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The availability of a technique for site-directed mutagenesis by gene replacement provides a powerful tool for genetic analysis in any bacterial species. We report here a general technique for gene replacement in *Pseudomonas aeruginosa*. Genes on fragments of cloned *P. aeruginosa* DNA, altered by transposon mutagenesis, can be transduced into a recipient strain and can replace homologous genes in the *P. aeruginosa* genome. In this study we applied this technique to the construction of recA mutants of *P. aeruginosa*. A cloned segment of *P. aeruginosa* FRD1 DNA was isolated which encoded a protein analogous to the recA gene product of *Escherichia coli*. The *P. aeruginosa* recA gene was able to complement several defects associated with recA mutation in *E. coli*. Transposon Tn1 and Tn501 insertions in the cloned recA gene of *P. aeruginosa* were used to generate chromosomal recA mutants by gene replacement. These recA strains of *P. aeruginosa* were more sensitive to UV irradiation and methyl methane sulfonate and showed reduced recombination proficiency compared with the wild type. Also examined was the effect of recA mutations on the expression of alginate, a virulence trait. Alginate is a capsulelike polysaccharide associated with certain pulmonary infections, and its expression is typically unstable. The genetic mechanism responsible for the instability of alginate biosynthesis was shown to be recA independent.

*Pseudomonas aeruginosa* is an opportunistic pathogen which causes a variety of disease manifestations. Many bacterial factors are believed to contribute to the pathogenesis of this organism, and the role of these virulence factors may vary with the site of infection. One extracellular product of *P. aeruginosa*, alginate, is uniquely associated with chronic pulmonary infections of patients with cystic fibrosis (13). Alginate is a capsulike polysaccharide and gives *P. aeruginosa* a mucoid phenotype. The role of alginate in pathogenesis appears to be complex (30). In general, mucoid strains of *P. aeruginosa* are rare among clinical isolates; however, up to 80% of the isolates from cystic fibrosis patients appear mucoid due to the production of alginate (10, 12). Strains which initially colonize the respiratory tract of cystic fibrosis patients are typically nonmucoid *P. aeruginosa*, but mucoid variants of these strains eventually emerge and become predominant in vivo (13). It appears that most *P. aeruginosa* strains have the genetic information for alginate production, but these genes are normally silent. In vitro studies have shown that alginate-producing (Alg⁺) variants can be selected from populations of nonmucoid cells with antibiotics (19), bacteriophages (27), or bacteriocins (this laboratory, unpublished data).

The Alg⁺ phenotype, whether selected in vivo or in vitro, is usually unstable, and nonmucoid colonies are frequently seen during normal laboratory passage of Alg⁺ strains (18, 37). Mutations responsible for the instability of the expression of alginate genes were mapped to one region of the chromosome in *P. aeruginosa* FRD, a strain originally isolated from a patient with cystic fibrosis (29). The mechanism responsible for the activation of genes involved in alginate biosynthesis and the instability of their expression is unknown. Several loci on the *P. aeruginosa* FRD chromosome are associated with alginate production (17, 29; this laboratory, unpublished data), but only one of these loci (29) appears to be involved in alginate instability. Mutants of *P. aeruginosa* FRD have been described which are stable for the production of alginate (17; this study).

In this study, we asked whether the recA gene product of *P. aeruginosa* promotes a recombinational mechanism responsible for the instability of the Alg⁺ phenotype. The recA gene product of *Escherichia coli* is known to be involved in homologous recombination (7), DNA repair (22), and proteolysis of specific regulatory proteins (32). Interspecific complementation was used to identify a DNA fragment containing the recA gene of *P. aeruginosa* FRD; this technique was recently used by Better and Helsinki (2) to isolate the recA gene of *Rhizobium meliloti*. Once a DNA fragment containing the recA gene of *P. aeruginosa* was isolated, transposon insertion mutagenesis was used to identify the physical location of the gene. Here we describe a general method for gene replacement in *P. aeruginosa* whereby a DNA fragment containing a transposon-inactivated gene (e.g., recA) can be used to construct the analogous chromosomal mutation in *P. aeruginosa*. Mutant bacteria generated by gene replacement were characterized for loss of recA-related functions. The effect of recA mutation on alginate instability was examined.

