JOURNAL OF BACTERIOLOGY, Oct. 1975, p. 578-581

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

Bacteriophage Mu-1-Induced Permeability Mutants in *Escherichia coli* K-12

ROBERT F. ALINE, JR., AND WILLIAM S. REZNIKOFF*

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706

Received for publication 9 June 1975

Apparent permeability mutations were produced in *Escherichia coli* K-12 by bacteriophage mu-1 mutagenesis. They are pleiotropic mutations showing sensitivity to a number of detergents and unrelated antibiotics, and presumably they affect cell wall or membrane biosynthesis. One of the mutations was genetically mapped at a site in or near the *acrA* and *mtc* loci at approximately 10.5 min on the Taylor and Trotter map (1972).

Antibiotics have been used extensively to study a variety of macromolecular processes such as deoxyribonucleic acid synthesis, ribonucleic acid synthesis, protein synthesis, and cell wall synthesis. In general, antibiotics which have a specific mode of action can be used to identify the genes which code for specific components involved in a given process (by isolating mutants resistant to these antibiotics) or to biochemically define the steps which constitute the process. Unfortunately *Escherichia coli* is resistant to a number of antibiotics which are active against gram-positive bacteria and which could prove useful in these studies. In many cases, this resistance is due to a permeability barrier which prevents the entry of the drugs into the cells (i.e., osmotically stabilized spheroplasts of *E. coli* and ethylenediaminetetraacetate-treated *E. coli* show an increased sensitivity to a number of these antibiotics [3, 9, 10, 14, 22]).

To permit the use of some of these antibiotics in studies on the mechanisms of protein or deoxyribonucleic acid synthesis in *E. coli*, we chose to isolate mutants that are sensitive to an expanded range of antibiotics. To produce mutants sufficiently stable for use in the isolation of secondary mutants specifically resistant to single antibiotics, mutants have been generated through the use of bacteriophage mu-1. Presumably these antibiotic-sensitive mutations have affected the permeability characteristics of *E. coli* in a manner similar to that reported for other antibiotic-sensitive mutants (1, 4, 6, 12, 17, 19) and thus could also be useful for studying the nature of *E. coli* permeability systems.

The antibiotic-sensitive mutants CF1, CV1, and CV3 were isolated from three independent cultures of A324-4 (described in reference 8) which had been exposed to bacteriophage mu-1 according to the technique of Taylor (20). These cultures were plated at various dilutions on TYE agar (15) and the resulting 1,720 colonies were replica plated onto TYE agar and TYE agar containing either clindamycin (100 μg/ml) or fusidic acid (100 μg/ml). Colonies that failed to grow on one or both of the antibiotic-containing plates were purified and tested for their sensitivity to a variety of compounds by a modification of the gradient plate method described by Szybalski (18). The results of these tests are described in Table 1. As indicated by these data, strain CF1 is sensitive to a larger number of compounds than either strain CV1 or CV3. Since CF1 shows the most pronounced sensitivities, all further experiments were performed with it alone.

In liquid medium, CF1 was found to be sensitive to Sarkosyl (0.2% final concentration) with cell lysis occurring over a 20-min period. The parental strain A324-4 continues to grow at this concentration of Sarkosyl.

Several permeability mutations in *E. coli* show altered resistance to phage T4 infection in relation to their parental strains (4, 5, 19). We tested the sensitivity of CF1 and A324-4 to phage T4 and found that CF1 has an increased sensitivity in relation to the parent A324-4. Phage T4 produced five to six times more plaques on CF1 than A324-4 (data not shown). This is probably due to an increased ability of T4 to adsorb to or inject its deoxyribonucleic acid into CF1.

To determine the approximate genetic location of the mu-1 insertion, strain CF1 was mated to a variety of *recA* F′ strains whose episomes carry genetic material which collectively covers the entire *E. coli* chromosome (11).

