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Fertility Regulation in F-Like Resistance Transfer Factors[1]

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Mutants of the R factor R100 have been isolated that mediate high-frequency transfer of the R factor during conjugation. Complementation tests revealed two classes of mutants, operator-constitutive and repressor-negative. Some of the latter class were suppressible by amber and ochre suppressors. The results support a simple model of regulation for the control of R-factor-mediated piliation.

The fi$^+$ or F-like R factors are known to inhibit F-factor-mediated fertility when in the *trans* configuration (13). In 1962 Egawa and Hirota (4) isolated a mutant of R factor R100 incapable of repressing fertility. They proposed an operator-repressor model for regulation of fertility and suggested that the mutant might produce a defective repressor. Meynell and Datta (6) have isolated additional mutants of F-like R factors incapable of repressing fertility. These mutants are believed to lack a functional repressor and have been called i^- (4) or *drd* for derepressed (6). Nishimura et al. (9) demonstrated that F pili are present on cells carrying a derepressed F-like R factor, whereas they were unable to detect pili on cells with a repressed wild-type F-like R factor by using the same techniques. The fertility inhibition of the F-like R factor is apparently related to this absence of pili.

If pili synthesis is regulated by a cytoplasmic repressor, then there should be at least two classes of *drd* mutants. These two classes would be equivalent to the repressor-negative (i^-) and operator-constitutive (o^c) classes of constitutive mutants found in the lactose operon (5). This paper reports the isolation and characterization of a series of *drd* mutants of the R factor R100. The object of this study was to test the operator repressor model of fertility regulation by looking for the o^c type of *drd* mutant and by looking for suppressible mutants of the i^- type (5).

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1.

R factors. The R factors and their origins are listed in Table 2.

Media. Cultures to be used in mating experiments were grown in L broth (2), and L agar was employed for the testing of clones for sensitivity to the F-specific phage, MS-2. The minimal salts medium of Sheppard and Englesberg (10) was used for selection and purification of recombinants. When necessary, it was supplemented with amino acids at 40 μg/ml each. Chloramphenicol (a gift from Parke, Davis & Co.) was used at a final concentration of 25 μg/ml, and tetracycline (a gift from Bristol Laboratories) was used at a final concentration of 12.5 μg/ml.

Mating experiments. Overnight cultures of the strains to be mated were diluted 1/20 in fresh L broth and were grown for 2.5 hr at 37 C on a shaker. The cultures were mixed in equal parts, placed at 37 C, and gently agitated for 15 min. Portions were then plated directly, or diluted and plated on the appropriate selective medium.

Mutant isolation. The *drd* mutants used in this study were isolated by a modification of the multiple-liquid mating technique developed by Edwards and Meynell (3). A typical isolation experiment is given below and an explanation for each step is given in Table 3.

Culture tubes containing 1 ml of L broth were inoculated with approximately 20 bacteria of strain UC5020 and were left standing overnight without aeration at 37 C. The cells were harvested by centrifugation, suspended in 1 ml of mutagenesis medium consisting of 1 mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml in tris(hydroxymethyl)aminomethane (Tris)-maleic buffer, *p*H 6.0 (1), and shaken for 30 min at 37 C. An 0.2-ml sample was then removed and washed on a membrane filter (0.45-nm pore size; Millipore Corp., Bedford, Mass.) with 5 ml of Tris-maleic buffer. The cells were suspended in 1 ml of L broth, grown at 37 C until a cell density of 3×10^8 to 5×10^8 cells/ml was obtained, and then mixed with 0.1 ml of strain UP1727 (3×10^9 to 5×10^9 cells/ml). After mating for 15 min, the cell mixture was diluted with 3 ml of 0.8% saline, separated from the filter disc, and then centrifuged and suspended in minimal glucose medium supplemented to permit only the growth of the recipient strain UP1727. These cultures were shaken at 37 C for 8 to 10 hr (2×10^9 cells/ml),

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TABLE 1. *Bacterial strains used*[a]

