JOURNAL OF BACTERIOLOGY, Sept. 1973, p. 1103-1107

Vol. 115, No. 3
Printed in U.S.A.

Isolation of a Mutant of *Escherichia coli* Defective in Cytosine-Specific Deoxyribonucleic Acid Methylase Activity and in Partial Protection of Bacteriophage λ Against Restriction by Cells Containing the N-3 Drug-Resistance Factor

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Received for publication 18 April 1973

A mutant (designated *mec*$^-$) of *Escherichia coli* F$^+$ 100 *endo* I$^-$ *su*$^+$ $r_K^-m_K^+$ has been isolated which is defective in cytosine-specific deoxyribonucleic acid (DNA) methylase activity. The DNA of this mutant, as well as the DNA of phages λ and fd propagated in it, is virtually devoid of 5-methyl-cytosine (MeC); in contrast, the mutation has no significant effect on the level of N^6-methyladenine in DNA. Phage λ grown on the *mec*$^-$ mutant is more strongly restricted by N-3-containing cells than is λ grown on the *mec*$^+$ parent. These results suggest that methylation of certain cytosine residues by the *E. coli* K-12 enzyme partially protects λ DNA from either the N-3 restriction nuclease or against secondary degradation subsequent to N-3-specific degradation. Analysis of the MeC level in viral and cellular DNA obtained from *mec*$^+$, *mec*$^+$ (m_{N3}^+), and *mec*$^-$ (m_{N3}^+) strains has led to the conclusion that the R-factor controlled DNA-cytosine methylase may be capable of methylating a sequence(s) which is a substrate for the K-12 enzyme.

Previous reports from this laboratory (11, 13) have shown that methylation of certain cytosine residues in deoxyribonucleic acid (DNA) appears to be involved in the restriction-modification system controlled by the *fi*$^-$ drug-resistance factor, N-3. The host specificity system (*hs*II; 3, 4) controlled by N-3 and related R factors affects a variety of double-stranded DNA phages, including coliphage λ (2-4, 11, 22, 23, 25). In contrast, the single-stranded DNA phages, M13 and fd, are not subject to *hs*II restriction (1, 5, 12); however, they are in vivo substrates for the factor-controlled cytosine-specific DNA methylase (12).

In the course of our studies, we noted that the degree of λ restriction by the N-3 factor was significantly influenced by the host strain in which the phage had been last grown (11); similar observations have been made by others (2, 4, 25) with the related R factor, R15 or RTF-2. It was suggested (11) that the reason phage λ is strongly restricted by N-3 after growth in *Escherichia coli* B strains is because the viral DNA lacks 5-methyl-cytosine (MeC) when it is propagaged in a DNA-cytosine methylase-deficient host (6-9, 11, 13, 16, 17); on the other hand, the relatively weak restriction of λ observed if the phage were grown on K-12 strains (able to produce DNA-cytosine methylase) is presumably the result of partial protection by the MeC residues present on the viral DNA. If this explanation is correct, then K-12 mutants deficient in DNA-cytosine methylase activity should also be defective in their ability to confer protection to phage λ against restriction by cells containing the N-3 factor. The present communication describes the isolation of just such a mutant.

MATERIALS AND METHODS

Phage and bacterial strains. Phage λ cI857 *ind* was obtained from B. Dottin; phage fd was from D. Marvin. *E. coli* 1100 *su*$^+$ *endo*I$^-$$B_1^-r_K^-m_K^+$ was obtained from H. Revel and made lysogenic for λ

cI857 and/or recipient of an F factor. The $r_{N3}^- m_{N3}^+$ factor is a mutant of the wild-type N-3 factor ($r_{N3}^+ m_{N3}^+$) which has lost the ability to restrict (Schlagman et al., in preparation); however, it still confers modification as well as resistance to drugs and ability to be transferred.

Media and chemicals. Minimal medium containing 0.1% Casamino Acids (wt/vol) was used in all labeling experiments (10). Cultures containing the R factor were grown overnight in the presence of 40 μg of streptomycin per ml; log-phase cells used for the labeling experiments were grown after 50- to 100-fold dilution of the overnight cultures into drug-free medium. [2-^3H]Adenine (Schwarz/Mann), L-[*methyl*-^3H]methionine (Amersham/Searle), 5-methylcytosine (Mann), N^6-methyladenine (MeAde; Calbiochem), streptomycin sulfate (Lilly), ethyl methane sulfonate (EMS; Eastman), and Triton X-100 (Rohm-Haas) were obtained from the firms indicated in parentheses. Phosphate buffer contained (per liter): 3 g of KH_2PO_4; 7 g of Na_2HPO_4; 0.5 g of NaCl; 1.0 g of NH_4Cl.

