lytic activity by spot tests*

Spot tests were carried out as follows: 0·01 ml of a logarithmically growing nutrient broth culture of the strain under examination (approximately 5×10^8 cells/ml) was delivered with a standard loop on to a marked area of nutrient agar. The culture was spread to a diameter of about 1 cm and allowed to dry. 0·01 ml of undiluted phage was then spotted on the inoculated area. The plates were read after incubation at 37 °C for 4–5 h. Readings were symbolized as follows: confluent (clear) lysis, CL; semi-confluent lysis, SCL; confluent lysis rendered opaque by heavy background growth of resistant organisms, OL; very opaque lysis, almost obscured by background growth, VOL.

(v) *Determination of efficiency of plating*

The classical agar-layer technique was used (Adams, 1959). One ml of broth culture of the indicator strain, incubated overnight at 37 °C, was inoculated into 100 ml of fresh nutrient broth. This was then incubated at 37 °C for 3 h with agitation, centrifuged at 7500*g* for 15 min, and resuspended in 20 ml of broth. Semi-solid agar, 2·5 ml, was inoculated with 0·1 ml of this suspension and 0·1 ml of phage undilution was added to the mixture, which was retained at 48 °C until poured on to normal nutrient agar underlay. After the surface agar had solidified, the plates were incubated at 37 °C overnight.

(vi) *Confirmation of fertility inhibition*

The effect of the *fi*$^+$ factors on F fertility was examined by introducing them into K12HfrH and crossing the resultant progeny with K12F$^-$. The method used in these crosses was that described by Clowes & Hayes (1968), the crosses being interrupted after 2 h.

3. RESULTS

The EOPs of phages μ 2 and ϕ 2 on the three strains into which the various factors were introduced are shown in Table 2. The results of the tests with these strains

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press

carrying the various R-factors are summarized in Tables 3 and 4. Table 3 shows results with K12F+.

As expected, the *fi*− factors did not impair lysis by phage μ 2 in K12F+. Moreover, in agar-layer tests, the morphology of plaques produced by this phage on K12F+ carrying the *fi*− factors was the same as that on K12F+ alone.

Table 2. *Efficiency of plating of phages μ 2 and ϕ 2 on K12F+, K12F− and K12F+def*

	Phage μ 2	Phage ϕ 2	
	EOP	EOP	Plaque morphology
K12F+	1	$1{\cdot}4 \times 10^{-2}$	Small clear
K12F+def	0	$1{\cdot}4 \times 10^{-2}$ to $3{\cdot}2 \times 10^{-3}$	Small clear
K12F−	0	1	Large clear, with halo

The *fi*+ factors produced varying degrees of inhibition of lysis of phage μ 2 in K12F+. When lysis was present it was turbid and was sometimes difficult to detect. Some *fi*+ factors, for example 256C and 334, totally inhibited visible lysis.

Observations on the visibility of individual plaques in agar layer were in good agreement with those on spot tests with concentrated phage suspension. As can be seen, there is a variation in the EOP of μ 2 on K12F+ carrying different *fi*+ factors, between K12F+ (125), which gave an EOP of $2{\cdot}3 \times 10^{-2}$, and factors such as 780 and 129, which gave an EOP of 1.

The *fi*+ factors were graded numerically, from 1 to 4, according to the degree to which they inhibited lysis of K12F+ by phage μ 2. Factors belonging to grade 4 totally inhibited visible lysis by μ 2. The phage-inhibitory gradings were added to the *fi*+ symbol to produce the following classes: fi^{+1}, fi^{+2}, fi^{+3} and fi^{+4}. The respective gradings are shown in the second column of Table 3.

Lysis by the 'female-specific' phage ϕ 2 was completely inhibited in K12F+ by all the *fi*− factors used in these experiments. Factors 683T and 256T are probably closely related to Δ, which explains their similar restriction of ϕ 2. One *fi*+ factor, x_5, also totally inhibited lysis by this phage. An *fi*− factor has not so far been detected in association with the x_5 complex, so that we have tentatively concluded that the *fi*+ factor x_5 is responsible for the ϕ 2 restriction.