TABLE 1. *Antibiotic and detergent sensitivities of bacterial strains on gradient plates*[a]

Antibiotic or detergent[b]	Maximum concn (μg/ml)	Growth length of the bacterial strains (cm)			
		A324-4	CF1	CV1	CV3
Acriflavin	250	9	2.5	NT[c]	NT
Actinomycin D	175	9	9	3.1	NT
Amicetin	250	9	9	9	9
Clindamycin	250	5	0	4.5	3.2
Erythromycin	100	8	0	8	8
Fusidic acid	250	9	0	7.4	9
Geldanamycin	250	9	9	9	9
Kasugamycin	250	9	4.4	5.8	5.1
Mitomycin C	2	3.2	0	NT	NT
Novobiocin	250	9	2.5	9	9
Nybomycin	250	9	0	9	9
Pactamycin	100	4.5	1.9	4.5	3
Penicillin G	100	3.4	3.2	4.1	4.5
Puromycin	250	4.9	2	4.9	3
Streptolydigin	250	9	9	9	9
Viomycin	250	8	5.5	2.5	2
Sodium deoxycholate	5×10^4	9	1	NT	NT
Sodium dodecyl sulfate	5×10^4	9	0	9	5.9

[a] Antibiotic (or detergent) gradient plates were prepared by a modification of the method outlined by Szybalski (18). Thirty milliliter of TYE agar containing the antibiotic were poured into a tilted (100 by 15 mm) square petri dish to form an agar slant. After solidification, 30 ml of TYE agar was poured over the leveled slant, producing a gradient of antibiotic. These plates were used within 12 h of their preparation. Overnight cultures of the bacterial strains were spread across the gradient plate with sterile filter strips and the growth lengths, after 24 h at 37 C, were measured and used as an indicator of the sensitivities of the strains to the antibiotic. Maximum possible length of growth is 9 cm.

[b] Sodium lauryl sarcosine (Sarkosyl) and sodium deoxycholate were purchased from Sigma Chemical Company. Sodium dodecyl sulfate was purchased from Matheson, Coleman and Bell. Amicetin, clindamycin, geldanamycin, novobiocin, nybomycin, pactamycin, and streptolydigin were products of the Upjohn Company. Actinomycin D, erythromycin, and puromycin were purchased from Sigma Chemical Company. Fusidic acid was obtained from E. R. Squibb and Sons, Inc. Actinobolin and viomycin were obtained from Parke, Davis and Company. Kasugamycin was a product of Bristol Laboratories. Penicillin G was a gift from J. Davies.

[c] NT, Not tested.

An F^- phenocopy or strain CF1 was produced by growth in LB. at 37 C for more than 30 h followed by 1 min of vortexing before use. Equal volumes of the F′ strain (grown to 3×10^8 cells/ml in minimal medium) and F^- phenocopy were mixed, incubated for 1 h at 37 C, and spotted onto T plates. These were incubated at 37 C for 6 h and then replica plated onto glucose minimal selective plates containing 100 μg of clindamycin per ml. Any resulting growth was presumably due to a complementation of the mu-1-induced mutation by markers carried on the episome. These clindamycin-resistant colonies were purified and checked for the presence of the mu-1 prophage by examining the culture for immunity to superinfection by mu-1 and by the ability to produce free phage.

Episomes F112 and F254 (Fig. 1) both produced clindamycin- and fusidic acid-resistant colonies which still contained the mu-1 prophage. Colonies produced from overnight cultures of the resistant colonies were tested for homogenotization of the mu-1 insertion onto the episome by replica plating onto TYE plates containing 100 μg of clindamycin per ml. All clindamycin-sensitive colonies were tested for retention of the F′ factor by testing for the ability to plaque the male-specific bacteriophage Qβ. It was found that only colonies containing F254 produced clindamycin-sensitive homogenotes, indicating that F254 covers the mu-1 insertion whereas F112 does not.

The reason episome F112 produced clindamycin-resistant colonies when transferred into CF1 is unclear at this time. It is possible that this resistance is a partial reversal of the permeability change produced by the mu-1 mutation and results from a gene dosage effect for a gene carried by the episome which codes for a component of the cell wall or membrane.