Determination of MeAde content and MeAde/MeC ratio. Growth and purification of labeled phage DNA were described earlier (11, 12), except no carrier phage was added. Hydrolysis of DNA in 70% perchloric acid and paper chromatographic analysis were as before (10, 13) with the following exception. Radioactivity determinations were done in a toluene-base scintillation fluid containing Triton-X100 (5:2, vol/vol).

Determination of MeAde/MeC in bacterial DNA was performed essentially as described earlier (13); however, in the present experiments, the cells were labeled for only 60 min, and alkaline hydrolysis was for 2 h at 42 C in 1 N NaOH.

To determine the MeAde content in bacterial DNA, cells were grown to 2×10^8 per ml, and [2-^3H]adenine (10 μCi/ml; 2 μg/ml) was added. After 120 min of incubation, the cells were taken for isolation of the DNA as above. The DNA was hydrolyzed in 1 N HCl for 60 min at 100 C. In a separate independent experiment, we observed little difference in the MeAde content obtained after hydrolysis by 70% perchloric acid.

EMS. EMS mutagenesis is based on a method previously devised to obtain plasmid mutants (Schlagman et al, in preparation). *E. coli* F^+ 1100 *mec*$^+$(λcI857) was grown in broth at 32 C to 5×10^8 cells per ml. The cells were harvested and washed several times by centrifugation in phosphate buffer, and suspended in phosphate buffer containing 0.3 M EMS at 32 C; non-EMS-treated control cells were run in parallel. Samples were removed at intervals to determine the titer of surviving colony formers and for dilution into minimal medium containing 5% sodium thiosulfate. The latter were harvested and washed by centrifugation; the pellets were resuspended in broth to approximately 10^8 cells per ml and grown overnight at 32 C. The overnight culture, which had been derived from cells exhibiting an 80 to 95% EMS inactivation of colony-formers, was serially diluted, and samples were spread on minimal agar to give isolated colonies.

Isolation of the mec$^-$ mutant. Isolated colonies, derived from the descendants of surviving EMS-treated cells, were picked with sterile toothpicks into 0.2 ml of phosphate buffer contained in separate wells (disposable U-plates, Scientific Products). By means of a specially constructed brass stamper, fractions were spotted onto agar plates seeded with λ-sensitive cells which harbored (or lacked) the $r_{N3}^+ m_{N3}^+$ factor. The plates were immediately placed at 42 C for 30 min (to induce the λcI857 prophage) and then shifted to 37 C for overnight incubation. Since the *mec*$^+$ parental strain confers λ with a partial protection against N-3 restriction, most of the induced clones produced a confluently lysed area on both indicator hosts. However, several clones were observed which lysed only the cells lacking the N-3 factor. Such clones were further purified by streaking on glucose-minimal agar (lacking Casamino Acids); this step selected against any mutants which may have also contained a secondary auxotrophic mutation. As described in the text, a strain (designated *mec*$^-$) was obtained in this fashion which is defective in DNA-cytosine methylase activity. Finally, a nonlysogen was derived from it by curing the procedure of Weisberg and Gallant (24); a 5-min induction period at 42 C was used prior to dilution and plating at 30 C for surviving colony formers.

RESULTS

An E. coli mutant unable to partially protect λ against restriction in R-factor-containing cells and deficient in DNA-cytosine methyl transferase. *E. coli* 1100 contains a DNA-cytosine methyl transferase activity (produces MeC residues at specific sites) and is capable of conferring partial protection to λ against restriction in N-3-factor-containing cells (11). Following EMS mutagenesis, a mutant was isolated which was defective in the latter property (see Materials and Methods). Progeny phage produced after heat induction in the parental strain (designated *mec*$^+$) plate with a relative efficiency of 0.01 to 0.02 on r_{N3}^+-factor-containing bacteria (Table 1); in contrast, phage λ produced in the mutant strain (designated *mec*$^-$) are strongly restricted by the r_{N3}^+-factor-containing cells (relative efficiency of plating is 10^{-5}). Also shown in Table 1 are the contents of methylated bases in phage λ DNA produced after growth in various *mec*$^+$ and *mec*$^-$ hosts. It is clear from these data that λ DNA from phage grown in the *mec*$^-$ host is devoid of MeC; in contrast, the MeAde content is not affected by growth in *mec*$^-$ cells. Separate experiments also showed that these phage have the normal K specificity conferred by the parental *mec*$^+$ strain (data not shown). The *mec*$^-$ mutation has no apparent influence on the ability of the N-3 factor to confer *hs*II modifica-