**MATERIALS AND METHODS**

Bacterial and phage strains. *P. aeruginosa* FRD strains used included FRD1 (prototroph, Alg⁺, cystic fibrosis isolate) (29), FRD40 (prot-3) (17, 29), and FRD140 (met-I trp-2 Alg⁺ stable) (this study). *P. aeruginosa* PA01 (prototroph) (23) was used. *E. coli* K-12 strains used included HB101 (hsdS20 recA13 ara-14 proA2 lacY1 galK2 mtl-1 xyl-5 mtl-1 supE44) (4), HfrH (thy-1 HfrH), and C600 (thy-1 thr-1 leuB6 lacY1 tonA21 supE44). Phages used were λ c1857, λ

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Tn5 (a b221 c1857 cII: Tn5 Oam Pam Sam) (1), and F116L (24).

Media. L medium, NY medium, and minimal medium have been described elsewhere (17). Antibiotics were used in selection media at the following concentrations (per milliliter) with *E. coli*: tetracycline, 10 μg; kanamycin, 25 μg; ampicillin, 50 μg; and streptomycin, 25 μg. Antibiotic concentrations (per milliliter) used with *P. aeruginosa* were: tetracycline, 100 μg; kanamycin, 500 μg; and carbenicillin, 300 μg. Mercuric chloride was used at 6 μg/ml in minimal agar and at 15 μg/ml in NY agar for both bacteria. Methyl methane sulfonate (MMS) agar was prepared by spreading 200 μl of a 2% solution on the surface of an L-agar plate.

Plasmids and DNA procedures. Plasmids used were pLAFR1 (IncP-1, ncl(b) Kmr) (14), RP4 (IncP1-Tnl) (5), R68.45 (IncP-1 chromosome mobilization) (21), pUW942 (ColE1-Tra+ Tn501) (35), and RSF1010 (IncQ Sm') (20). Plasmid DNA was isolated from *E. coli* by the method of Birnboim and Doly (3) and purified by ethidium bromide-cesium chloride centrifugation. Plasmid DNA was used to transform calcium-treated (8) *E. coli* HB101. Genomic DNA was isolated as previously described (17). Restriction endonucleases and T4 ligase were purchased from New England Biolabs, Inc., and reaction conditions were as specified by the manufacturer. Restriction fragments were examined by agarose gel electrophoresis as previously described (17). Transposon Tn1, Tn5, and Tn501 insertions in DNA were physically mapped by their effect on the restriction fragment pattern.

RSF1010::Tn5 was isolated by infecting HB101(RSF1010) with a Tn5 and selecting for the kanamycin resistance (Km') phenotype; approximately 2000 Km' colonies were pooled, and plasmid DNA was isolated and used to transform *E. coli* HB101 to Km'. RSF1010::Tn5 was distinguished in a transformant by its altered electrophoretic mobility and restriction pattern. To obtain RSF1010::Tnl, plasmid DNA was isolated and purified from *E. coli* HB101 (RSF1010, RP4), treated with HindIII to cut the RP4 molecules (but not RSF1010 or Tn5), and used to transform *E. coli* HB101 to Ap'. RSF1010::Tnl was distinguished in a transformant by its electrophoretic restriction pattern. To obtain RSF1010::Tn501, plasmid DNA from *E. coli* HB101(RSF1010, pUW942) was treated with BamHI to cut the pUW942 molecules (but not RSF1010 or Tn501) and used to transform *E. coli* HB101 to Hg'. RSF1010::Tn501 was distinguished by its electrophoretic pattern.

