Explanation of the Apparent Association of Host Specificity Determinants with fi^+ R Factors

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SUMMARY

Two strains of *Escherichia coli* K12 carried R factors associated with the $hsII$ host specificity determinants although they were apparently fi^+ , while all other R factors carrying this host specificity have been found to be fi^- . When these two strains were used as donors of drug resistance, separation of two R factors from each strain was observed. The separated R factors were tested for fi character, and it was found that in both cases the original strains carried an fi^+ and an fi^- R factor, and it was the fi^- R factor which carried the $hsII$ determinants.

INTRODUCTION

Transmissible drug resistance in the Enterobacteriaceae is determined by drug resistance factors (R factors) which are divisible into two classes, fi^+ and fi^- , on the basis of the repression of the expression of the sex factor F (see Watanabe, 1963), as well as by the structure of their sex pili (see Meynell, Meynell & Datta, 1968). An apparent third, distinguishing feature between the fi^- and fi^+ R factors, was that the presence of certain fi^- R factors led to a reduction in efficiency of plating (e.o.p.) of a number of bacteriophages such as λ and T1 (Watanabe, Takano, Arai, Nishida & Sato, 1966).

In a survey of 153 R^+ *Escherichia coli* K12 strains this affect was observed with both fi^- and fi^+ R factors (Bannister & Glover, 1968). In some instances, a reduction in e.o.p. may be due to the operation of a host-controlled modification, the host range of a bacteriophage being altered by a single cycle of growth in a new host strain (Luria, 1953; Arber & Dussoix, 1962). In 12 R^+ strains, the reduction in e.o.p. of a number of bacteriophages was found to be due to R factor-associated host controlled modification, and two different host specificities were observed. One of these, termed hsI , was present only in one R^+ strain, carrying the fi^+ R factor R124, while the second, $hsII$, was found in the remaining 11 strains (Bannister & Glover, 1968), and was also shown to be identical to that found in two strains with fi^- R factors by Watanabe *et al.* (1966). Careful examination of the fi character of the 11 $hsII^+$ strains showed that two of them were apparently exceptional in that they carried fi^+ R factors. This paper presents the results of an investigation of these two strains.

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METHODS

Media. The media have been described previously (Bannister, 1970; Bannister & Glover, 1970).

Antibiotics. Tetracycline: (achromycin hydrochloride from Cyanamid of Great Britain, Bush House, London, W.C.2) used at a final concentration of 50 µg./ml. Streptomycin: (streptomycin sulphate BP from Glaxo Laboratories Ltd., Greenford, Middlesex) used at a final concentration of 20 µg./ml. Sulphonamide: (sulphamezathine from ICI Pharmaceuticals Division, Macclesfield, Cheshire) used at a final concentration of 20 µg./ml. Chloramphenicol: (chloromycetin from Parke Davis, Hounslow, Middlesex) used at a final concentration of 20 µg./ml. Kanamycin: (kanamycin sulphate from Bayer Products Co., Surbiton, Surrey) used at a final concentration of 20 µg./ml.

Bacterial strains: The following *Escherichia coli* strains were used: C600 *thr leu thi lac* (Appleyard, 1954) from the collection of Dr S. W. Glover, and J5-3 *pro met* (Clowes & Rowley, 1954), J5-3 F⁺ and J5-3 R⁺ strains kindly provided by Dr Elinor Meynell and Dr Naomi Datta.

Bacteriophages. Phage λvir (Jacob & Wollman, 1954); MS2 (Davis, Strauss & Sinsheimer, 1961); T3.

Techniques. These are described elsewhere (Bannister, 1970; Bannister & Glover, 1970).

RESULTS AND DISCUSSION

*Association of *hsII* with the *fi*⁻ character.* Two strains apparently carrying *fi*⁺ R factors associated with *hsII* were examined J5-3 (R269), which was resistant to the following antibiotics; tetracycline (Tc), streptomycin (Sm), sulphonamide (Su), and kanamycin (Km); and J5-3 (R348), resistant to Tc, Sm, Km and chloramphenicol (Cm). These two strains were used as donors of drug resistance to C600, and exconjugants selected for one resistance were scored for the others, and for the presence of the *hsII* determinants. The results of two such experiments are presented in Table 1. With R269, three classes of exconjugants were observed: those resistant to Tc, Sm, Su and *hsII*⁺; those resistant to Tc, Sm, Su, Km and *hsII*⁺, and those resistant to Sm, Su, Km and not carrying the *hsII* determinants. The most likely explanation of these findings is that two R factors were present in the original strain, one carrying resistance to Tc, Sm, Su and *hsII*⁺ determinants, and the other carrying resistance to Sm, Su and Km. Those exconjugants carrying all the determinants of the parent strain would then arise by transfer of both R factors to the recipient. Similarly, three types of exconjugant were found for R348: those resistant to Tc, Sm and *hsII*⁺; those resistant to Tc, Km and Cm, and those resistant to Tc, Sm, Km and Cm, and also carrying *hsII*⁺. Again, the result can be explained in terms of the presence of two R factors in the original strain, one carrying resistance to Tc and to Sm, and also carrying the *hsII* determinant, the other carrying resistance to Tc, Km and Cm.