Strain	Important properties[b]	Source[c]
Escherichia coli		
DC487 B	*met, gal, recA1, r^-, su^-*	D. P. Kessler
HB45 B/r	*thr, leu, pro, try, his, arg, met, Sm^r, su^-*	D. P. Kessler
J5-3 K12	*pro, met, su^+* amber	E. Meynell
JE254 K12	R100/*met*	Y. Hirota
KL16-99 K12	Hfr, B_1, *recA1*	B. Low
SB5004 B/r	*leu, arg, su^-*	D. P. Kessler
UP1727 B/r	*thr, leu, tyr, su^-*	D. P. Kessler
UC1 K12	*try* AE1, *su^-*	D. P. Kessler
UC3 K12	*try* AE1, *su^+ II* amber	C. Yanofsky
UC7 K12	*try* AE1, *su^+ C* ochre	C. Yanofsky
UC17 K12	*his, arg, Sm^r, su^+* amber	UCD Bacteriology Collection
UC31 K12	R100/J5-3	J5-3 × JE254
UC46 K12	*pro, met, gal^-, su^+* amber	J5-3 by DES mutagenesis
UC59 K12	*pro, met, gal^-, his su^+* amber	UC46 by DES mutagenesis
UC61 K12	*pro, met, gal^-, su^+* amber, *recA1*	UC59 × KL16-99
UC93 K12	UCR12/UC61	UC61 × UC137
UC133 K12	F^1*gal^+, su^+ 812* ochre/UC61	UC61 × SL4040
UC134 K12	UCR/UC61	UC61 × UC136
UC136 K12	UCR4/UC17	UC17 × UC141
UC137 K12	UCR12/UC17	UC17 × UC147
UC141 K12	UCR4/J5-3	Spontaneous from UC31
UC147 K12	UCR12/J5-3	By P1 trandsuction from UC31
UC5020 B/r	UCR4/HB45	HB45 × UC141
UC5021 B/r	UCR12/HB45	HB45 × UC147
UC5022 B	UCR12/DC487	DC487 × UC141
UC5023 B	UCR4/DC487	DC487 × UC141
Salmonella typhimurium		
SL4040 LT2	F^1*gal^+, su^+ 812* ochre/*met, try, gal^-, Sm^r*	P. Vary

[a] Amino acid requirements: *arg*, arginine; *leu*, leucine; *pro*, proline; *try*, tryptophan; B_1, vitamin B_1; *his*, histidine; *met*, methionine; *tyr*, tyrosine; *thr*, threonine.
[b] *Gal^-* or *gal^+*, inability or ability, respectively, to utilize galactose as a carbon source; r^-, inability to restrict foreign deoxyribonucleic acid; *Sm^r*, chromosomal resistance to streptomycin; *recA1*, mutation preventing formation of normal recombinants; *su^-*, lacks suppressor activity; *su^+* amber, carries amber suppressor; *su^+* ochre, carries ochre suppressor.
[c] UCD, University of California, Davis; DES, diethyl sulfate.

TABLE 2. *R factors and their derivatives*[a]

R factor	Resistance markers	Origin
R100	*Cm^r, SMA^r, Tc^r*	Y. Hirota JE254
UCR4	— *SMA^r, Tc^r*	Spontaneous from R100
UCR7	— — *Tc^r*	Spontaneous from R100
UCR12	*Cm^r, SMA^r* —	P1 transduction from R100
R100 *drd-49* through R100 *drd-62*	Constitutive derivatives of UCR7 in UC17	NTG mutagenesis
R100 *drd-63* through R100 *drd-78*	Constitutive derivatives of UCR12 in UC17	NTG mutagenesis
R100 *drd-79* through R100 *drd-93*	Constitutive derivatives of UCR4 in SB5004	NTG mutagenesis
R100 *drd-94* through R100 *drd-102*	Constitutive derivatives of UCR12 in SB5004	NTG mutagenesis

[a] Abbreviations: *Cm^r*, resistance to chloramphenicol; *SMA^r*, resistance to both streptomycin and sulfanilamide; *Tc^r*, resistance to tetracycline; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; *drd*, derepressed mutant.

