Properties of pPE1572(Hys-Raf), a Plasmid Governing Hydrogen Sulphide Production and Raffinose Fermentation in *Escherichia coli*

By M. MAGALHÃES AND ADELMA VÉRAS

*Departamento de Microbiologia, Faculdade de Medicina, Cidade
Universitária, 50000 Recife, Brazil*

(Received 7 September 1976)

INTRODUCTION

The transmissible nature of the extrachromosomal genetic element which mediates hydrogen sulphide production (Hys) in *Escherichia coli* was recognized by Lautrop, Ørskov & Gaarslev (1971). Since then, Ørskov & Ørskov (1973) have reported that some H₂S-positive strains are also able to transfer the determinant responsible for raffinose-fermenting ability (Raf), and have shown that both markers are transmitted as a single linkage group. Thus, raffinose utilization has been added to the list of those bacterial degradative characters that are governed by plasmids.

This paper reports the genetic characterization of pPE1572(Hys-Raf), which has been isolated in Brazil from a naturally occurring human strain of *E. coli*.

METHODS

Bacteria, plasmids and phages. pPE1572(Hys-Raf) and nine other Hys-Raf plasmids, isolated from human faecal *E. coli* strains, were carried into *E. coli* 20R764 F⁻ *rif* (K12) or *E. coli* KLI10 F⁻ *str recA* (KLI10).

To test for the Fi character and for the physical independence of plasmids, respectively, *E. coli* 27R207 HfrH *nal* and *E. coli* 14R525 F⁻ *nal* were used. Standard R plasmids of different incompatibility groups (R386, R538*drd1*, R1*drd19*, R124, R144, R621a, R446b, RN3 and RP4) were transferred into *E. coli* 153-1 or *E. coli* AB1553. Donor-specific and female-specific phages were R17 and ϕ 2.

Matings and plasmid interaction. These were done as described by Smith *et al.* (1973). In superinfection studies, standard R plasmids were introduced into KLI10 or K12 containing pPE1572(Hys-Raf). The *recA* mutant strain was preferred, and strain K12 was only used if the plasmid under test included genes for streptomycin resistance. Donors were counter-selected by streptomycin, rifampicin (100 μ g ml⁻¹) or nalidixic acid (40 μ g ml⁻¹), and recipients by streptomycin, chloramphenicol or tetracycline (20 μ g ml⁻¹), which were incorporated into MacConkey agar. Three transconjugant colonies derived from each cross were purified on selective media, cultured overnight in broth, and then master plates were prepared. These master plates were replicated on to MacConkey agar containing the relevant antibiotic, on to a minimal medium supplemented with raffinose as the sole source of carbon, and on to peptone iron agar. Plates were analysed after 48 h. When strain K12 was the recipient, the physical independence of the plasmids was further investigated by performing retransfer tests.

Table 1. *Incompatibility of pPE1572(Hys-Raf)**

R plasmid	Incompatibility group	Analysis of transconjugant colonies†			
		No. on master plate	No. with pPE1572	No. with R plasmid	No. with both plasmids
R386	FI	360	4	356	0
R538 <i>drd1</i>	FII	180	180	180	180
R124	FIV	160	160	155	155
R144	Ia	200	200	184	184
R621a	Iy	180	180	180	180
RP4	P	180	180	172	172
R446b	M	200	200	200	200
RN3	N	190	190	190	190

* pPE1572 was the resident plasmid.

† Derived from three purified 'double' clones.

RESULTS AND DISCUSSION

When *E. coli* J53-1 carrying standard R plasmids was used as donor and crossed with recipients which either contained pPE1572(Hys-Raf) or did not, no significant difference in the frequency of transfer was observed, although transconjugant colonies derived from the cross involving R386 were very small. This, *per se*, suggested incompatibility. In fact, pPE1572(Hys-Raf) coexisted stably with a number of R plasmids, but not with R386 (Table 1). The other nine Hys-Raf plasmids were therefore tested against R386. Results were uniform, indicating that all belonged to incompatibility group FI. Analysis of more than 200 colonies derived from three hybrid lines of each cross, showed that Hys-Raf plasmids were more readily eliminated from 'doubles'. Indeed, derepressed plasmids (like R386) due to their higher donor efficiency have the greatest likelihood of being retained (Falkow, 1975).

