JOURNAL OF BACTERIOLOGY, June 1978, p. 875-883
0021-9193/78/0134-0875$02.00/0

Vol. 134, No. 3
Printed in U.S.A.

Characterization of *Pseudomonas aeruginosa* Mutants Deficient in the Establishment of Lysogeny

ROBERT V. MILLER* AND CHAO-MIN C. KU

Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37916

Received for publication 15 September 1977

Mutants of *Pseudomonas aeruginosa* with impaired ability to establish a lysogenic relationship with temperate bacteriophage (Les$^-$) have been isolated. These *les* mutations map to two areas of the *P. aeruginosa* chromosomal map as determined by conjugational and transductional analyses. Two phenotypic classes of Les$^-$ mutants were identified. One class of mutations has pleiotropic effects on DNA metabolism. These mutants are unable to recombine genetic material acquired as a result of either conjugation or transduction (Rec$^-$). In addition, the ability of these Les$^-$ Rec$^-$ mutants to repair UV-induced damage to bacteriophage is reduced (host-cell reactivation deficient, Hcr$^-$). Mutants of the second class are Les$^-$, Rec$^+$, and Hcr$^+$.

In 1966, Holloway reported the existence of a phenotypic class of *Pseudomonas aeruginosa* which exhibited the inability to be lysogenized by temperate bacteriophage (Les$^-$). Holloway's original report indicated that recombination in these mutants was reduced after both conjugation and transduction (8, 21). Recombinationally deficient (Rec$^-$) mutants of *P. aeruginosa* were sought as mutants deficient in the ability to undergo lysogeny, on the argument that a bacterial recombination function might be necessary for the integration of prophage. It is now recognized that the discovery of Les$^-$ Rec$^-$ mutants was fortuitous, since the integration of many prophages, such as λ in *Escherichia coli*, into the host genome appears to be solely a function of the phage genome (20). Furthermore, Rec$^-$ mutants of *E. coli* can be lysogenized with the same frequency as those of the Rec$^+$ form (3). Holloway suggested that it is more likely that changes in the substrate of the enzyme(s) involved in recombination cause the altered recombination phenotype of these mutants rather than changes in the enzymes themselves (9).

The deficiency in the establishment of lysogeny provides an approach to the examination of the cellular mechanisms for dealing with newly acquired DNA elements. To date, very few examples of host control of the establishment of prophage have been reported (11). Previous research was focused on the extrachromosomal factor's influence on its own establishment (1, 6). Exploitation of the Les$^-$ phenotype will allow examination of the host cell's contribution to the establishment of extrachromosomal elements and their maintenance. To this end, we have isolated Les$^-$ mutants, characterized them to phenotypic classes, and mapped them genetically.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. The strains of *P. aeruginosa* used in these experiments are shown in Table 1. All were derived from strain PAO (9). F116, G101, and D3 lysates were obtained from B. Holloway (7, 8). Clear-plaque mutants of each of the phages were selected from the lysates.

Chemicals. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was from Aldrich Chemical Co., Inc., Milwaukee, Wis. All amino acids were from Eastman Kodak Co., Rochester, N.Y. All inorganic chemicals were from Fisher Scientific Co., Fair Lawn, N.J. Tryptone, yeast extract, and agar were from Difco Laboratories, Detroit, Mich. Streptomycin sulfate was from Sigma Chemical Co., St. Louis, Mo.

Growth and culture conditions. Cultures were grown in Luria complete broth (LB: 15 g of tryptone [Difco], 5 g of yeast extract, 10 g of NaCl, 80 mg of NaOH, and 1 liter of water) or plated on Luria agar (L-agar). L-agar was prepared by adding 15 g of agar to 1 liter of LB. Bacterial matings were analyzed by using selective media which were prepared with *Pseudomonas* minimal medium [PMM: 7 g of K_2HPO_4, 3 g of KH_2PO_4, 0.5 g of sodium citrate, 1 g of $MgSO_4 \cdot 7H_2O$, 1 g of $(NH_4)_2SO_4$, 4 g of glucose, and 1 liter of water] supplemented with the appropriate growth requirements. Amino acids were supplied at 50 μg/ml, and nucleotides were supplied at 20 μg/ml. Streptomycin was used at a concentration of 650 μg/ml, and $HgCl_2$ was used at a concentration of 11.5 μg/ml. Liquid cultures were grown at 37°C in a shaking water bath, and plates were incubated at 37°C.

Preparation of phage stocks. Phage stocks were prepared by plating sufficient phages to give confluent lysis of an indicator strain (PAO1) on L-agar plates overlaid with 2.5 ml of lambda top agar (10 g of tryptone, 5 g of NaCl, 6.5 g of Difco agar, and 1 liter of