TABLE 3. *Protocol for mutant isolation*

Step in protocol	Explanation
(1) UC5020 (containing UCR4) diluted to 20 cells/tube	Reduces possibility of having same spontaneous mutant in all tubes
(2) Overnight standing culture—no aeration	Provides low density cultures (approximately 5×10^8)
(3) Mutagenesis and taking 0.2-ml samples	Survival from mutagenesis is approximately 1%, and 0.2 ml would contain about 10^6 viable cells. If *drd* mutations occur at a frequency of 10^{-6}, on the average each tube should have one mutant.
(4) Growth period in L broth	Survivors grow after a long lag (density 3×10^8 to 5×10^8). Complex medium will not select against chromosomal mutations resulting in auxotrophy.
(5) UC5020 mating with UP1727	The *drd* mutants mate at a high frequency, whereas nonmutant R factors give recombinants at a frequency of 10^{-3} to 10^{-5} per donor.
(6) Growth period (8–10 hr)	UP1727 has a long lag period following the shift from L broth to supplemented minimal medium. The *drd* R factors contained by some UP1727 cells can infect a large number of the uninfected cells during this period. The nonmutant R factors, although infectious initially, lose fertility during prolonged growth.
(7) SB5004 mating with mixed population of UC5020 and UP1727	Short mating period selects for high-frequency transfer of the mutant R factor from UP1727 to SB5004.
(8) Final plating	Selects for SB5004 that has received the mutant R factor. All other donors and recipients will not grow.

after which 0.2-ml samples were mated for 15 min with an equal volume of strain SB5004 that had previously been grown in supplemented minimal medium to about 8×10^8 cells/ml. Mating mixtures were diluted (10^{-1} and 10^{-2}) and plated on minimal agar selecting for Tc^r recombinants of SB5004. These colonies were tested for constitutive pili synthesis as described below. The mutants were then purified once and stored in L-agar deeps. Only one mutant was retained per mutagenized culture.

Test for derepression. The suspected *drd* mutants were streaked across an L-agar plate at right angles to an area seeded with MS-2 phage (titer 3.2×10^{11}, obtained from C. E. Dowell). The *drd* mutants were partially lysed in the area where the streak contacted phage. When hetero-R clones were tested for MS-2 sensitivity, both chloramphenicol and tetracycline were added to the L-agar plates to maintain selection for nonsegregants.

Test for dominance. Two R factors of the same type cannot exist stably in the same cell (8). When two similar R factors are forced to remain together, by selection with drugs, they will yield stable recombinants (8, 15). Unpublished work done in this laboratory indicates that the presence of the *recA1* mutation reduces to an undetectable level recombination between R factors; thus, stable hetero-R clones can be constructed and maintained on the appropriate selective medium.

The *drd* mutants were mated with *recA1* containing either strain UC61 or DC487, each of which carries a derivative of R100 having a drug pattern complementary to that of the mutant being tested (UC93, UC134, UC5022, UC5023). Hetero-R recombinants were constructed by selecting for chloramphenicol and tetracycline-resistant colonies on minimal medium selective for the recipient used.

Segregation analysis. Ten hetero-R recombinants were tested for segregation to confirm the hetero-R state. The strains were inoculated into L broth, grown overnight, and streaked onto L-agar plates. About 300 of the resulting colonies were then tested on L-agar containing either tetracycline or chloramphenicol, or both drugs together. Segregants were tested for MS-2 sensitivity as described earlier.

Test for suppression. The mutant R factors R100 *drd-79* through R100 *drd-102* were isolated in strains that are su^-. These *drd* R factors were transferred by conjugation to UC133 (su^+amber, su^+ochre) and tested for MS-2 sensitivity by cross-streaking on L-agar. Any R factor showing some suppressor response (i.e., resistance to MS-2 phage) was crossed to UC61 (su^+amber), UC3 (su^+II), UC7 (su^+C), and UC1 (su^-) and rechecked for phage sensitivity. The cross to UC1 served as a control.

Transduction with phage P1kc. P1 transduction was done by the methods of Watanabe et al. (14). Transduction of R factors with phage P1 generally results in the transduction of the complete R factor, and partial transductants are rare (14).

RESULTS

Dominance test. Of a total of 54 mutants characterized by using the dominance test for MS-2 phage sensitivity, 40 were recessive to the wild-type R factor and the remaining 14 were dominant. Table 4 illustrates the results obtained for

one mutant of each type. Line 4 shows the behavior of a recessive mutant; its response is characteristic of that class of mutants that might produce an inactive regulatory gene product. Line 5 shows the results obtained with a dominant mutant. This response is typical of mutants having a modified operator element.