Like R386 and F, pPE1572(Hys-Raf) appears to determine pili of the F type. When a broth culture of K12(pPE1572) was infected with R17, the phage titre was increased by a factor of 10^3 . However, in contrast to R386 and F, Hys-Raf plasmids did not determine restriction of the female-specific phage $\phi 2$, and behaved in matings as self-repressed conjugative plasmids.

All of our Hys-Raf plasmids were Fi^+ , and the specificity of F inhibition by pPE1572(Hys-Raf) was evident. When R1*drd19*, a plasmid belonging to incompatibility group FII (genotype *finO traO*⁺), was introduced by conjugation into K12(pPE1572), stable 'double' clones were formed. The frequency of transfer of R1*drd19* from these was reduced by a factor of 10^3 compared with its transfer from the original R⁺ host. Simultaneously, bacterial host susceptibility to phage R17 was abolished. On the other hand, there was a lack of a fertility inhibition effect in K12(pPE1572) harbouring the plasmid R583*drd1* (genotype *finO*⁺ *traO*^o), which is resistant to fertility inhibition by *finO*⁺ plasmids. These findings confirm that the genotype of pPE1572(Hys-Raf) is *finO*⁺.

Like Ørskov & Ørskov (1973) we have observed a close linkage between Hys and Raf determinants. All transconjugant colonies obtained during this investigation either retransferred Hys and Raf together or lost them together. This kind of association is not unique in nature. Recently, Smith & Parsell (1975) found, among pathogenic swine strains of *E. coli*, a close relationship between the transfer of Raf and the ability to transfer the K88 plasmid. None of their strains transferred the raffinose-fermenting character in the absence of the K88 plasmid. K88 has its own transfer genes and the potentiality to mobilize nonconjugative plasmids such as Sm-Su (Guerry, van Embden & Falkow, 1974). To ascertain whether Hys

also has the capacity to mobilize nontransmissible genetic determinants, the triparental method of Anderson (1965) was tried. Out of 30 wild-type H_2S -positive and raffinose-nonfermenting strains of *E. coli* that were used as donor, one carried a Hys plasmid which was able to mobilize Sm-Su from the intermediary recipient. The resulting plasmid aggregate pPE1777(Hys-Sm-Su) behaved stably when introduced into K12 and was incompatible with R386, like our Hys-Raf plasmids. Recombination between transfer genes and nonconjugative plasmids may take place, and this presumably represents one means by which existing R plasmids may acquire new resistance determinants (Guerry *et al.* 1974). Nonetheless, whether Raf exists in nature as a nontransmissible genetic element before being mobilized and eventually integrated into a conjugative plasmid such as Hys or K88, remains to be seen. If this should be the case, the properties now ascribed to Hys-Raf might really be due to the transfer genes of the plasmid Hys.

We are grateful to E. S. Anderson and D. S. Santos who kindly sent us standard organisms.

REFERENCES

- ANDERSON, E. S. (1965). A rapid screening test for transfer factors in drug-sensitive Enterobacteriaceae. *Nature, London* **208**, 1016-1017.
- FALKOW, S. (1975). *Infectious Multiple Drug Resistance*, 1st edn, p. 157. Edited by S. Falkow. London: Pion Ltd.
- GUERRY, P., VAN EMBDEN, J. & FALKOW, S. (1974). Molecular nature of two non-conjugative plasmids carrying drug resistance genes. *Journal of Bacteriology* **117**, 619-630.
- LAUTROP, H., ØRSKOV, I. & GARRSLEV, K. (1971). Hydrogen sulphide producing variants of *Escherichia coli*. *Acta pathologica et microbiologica scandinavica* **B79**, 641-650.
- ØRSKOV, I. & ØRSKOV, F. (1973). Plasmid-determined H_2S character in *Escherichia coli* and its relation to plasmid-carried raffinose fermentation and tetracycline resistance characters. *Journal of General Microbiology* **77**, 487-499.
- SMITH, H. R., GRINDLEY, N. D. F., HUMPHREYS, G. O. & ANDERSON, E. S. (1973). Interactions of group H resistance factors with the F factor. *Journal of Bacteriology* **115**, 623-628.
- SMITH, H. W. & PARSELL, Z. (1975). Transmissible substrate-utilizing ability in Enterobacteria. *Journal of General Microbiology* **87**, 129-140.