

Further Characterization of the Recipient Ability of *Escherichia coli* K-12 Bacteriophage-Resistant Mutants

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Received for publication 13 October 1976

We extended the study of *Escherichia coli* mutants defective in conjugation and showed that the mutants with altered lipopolysaccharide, which are defective as recipients with F-like donors, are also defective with the I-like plasmid R64-11. However, the extent of reduction in recipient ability for I-like donors does not correlate either with the effect on recipient ability for F-like donors or with the degree of alteration to the lipopolysaccharide.

Several classes of *Escherichia coli* K-12 mutants that have been isolated as resistant to bacteriophages are also defective as recipients in conjugation. These include: ϕ W-resistant mutants isolated by Monner et al. (9); the mutants of Reiner (10), selected as resistant to the single-stranded deoxyribonucleic acid phage ST-1; the *ompA* mutants of Skurray et al. (11), selected as resistant to bacteriophage K3; and the mutants of Havekes et al. (3), isolated as resistant to bacteriophages T3, T4, and T7.

We showed previously (7) that among the set of bacteriophage-resistant mutants isolated by Hancock and Reeves (1) were a number of mutants defective as recipients in conjugation with an F' donor strain. We also demonstrated that the *ompA* mutants are defective as recipients with F', Hfr, and most F-like R-factor donors, but are competent with R100-1, the closely related R136/*fn*⁻, and a number of I-like R-factors including R64-11 (8). In this note we examine the remainder of these mutants for recipient ability with a strain carrying the I-like plasmid R64-11 and with an Hfr strain, to enable us to make more general comparisons.

All of the strains were derivatives of *E. coli* K-12; all of the bacteriophage-resistant mutants were derived directly from strain P400 and have been described elsewhere (1). The I-like plasmid R64-11 was maintained in strain JC6256. Cultures were grown in nutrient broth, and the matings were carried out as described previously (7) except that Hfr matings were allowed to proceed for 60 min before interruption by blending.

The results of the matings are summarized in Table 1; as done previously (7), the mutants yielding less than 50% recombinants relative to

the controls are considered to be sufficiently reduced in their recipient ability to be classed as defective.

If we compare the number of recombinants obtained with HfrH to those previously observed with an F'*lac pro* donor (7), then essentially the same mutants are classified as defective as recipients, although the defects observed with the HfrH are, in general, not as great as for the F'*lac pro* strain. The exceptions to this were the Bar-2, Bar-4, and Ktw-3 mutants, which we previously scored as defective with an F'*lac pro* donor but are now above our cutoff point of 50% reduction. However, these discrepancies are probably not significant. Mutants defective with HfrH are the Bar-3, Bar-5, Bar-7, Bar-8, Wrm-1, and Wrm-2 mutants, all of which have been shown to have defects in their lipopolysaccharide (LPS) (2).

The effect of an alteration on the recipient functions in matings with donors with F-like pili is at least approximately proportional to the extent of the LPS alteration, be it to decrease the transfer frequency (F' and Hfr crosses) or to increase it (R100-1 crosses) (7).

In the case of the I-like plasmid R64-11, which produces an antigenically unrelated pilus (5), the effect on recipient function is different in that it is not correlated with the extent of LPS alteration. Thus, mutants such as P489 (Bar-8) and P435 (Wrm), which retain little other than lipid A and ketodeoxyoctonate in their LPS and are greatly affected as recipients for F-like donors, are affected to a much lesser extent as recipients for an I-like donor. In contrast P495 (Bar-3), with a lesser defect in its LPS, is more affected in recipient ability with the I-like donor.

None of the defects observed with these mutants is as extreme as the defect in recipient ability observed using *ompA* mutants with F'

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TABLE 1. Recipient ability in conjugation of bacteriophage-resistant mutants

Mutant class ^a	Mutant	Recipient ability ^b with:	
		HfrH	R64-11
Bar-1	P455	1.35 ± 0.16	1.04 ± 0.041
Bar-2	P492	0.95 ± 0.24	0.64 ± 0.027
Bar-3	P495	0.17 ± 0.054	0.041 ± 0.0084
Bar-4	P436	0.63 ± 0.23	0.090 ± 0.016
Bar-5	P402	0.31 ± 0.078	0.058 ± 0.017
Bar-6	P451 ^c		
Bar-7	P487	0.17 ± 0.071	0.018 ± 0.0093
Bar-8	P489	0.083 ± 0.048	0.15 ± 0.064
Ktw-1	P456	1.09 ± 0.24	1.90 ± 0.46
Ktw-2	P476	0.94 ± 0.12	4.65 ± 0.60
Ktw-3	P240	0.85 ± 0.42	0.40 ± 0.017
Ttk-1	P429	0.81 ± 0.10	0.42 ± 0.049
Ttk-2	P423	0.96 ± 0.19	0.56 ± 0.073
Ttk-3	P425	1.17 ± 0.19	0.23 ± 0.041
Ttk-4	P474	1.23 ± 0.15	0.35 ± 0.065
Wrm-1	P435	0.17 ± 0.067	0.22 ± 0.043
Wrm-2	P424	0.45 ± 0.14	0.064 ± 0.018

^a The mutant classes are defined in reference 1. None of the *bfe*, *efr*, *ktn*, *Misc(1-5)*, *tonA*, and *tonB*, and *tsx(1, 2)* mutants used previously (7) were included since they showed no significant alteration in recipient ability.

^b Recipient ability is given with respect to P400, the parent recipient strain, which is taken as 1. HfrH gave 19.8 ± 3.78% transfer, selecting for *thr*⁺ recombinants, and R64-11 gave 0.38 ± 0.063% transfer, selecting for tetracycline resistance, using 20 µg of tetracycline per ml. Streptomycin (1,000 µg/ml) was used for contraselection in both cases, and the number of recombinants or transconjugants was recorded as a percentage of the input donor cell number. All results are the mean of at least three matings, and the standard deviations are given.

^c Strain P451 was not included since it has been lost.

and Hfr donors (7, 8, 11).

ompA mutants are so markedly affected in their recipient ability that it seems that outer membrane protein 3A (absent in *ompA* mutants) plays an essential role in recipient functions for at least some F-like donors (7, 8, 11). Clearly, LPS also plays a role in recipient functions, but the role is apparently complex. The limited magnitude of effect on recipient ability observed in this study provides no evidence that LPS acts as the major determinant for specificity for either F-like or I-like pilus attachment, although other authors (3, 9, 10) have observed a greater reduction in recipient ability in similar mutants. The reason for this discrepancy is not apparent, although one of the mutants had a large deletion (3). It is known that mutations

affecting primarily the synthesis of LPS can lead to the loss of outer membrane proteins (4, 6), and it is possible that LPS mutations affect recipient functions by altering the local environment of protein 3A or other receptor in the outer membrane, thereby modifying its function.

ADDENDUM IN PROOF

Since submitting this note, we learned that other mutants have been isolated that are defective as recipients with an I-like R-factor (L. Havekes and W. P. M. Hoekstra, personal communication). These mutants also have a defective LPS structure.

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