None of the other *fi*+ factors produced serious reduction in the EOP of phage ϕ 2 on K12F+. However, two factors, 125 and 780, resulted in the production of turbid plaques with this phage. These factors reduced lysis by ϕ 2 to the extent that none was visible on spot tests.

(i) *Effects of transfer factors on* K12F− and K12F+def

The results of these experiments are shown in Table 4.

Neither K12F− nor K12F+def yielded visible lysis with phage μ 2, irrespective of the factors carried. So far as lysis with the 'female-specific' ϕ 2 phage is concerned, K12F− produced a CL reaction on spot test with this phage and an EOP

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press

Table 3. *Effect of various fi⁺ and fi⁻ factors in K 12 F⁺*

		Phage μ 2 (male-specific)			Phage ϕ 2 ('female-specific')		
Factor	*fi* character	Spot test	EOP μ 2 (agar-layer)	Plaque morphology (agar-layer)	Spot test	EOP ϕ 2 (K 12 F⁻ = 1)	Plaque morphology
F	.	CL	1	Small clear	OL	$1{\cdot}4 \times 10^{-2}$	Small, clear
Δ*	*fi*⁻	CL	1	As F⁺	—	0	—
683 T	*fi*⁻	CL	1	As F⁺	—	0	—
256 T	*fi*⁻	CL	1	As F⁺	—	0	—
129	*fi*$^{+1}$	OL	1	Turbid	OL	$1{\cdot}8$ to $7{\cdot}8 \times 10^{-3}$	Very small, clear
130	*fi*$^{+1}$	OL	4×10^{-1}	Turbid	OL	$1{\cdot}9$ to $3{\cdot}4 \times 10^{-3}$	Very small, clear
780	*fi*$^{+1}$	OL	1	Turbid	—	$7{\cdot}5 \times 10^{-3}$	Very small, turbid
118	*fi*$^{+2}$	VOL	$4{\cdot}5 \times 10^{-2}$	Very turbid	OL	$1{\cdot}4 \times 10^{-3}$	Very small, clear
125	*fi*$^{+2}$	VOL	$2{\cdot}3 \times 10^{-2}$	Very turbid	—	$7{\cdot}5 \times 10^{-3}$	Very small, turbid
x_5	*fi*$^{+3}$	VOL to —	$1{\cdot}3$ to $1{\cdot}6 \times 10^{-1}$	Very turbid, difficult to count	—	0	No detectable plaques
256 C	*fi*$^{+4}$	—	0	No detectable plaques	SCL	$9{\cdot}3 \times 10^{-3}$	Small, clear
334	*fi*$^{+4}$	—	0	No detectable plaques	SCL	8×10^{-3}	Small, clear

0 = no detectable plaques on agar-layer.
— = no detectable lysis on spot test.
* Anderson & Lewis (1965*b*).

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press

Table 4. *Effect of* fi⁺ *and* fi⁻ *factors in K 12 F⁻ and K 12 F⁺def on sensitivity to phage* ϕ *2*

