SHORT COMMUNICATIONS

Fertility Inhibition of an N Group R Factor by a Group X R Factor, R6K

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INTRODUCTION

R factors that are fi^+ repress F-mediated functions such as fertility and sensitivity to sex-specific phages (Meynell, Meynell & Datta, 1968), and this is taken to indicate their close relationship to the F factor (Meynell & Datta, 1965). However, not all fi^+ R factors are F-like (Lawn, Meynell, Meynell & Datta, 1967; Grindley & Anderson, 1971; Meynell, 1973). Indeed, Hedges, Datta, Coetzee & Dennison (1973) have shown that two fi^+ X group R factors reduce the transfer of R386 (group F1), which is not susceptible to repression by normal F-like plasmids. They suggest that some R factors, not related to F, may possess a mechanism of F pilus repression that is distinct from that of true F-like plasmids. We present evidence that fertility inhibition by X group R factors is even more diverse: not only does R6K, the prototype of this group, repress the transfer of an N group R factor (R46), but it also reduces the propagation of the N group-specific phage IKE on strains that harbour R46.

METHODS

R factor transfer. Strains containing the two R factors, R46 (which confers resistance to ampicillin, Am, streptomycin, Sm, tetracyclines, Tc, and sulphonamides, Su) and R6K (resistance to Am and Sm), were constructed in *Escherichia coli* J53 *pro met* by mating with *E. coli* J62 *pro his trp lac* donors. Although both resistances conferred by R6K are also mediated by R46, it is possible to differentiate the two R factors because the R6K resistance levels are much higher than their R46 counterparts. Transfer frequencies from J53 strains harbouring both plasmids were estimated from matings with strain J62 as recipient. Logarithmic phase donor cultures were mixed with stationary phase recipients in a ratio of 1 to 10 in 2.5 ml nutrient broth at 37 °C. After 40 min, cells that had received the R factor were selected by the method of Smith (1969). Tetracycline at 10 µg/ml was used for the selection of R46 and Am at 400 µg/ml for R6K.

Plaque formation by phage IKE. Both IKE (Khatoon, Iyer & Iyer, 1972) and its indicator strain *Escherichia coli* MA50(RM98) were kindly supplied by Dr R. V. Iyer. High titre phage stocks were prepared by the confluent lysis technique on strain MA50(RM98). Diluted phage suspension (0.1 ml) was mixed with 0.1 ml of a logarithmic culture of the particular R^+ strain being tested in 1.8 ml of top agar (0.35 g Ionagar and 2.25 g Oxoid No. 2 broth powder in 90 ml distilled water). Bottom layers consisted of 20 ml Oxoid blood agar base. Plaque formation was most distinct after overnight incubation at 25 °C.

Phage IKE propagation. Mid-logarithmic phase nutrient broth cultures of J53 R^+ strains

Table 1. *Inhibition of R46-mediated functions by R6K*

Donor strain	Recipient strain	Transfer frequency of R factor*		Action of IKe on donor	
		R46	R6K	Plaque formation	Propagation†
J62(R46)	J53	3.7×10^{-8}	—	+	n.d.
	J53(R6K)	2.3×10^{-8}	—		
J62(R6K)	J53	—	1.1×10^{-3}	—	n.d.
	J53(R46)	—	1.1×10^{-3}		
J53(R46)	J62	6.5×10^{-8}	—	+	1.5×10^8
J53(R6K)	J62	—	6.4×10^{-4}	—	1
J53(R46, R6K)	J62	2.2×10^{-8}	2.9×10^{-4}	—	50
J53(R6K, R46)	J62	9.0×10^{-8}	2.5×10^{-4}	—	35

* Expressed as frequency per input donor.

† Phage propagation is expressed as the ratio of the phage titre after 2 h incubation in the presence of the test strain divided by the input titre. +, Plaque formation; —, no plaque formation; n.d., not done.

were mixed at 37 °C with phage IKe at a multiplicity of infection of 20. Five minutes were allowed for phage adsorption and 0.5 ml of the mixture was then diluted into 4.5 ml of a 10^{-3} broth dilution of IKe antiserum ($K = 1 \times 10^4$). After incubation at 37 °C for 5 min the cells were removed by membrane filtration and washed twice with 10 ml quantities of warm nutrient broth. The membranes were placed in 10 ml warm nutrient broth and vortexed to detach the cells. A sample of the bacterial suspension was removed immediately, filter sterilized, and the filtrate assayed for IKe. The remainder of the suspension was incubated at 37 °C for 2 h, filtered and reassayed for IKe.