*The *fi* character of the separated R factors from J5-3 (R269) and J5-3 (R348).* A number of R⁺ strains have been shown to carry both an *fi*⁺ and an *fi*⁻ R factor, though the presence of the *fi*⁻ R factor was masked by the *fi*⁺ R factor when the strains were tested originally for their ability to plate the F-specific phage MS2 (Romero & Meynell, 1969). Since two *fi*⁺ R factors do not co-exist stably (Watanabe *et al.* 1964), it was pre-

Table 1. *Properties of exconjugants obtained after mating R⁺ strains with strain C600.*

An overnight culture of the J5-3 R⁺ donor was diluted 1 in 10 into fresh broth, grown on an inclined rotor at 37° for 90 min., then diluted 1 in 10 into an overnight culture of the recipient and kept at 37° for 30 min. This mating mixture was diluted and samples plated in soft agar overlays on supplemented VB agar plates, which were incubated at 37° for 36 to 48 hr. Colonies were purified by stabbing on to selective VB agar and grown overnight at 37° before replica-plating to determine drug resistance. The *hsII* character was determined by streaking colonies on Difco agar plates which were spotted with dilutions of phage λ vir grown on K, and incubated overnight at 37°.

R factor and determinants carried	Drug resistance selected	Determinants found*†	Nos. observed	
			Expt. 1	Expt. 2
R 269 Tc Sm Su Km <i>hsII</i> ⁺	Tc	Tc Sm <i>hsII</i>	97	35
		Tc Sm Km <i>hsII</i>	30	117
	Sm	Tc Sm <i>hsII</i>	15	2
		Tc Sm Km <i>hsII</i>	40	46
		Sm Km	15	96
	Su	Tc Sm Su <i>hsII</i>	0	2
		Tc Sm Su Km <i>hsII</i>	2	39
		Sm Su Km	128	102
	Km	Tc Sm Km <i>hsII</i>	25	36
		Sm Km	120	99
	R 348 Tc Sm Km Cm <i>hsII</i> ⁺	Tc Sm <i>hsII</i>	16	112
		Tc Km Cm	97	27
		Tc Sm Km Cm <i>hsII</i>	8	2
		Sm	75	68
		Km	19	4
		Tc Km Cm	109	33
	Cm	Tc Km Cm	123	51
		Tc Sm Km Cm <i>hsII</i>	33	3

* At least 20 colonies of each tested for *hsII* where available.

† It is not possible to score Su resistance by replica-plating, hence Su resistance is not recorded for R 269, except where Su resistance was selected directly.

Table 2. *Phage MS2 plating and presumed fi character of F⁺R⁺ strains*

Parent R factor	Drug resistances present	<i>hsII</i>	MS 2 plating*	<i>fi</i> character deduced	No. of colonies
R 269	Tc Sm Su	+	+	—	3
R 269	Sm Su Km	—	—	+	6
R 348	Tc Sm	+	+	—	3
R 348	Tc Cm Km	—	—	+	7

* MS 2 plating was determined by spotting 0.01 ml. of an MS 2 lysate on to the F⁺ R⁺ strain in soft agar on Difco agar plates.

sumed that the two strains J5-3 (R 269) and J5-3 (R 348), had both harboured an *fi*⁺ and an *fi*⁻ R factor. To test this assumption the presumed separated R factors were transferred by conjugation to J5-3 F⁺ strain, which was then tested for lysis with the F-specific phage MS2. The results are presented in Table 2. Lysis was abolished by the segregant of R 269 carrying resistance to Sm, Su and Km, and similarly by the segregant of R 348 carrying resistance to Tc, Km and Cm. Tests which showed a re-

duced efficiency of plating of phage T3 (Schell *et al.* 1963) confirmed that F was still present and thus that these R factors were acting by preventing its expression, and were therefore fi^+ . The remaining two components of the original R269 and R348 factors, which carried the $hsII^+$ character, were both fi^- .

There have been three previous reports of R factor associated host specificities. For two fi^- R factors described by Watanabe *et al.* (1966), it had been possible to identify the host specificity since one of the R factors, R15, was made available for this purpose, and the host specificity found was $hsII$. Yoshikawa & Akiba (1962) also found a host specificity associated with fi^- R factors, while the third report (Molina, 1964) did not specify the fi character nor give details of the host specificity. There remains, therefore, only one instance of an fi^+ R factor carrying host specificity determinants, this being the association of R124 with the determinants for hsI , a host specificity distinct from $hsII$ (Bannister, 1969). It is possible that once again the fi^+ R124 masks the presence of a second fi^- R factor which carries the hsI determinants, since it is not unusual for an fi^- R factor to be present in an apparently fi^+ strain (Romero & Meynell, 1969). However, it has not been possible to separate hsI from the fi^+ character and resistance to Tc, the only drug resistance carried by this R factor (Bannister, 1969).

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