Factor	*fi* character	Strain	Phage ϕ 2: Spot test	EOP (agar layer): Compared with K 12 F⁻	EOP (agar layer): Compared with K 12 F⁺def	Plaque morphology (agar layer)
None	—	K 12 F⁻	CL	1	—	Large, clear plaques with turbid haloes
Defective F	—	K 12 F⁺def	SCL	$3 \cdot 2 \times 10^{-3}$ to $1 \cdot 4 \times 10^{-2}$	1	Small, clear
Δ	*fi*⁻	K 12 F⁻	OL	$1 \cdot 5 \times 10^{-2}$	—	Small, clear
		K 12 F⁺def	—	0	0	—
683 T	*fi*⁻	K 12 F⁻	OL	$9 \cdot 5 \times 10^{-3}$	—	Small, clear
		K 12 F⁺def	—	0	0	—
256 T	*fi*⁻	K 12 F⁻	OL	2×10^{-2}	—	Very small, clear
		K 12 F⁺def	—	0	0	—
129	*fi*⁺¹	K 12 F⁻	CL	1	—	As K 12 F⁻
		K 12 F⁺def	SCL	$8 \cdot 3 \times 10^{-4}$	$2 \cdot 3 \times 10^{-1}$	Small, turbid
130	*fi*⁺¹	K 12 F⁻	CL	1	—	Small, clear
		K 12 F⁺def	SCL	2×10^{-3} to $9 \cdot 1 \times 10^{-4}$	$1 \cdot 2 \times 10^{-1}$ to $6 \cdot 3 \times 10^{-1}$	Small, turbid
780	*fi*⁺¹	K 12 F⁻	CL	1	—	Small, clear
		K 12 F⁺def	—	0	0	—
118	*fi*⁺²	K 12 F⁻	CL	1	—	Small, clear
		K 12 F⁺def	SCL	$4 \cdot 7 \times 10^{-2}$	$3 \cdot 6 \times 10^{-1}$	Small, turbid
125	*fi*⁺²	K 12 F⁻	CL	1	—	Small, clear
		K 12 F⁺def	OL to —	$3 \cdot 7 \times 10^{-3}$ to 0	$2 \cdot 6 \times 10^{-1}$ to 0	Very small, turbid
x_5	*fi*⁺³	K 12 F⁻	CL	1	—	Large, turbid
		K 12 F⁺def	—	0	0	—
256 C	*fi*⁺⁴	K 12 F⁻	CL	1	—	As K 12 F⁻
		K 12 F⁺def	SCL	$9 \cdot 3 \times 10^{-3}$	2×10^{-1}	As K 12 F⁺def
334	*fi*⁺⁴	K 12 F⁻	CL	1	—	As K 12 F⁻
		K 12 F⁺def	OL	5×10^{-2}	1	As K 12 F⁺def

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press

of 1. K12F+def produced SCL on spot test and an average EOP of about 10^{-2}. The introduction of the *fi*⁻ factors Δ, 256T and 683T into K12F⁻ reduced the EOP by about the same amount, 10^{-2}. All three of these factors abolished lysis by ϕ 2 when they were introduced into K12F+def.

The EOP of ϕ 2 was not reduced in K12F⁻ by any of the *fi*+ factors. When introduced into K12F+def, the plating efficiency was not reduced significantly by 129, 130, 118, 256C or 334. In contrast, factors x_5 and 780 abolished ϕ 2 sensitivity in K12F+def. In this respect the effect of 780 in K12F+def differed from the restriction it produced in K12F+ (see Table 3). The effect of 125 varied, probably because the development of plaques, which were very small and turbid even in agar-layer tests, was sensitive to slight variations in cultural conditions. With the exception of 780, the effects of the *fi*+ factors on K12F+def were similar to those they produced on K12F+.

As can be seen from Table 4, all the *fi*⁻ factors produced OL on spot tests with ϕ 2 when they were carried by K12F⁻; they modified both the results of spot tests and the morphology of the plaques produced in agar layer.

The *fi*+ factors produced variable results in these respects, as Table 4 shows.

(ii) *Effects of transfer factors on K12HfrH*

The effects of the various transfer factors on K12HfrH are shown in Table 5.

(*a*) *Phage sensitivity*

Only the results with phage μ 2 are presented in Table 5. They show an important difference from those with K12F+, in that lysis by μ2 in spot tests is totally inhibited by the *fi*+ factors studied, although with three of the factors, 129 and 130, both *fi*+1, and 125 (*fi*+2), turbid plaques were visible in agar-layer tests.