TABLE 1. *Bacterial strains*

Strain	Genotype[a]	Reference
PAO1	Prototrophic, FP$^-$	9
PAO283	*his-3 lys-56 met-28 trp-6* FP$^-$	18
PAO303	*argB18* FP$^-$	18
RM5	*argB18 arg-304*[b] *lesB905* FP$^-$	This paper
RM7	*argB18 lesB907* FP$^-$	This paper
RM8	*argB18 lesB908* FP$^-$	This paper
RM40	*lys-56 met-28 trp-6 pur-600 str-901* FP$^-$	This paper[c]
RM224	*lys-56 met-28 trp-6 pur-600 str-901 lesA924* FP$^-$	This paper
RM231	*lys-56 met-28 trp-6 pur-600 str-901 les-931* FP$^-$	This paper
RM235	*lys-56 met-28 trp-6 pur-600 str-901 lesA935* FP$^-$	This paper
OT101	*his-6 ilvB112 leu-1 str-2* FP$^-$	14
OT94	*ilvB112 leu-1 ser-5 str-2* FP$^-$	14
JC9005	*pur-600* FP2$^+$	17
PAO381	*leu-38 str-2* FP2$^+$	18
RM18	*argB18 lesB908* FP2$^+$	This paper
RM21	*argB18 lesB907* FP2$^+$	This paper
RM23	*argB18 arg-304 lesB905* FP2$^+$	This paper
RM209	*argB18* FP2$^+$	This paper
RM225	*lys-56 met-28 trp-6 pur-600 str-901 lesA924* FP2$^+$	This paper
RM232	*lys-56 met-28 trp-6 pur-600 str-901 les-931* FP2$^+$	This paper
RM236	*lys-56 met-28 trp-6 pur-600 str-901 lesA935* FP2$^+$	This paper
RM240	*lys-56 met-28 trp-6 pur-600 str-901* FP2$^+$	This paper

[a] FP$^+$ designates a donor in conjugation. FP2 sex factor confers resistance to mercuric salts on bacterial strains. FP$^-$ designates a recipient strain. Abbreviations: *arg*, arginine; *his*, histidine; *ilv*, isoleucine-valine; *les*, lysogeny-establishment; *leu*, leucine; *lys*, lysine; *met*, methionine; *pur*, purine (adenine); *ser*, serine; *str*, streptomycin; *trp*, tryptophan. When present in a genotype, these abbreviations indicate auxotrophy for the amino acid or purine. When *str* is present in the genotype, the strain is resistant to this antibiotic, and when *les* is present the strain is lysogeny-establishment deficient.

[b] *arg-304* is a mutation in a gene which codes for an enzyme late in arginine biosynthesis. Ornithine will not fulfill the requirement for arginine for strains carrying *arg-304*. Therefore, this mutation may be in *argF*, which maps later than 50 min (6). *arg-304* arose as a secondary mutation in RM5 following the nitrosoguanidine mutagenesis of PAO303, which yielded *lesB905*.

[c] RM40 is an exconjugant from a cross between JC9005 and PAO283.

water). After overnight incubation, the top-agar layer was removed and placed into a tube with 5 ml of LB. After centrifugation (5,000 × *g*, 10 min) to remove agar and cellular debris, the phage suspensions were passed through membrane filters (0.45-μm mean pore size; Millipore Corp.) to eliminate bacteria. Phage stocks were stored at 4°C.

Mutagenesis and selection of Les$^-$ mutants. Strains were grown overnight in 8 ml of LB at 37°C, diluted with fresh LB to a turbidity reading of 10 Klett units (read at 660 nm), and reincubated at 37°C in a shaking water bath until the cells were in logarithmic growth phase (ca. three generations; 2 to 4 h). These cultures were centrifuged (5,000 × *g*, 10 min) and resuspended in citrate buffer (0.1 M, pH 5.5). *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1,000 μg/ml) was added to a final concentration of 100 μg/ml, and cultures were incubated for 30 min. Cells were centrifuged, resuspended in LB, and incubated for 4 additional h with shaking. Cultures were diluted to a concentration which gave 100 colonies per plate and were then plated on L-agar plates. Plates were incubated for 48 h before colonies were picked for testing for Les$^-$ phenotype.

To test for the Les$^-$ phenotype, isolated colonies of bacteria were picked and streaked with sterile toothpicks perpendicularly to streaks of phages (10^9 plaque-forming units per ml) made with a 0.2-ml pipette on L-agar or selective medium plates. After 12 to 15 h of incubation, the Les$^-$ phenotype could be recognized by the reduced growth at the phage-bacterial intersection (8).

Efficiency of lysogenization of Les$^-$ strains. Strains to be tested for their ability to be lysogenized by temperate phages were grown overnight, diluted, reincubated for 2 to 4 h, centrifuged, and resuspended in TNM buffer [15 mM NaCl, 10 mM $MgSO_4 \cdot 7H_2O$ in 10 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.4]. The cells were mixed with the phage at various concentrations and incubated at 37°C for 10 min. Cells were then sedimented and resuspended in medium 56/2 [0.1 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $(NH_4)_2SO_4$, 5 mg of $Ca(NO_3)_2$, and 0.25 mg of $FeSO_4 \cdot 7H_2O$ per liter of 0.1 M sodium-potassium phosphate buffer, pH 7.0], diluted, and plated on L-agar plates. After overnight growth at 37°C, colonies were counted and survival was determined. In addition, the titer of the phage preparation used was checked so that the exact multiplicity of infection (MOI) for each concentration of phage used could be determined. Fifty colonies were picked from the survivors at each MOI, and their lysogeny phenotype was determined by cross-streaking them against the original infecting phage. Lysogenic isolates gave no killing response when streaked across phage, whereas nonlysogenized isolates showed reduced growth at the cell-phage intersection.

Radiation sensitivity (UV and X-ray). In quantitative tests for UV sensitivity, strains to be tested were grown overnight, diluted in fresh medium, and reincubated for 2 to 4 h. The entire 5 ml of prepared culture was centrifuged (5,000 × *g*; 10 min), resuspended in 5 ml of medium 56/2, and transferred to a glass petri dish. The cells were exposed to UV irradiation (at 40 ergs cm^{-2} s^{-1}) by using a General Electric germicidal lamp for various periods of time. After exposure to UV, samples were removed, diluted in medium 56/2, and plated on L-agar plates. Care was taken to avoid photoreactivation by incubating plates in the dark. Surviving colonies were counted after 24 h. X-ray sensitivity was determined by following the same procedure. X irradiation was performed with a GE Maxitron 300 at 160 kV, 20 mA at a distance of 22 cm. No filtration was used. Samples were irradiated with 4,000 R/min for 2.5, 5.0, and 7.5 min.