Transposon mutagenesis of cloned DNA. An adaptation of a procedure described by White et al. (36) was used to isolate recombinant plasmids containing transposon insertions. This technique takes advantage of the ability of lambda phage to package cosmid molecules (e.g., pLAFR1) in vivo during propagation on *E. coli*. *E. coli* HB101 containing a target pLAFR1 recombinant plasmid was first transformed with RSF1010::Tnl, RSF1010::Tn5, or RSF1010::Tn501 plasmid DNA. Overnight cultures of *E. coli* containing both plasmids were used to prepare plate lysates of λ c1857 (26); such lysates were used to transduce recombinant plasmids into *E. coli* HB101. Plasmids which sustained a transposon insertion from the appropriate RSF1010-transposon vector were isolated by selection for transductants on NY agar containing ampicillin (Tn1), kanamycin (Tn5), or mercuric chloride (Tn501).

Construction and mobilization of recombinant plasmids. We previously described (17) a clone bank of *P. aeruginosa* FRD1 which was constructed by ligating a size-fractionated EcoRI partial digest of genomic DNA into the EcoRI site of pLAFR1, a broad-host-range cosmid derivative of pRK290 (15). This DNA was packaged in vitro into phage lambda particles and transfected into *E. coli* HB101 (17). To mobilize these recombinant plasmids into *P. aeruginosa*, triparental matings were performed as previously described (17), with the conjugative functions of *E. coli* HB101(pRK2013) (14) acting upon the relaxation complex site (rlx) of pLAFR1 for conjugal transfer.

UV irradiation. Bacteria from log-phase liquid cultures were diluted appropriately and spread onto the surface of L-agar plates. These plates were irradiated with UV light (predominantly 254 nm) from a germicidal lamp at the indicated doses at the rate of 0.8 J/m². After irradiation, bacteria were kept in the dark to avoid photoreactivation.

Bacterial conjugation and transduction. The ability of *E. coli* strains to form recombinants was tested in L broth (5 ml) with a donor/recipient ratio of 1:20 and a total cell concentration of 2 × 10⁸ cells per ml. The mixture was incubated at 37°C for 60 min before it was plated on minimal agar (with appropriate nutritional supplements) containing streptomycin to counterselect against the donor. The ability of *P. aeruginosa* strains to form recombinants was tested with R68.45 to mobilize the chromosome as previously described (29); recombinants were selected on appropriately supplemented minimal agar. Transduction tests in *P. aeruginosa* were performed with the generalized transducing phage, F116L (23, 24). Plate lysates of F116L were prepared on *P. aeruginosa* PAO1 strains containing recombinant plasmids; a 0.2-ml sample of a lysate (ca. 5 × 10¹⁰ PFU/ml) was placed atop 10⁹ cells of *P. aeruginosa* FRD immobilized on a membrane filter (0.45 μm, 25-mm diameter) on the surface of an L-agar plate. After the mixture was incubated at 30°C overnight, cells were suspended in 5 ml of NY broth and spread (0.1 ml) on NY agar with appropriate antibiotic supplements.

Southern blot hybridization. Genomic DNA (10 μg per lane) or plasmid DNA (0.1 μg per lane) was digested with EcoRI, fractionated on 0.7% agarose slab gels, and blotted to nitrocellulose (34). Hybridizations were performed as described previously (26), with probes radiolabeled in vitro by nick translation (31) with [32P]dCTP.