TABLE 1. *Analysis of methylated bases and plating ability of λ produced in various F⁺ 1100 strains*[a]

Phage	Relative[b] plating efficiency	Mol[c] % MeAde	Calculated[d] MeAde/DNA	MeC/MeAde[c]	Calculated[d] MeC/DNA
λcI·*mec*⁺	0.01–0.02	0.93	233	0.34	79
λcI·*mec*⁺($r_{N3}^-m_{N3}^+$)	1.0	0.74	185	0.92	171
λcI·*mec*⁻	10^{-5} to 2×10^{-5}	0.90	225	≤0.003	≤1
λcI·*mec*⁻($r_{N3}^-m_{N3}^+$)	1.0	0.89	223	0.83	185

[a] See the Materials and Methods. The F⁺ 1100 strains were each lysogenic for the λcI857 *ind*⁻ prophage; the phage produced was obtained by heat induction at 42 C for 15 min., followed by growth at 37 C.

[b] The ratio of plaque-forming units per milliliter measured on F⁺1100 *mec*⁺ ($r_{N3}^+m_{N3}^+$) versus F⁺1100 *mec*⁺ host cells.

[c] The figures listed here are the mean values obtained from analyses on, at least, two independently labeled and purified phage preparations; in most cases, each preparation was subjected to duplicate chromatographic analyses. The variation was generally ±10% for both the MeC/MeAde ratio and the mole percent MeAde (in one or two instances, certain hydrolysates deviated by as much as 20% from the mean value). In this paper, mole percent MeAde refers to the percentage of adenine bases which are MeAde.

[d] The number of MeAde/DNA was calculated on the basis of 25% adenine content and 10^5 total bases/DNA; the MeC/DNA was calculated from the MeAde/DNA and the MeC/MeAde ratio.

tion; e.g., λ·*mec*⁻ ($r_{N3}^-m_{N3}^+$) phage are fully protected against restriction in r_{N3}^+ cells (Table 1). These data are similar to those obtained with λ grown in *E. coli* B strains (11) which are naturally occurring *mec*⁻ bacteria. In summary, the foregoing results demonstrate that the *mec*⁻ strain is unable to partially protect λ against restriction in N-3 containing cells and is also unable to methylate DNA-cytosine residues.

It is interesting to compare the number of MeC per λ DNA determined for the various phage preparations (Table 1). For example, presence of the N-3 factor in *mec*⁺ cells leads to a specific increase in approximately 90 MeC residues per λ DNA. If the N-3 and *mec*⁺ DNA-cytosine methylases act independently at different sites, then one would expect to observe only 90 MeC per λ·*mec*⁻ (m_{N3}^+) DNA; however, this was not the case, and we observe this DNA to contain about 185 MeC residues (Table 1). Thus, after growth of λ in *mec*⁻ (m_{N3}^+) cells, the number of MeC residues per DNA is virtually the same as after growth in the *mec*⁺ (m_{N3}^+) host. One explanation for this situation is that the N-3 methylase, in addition to recognizing certain specific sites, is also capable of recognizing the K-12-specific sites; the ability of the K-12 DNA-cytosine methylase to partially protect λ against restriction in N-3-containing cells may also suggest the possibility that some of the N-3 and K-12 recognition sites (and enzymes?) are related.

The analysis of methylated bases in DNA was extended to host bacterial cells. As shown in Table 2, the *mec*⁻ strain contains at least a 50-fold reduction in the MeC/MeAde ratio; however, the MeAde content remains the same as in the *mec*⁺ parent. It is also interesting to note that presence of the ($r_{N3}^-m_{N3}^+$) factor in *mec*⁺ cells produces a slight, but reproducible, increase in the MeC/MeAde ratio. A similar observation was made in *mec*⁺ strains of *Salmonella typhimurium* (13). Thus, compared to λ DNA, it would appear that the cell DNA has relatively fewer N-3 recognition sites than sites for the K-12 cytosine methylase. In this respect, it is noteworthy that the *mec*⁺(m_{N3}^+) and *mec*⁻ (m_{N3}^+) DNAs have almost the same MeC/MeAde ratio. As in the case of phage λ DNA, the N-3 DNA-cytosine methylase appears to have also methylated the sites usually recognized by the K-12 enzyme; alternative explanations are not yet ruled out.