RESULTS

R factor transfer

Transfer frequencies obtained during construction of J53 strains harbouring both R46 and R6K showed the transfer of either R factor was unaffected by the presence of the other in the recipient (Table 1). R factor compatibility was further demonstrated by the fact that both sets of resistances were retained during subculture in antibiotic-free media and on prolonged storage at 4 °C.

When transfer frequencies from strains containing both R factors were compared with those from strains harbouring the plasmids individually, it was found that the frequency of R6K transfer from J53(R46, R6K) or J53(R6K, R46) was approximately half of that from J53(R6K) (Table 1). Analysis of exconjugants showed that selection for the transfer of R6K from J53(R46, R6K) resulted in 23 out of 24 tested receiving R6K alone: one clone exhibited the resistance pattern of both R factors. Co-transfer of R46 with R6K was not observed in the 24 clones examined after selection of R6K recipients from the J53(R6K, R46) donor.

In contrast, it was found that the transfer frequency of R46 was reduced several hundred-fold by the presence of R6K in the donor (Table 1). Examination of recipients selected on Tc for R46 transfer from the J53(R46, R6K) donor showed that 23 out of 24 clones also received the Sm resistance level conferred by R6K, 17 exhibited the high Am resistance characteristic of R6K, and six were no longer Su resistant. Analogous figures for 24 recipients selected on Tc after mating with the J53(R6K, R46) donor were: 19 resistant to high levels of Sm, 9 to high levels of Am, and 12 that were Su sensitive. Many of these segregants were

unstable, which has been shown to occur when two dissimilar R factors recombine (Yoshikawa & Sakai, 1972). The results suggest that not only does R6K repress R46 transfer to a rate much less than that of R6K itself, but that recombination occurs between the two R factors. Indeed, it may be inferred from the high proportion of Tc resistant exconjugants that also exhibit R6K conferred resistances, that recombination may be a prerequisite of transfer.

Plaque formation by IKE

Sex-specific phages do not form plaques on strains containing repressed F or I group plasmids (Meynell *et al.* 1968). However, Dennison & Baumberg (1973) have reported that, although N group R factors transfer at a repressed rate, the group-specific phage IKE will plaque on N⁺ strains. We have confirmed this with R46 (Table 1). Phage IKE had an efficiency of plating approaching unity on strains J62(R46) or J53(R46) when compared with plaque formation on strains MA50(RM98) or J53(RM98). Introduction of R6K into strains already harbouring R46 abolished plaque-forming ability. No plaques were seen in any dilution of IKE plated, which suggests that IKE is not restricted by R6K but is inhibited by some other mechanism, perhaps by prevention of phage adsorption.

Propagation of phage IKE

A more quantitative estimate of phage repression was obtained by testing the ability of IKE to propagate on strains J53(R46), J53(R46,R6K) and J53(R6K,R46). It was found that after 2 h incubation with strain J53(R46), the input titre of IKE increased by more than one millionfold. However, when R6K was also present in the strain, the titre increased by only 50 and 35 times, respectively, in the same period (Table 1).

DISCUSSION

Filamentous phages, specific for bacteria harbouring F- or I-like plasmids, adsorb to the respective type of pilus. When pilus synthesis is repressed, plaque formation is inhibited and transfer rates are reduced (Meynell *et al.* 1968). No definitive evidence is available to show that sex pili are necessary for the transfer of N group plasmids, but Dennison & Baumberg (1973) have reported that the IKE receptor functions as if it were a transfer component. In agreement with this view, we have shown that repression of R46 transfer by R6K is paralleled by inhibition of IKE propagation. This situation is analogous to the interactions between F and *fi*⁺ R factors (Meynell & Datta, 1965), and it is possible that a repressor, mediated by a regulator gene of R6K, also acts on the R46 genome. However, Meynell (1973) has shown that the *fi*⁺ character of R62(I) is not related to specific repression, and Hedges *et al.* (1973) have suggested that *fi*⁺ R factors that are non-F-like may have evolved a mechanism for the inhibition of F piliation unlike the repression mechanism of true F-like plasmids. The inhibition of both R46 transfer and IKE propagation may therefore be mediated by such a non-specific mechanism.

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