Determination of host cell reactivation. A lysate (10^8 plaque-forming units per ml) was prepared by diluting a stock lysate of a clear-plaque mutant of phage D3 with medium 56/2. This lysate was exposed to UV irradiation (40 ergs cm^{-2} s^{-1}) for various periods of time. After irradiation, dilutions of the lysate and

Les⁻ mutant strains were added to an overlay of lambda top agar, which was poured onto an L-agar plate and incubated overnight. The repair of the UV-damaged phage was measured by plaque-forming ability on the strains tested (7).

Bacterial matings and determination of recombinational proficiency. Cultures of donor and recipient strains in logarithmic growth phase were harvested, washed in medium 56/2, and resuspended in medium 56/2 at a concentration of ca. 8×10^8 cells per ml. One milliliter of the donor suspension was added to 1.2 ml of the suspension of recipient cells and incubated at 37°C. Most matings were uninterrupted plate matings. For these experiments, the mating mix was allowed to incubate for 30 min at 37°C before plating. When interrupted matings were performed, the mating pairs were interrupted at appropriate times by agitating for 2 min at the top speed of a Vortex Genie mixer (Scientific Industries, Springfield, Mass.). The original mating mixture and dilutions of 10^{-1} and 10^{-2} were prepared in 56/2 buffer, and 0.1-ml samples were plated on selective agar plates. Streptomycin (750 μg/ml) was used as a contraselecting agent where possible. In those matings where streptomycin could not be used, contraselection was by the omission of a donor nutritional requirement (adenine for JC9005; methionine for RM225, RM232, RM236, and RM240; and leucine for PAO381). Control samples of the donor and recipient strains were also plated on selective plates at the same dilutions. Plates were incubated at 37°C for 24 to 48 h and scored for recombinants.

Determination of recombinational ability after transduction and transductional analysis. Strains were grown overnight, diluted, and reincubated for 2 to 4 h until logarithmic growth had been initiated (ca. three generations). The cultures were then centrifuged and resuspended in TNM buffer. A portion (0.2 ml) of the cell suspension was added to a volume of phage F116 (10^9 to 10^{10} plaque-forming units per ml) at a multiplicity of 40 phage particles per cell (2). These mixtures were incubated for 15 min in TNM buffer, and 0.1-ml samples were plated on selective medium plates. The plates were incubated at 37°C for 48 to 72 h and scored for transductants.

Determination of percent viability. A Petroff-Hausser bacterial counting chamber was used to determine the total number of cells in a milliliter of culture medium under microscopic observation. Viable cell counts were carried out by diluting and plating on L-agar plates. The ratio of the concentration of viable cells to the concentration of total cells was determined. In each experiment, duplicate dilutions were prepared and each dilution was plated in triplicate.

RESULTS

Mutant isolation. Two strains derived from strain PAO of *P. aeruginosa* were mutagenized to obtain Les⁻ mutants. PAO303 is an arginine auxotroph carrying the allele *argB18*. RM40 is a multiple auxotrophic strain requiring adenine (*pur-600*), methionine (*met-28*), tryptophan (*trp-6*), and lysine (*lys-56*). Approximately 6,600 survivors of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine treatment from each strain were screened for their ability to be lysogenized by cross-streaking against phage D3. Thirty-eight Les⁻ mutants were isolated. Three nonsibling mutants from RM40 (RM224, RM231, and RM235) and three derived from PAO303 (RM5, RM7, and RM8) were used for further studies.

Efficiency of lysogenization of Les⁻ strains. The Les⁻ strains were subjected to infection by the temperate phages D3, G101, and F116 at various MOI ranging from 0.5 to 38 phage per bacterium, and the percentage of surviving cells lysogenized was determined. At each MOI the fraction of cells surviving was determined, and the survivors were then scored (by cross-streaking against the phage with which they were originally infected) to determine whether they were lysogenized (Fig. 1). All of the Les⁻ mutants except RM231 demonstrated reduced ability to be lysogenized as compared with the parental strains.

Two phenotypic subclasses were recognized among the mutants deficient in the establishment of lysogeny with respect to their ability to be lysogenized at a higher MOI. The first of these classes exhibited little or no lysogenization (absolute Les⁻). The second class demonstrated decreased ability to be lysogenized when compared to the parent but supported significant levels of lysogenization at higher MOI (intermediate Les⁻). The response of these mutants was dependent upon the infecting phage. Thus, "absolute" or "intermediate" designations of a particular Les⁻ mutant were defined only with respect to a specific phage. Each of these experiments was repeated at least four times, and the designations, which are evident from Fig. 1, with respect to each phage did not vary. All Les⁻ strains were found to exhibit absolute Les⁻ characteristics when infected with phage F116. Strains RM7, RM8 and RM224 were absolute and strains RM5 and RM235 were intermediate when infected with bacteriophage G101. Strains RM5 and RM224 were absolute when infected with phage D3 while strains RM7, RM8, and RM235 were in the intermediate class with respect to this phage.