RESULTS

Isolation of the recA gene from *P. aeruginosa*. A clone bank from *P. aeruginosa* FRD1 was constructed with the cosmid vector pLAFR1 (15) and stored in *E. coli* HB101 as previously described (17). Strain HB101 is a recA mutant and, thus, sensitive to DNA-damaging agents such as MMS. When the HB101(pLAFR1-FRD1) clone bank was plated onto L agar supplemented with MMS (ca. 10³ cells per plate), MMS' colonies were obtained at a frequency of ca. 10⁻³. One MMS' isolate, HB101(pMW1), was examined further. When pMW1 DNA was isolated and transformed into HB101 by selection for Te', the MMS' phenotype was conferred. pMW1 contained two EcoRI fragment insertions of 17 and 10 kilobases (kb). To verify that the cloned DNA was *P. aeruginosa* origin, ³²P-labeled pMW1 DNA was shown to hybridize with two restriction fragments (17 and 10 kb) on a Southern blot of EcoRI-digested *P. aeruginosa* FRD1 genomic DNA (data not shown). pMW1 was partially digested with EcoRI, religated, and transformed into *E. coli* HB101. Among the Te' transformants, HB101(pMW1) carried the 10-kb EcoRI fragment but was MMS sensitive (MMS'); HB101(pMW11) carried the 17-kb fragment and retained the MMS' phenotype. pMW11 was further characterized.

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Restriction mapping and transposon mutagenesis of pMW11. A preliminary restriction map of the 17 kb of cloned P. aeruginosa DNA in pMW11 was constructed (Fig. 1). To determine the location of the presumptive P. aeruginosa recA gene in this relatively large DNA fragment, plasmids with random transposon insertions were isolated (see above). Transposon Tn1, Tn5, and Tn501 insertions in pMW11 were mapped, and their effect on the MMSr phenotype in E. coli HB101 was examined (Fig. 1). Transposon insertions Tn1-18, Tn5-1, Tn5-8, and Tn501-7 in pMW11 (Fig. 1) all inactivated the MMSr phenotype conferred by the plasmid and were clustered in a 1.1-kb region on the cloned P. aeruginosa DNA.

Expression of the P. aeruginosa recA gene in E. coli. Further analyses indicated that pMW11 contained the recA gene of P. aeruginosa and was able to complement other defects associated with the recA mutation in E. coli. pMW11 suppressed the UV sensitivity phenotype of the recA mutation in E. coli HB101 (Fig. 2). HB101(pMW11) displayed an approximately 4-log-higher level of UV resistance than did HB101 without a plasmid. Compared with E. coli C600 recA\(^{+}\), HB101(pMW11) was nearly as UV resistant (i.e., only about a threefold difference was observed). All transposon insertions which had blocked the ability of pMW11 to confer the MMSr phenotype also blocked the UVr phenotype in recA mutant E. coli. The UV resistance of HB101 carrying plasmids with Tn1-18 and Tn501-7 insertions was the same as that of parental HB101 (Fig. 2); Tn5-1 and Tn5-8 insertions in pMW11 produced comparable results (data not shown). Transposon insertions which did not affect the ability of pMW11 to promote MMS resistance in E. coli HB101 had no effect on UV resistance (data not shown).

pMW11 was also able to promote homologous recombination in recA mutant E. coli (Table 1). The frequency of recombination in E. coli HB101 increased approximately 100-fold when the plasmid-borne P. aeruginosa recA\(^{+}\) gene was present in the HB101 recA mutant host, compared with frequency of recombination in cells which carried pLAFR1. Transposon insertions in pMW11 which prevented the expression of MMSr and UVr phenotypes in HB101 also blocked recombogenic activity promoted by pMW11 (Table 1).

