JOURNAL OF BACTERIOLOGY, Oct. 1975, p. 576-577

Vol. 124, No. 1
Printed in U.S.A.

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

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Received for publication 5 May 1975

The ability of a wide range of bacteriophage-resistant mutants to act as recipients in conjugation with F'*lac pro* and R100-1 donors has been studied. A number of mutant types defective in recipient ability with F'*lac pro*, as well as mutants which were hyperreceptive with R100-1, have been detected.

It has been recently shown that a number of mutants isolated as resistant to certain bacteriophages are reduced in their ability to act as recipients in conjugation with F' and Hfr strains (3, 4, 7). The Con mutants have been shown to lack protein 3a (7; Manning and Reeves, manuscript in preparation), using the nomenclature of Schnaitman (6). The type A and B conjugation-defective mutants of Reiner (4), selected as resistant to single-stranded deoxyribonucleic acid phage, probably have alterations in their lipopolysaccharide (LPS), as do the conjugation-defective mutants of Monner and co-workers (3), which were selected as ϕW-resistant mutants of an ampicillin-resistant strain. Lugtenberg and associates (personal communication) have isolated conjugation-defective mutants by selecting for resistance to phages T3, T4, and T7. These mutants have altered cell walls, their LPS being defective in heptose and there being reduced amounts of outer membrane proteins. Thus we have a precedent for a number of mutants, selected as phage resistant and having altered cell walls, being defective in conjugation. In this study we tested the type strains of all the bacteriophage-resistant mutant classes isolated in a recent study (2). Their recipient ability was assessed using both F'*lac pro* and R100-1-bearing donor strains. We were interested in F' and R100-1 transfer, since the Con mutants were not defective with respect to the R100-1 factor, but were with F' and Hfr strains (7).

The strains used were all derivatives of *Escherichia coli* K-12 and are listed in Table 1. The donor strains (CSH23 and JC6256/R100-1) were used as standing overnight cultures grown in nutrient broth at 37 C and diluted to 2×10^8 cells/ml. The recipient strains were grown in nutrient broth with vigorous aeration at 37 C, for at least four generations, to 2×10^8 cells/ml. A volume of 0.1 ml of donor culture was added to 1.0 ml of recipient culture and incubated for 30 min at 37 C, after which the mating mixture was diluted and plated out. F'*lac pro* transfer was measured on minimal plates selecting for proline synthesis and utilization of lactose (1%) as carbon source and with streptomycin (100 μg/ml) for contraselection. R100-1 transfer was measured on nutrient plates (Difco, blood base agar) containing 20 μg of tetracycline and 1,000 μg of streptomycin per ml.

The results of the various matings, each of which is the average of three or more experiments, are summarized in Table 2.

If we consider 50% of the number of recombinants formed with the parent to be the cut-off point, then both the Wrm mutants and all Bar

TABLE 1. *Bacterial strains used*[a]

Strain	Characteristics	Source
AB1133	F⁻/*thi,argE,his,proA,thr,leu, mtl,xyl,ara,galK,lacY,str, supE*,λ⁻	B. Bachmann
P400	*his*⁺*non*9 transductant of AB1133	R. E. W. Hancock (2)
CSH23(E5014)	F'*lac*⁺ *proA*⁺,*B*⁺/Δ(*lac pro*), *supE,spc,thi*	Cold Spring Harbor
JC6256/R100-1	R100-1/*trp,lac*Δ	N. Willetts

[a] All mutations for bacteriophage resistance were selected in P400 and are described in Hancock and Reeves (2).

TABLE 2. *Recipient ability of the bacteriophage-resistant mutants for transfer of F'lac pro and R100-1 plasmids*

Mutant classes[a]	Mutant	F'*lac pro* transfer[b]	R100-1 transfer[b]
Bar 1	P455	1.08	1.11
2	P492	0.44	1.85
3	P495	0.19	3.92
4	P436	0.24	2.59
5	P402	0.16	2.30
6	P451	0.45	1.21
7	P487	0.15	1.63
8	P489	0.017	3.11
Bfe	P445	0.70	0.58
Con	P460	0.0005	1.10
Efr	P448	0.89	1.14
Ktn[c]	P466	1.28	0.77
Ktw 1	P456	0.96	3.30
2	P476	1.12	1.65
3	P240	0.40	2.66
Misc 1	P491	1.33	0.69
2	P443	1 12	1.66
3	P498	1.11	0.53
4	P237	1.00	0.52
5	P493	1.13	0.83
Ton A	P417	0.74	0.63
B	P442	1.14	0.38
Tsx 1	P407	0.69	0.68
2	P433	0.93	1.68
Ttk 1	P429	1.05	0.66
2	P423	0.88	1.71
3	P425	0.86	1.03
4	P474	1.15	3.76
Wrm 1	P435	0.079	6.65
2	P424	0.34	2.99

[a] The mutant classes are defined in reference 2.

[b] Transfer was measured as a percentage of input donor cells and given with respect to P400, the parent recipient strain, which is taken as 1. F'*lac pro* gave 25.5% transfer and R100-1 gave 9.86% transfer to P400. All matings were carried out at least three times.

[c] Ktn is now believed to be a typical λ-resistant mutant.

mutants other than Bar (1), as well as the Con and Ktw (3) mutants, are conjugation defective with respect to F'*lac pro*. All these mutants, with the exception of Con mutants, have LPS alterations, and are thought to be receptor mutants defective in the adsorption of phage which use regions of the LPS as their receptor (R. E. W. Hancock, Ph.D. thesis, Univ. of Adelaide, Australia, 1974; Hancock and Reeves, manuscript in preparation). The only apparent structural defect in the Con mutants is the lack of protein 3a (7; Manning and Reeves, unpublished data) and these mutants have been shown to be defective in the adsorption of phage K3 (7).

The defect in recipient ability of these two classes of mutants differs in that the LPS mutants are not as defective with respect to F' transfer as are the Con mutants, while their recipient ability with respect to R100-1 is increased and that of the Con mutants is normal.

The results suggest that there are two classes of cell wall-defective mutants, altered in either LPS or protein composition, which differ in the nature of their defect in recipient ability. In the case of the Con mutants, the evidence suggests that mating pair formation is the stage affected (7). It is not shown at what stage of conjugation the defect occurs in conjugation-defective mutants with LPS alterations, but, since the LPS is a cell wall constituent, we suggest they may also be affected in the early stages of conjugation, specifically the formation of either preliminary or effective mating pairs.

The results also confirm the difference in specificity of recipient ability for F' and R100-1 factors.

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