Strain RM231 demonstrated a more complicated response. This strain appeared to be resistant to phage G101 (therefore not included in Fig. 1C) and showed high levels of survival after infection with the other phages (Fig. 1A and E). There was no apparent reduction in the efficiency of lysogenization as compared with the parent (RM40) when the data were graphed as in Fig. 1. However, the percentage of total lysogenized cells (percent survivors lysogenized × percent survival) of RM231 at various MOI for

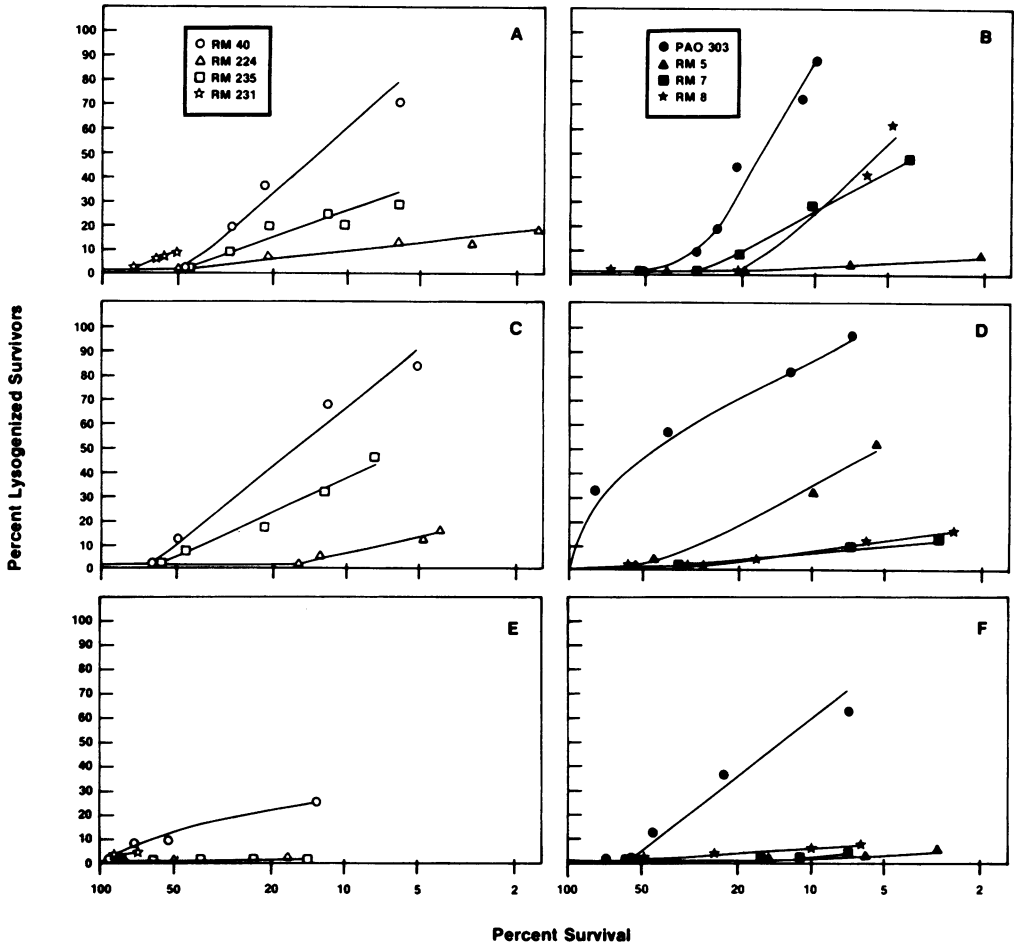

FIG. 1. *The efficiency of lysogenization of Les⁻ mutants. Mutant strains were infected with temperate phage at various MOI, and the percentage of surviving cells lysogenized was measured and compared to the parental strains. (A) and (B) are representative data for infection with phage D3; (C) and (D) are representative data for infection with phage G101; and (E) and (F) are representative data for infection with phage F116.*

D3 or F116 was lower than that of its parent strain RM40 (Fig. 2).

Viability of Les⁻ strains. We observed that the Les⁻ mutant strains grew more slowly than did their parents and other wild-type strains. This led us to investigate the possibility that the viability of these strains is reduced. The viability of Les⁻ strains and their parents was assayed as described above. The viability of strains RM7, RM231, and RM235 was reduced 44, 50 and 62%, respectively, when compared with their Les⁺ parents. The viability of the other Les⁻ strains was not significantly reduced.

Chromosomal localization of *les* mutations. Preliminary mapping of *les* mutations was undertaken by measurement of coinheritance of the donor Les phenotype with selected nutritional markers after conjugation (Table 2). The mutations *les-924* and *les-935* are coinherited at a high frequency with markers around 20 min on the *P. aeruginosa* map (19); whereas *les-905, les-907,* and *les-908* are coinherited with *leu-1* (=*leu-38,* A. Emerich and A. J. Clark, personal communication) at 48 min (5). In the case of *les-905,* a high linkage with *arg-304* which enters later than *leu-38* in conjugation was also observed.

Transducing lysates of phage F116 were produced on each of the Les⁻ strains. These were used to transduce strain OT101. *les-905, les-907,* and *les-908* are 60, 53, and 33% cotransducible with *leu-1,* respectively. *les-924* and *les-935* are not cotranducible (0%) with *leu-1.*

In the experiments described above, when the donor strain was Les⁺ in matings involving any of the Les⁻ mutants, the Les⁺ phenotype of the

donor was coinherited from 84 to 98% with mercury resistance (determined by genes on the plasmid FP2; 13). In the reverse situation (i.e., Les⁻ donor and Les⁺ recipient), no inheritance of the Les⁻ phenotype of the donor with mercury resistance was found. This was true even when 10-min interrupted matings were performed and may indicate the presence of a suppressor-like activity with respect to Les⁻ phenotype. This suppressor-like activity may artificially increase the apparent coinheritance of Les⁺ with other chromosomal markers in crosses between Les⁺, FP2⁺ donor, and Les⁻, FP2⁻ recipient strains. Loutit has found that FP2 is concomitantly inherited with chromosomal DNA during conjugation (15).