TABLE 2. *Analysis of methylated bases in bacterial DNA of various F⁺ 1100 strains*[a]

Bacterial[b] strain	MeC/MeAde[c]	Mol % MeAde[c]
mec⁺	0.45	1.57
mec⁺($r_{N3}^-m_{N3}^+$)	0.56	1.63
mec⁻	≤0.005	1.58
mec⁻($r_{N3}^-m_{N3}^+$)	0.59	1.30

[a] See Materials and Methods.

[b] The bacterial strains used in these experiments were all lysogenic for λcI857 *ind*⁻; these strains are the same as those used as hosts for heat induction of λ (Table 1). Growth and labeling were all performed at 32 C (to avoid induction of the prophage).

[c] See Table 1, footnote *c*.

Properties of phage fd grown in mec⁻ bacteria. It is known that the single-stranded DNA phages M13 and fd are not subject to *hs*II restriction (1, 5, 12). However, the phage stocks used in the studies cited were generally propagated on hosts which were *mec*⁺; consequently,

the phage DNA must have contained approximately one to two MeC residues per DNA molecule (12). In view of the fact that K-12 cytosine-specific DNA methylase appears to afford partial protection to λ DNA against *hs*II restriction, it was not ruled out that fd is similarly protected. To test this possibility, it was necessary to obtain fd devoid of MeC and analyze its ability to grow in r_{N3}^+ cells. Therefore, the plating properties and content of methylated bases of fd grown in various *mec*$^+$ and *mec*$^-$ strains were analyzed. As shown in Table 3, fd·*mec*$^-$ phage are, indeed, devoid of MeC, but they are still not restricted by the N-3 plasmid. The level of MeAde is not affected by the *mec*$^-$ mutation or by the presence of the ($r_{N3}^-m_{N3}^+$) plasmid. The level of MeC is the same for both fd·*mec*$^-$ (m_{N3}^+) and fd·*mec*$^+$ (m_{N3}^+); and this level is higher than that for the fd·*mec*$^+$. Thus, as for the cellular and λ DNAs, it appears that the N-3 methylase may also be able to methylate the K-12 site(s) on fd DNA.

DISCUSSION

We have been able to select a mutant of *E. coli* K-12 which is unable to confer phage λ with partial protection against restriction by N-3 factor-containing cells and unable to methylate DNA-cytosine residues. However, this strain appears to be normal in its ability to methylate DNA-adenine residues and to confer K-specific modification. Mamelak and Boyer (18) had shown earlier that the lack of MeC on λ DNA has no effect on K specificity. Our success in isolating such mutant supports our original proposal (11) that the *E. coli* K-12 cytosine-specific DNA methylase partially protects λ DNA against restriction in N-3 factor-containing cells. It is not known at the present time whether this protection is against the primary action of the N-3 restriction nuclease or against some secondary degradation; e.g., protection against secondary degradation systems (21) might allow repair of the initial N-3 nuclease damage. In any event, it is clear that MeC residues produced by the K-12 host enzyme protect λ after infection of N-3-containing cells. Mutants of *E. coli* K-12 defective in cytosine-specific DNA methylase activity have recently been isolated by a different selection procedure (19).

We have utilized the *mec*$^-$ mutant to produce fd phage which lack MeC; this phage is still not restricted by the N-3 factor (Table 3). Thus, our failure to observe N-3 restriction of fd·*mec*$^+$ is not due to any protection of fd by the K-12 DNA-cytosine methylase. The absence of N-3 restriction still remains somewhat mysterious, since the N-3 methylase increases the MeC content of fd DNA grown in both *mec*$^+$ and *mec*$^-$ hosts (Table 3; 12). This plasmid-controlled methylation is probably not due to a second cytosine-methylating enzyme (Schlagman et al., in preparation). Therefore, the MeC observed in fd DNA may be in a sequence(s) which is not a cleavage site(s) (analogous to the *hs*B system [15]) or may be in a sequence(s) which is not a true *hs*II-recognition site(s). This question still remains open.