Table 5. *Effect of* fi+ *transfer factors on K12HfrH*

Factors	*fi*+ character	Lysis by μ 2: Spot tests	Lysis by μ 2: Agar layer	Transfer of *pro* (K12HfrH = 1)
129	+1	—	Very turbid	$7{\cdot}7 \times 10^{-3}$
130	+1	—	Very turbid	$3{\cdot}9 \times 10^{-2}$
125	+2	—	Very turbid	$1{\cdot}7 \times 10^{-2}$
503	+2	—	—	$6{\cdot}5 \times 10^{-4}$
x_5	+3	—	—	$2{\cdot}7 \times 10^{-4}$
334	+4	—	—	$8{\cdot}8 \times 10^{-4}$
256C	+4	—	—	$5{\cdot}5 \times 10^{-4}$

From the point of view of distinguishing between the four classes of *fi*+ factors by inhibition of lysis by μ 2, therefore, K12F+ is a more useful host than K12HfrH.

(*b*) *Confirmation of fertility inhibition*

The *fi*+ character of the factors was confirmed by examining their effects on the transfer of *pro* from K12HfrH to K12F⁻. The results shown in Table 5 are

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press

calculated as a fraction of the transfer frequency of *pro* by HfrH. Both fi^{+1} and one of the fi^{+2} factors inhibited transfer less than the remainder. It was precisely these three factors that showed lysis of HfrH with phage μ 2 in agar-layer tests.

4. DISCUSSION

The observations described above suggest that the fi^{+} property of transfer factors can be subdivided according to the various degrees of inhibition they impose on lysis of K12F+ by the male-specific phage μ 2. A provisional classification is given in Table 6.

In this table, the first class of fi^{+} factors is designated fi^{+1}. When these factors are introduced into K12F+, they do not completely inhibit lysis by phage μ 2. The lysis produced is easily visible, and is turbid in spot and agar-layer tests. Examples of such factors are 129 and 130.

Table 6. *Effect of transfer factors on lysis of K12F+ by phage μ 2*

		Lysis by phage μ 2	
fi character	Representative factors	Spot test	Plaque morphology (agar-layer)
fi^{-}	Δ, 683T, 256T	CL	Clear
fi^{+1}	129, 130, 780	OL	Turbid
fi^{+2}	118, 125	VOL	Very turbid
fi^{+3}	x_5	–	Very turbid (almost invisible)
fi^{+4}	256C, 334	–	–

– = no lysis

The second class of fi^{+} factors is designated fi^{+2}. K12F+ carrying such factors shows very turbid lysis in spot tests and plaques are very turbid but still countable in agar-layer experiments. Examples of such factors are 118 and 125.

The third class of factors is fi^{+3}. In K12F+ these factors completely inhibit visible lysis in spot tests with phage μ 2. However, lysis is still detectable in agar-layer tests, although the plaques are very faint and difficult to count. The example in the present series is x_5.

Factors producing the maximum inhibition of lysis are shown as fi^{+4}. With K12F+ carrying these factors, both spot and agar-layer tests with phage μ 2 are negative. Examples of this class are 256C and 334.

When the factors were introduced into K12HfrH, lysis by μ 2 in spot tests was abolished. However, with three factors, 129, 130 and 125, turbid plaques were obtained in agar-layer tests, and it is significant that fertility, as indicated by *pro* transfer from HfrH, was inhibited least by these factors. It can thus be concluded that they inhibit formation of sex fimbriae—which are the male-specific phage receptors and are also active in conjugation—less than the remaining transfer

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press

factors in HfrH. This conclusion is also in agreement with their classification as fi^{+1} and fi^{+2} factors according to their effects on lysis of K12F+ by μ 2. One fi^{+2} factor, 118, gave discordant results in this respect: although yielding visible plaques with μ 2 on K12F+, it totally inhibited visible lysis of K12HfrH, and also inhibited *pro* transfer to a greater extent than 125, the other fi^{+2} factor.

Reconstruction experiments showed that visible lysis by μ 2 was no longer detectable when the proportion of F+ to F− cells was less than about 1 to 5. This indicates that the degree of fertility inhibition in HfrH was greater than would be suggested by the inhibition of lysis by μ 2 by classes fi^{+1}, fi^{+2} and fi^{+3} in K12F+, and by classes fi^{+1} and fi^{+2} in HfrH, although, of course, the conditions of test for fimbrial synthesis are different in conjugation experiments from those in phage lysis tests.