Experiments to test the coinheritance of mercury resistance with each of these chromosomal

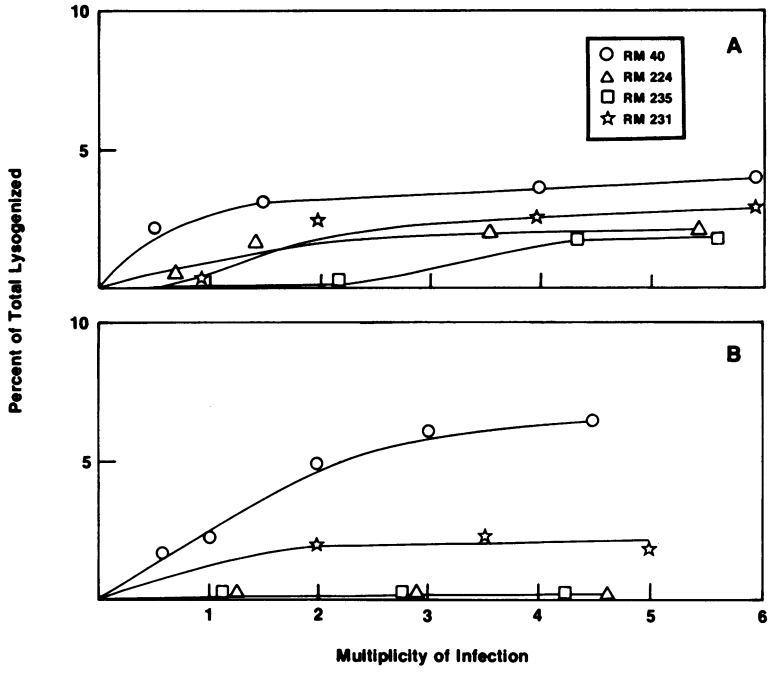

FIG. 2. *Alternate representation of the efficiency of lysogenization of Les⁻ mutants isolated from P. aeruginosa RM40. Mutant strains were infected with temperate phage at various MOI, and the percentage of total input cells lysogenized was measured. (A) The infecting phage was D3; (B) the infecting phage was F116.*

TABLE 2. *Coinheritance of Les phenotype of donor with selected chromosomal markers*

Donor[a]	Recipient	% Coinheritance with selected marker[b]								
		ilvB112 (2)	*his-6* (12)	*ser-5* (13)	*lys-56* (19)	*argB18* (21)	*met-28* (28)	*trp-6* (32)	*leu-1* (48)	*arg-304* (late)
PAO381 (*les*⁺)	RM224 (*les-924*)	—[c]	—	—	100	—	95	86	—	—
RM225 (*les-924*)	PAO303 (*les*⁺)	—	—	—	—	89	—	—	—	—
PAO381 (*les*⁺)	RM235 (*les-935*)	—	—	—	95	—	86	80	—	—
RM236 (*les-935*)	PAO303 (*les*⁺)	—	—	—	—	71	—	—	—	—
RM18 (*les-908*)	OT101 (*les*⁺)	0	2	—	—	—	—	—	24	—
RM18 (*les-908*)	OT94 (*les*⁺)	0	—	0	—	—	—	—	38	—
RM21 (*les-907*)	OT101 (*les*⁺)	0	0	—	—	—	—	—	23	—
RM21 (*les-907*)	OT94 (*les*⁺)	0	—	0	—	—	—	—	30	—
RM23 (*les-905*)	OT101 (*les*⁺)	0	0	—	—	—	—	—	25	—
RM23 (*les-905*)	OT94 (*les*⁺)	0	—	0	—	—	—	—	34	—
JC9005 (*les*⁺)	RM5 (*les-905*)	—	—	—	—	—	—	—	—	76

[a] *les* genotype of strains is given in parentheses.

[b] Parenthetical numbers placed after the genotype designation indicate its map position in minutes. Each percentage is the average of two experiments for a total of 200 selected exconjugants for each marker.

[c] —, Recipient is prototrophic for the marker in question.

markers suggested that, in fact, mercury resistance was inherited with each of the markers. The coinheritance frequency increased with distance of the selected marker from the origin (12% with *ilvB112* to 50% with *leu-1*). It is therefore likely that linkage of the *les*$^+$ allele with auxotrophic markers is spuriously high in crosses of PAO381 with RM224 and RM235 (Table 2, lines 1 and 3) because of this suppressor-like activity of FP2. Consistent with this hypothesis is the finding that, when lysates of F116 prepared in PAO1 were used to transduce RM224 and RM235, the *les*$^+$ allele was not cotransduced with prototrophic alleles for *lys-56*, *met-28*, and *trp-6*. This indicates that the *les* mutations in these strains are probably located in the 21- to 26-min region of the *P. aeruginosa* map (19).

Characterization of Les$^-$ mutants. Holloway has suggested that the processes of repair of DNA damaged by exposure to UV light, the ability to recombine DNA, and the ability to be lysogenized are closely related functions in *P. aeruginosa* (10). We wished to test the possible relationships of these functions in our mutants.