The observation that the *E. coli* K-12 cytosine-specific DNA methylase affords protection to λ against restriction in N-3-containing cells suggested, among other alternatives, that it may recognize some site(s) which is methylated by the N-3 modification (cytosine) methylase. Conversely, the plasmid-specified enzyme might also be able to methylate a sequence(s) which is substrate for the K-12 enzyme. The latter possibility is supported by the fact viral (and cellular) DNAs derived from *mec*$^+$ (m_{N3}^+) and *mec*$^-$ (m_{N3}^+) strains have similar MeC contents (Tables 1-3). The most striking example is in bacterial DNA where the MeC content is increased by, at most, 20% in *mec*$^+$ (m_{N3}^+)

TABLE 3. *Analysis of methylated bases and plating properties of fd grown in various F*$^+$ *1100 hosts*[a]

Phage	Mol %[b] MeAde	Calculated[c] MeAde/DNA	MeC/MeAde[b]	Calculated[d] MeC/DNA	Relative[e] plating efficiency
fd·*mec*$^+$	0.31	4.8	0.36	1.7	1.0
fd·*mec*$^+$($r_{N3}^-m_{N3}^+$)	0.27	4.3	0.73	3.1	1.0
fd·*mec*$^-$	0.30	4.8	<0.002	<0.01	0.9
fd·*mec*$^-$($r_{N3}^-m_{N3}^+$)	0.27	4.3	0.69	3.0	1.0

[a] See Materials and Methods.

[b] See Table 1, footnote c.

[c] The number of MeAde residues per DNA molecule was calculated assuming 6,600 nucleotide bases per DNA molecule (14) and an adenine content of 24% (20).

[d] The number of MeC per DNA molecule was calculated from the MeC/MeAde ratio and the calculated number of MeAde per DNA molecule.

[e] The ratio of plaque-forming units per milliliter measured on F$^+$ 1100 *mec*$^+$($r_{N3}^+m_{N3}^+$) versus F$^+$ 1100 *mec*$^+$ host cells.

versus *mec*$^+$ cells; yet, *mec*$^-$ (m_{N3}^+) DNA has the same MeC content as the *mec*$^+$ (m_{N3}^+) strain. If the N3 and K-12 enzymes act independently at unrelated sites, then the *mec*$^-$ (m_{N3}^+) DNA should have exhibited a relatively low MeC content.

It is not known whether the additional sites methylated by the plasmid enzyme in *mec*$^-$ cells are those normally substrate for the K-12 enzyme. Preliminary studies suggest that this may, in fact, be the case; e.g., the distribution of MeC residues among oligo-pyrimidine tracts (isopliths) of defined length and base composition seem to be the same for $\lambda \cdot mec^+$ and $\lambda \cdot mec^-$ (m_{N3}^+) (May and Hattman, in preparation); this is true for fd$\cdot mec^+$ and fd$\cdot mec^-$ (m_{N3}^+) DNAs as well. The sequence for each isoplith has not yet been defined, nor is anything known about the purine residues adjacent to the 3′ and 5′ ends of each pyrimidine tract; however, the preliminary observations do indicate a relationship in the site specificity of the host- and plasmid-controlled enzymes. Thus, the genes controlling DNA-cytosine methylase activity may not have evolved independently on the host and plasmid DNAs. If the N-3 enzyme methylates the K-12 sites, it means that it is capable of recognizing several kinds of sequences. Such an expanded range of sequence recognition could have been produced by mutation; e.g., it has been shown that a single mutation can increase the site recognition of the DNA-adenine methylase specified by phage T2 (10). Experiments are in progress to study this possibility.

ACKNOWLEDGMENTS

The excellent technical assistance of Diane Kuharik is gratefully acknowledged.

This investigation was supported by Public Health Service grants AI-10864 and AI-08738, from the National Institute of Allergy and Infectious Disease, by grant GB-32125 from the National Science Foundation, and by a Public Health Service Research Career Development Award KO4 AI-28022 to S.H. One of us (S.S.) is a pre-doctoral trainee supported in part by Public Health Service training grant no. 5T01-GM06658-12 from the National Institute of General Medical Sciences, and one of us (L.C.) is a pre-baccalaureate student.