It is not yet known to what extent the various degrees of inhibition of μ 2 lysis in K12F+ reflect similarity of the respective transfer factors to the F factor.

The fi^{-} factors in this investigation were Δ and factors related to it. When these factors are present in K12F+ they do not inhibit lysis by male-specific phage. In contrast, lysis by the 'female-specific' phage ϕ 2 is abolished and the efficiency of plating of this phage is zero (Anderson, 1966).

Other fi^{-} factors have been identified which, when introduced into K12F+, do not inhibit lysis by phage ϕ 2 to the same extent as the transfer factors of which Δ is the prototype.

It is thus possible to subdivide fi^{-} factors on the basis of their range of restrictive effects on phage ϕ 2.

It is worth-while recalling that phage-restriction effects in salmonellae can be added to those in K12 for the additional subdivision of transfer factors or R-factors (Anderson, 1966).

One of us (J. S. Pitton) is grateful to the Fonds National Suisse pour la Recherche Scientifique for a grant.

REFERENCES

Adams, M. H. (1959). *Bacteriophages*. London and New York: Interscience Publishers Inc.

Anderson, E. S. (1965*a*). Origin of transferable drug-resistance factors in Enterobacteriaceae. *British Medical Journal* ii, 1289–1291.

Anderson, E. S. (1965*b*). A rapid screening test for transfer factors in drug-sensitive Enterobacteriaceae. *Nature* **208**, 1016–1017.

Anderson, E. S. (1966). Influence of the Δ transfer factor on the phage sensitivity of Salmonellae. *Nature* **212**, 795–799.

Anderson, E. S. & Lewis, M. J. (1965*a*). Drug resistance and its transfer in *Salmonella typhimurium*. *Nature* **206**, 579–583.

Anderson, E. S. & Lewis, M. J. (1965*b*). Characterization of a transfer factor associated with drug resistance in *Salmonella typhimurium*. *Nature* **208**, 843–849.

Clowes, R. C. & Hayes, W. (1968). *Experiments in Microbial Genetics*. Oxford and Edinburgh: Blackwell Scientific Publications.

Cuzin, F. (1965). Un bactériophage spécifique du type sexuel F− d'*Escherichia coli* K12. *Comptes Rendus Académie des Sciences, Paris* **260**, 6482–6485.

Dettori, R., Maccacaro, G. A. & Piccinin, G. L. (1961). Sex-specific bacteriophages of *Escherichia coli* K 12. *Giornale di Microbiologia* **9**, 141–150.

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press

Hayes, W. (1953). The mechanism of genetic recombination in *Escherichia coli*. *Cold Spring Harbour Symposium on quantitative Biology* **8**, 75–83.

Meynell, E. & Datta, N. (1966). The relation of resistance transfer factors to the F-factor (sex-factor) of *Escherichia coli* K 12. *Genetical Research* **7**, 134–140.

Watanabe, T. & Fukasawa, T. (1962). Episome-mediated transfer of drug resistance in Enterobacteriaceae. IV. Interactions between resistance transfer factor and F-factor in *Escherichia coli* K 12. *Journal of Bacteriology* **83**, 727–735.

Watanabe, T., Fukasawa, T. & Takano, T. (1962). Conversion of male bacteria of *Escherichia coli* K 12 to resistance to f phages by infection with the episome 'Resistance Transfer Factor'. *Virology* **17**, 217–219.

Watanabe, T., Nishida, H., Ogata, C., Arai, T. & Sato, S. (1964). Episome-mediated transfer of drug resistance in Enterobacteriaceae. VII. Two types of naturally occurring R factors. *Journal of Bacteriology* **88**, 716–726.

https://doi.org/10.1017/S0016672300002433 Published online by Cambridge University Press