Recombination ability of Les$^-$ mutants was examined (i) after conjugation by crossing with PAO381 (FP2$^+$, *leu-38*, *str-2*) and (ii) after transduction with phage F116 (the transducing lysate was prepared by growth of the phage on PAO1). With PAO303 and its Les$^-$ progeny, recombination at the *argB18* locus was assayed, and with the RM40 strains recombination at the *lys-56* locus was determined. The results of these experiments are shown in Table 3. RM8 and RM231 show a pronounced Rec$^-$ phenotype after the transfer of genetic material by both conjugation and transduction, whereas the remaining Les$^-$ mutants are recombinationally proficient.

TABLE 3. *Recombinational proficiency among Les$^-$ strains*

Strain	Recombination proficiency[a]	
	Conjugation[b]	Transduction[c]
PAO303 series mutants		
RM5	0.7	0.5
RM7	0.3	0.2
RM8	<0.001	<0.009
RM40 series mutants		
RM224	0.4	0.6
RM231	<0.006	<0.009
RM235	0.8	0.8

[a] Recombinational proficiency after conjugation is the recombination frequency of the Les$^-$ mutant strain divided by the recombination frequency of the Les$^+$ parental strain. Recombinational proficiency after transduction is the transduction frequency of the Les$^-$ mutant strain divided by the transduction frequency of the Les$^+$ parental strain.

[b] Exogenote was acquired through FP2-mediated chromosomal transfer (see text).

[c] Exogenote was acquired through F116-mediated transduction (see text).

Sensitivity to UV irradiation was determined by comparing the survival of Les$^-$ mutants to that of their parents after UV irradiation at various doses. Only strain RM8 was found to have an increased sensitivity to UV irradiation.

Strains of *E. coli* sensitive to UV light are often sensitive to X rays (12). We investigated the X-ray sensitivity of the Les$^-$ strains. Strain RM8 was found to be more sensitive to X rays that its Les$^+$ parent. The other mutants are as resistant to X irradiation as their parents.

The ability of a bacterial strain to repair UV-damaged phage DNA when the viral DNA is exposed to UV irradiation while in the virion is termed host-cell reactivation. In order to assay the host-cell reactivation (Hcr) phenotype of our Les$^-$ mutants, we irradiated a clear-plaque mutant of phage D3 with various amounts of UV light. The Les$^-$ strains and their parents were then used as hosts for these irradiated preparations, and the percentage of the original number of plaque-forming units recovered was assessed (Fig. 3). UV-irradiated bacteriophage are reactivated almost as effectively in the Les$^-$ Rec$^+$ mutants, RM5, RM7, RM224, RM235, as they are in Les$^+$ strains. The Les$^-$ Rec$^-$ strains, RM8 and RM231, appear to have a diminished ability to repair the UV-damaged DNA of such phage.

Fertility of male derivatives of Les$^-$ mutants. Chromosome transfer mediated by the sex factor F in *E. coli* is dependent upon bacterial recombination functions (17). Chandler and Krishnapillai (4) have demonstrated that although the absence of a functional recombination system (Rec$^-$) in a mutant strain of *P. aeruginosa* did not affect its ability to transfer the sex factor FP2, the *rec* mutation markedly reduced the ability of this plasmid to mediate chromosome transfer. To test whether our mutants could be donors, we constructed FP2$^+$ derivatives of the Les$^-$ mutants and their Les$^+$ parents, which were used as donors in conjugal matings.

FP2$^+$ derivatives (Table 1) were prepared by crossing each Les$^-$ FP$^-$ strain with a donor (JC9005) strain in a 15-min interrupted mating. FP2$^+$ exconjugants of the Les$^-$ strains were selected on medium containing $HgCl_2$ (see Materials and Methods), and the Les phenotype of the newly derived donors was determined by cross-streaking the transconjugants against phage D3.

The fertility of each donor was determined by mating each FP2$^+$ Les$^-$ derivative strain and an

$FP2^+$ derivative of its parent with OT101 (FP^- *ilvB112 his-6 leu-1 str-2* Les^+) and measuring the ratio of the frequency of sex factor transfer to chromosomal transfer (*ilvB112* prototrophy was selected, Table 4). Certain Les^- strains, when harboring the FP2 factor, appear to stimulate the transfer of this sex factor significantly above the level of transfer observed in their parental strains (Table 4, columns 1 and 2). This is especially apparent in strain RM18. When the number of conjugal events necessary to initiate a chromosomal transfer event is considered, it is apparent that chromosomal transfer is significantly inhibited in the $FP2^+$ Les^- Rec^- strains. From these data, it appears that the recombination system which is inhibited in the Les^- Rec^- mutants is necessary for chromosome transfer mediated by the sex factor FP2.

DISCUSSION

We isolated and characterized several mutants of *P. aeruginosa* which are unable to be lysogenized by temperate bacteriophage. The degree to which lysogenization is reduced in these strains is related to the ratio of infectious viral particles per susceptible bacterium (MOI). Two