Development of a gene replacement technique and construction of recA mutants in P. aeruginosa. To establish that pMW11 contained a recA gene which was functional in P. aeruginosa and to construct recA mutants, we developed a general method for the replacement of P. aeruginosa genomic DNA with homologous sequences altered by transposon mutagenesis. The experimental approach used for gene replacement is shown in Fig. 3. The recA\(^{+}\):Tn501 and recA18::Tnl mutations in pMW11 were chosen for recA gene replacement in P. aeruginosa because these trans-
FIG. 3. General scheme used to introduce specific transposon (Tn) insertions into the *P. aeruginosa* FRD genome. (A) Preparation of an F116L transducing lysate which includes phage particles that contain transposon-modified *P. aeruginosa* DNA. *P. aeruginosa* FRD DNA in a recombinant plasmid (pLAFR1-FRD) was mutagenized by transposon Tn5 or Tn501 insertion mutagenesis (see the text). Transposon insertions were characterized for their effect on plasmid trans-acting activity (e.g., Rec phenotype) and for their physical insertion site. *E. coli* HB101(pRK2013) was used in triparental matings (17) to transfer transposon-altered plasmids to *P. aeruginosa* PAO. Strain PAO is a replicative host for the generalized transducing phage F116L, and plate lysates with this phage were prepared. (B) Gene replacement by transduction. The DNA contained in the F116L transducing lysates just described included plasmid fragments of *P. aeruginosa* DNA altered by transposon insertion (pLAFR1-FRD::Tn). *P. aeruginosa* FRD was infected with these lysates, and recombination occurred between homologous *P. aeruginosa* FRD DNA on plasmid fragments and the genome. The regions of homologous DNA are shown in heavy lines. Transductants inheriting the transposon were obtained by selection for Hg' (Tn501) or Cb' (Tn1). Strains of *P. aeruginosa* FRD inheriting the transposon by homologous recombination were detected in tests indicating loss of the plasmid cloning vector (Tc').

**TABLE 2. *P. aeruginosa* recA mutant strains constructed**

<table>
<thead>
<tr>
<th>recA' parental strain</th>
<th>Genotype or phenotype</th>
<th>recA' mutants constructed by gene replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>recA</strong></td>
<td><strong>recA</strong></td>
<td><strong>recA</strong></td>
</tr>
<tr>
<td>FRD1</td>
<td>Prototroph, Alg'</td>
<td>FRD280</td>
</tr>
<tr>
<td>FRD40</td>
<td>pro-3 Alg'</td>
<td>FRD282</td>
</tr>
<tr>
<td>FRD140</td>
<td>met-I arg-1 trp-2 Alg'</td>
<td>FRD284</td>
</tr>
</tbody>
</table>

*recA* allele designations, i.e., recA18 and recA7, were assigned to recA mutants of *P. aeruginosa* constructed by gene replacement with pMW11::Tn1-18 and pMW11::Tn501, respectively.
and FRD283) and their parental strains were also tested for UV sensitivity; the same results as indicated above were obtained (data not shown). However, we were unable to maintain pMW11, or even pLAFR1, in any recA mutant derived from FRD1 or FRD40; pLAFR1-based plasmids are easily maintained in their Rec+ parental strains. Our ability to stably maintain pLAFR1-based plasmids in recA mutants of FRD140 may be related to the uncharacterized alginate stability mutation possessed by this strain.

In addition to increased sensitivities to MMS and UV light, recA mutants of P. aeruginosa were shown to be deficient in homologous recombination. The average recombination proficiency of recA mutants FRD284 and FRD285 was reduced to approximately 5% of that seen in the recA+ parental strain (Table 3). Similar results were obtained with FRD282 and FRD283 (data not shown). pMW11 was able to complement the recombination defect in strains FRD284 and FRD285 to produce recombination frequencies greater than 60% of that seen with recA+ FRD140(pMW11) (Table 3).

Effect of recA mutation on alginate stability. Strains FRD1 and FRD40 were compared with their recA mutant derivatives with respect to the formation of spontaneous Alg- cells in culture. In general, Alg+ P. aeruginosa FRD strains incubated in liquid culture for 48 h revert to the nonmucoid form at a regular frequency, and such cultures usually contain approximately 50% Alg- cells (29). In this study, both recA mutant and recA+ cultures contained approximately the same fraction (~50 to 70%) of Alg- cells (data not shown). Thus, no apparent effect on the stability of the Alg- phenotype could be demonstrated by the presence of a recA mutation.