The segregation analysis for mutants *drd-52* and *drd-56* is given in lines 4 and 5 of Table 4. These data are representative of those obtained with the other mutants tested.

Suppression test. Of the twenty-four mutants tested for suppressor sensitivity, four were sensitive (see Table 5). None of the mutants characterized as operator constitutives were sensitive to the suppressors used.

Further analysis of o^c-like mutants. Two o^c mutants, *drd-56* and *drd-59*, were checked to determine whether they still produced a functional repressor. MS-2 resistant derivatives of these two mutants were isolated in the host UC61 (*recA1*). [We presume the mutation to resistance is localized in the pili structural gene(s).] If the repressor is functional in these o^c pil^- R factors, then it should inhibit expression of an intact pili gene located on an R factor carrying a repressor-negative mutation. Hetero-R recombinants having the genotype I^+ o^c56 pil^- Cm^s $Tc^r/I67$ O^+ pil^+ Cm^r Tc^s were constructed and found to be MS-2 resistant; thus the repressor gene(s) *cis* to these o^c mutations is intact.

Two control experiments were carried out to establish whether the mutation to MS-2 resistance was episomal. In the first experiment a hetero-R strain of the genotype I^+ o^c56 pil^- Cm^s Tc^r/I^+ o^c63 pil^+ Cm^r Tc^s was constructed and found to be MS-2 sensitive; this indicates that the host does not interfere with the synthesis of pili. The second experiment involved the P1-mediated transduction of the R factor from the MS-2-resistant strain back into the original host, UC61. Of the 20 Tc^r transductants tested, all were MS-2-resistant. These experiments are consistent with the hypothesis that the MS-2-resistant mutation is localized in the pili structural gene(s).

TABLE 5. *Results of suppression test*

Mutants	MS-2 response in strain[a]			
	su^-	su^+am[b]	su^+II[c]	su^+C[d]
3	s	s	s	s
drd-81	s	r	r	r
drd-99	s	r	r	r
drd-79	s	s	s	r
drd-96	s	s	s	r

[a] s, Sensitive; r, resistant to MS-2 phage.
[b] This suppressor in strain UC61 has a suppression pattern like su^+I.
[c] See Table 1 for the suppressor type.
[d] Twenty-four mutants were tested; 20 of these did not respond to any suppressor. All eight o^c-like mutants were in this grouping.

DISCUSSION

It is apparent from the results obtained by using the classical *cis-trans* test that the operator constitutive-like *trans*-dominant class of derepressed fertility mutants exists. The occurrence of nonsense-suppressor-sensitive mutants in the *trans*-recessive class of derepressed mutants indicates that these mutants are defective in a protein (11). These data are consistent with the proposed operator-repressor model for regulation of fertility by control of pili synthesis.

The use of the hetero-R state in a *recA* host has been very useful in studying the *cis-trans* responses of the regulatory genes for pili synthesis. The data obtained from segregation analysis of hetero-R clones demonstrate that the genetic properties of the individual R factors are un-

TABLE 4. *Results of dominance test for MS-2 phage sensitivity*

Haploid or diploid condition		Analysis of R-factor marker segregation				
Genotype	MS-2 response	Tc^r Cm^r (parental)	Tc^r	Cm^r	MS-2 sensitivity, of segregants: MS-2-sensitive Tc^r (Tc^r tested)	MS-2 sensitivity, of segregants: MS-2-sensitive Cm^r (Cm^r tested)
(1) I^+, 0^+	r					
(2) *152*, 0^+	s					
(3) I^+, o^c56	s					
(4) *152*, 0^+, Cm^s, Tc^r/I^+, 0^+, Cm^r, Tc^s	r	34	63	181	63/63	0/48
(5) I^+, o^c56, Cm^s, Tc^r/I^+, 0^+, Cm^r, Tc^s	s	37	72	227	72/72	0/68

changed, and recombination is not a problem when the hetero-R state is forced in a *recA* host. The high proportion of Tc^r segregants occurs consistently in hetero-R clones formed with UCR12. This may be due to an alteration of the superinfection immunity properties of this derivative of R100.

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