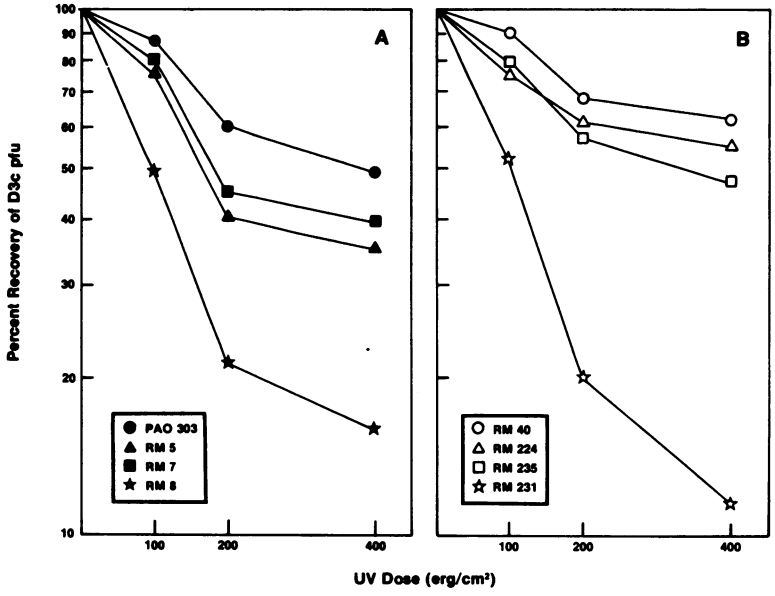

FIG. 3. *Host cell reactivation of P. aeruginosa Les^- mutants. The repair of UV-damaged phage D3c was determined by plaque-forming ability on mutants as described in the text. All points are the average of at least three experiments. pfu, Plaque-forming units.*

TABLE 4. *Fertility of $FP2^+$ derivatives of Les^- strains*

$FP2^+$ strain	FP2[a]		Chromosomal[b]		Ratio (Transfer/Recombination[d])
	% Transfer	Relative proficiency[c]	% Recombination	Relative proficiency[c]	
RM209	7×10^{-4}		3×10^{-4}		2
RM23	4×10^{-3}	5.7	1×10^{-4}	0.3	40
RM21	2×10^{-2}	28.6	3×10^{-4}	1.0	67
RM18	4×10^{-2}	57.1	2×10^{-4}	0.7	200
RM240	5×10^{-4}		2×10^{-5}		25
RM225	3×10^{-4}	0.6	1×10^{-5}	0.5	30
RM236	2×10^{-4}	0.4	8×10^{-6}	0.4	25
RM232	7×10^{-5}	0.2	1×10^{-7}	0.005	700

[a] Frequency of FP2 transfer was assayed by the acquisition of mercury resistance by the recipient strain.
[b] Frequency of chromosomal transfer was assayed by the acquisition of isoleucine-valine prototrophy by the recipient strain.
[c] Relative proficiency is the frequency in the Les^- mutant divided by the frequency in the Les^+ parent.
[d] Ratio is the number of FP2 transfer events divided by the number of chromosomal transfer events.

classes of the Les⁻ phenotype can be identified. The first or absolute class appears to support little or no lysogeny even at a high MOI. The second or intermediate class shows a decreased ability to form lysogens at a high MOI in comparison to the parent. The class to which a particular mutant belongs appears to be dependent not only upon the *les* allele present, but also upon the strain of infecting bacteriophage.

Since all the Les⁻ strains studied are absolutely deficient in establishing lysogeny when infected by F116, we suggest that F116 be employed when testing the Les⁻ phenotype. This observation may also indicate the increased dependence of F116 on the gene products of the *les* genes in induction of the temperate response. It is possible that the products of the *les* genes described here are important in the establishment and/or stable maintenance of extrachromosomal elements such as the nonintegrating F116 prophage (16). However, the role of the *les* gene product is not limited to establishment and/or maintenance of extrachromosomal elements, as it also limits the establishment or maintenance of D3, an integrated prophage (Miller, unpublished data).

Strain RM231 is exceptional in its response to phage infection. Both the lytic and temperate responses to phage infection are inhibited in this strain. Although sensitivity to the lytic response can be recovered after growth of the strain at 43°C (Ku, unpublished data), the Les⁺ phenotype is not restored. This phenomenon may be due to a secondary mutation or pleiotropic effect of the *les-931* allele, which reduces the ability of phages to adsorb to this strain.

The *les* alleles reported here map to two locations on the *P. aeruginosa* chromosome: *les-924* and *les-935* are coinherited with genes in the 21- to 26-min region of the genetic map; and *les-905, les-907,* and *les-908* are inherited with markers later than 45 min. These results indicate that at least two loci affecting lysogeny-establishment are represented among the mutants studied. We propose that mutations mapping in the region near *argB18* be designated *lesA* (i.e., *lesA924* and *lesA935*), and that the mutations which map near *leu-1* be assigned to the gene *lesB* (i.e., *lesB905, lesB907,* and *lesB908*).

High coinheritance of mercury resistance and the Les⁺ phenotype in conjugation between a Les⁺ donor and a Les⁻ recipient was observed in this study. In crosses between a Les⁻ donor and a Les⁺ recipient, the Les⁺ phenotype was always retained when selection for mercury resistance was made. These results may indicate that an indirect suppressor of the Les⁻ phenotype is present either as part of the FP2 sex factor itself or as part of the *P. aeruginosa* chromosome which is coinherited with very high frequency with FP2-coded mercury resistance. If there is indeed a requirement for the functions encoded in the *les* genes for the stable maintenance of extrachromosomal DNA, it is possible that the fertility factor FP2 itself can only be established and/or stably maintained in the Les⁻ cell because of the presence of this suppressor.