Strain FRD140 had a mutation which conferred alginate stability, and cultures of this strain incubated under the same conditions as described above contained less than 0.5% Alg- cells. The recA mutant derivatives of FRD140 (FRD284 and FRD285) were the same as the parental strain with respect to the frequency of conversion to the Alg- phenotype (data not shown). In addition, hundreds of colonies isolated in the cultures above were tested for the presence of the recA mutation due to Tn501 (HgT') or Tn1 (CbT') in the chromosome; spontaneous loss of the transposons was never detected.

**DISCUSSION**

The versatile broad-host-range cloning system based on pRK290/RK2 plasmids (11) has been used with particular success in R. meliloti (15, 33) and Agrobacterium tumefaciens (36). Genes associated with the pathogenesis of P. aeruginosa have recently become more amenable to molecular analysis by the introduction of this cloning system to P. aeruginosa (9, 17). However, the study of spontaneous DNA from P. aeruginosa has been limited by the difficulty in using cloned DNA fragments for site-directed mutagenesis. The modification of cloned DNA, followed by exchange of the altered genes for wild-type alleles in the bacterial genome, has numerous and powerful applications. Here, we describe the general application of both of these techniques to the study of P. aeruginosa. Ruvkun and Ausubel (33) described a method for site-directed mutagenesis to exploit cloned DNA for the generation of mutants in R. meliloti. In their system, transposon insertions in cloned DNA replaced wild-type genomic DNA by the introduction of IncP-1 plasmids incompatible with the vector (pRK290), followed by concomitant selection for the rescue of the transposon through homologous recombination with the genome. When we applied this general technique for gene replacement in P. aeruginosa, difficulties were encountered during the selection for the incompatible plasmid (e.g., spontaneous resistance to selective markers) which made this approach seem unfeasible (unpublished data). In this study, we describe the development of an alternative strategy for gene replacement in P. aeruginosa in which fragments of cloned P. aeruginosa DNA, previously transposon mutated, were transduced from one strain of P. aeruginosa to another with the generalized transducing phage F116L. Transductants selected for the transposon antibiotic resistance marker, which did not co-inherit the pLAFR1 (Tc') marker, were found to be the products of gene replacement. Mutants constructed by this method of gene replacement were shown to contain no
pLAFR1 DNA, as determined by Southern blot hybridization.

The use of transposon mutagenesis of cloned *P. aeruginosa* DNA has not been previously described. We have developed a system of transposon mutagenesis based on a technique described by White et al. (36). Transposons on a plasmid vehicle (RSF1010) which randomly transpose to the compatible target plasmid (pLAFR1 plus insert DNA) were isolated with lambda phage to package these cosmids in vivo and transduce them into *E. coli*. Plasmid RSF1010 vehicles carrying transposons Tn1, Tn5, and Tn501 were constructed for this purpose. Tn501 has generally been our transposon of choice for most studies in *P. aeruginosa*, because spontaneous Hg"*P. aeruginosa* is rare. We were also successful in this study with Tn1 mutagenesis in *P. aeruginosa*. However, the maintenance of pLAFR1 plasmids carrying Tn1 in *P. aeruginosa* was often difficult and resulted in poor growth even in the presence of selective antibiotics (unpublished data); the reason for this phenomenon is not understood. Tn5 was a valuable tool to study *P. aeruginosa* DNA in *E. coli*, but this transposon was not used in *P. aeruginosa* because of the high frequency of Km" cells after selection on this antibiotic.