The location of the mu-1 prophage in CF1 was more closely defined by conjugation experiments with two Hfr's (D7011 and CSH68), whose origins of transfer are within the region

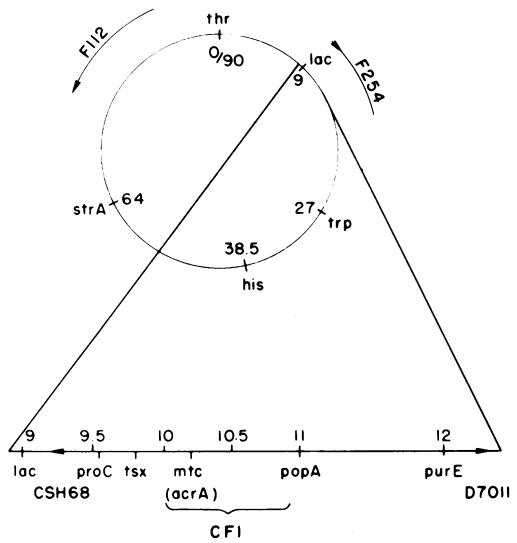

FIG. 1. *Genetic map of E. coli showing the F' factors F112 and F254, the Hfr strains used, and the approximate position of the mu-1 insertion in strain CF1. Origin and direction of transfer are indicated by arrows.*

covered by F254 (11; Fig. 1). These conjugation experiments were performed by mixing equal volumes of the Hfr strain and the F^- phenocopy of CF1 and incubating at 37 C for 15 min. Mating pairs were disrupted by vortexing for 1 min and were diluted 1:10 into M63. Aliquots of 0.1 ml were then plated directly onto glucose minimal selective plates containing 200 μg of streptomycin per ml and 100 μg of clindamycin per ml. The resultant colonies were purified and tested for the presence of the mu-1 prophage. Clindamycin-resistant recombinant colonies which were no longer mu-1 lysogens resulted from matings with both Hfr strains, indicating that the mu-1 prophage in CF1 is located between 9.5 and 12 min on the Taylor and Trotter map (21; see Fig. 1).

The mu-1 prophage in CF1 was also tested for linkage to *proC* and *purE* by $P1_v$ transduction analysis. $P1_v$ grown on CF1 was used to transduce a $proC^-$, $purE^-$ strain to either $proC^+$ or $purE^+$ using techniques described previously (16). Of 312 $proC^+$ transductants found, 2 (0.6%) were found to be clindamycin sensitive (and therefore to have received the mu-1 prophage). No (0/312) $purE^+$ clindamycin-sensitive transductants were found. Previous workers have found that, when a mu-1 mutation is transduced by $P1_v$, a co-transduction frequency of 0.5% is equivalent to approximately 1 min on the *E. coli* genetic map (2). Although no $purE^+$ clindamycin-sensitive transductants were found, we believe that the mu-1 prophage lies about 0.5 to 1.5 min away from *proC* between *proC* and *purE* and probably not between *lac* and *proC* because of the results of the Hfr and F' mapping experiments.

There are two known permeability mutations located in this same region of the *E. coli* chromosome, the *acrA* locus (12) and the *mtc* locus (6, 13). These mutations show increased sensitivity to acriflavine and mitomycin C, respectively, as well as to some basic dyes. Although neither mutation has been analyzed for other antibiotic sensitivities, and neither were available for examination, it is possible that the CF1 mutation is in the *acrA* or *mtc* locus. This is supported by the observation that CF1 shows sensitivities to acriflavin and mitomycin C similar to those found with *acrA* and *mtc* mutants.

In attempting to produce spontaneous clindamycin-resistant mutants, it was discovered that revertants of strain CF1 could be found. These revertants were no longer mu-1 lysogens, were mu-1 sensitive, and appeared to have wild-type permeability characteristics, showing antibiotic sensitivities characteristic of the parental strain A324-4. This spontaneous reversion occurred with a frequency of 10^{-10} to 10^{-11} revertants/colony-forming units, and treatment with nitrosoguanidine did not increase the frequency. Previous workers have also found that reversion frequencies of mu-1-induced mutations are less than 10^{-10}, and that treatment with mutagens did not increase the frequency (7).

We wish to thank J. Davies for the use of various antibiotics and for his helpful discussions during the course of this research.