When the recombinational ability of these Les⁻ mutants is considered, *les* mutations fall into two classes. One class appears to have a pleiotropic effect on the recombinational ability of the mutant strain. This is shown by reduction in the frequency of viable recombinants to less than 1% of the levels acquired with the parental strain after conjugation or transduction. The recombination-deficient mutants (Rec⁻) are markedly affected in their ability to repair damage produced by exposure of phage DNA to UV irradiation, indicating that the process of host cell reactivation is impaired. Previously reported Rec⁻ mutants, whether Les⁺ or Les⁻ (4, 8, 21), have been less pleiotropic in their effects on DNA metabolism. As the Les⁻ Rec⁻ mutants reported in our study were obtained from nitrosoguanidine mutagenesis, it is possible that the pleiotropic effects noted here are due to a secondary mutation. If separable, this *rec* gene must be closely linked to *les,* as several isolates in this study, as well as those reported earlier (8, 21), have lead to the concomitant loss of recombinational ability with acquisition of the Les⁻ phenotype.

Our studies on the ability of Les⁻ Rec⁻ strains to act as donors when the sex factor FP2 is present within the cell revealed that the ability to donate the chromosome was impaired in these strains if one considers the number of chromosomal transfer events per FP2 transfer event (or mating). We conclude from these observations that the product or products of the mutations in RM8 and RM231 are necessary for successful FP2-mediated chromosomal transfer. A similar role has been indicated for the product of the recombinational gene inactivated in the Rec⁻ mutants of *P. aeruginosa* reported by Chandler and Krishnapillai (4) and for the *recA* product in *E. coli* (17).

ACKNOWLEDGMENTS

We are indebted to Alvin J. Clark and W. Stuart Riggsby for valuable discussions during the course of these studies.

This work was aided by grant VC-206 from the American Cancer Society.

LITERATURE CITED

1. **Achtman, M., N. Willetts, and A. J. Clark.** 1971. Beginning a genetic analysis of conjugational transfer determined by the F Factor in *Escherichia coli* by isolation and characterization of transfer-deficient mutants.

J. Bacteriol. **106**:529–538.

2. **Booker, R. J., and J. S. Loutit.** 1974. The order of replication of chromosomal markers in *Pseudomonas aeruginosa* strain 1. I. Marker frequency analysis by transduction. Genet. Res. **23**:145–153.
3. **Brookes, K., and A. J. Clark.** 1966. Behavior of λ bacteriophage in a recombination deficient strain of *Escherichia coli*. J. Virol. **1**:283–293.
4. **Chandler, P. M., and V. Krishnapillai.** 1974. Isolation and properties of recombination-deficient mutants of *Pseudomonas aeruginosa*. Mutat. Res. **23**:15–23.
5. **Haas, D., and B. W. Holloway.** 1976. R factor variants with enhanced sex factor activity in *Pseudomonas aeruginosa*. Mol. Gen. Genet. **144**:243–251.
6. **Herskowitz, I.** 1973. Control of gene expression in bacteriophage lambda. Annu. Rev. Genet. **7**:289–324.
7. **Holloway, B. W.** 1966. Radiation-sensitive mutants of *Pseudomonas aeruginosa* with reduced host-cell reactivation of bacteriophages. Mutat. Res. **3**:167–171.
8. **Holloway, B. W.** 1966. Mutants of *Pseudomonas aeruginosa* with reduced recombination ability. Mutat. Res. **3**:452–455.
9. **Holloway, B. W.** 1969. Genetics of *Pseudomonas*. Bacteriol. Rev. **33**:419–443.
10. **Holloway, B. W., M. Monk, L. M. Hodgins, and B. Fargie.** 1962. Effects of radiation on transduction in *Pseudomonas aeruginosa*. Virology **18**:89–94.
11. **Hong, J., G. R. Smith, and B. N. Ames.** 1971. Adenosine 3:5-cyclic monophosphate concentration in the bacterial host regulates the viral decision between lysogeny and lysis. Proc. Natl. Acad. Sci. U.S.A. **68**:2258–2262.
12. **Horii, Z.-I., and A. J. Clark.** 1973. Genetic analysis of the Rec F pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. J. Mol. Biol. **80**:327–344.
13. **Loutit, J. S.** 1970. Investigation of the mating system of *Pseudomonas aeruginosa* strain I. IV. Mercury resistance associated with the sex factor (FP). Genet. Res. **16**:29–36.
14. **Loutit, J. S., and M. G. Marinus.** 1968. Investigation of the mating system of *Pseudomonas aeruginosa* strain I. II. Mapping of a number of early markers. Genet. Res. **12**:37–44.
15. **Loutit, J. S., M. G. Marinus, and L. E. Pearce.** 1968. Investigations of the mating system of *Pseudomonas aeruginosa* strain I. III. Kinetic studies on the transfer of the sex factor (FP). Genet. Res. **12**:139–145.
16. **Miller, R. V., J. M. Pemberton, and A. J. Clark.** 1977. Prophage F116: evidence for extrachromosomal location in *Pseudomonas aeruginosa* strain PAO. J. Virol. **22**:844–847.
17. **Moody, E. E. M. and W. Hayes.** 1972. Chromosome transfer by autonomous transmissible plasmids: the role of the bacterial recombination (*rec*) system. J. Bacteriol. **111**:80–85.
18. **Pemberton, J. M., and A. J. Clark.** 1973. Detection and characterization of plasmids in *Pseudomonas aeruginosa* strain PAO. J. Bacteriol. **114**:424–433.
19. **Pemberton, J. M., and B. W. Holloway.** 1972. Chromosome mapping in *Pseudomonas aeruginosa*. Genet. Res. **19**:251–260.
20. **Signer, E. R.** 1968. Lysogeny—the integration problem. Annu. Rev. Microbiol. **22**:451–488.
21. **van de Putte, P., and B. W. Holloway.** 1968. A thermosensitive recombination deficient mutant of *Pseudomonas aeruginosa*. Mutat. Res. **6**:195–203.