In this study we isolated a cloned segment of *P. aeruginosa* FRD DNA which encoded a protein analogous to the recA gene product of *E. coli*. This work was accomplished by complementation of the MMS sensitivity phenotype of the recA mutant, *E. coli* HB101, with DNA from a *P. aeruginosa* gene bank. Growth on MMS, a DNA-methylating agent, requires a recA+ gene and thus provides a positive selection. Better and Helinski (2) isolated the recA gene of *R. mellioti* in a similar fashion. Other defects associated with recA mutation in *E. coli* were also suppressed by the *P. aeruginosa recA* protein expressed from pMW11. *E. coli* HB101(pMW11) was almost as UV resistant as a recA+ strain of *E. coli*. *E. coli* HB101(pMW11) formed recombinants in Hfr crosses at frequencies approximately 100-fold greater than that of the recA mutant parental strain. Transposon Tn1, Tn5, and Tn501 insertion mutagenesis of pMW11 was used to inactivate the recA complementation activity in *E. coli*; all such transposon insertions were clustered in a 1.1-kb region and centrally located on a 17-kb fragment of cloned *P. aeruginosa* DNA. The plasmids containing recA::Tn1 and recA::Tn501 insertions were then used to generate chromosomal recA mutants of *P. aeruginosa* FRD strains by the gene replacement technique described above. These recA mutations in *P. aeruginosa* had pleiotropic effects similar to those of recA mutations in *E. coli*. The recA mutant strains of *P. aeruginosa* FRD were more sensitive to MMS and UV irradiation than were their parental strains; recA strains were also recombination deficient. All pleiotropic defects due to the chromosomal recA::Tn1 or recA::Tn501 mutations were complemented in trans by the plasmid-borne *P. aeruginosa recA*+ gene on pMW11.

Several *P. aeruginosa* mutants have been described which are recombination deficient. The mms-13 marker (25) caused reduced (3- to 10-fold) recombination proficiency, increased UV sensitivity, and loss of an ATP-dependent DNase, and it mapped at about 20 min on the strain PAO chromosome. The lesB908 marker (28) caused reduced (100-fold) recombination proficiency and a lysogeny defect, and it mapped at about 40 min on the PAO chromosome. The rec-102 mutation (16) caused reduced (10- to 100-fold) recombination proficiency, increased UV sensitivity, and lysogeny defects, and it mapped in the 45-min region of the PAO chromosome. The rec-2 mutation (6) also caused severely reduced (103-fold) recombination proficiency and showed no linkage to the other three markers associated with recombination defects (16). Although some of these recombination-deficient mutants of *P. aeruginosa* phenotypically resemble recA mutants of *E. coli* K-12, it had not previously been possible to ascertain which (if any) actually had a mutation in recA. Our isolation of the recA gene from *P. aeruginosa* (which efficiently complements a recA mutation in *E. coli*) should now make it possible to study the role of the recA gene product in *P. aeruginosa* DNA metabolism. Our development of a system for gene replacement has permitted us to construct recA mutants of *P. aeruginosa* and will make it possible to study the relationship between recA and other genes which are apparently involved in homologous recombination. The fact that our *P. aeruginosa* recA mutants still retained a recombinational proficiency of approximately 5% in conjugation (compared with that of Rec' parental strains) suggests that other systems for homologous recombination are present in *P. aeruginosa*. Also, survival of recA mutant *P. aeruginosa* after UV irradiation (Fig. 4) was considerably higher than that of recA mutant *E. coli* (Fig. 2) and suggests that recA-independent DNA repair mechanisms possessed by the two organisms are dissimilar. *P. aeruginosa* may have a number of gene products which accomplish a spectrum of DNA repair mechanisms.

One of our major interests in this study was to investigate the nature of the genetic instability associated with alginate biosynthetic genes. Most *P. aeruginosa* strains do not produce alginate, even though they have the genetic information for alginate production. However, strains of *P. aeruginosa* in which these genes are active are very common in respiratory tract infections of cystic fibrosis patients; Alg" variants can also be isolated by in vitro enrichments (19, 27). The Alg" phenotype is unstable, and these strains revert to the typical mucoid form during passage in the laboratory. Such mutations usually occur in one region of the chromosome (29). Mutants have been isolated (17; this study) which are stable for alginate production and rarely form nonmucoid colonies. In this study we determined that the genetic instability characteristic of the Alg" phenotype in *P. aeruginosa* FRD (a strain originally isolated from a cystic fibrosis patient) was not affected by insertional inactivation of the recA gene. Thus, alginate instability in *P. aeruginosa* FRD is apparently independent of the recA gene product.

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LITERATURE CITED