LITERATURE CITED

1. Arber, W. 1966. Host Specificity of DNA produced by *Escherichia coli* 9. Host controlled modification of bacteriophage fd. J. Mol. Biol. **20**:483–496.
2. Arber, W., and M. L. Morse. 1965. Host specificity of DNA produced by *Escherichia coli*. VI. Effects on bacterial conjugation. Genetics **51**:137–148.
3. Bannister, D., and S. W. Glover. 1968. Restriction and modification of bacteriophages by R$^+$ strains of *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **30**:735–738.
4. Bannister, D., and S. W. Glover. 1970. The isolation and properties of non-restricting mutants of two different host specificities associated with drug resistance factors. J. Gen. Microbiol. **61**:63–71.
5. Bickle, T., and W. Arber. 1969. Host-controlled restriction and modification of filamentous I- and F-specific bacteriophages. Virology **39**:605–607.
6. Doskočil, J., and Z. Šormová. 1965. The occurrence of 5-methyl-cytosine in bacterial deoxyribonucleic acids. Biochim. Biophys. Acta **95**:513–515.
7. Doskočil, J., and Z. Šormová. 1965. The sequences of 5-methylcytosine in the DNA of *Escherichia coli*. Biochem. Biophys. Res. Commun. **20**:334–339.
8. Fujimoto, D., P. R. Srinivasan, and E. Borek. 1965. On the nature of the deoxyribonucleic acid methylases. Biological evidence for the multiple nature of the enzymes. Biochemistry **4**:2849–2855.
9. Gough, M., and S. Lederberg. 1966. Methylated bases in the host-modified deoxyribonucleic acid of *Escherichia coli* and bacteriophage λ. J. Bacteriol. **91**:1460–1468.
10. Hattman, S. 1970. DNA methylation of T-even bacteriophages and of their nonglucosylated mutants: its role in P1-directed restriction. Virology **42**:359–367.
11. Hattman, S. 1972. Plasmid-controlled variation in the content of methylated bases in bacteriophage lambda deoxyribonucleic acid. J. Virol. **10**:356–361.
12. Hattman, S. 1973. Plasmid-controlled variation in the content of methylated bases in single-stranded DNA phages M13 and fd. J. Mol. Biol. **74**:749–752.
13. Hattman, S., E. Gold, and A. Plotnik. 1972. Methylation of cytosine residues in DNA controlled by a drug resistance factor. Proc. Nat. Acad. Sci. U.S.A. **69**:187–190.
14. Hoffmann-Berling, H., D. A. Marvin, and H. Dürwald. 1963. Ein fädiger DNS-Phage (fd) and ein sphärischer RNS-Phage (fr), wirtsspezifisch für männliche Stämme von *E. coli*. I. Präparation und chemische Eigenschaften von fd und fr. Z. Naturforsch. **18b**:876–883.
15. Horiuchi, K., and N. D. Zinder. 1972. Cleavage of bacteriophage f1 DNA by the restriction enzyme of *Escherichia coli* B. Proc. Nat. Acad. Sci. U.S.A. **69**:3220–3224.
16. Lederberg, S. 1966. 5-methylcytosine in the host-modified DNA of *Escherichia coli* and phage λ. J. Mol. Biol. **17**:293–297.
17. Ledinko, N. 1964. On the occurrence of 5-methyldeoxycytidylate in the DNA of phage λ. J. Mol. Biol. **9**:834–835.
18. Mamelak, L., and H. W. Boyer. 1970. Genetic control of the secondary modification of deoxyribonucleic acid in *Escherichia coli*. J. Bacteriol. **104**:57–62.
19. Marinus, M. G., and N. R. Morris. 1973. Isolation of deoxyribonucleic acid methylase mutants of *Escherichia coli* K-12. J. Bacteriol. **114**:1143–1150.
20. Marvin, D. A., and B. Hohn. 1969. Filamentous bacterial viruses. Bacteriol. Rev. **33**:172–209.
21. Simmon, V. F., and S. Lederberg. 1972. Degradation of bacteriophage lambda deoxyribonucleic acid after restriction by *Escherichia coli* K12. J. Bacteriol. **112**: 161–169.
22. Takano, T., T. Watanabe, and T. Fukasawa. 1968. Mechanism for host-controlled restriction of bacteriophage λ by R-factors in *Escherichia coli* K12. Virology **34**:290–302.
23. Watanabe, T., T. Takano, R. Arai, H. Nishida, and S. Sato. 1966. Episome-mediated transfer of drug-resistance in *Enterobacteriaceae*. X. Restriction and modification of phages by *fi*$^-$ R-factors. J. Bacteriol. **92**:477–486.
24. Weisberg, R. A., and J. A. Gallant. 1967. Dual function of the prophage repressor. J. Mol. Biol. **25**:537–544.
25. Yoshimori, R., D. Roulland-Dussoix, and H. W. Boyer. 1972. R factor-controlled restriction and modification of deoxyribonucleic acid: restriction mutants. J. Bacteriol. **112**:1275–1279.