This work was supported in part by the College of Agriculture and Life Sciences and by Public Health Service grant 1-R01-GM-19670 from the National Institute of General Medical Sciences, by NSF grant GB-20462, and by the Wisconsin Alumni Research Foundation. W. S. R. was supported by Public Health Service career development award 5-K02-GM-30970 from the National Institute of General Medical Sciences, and R. F. A. was supported by NSF training grants GZ-2162, GZ-2522, and GZ-2813.

LITERATURE CITED

1. Apirion, D. 1967. Three genes that affect *Escherichia coli* ribosomes. J. Mol. Biol. **30**:255-275.
2. Bukhari, A. I., and A. L. Taylor. 1971. Genetic analysis of diaminopimelic acid- and lysine-requiring mutants of *Escherichia coli*. J. Bacteriol. **105**:844-854.
3. Ennis, H. 1967. Bacterial resistance to the synergistic antibiotics of the PA114, streptogramin, and vernamycin complexes. J. Bacteriol. **93**:1881-1887.
4. Ennis, H. 1971. Mutants of *Escherichia coli* sensitive to antibiotics. J. Bacteriol. **107**:486-490.
5. Ericksson-Grennberg, K. G., K. Nordström, and P.

Englund. 1971. Resistance of *Escherichia coli* to penicillins. IX. Genetics and physiology of class II ampicillin-resistant mutants that are galactose negative or sensitive to bacteriophage C21, or both. J. Bacteriol. **108:**1210-1223.
6. Imae, Y. 1968. Mitomycin C-sensitive mutant of *Escherichia coli* K-12. J. Bacteriol. **95:**1191-1192.
7. Jordan, E., H. Saedler, and P. Starlinger. 1968. 0° and strong polar mutations in the gal-operon are insertions. Mol. Gen. Genet. **102:**353-363.
8. Kennedy, E. P. 1970. The lactose permease system of *E. coli*, p. 49-92. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor, N.Y.
9. Lieve, L. 1968. Studies on the permeability change produced in coliform bacteria by EDTA. J. Biol. Chem. **243:**2373-2380.
10. Mach, B., and E. L. Tatum. 1963. Ribonucleic acid synthesis in protoplasts of *Escherichia coli*: inhibition by actinomycin D. Science **139:**1051-1052.
11. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor, N.Y.
12. Nakamura, H. 1968. Genetic determination of resistance to acriflavine, phenethyl alcohol, and sodium dodecyl sulfate in *Escherichia coli*. J. Bacteriol. **96:**987-996.
13. Otsuji, N. 1968. Properties of mitomycin C-sensitive mutants of *Escherichia coli* K-12. J. Bacteriol. **95:**540-545.
14. Reid, P., and J. Speyer. 1970. Rifampicin inhibition of ribonucleic acid and protein synthesis in normal and ethylenediaminetetraacetic acid-treated *Escherichia coli*. J. Bacteriol. **104:**376-389.
15. Reznikoff, W. S., J. H. Miller, J. G. Scaife, and J. R. Beckwith. 1969. A mechanism for repressor action. J. Mol. Biol. **43:**201-213.
16. Reznikoff, W. S., and K. P. Thornton. 1972. Isolating tryptophan regulatory mutants in *Escherichia coli* by using a *trp-lac* fusion strain. J. Bacteriol. **109:**526-532.
17. Sekiguichi, M., and S. Iida. 1967. Mutants of *Escherichia coli* permeable to actinomycin. Proc. Natl. Acad. Sci. U.S.A. **58:**2315-2320.
18. Szybalski, W. 1952. Gradient plate technique for study of bacterial resistance. Science **116:**46-48.
19. Tamaki, S., T. Sato, and M. Matsuhashi. 1971. Role of lipopolysaccharides in antibiotic resistance and bacteriophage adsorption of *Escherichia coli* K-12. J. Bacteriol. **105:**968-975.
20. Taylor, A. L. 1963. Bacteriophage-induced mutation in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **50:**1043-1051.
21. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36:** 504-524.
22. Weisser, R., A. W. Asscher, and J. Wimpenny. 1968. *In vitro* reversal of antibiotic resistance by EDTA. Nature (London) **219:**1365-1366.