Journal of Bacteriology, Oct. 1972, p. 200-205
Vol. 112, No. 1
Printed in U.S.A.
Copyright © 1972 American Society for Microbiology

Klebsiella aerogenes Strain Carrying Drug-Resistance Determinants and a *lac* Plasmid

JEAN E. BRENCHLEY[1] AND BORIS MAGASANIK

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received for publication 13 July 1972

In addition to carrying determinants conferring resistance to at least two antibiotics, chloramphenicol and streptomycin, a *Klebsiella aerogenes* strain contains a plasmid responsible for increased β-galactosidase activity. The plasmid can be transferred to *Escherichia coli* and *Salmonella typhimurium* strains. *K. aerogenes* segregants without the plasmid grow on lactose one-half as fast as the parent strain and contain only one-tenth to one-fifth as much β-galactosidase.

Although numerous plasmids, particularly those for drug resistance markers, have been described (1, 9), reports of naturally occurring factors carrying genes normally associated with the bacterial chromosome are more rare. Factors have been reported for *Salmonella typhosa* (3) and *Proteus rettgeri* (4, 14) which allowed these strains to grow on lactose. The detection of these *lac* factors was based on the isolation from clinical specimens of organisms with properties normally associated with *Salmonella* or *Proteus* except that they were capable of fermenting lactose. Characterization of these factors suggested that they originated from organisms other than *Salmonella* or *Proteus*. One other *lac* plasmid, itself devoid of transfer ability but capable of being transferred with known F-primes, has been described for *Klebsiella* (11, 12). In this case the parent strain was assumed to be lactose positive, but a derivative without this plasmid was not characterized to determine the effect of the plasmid on the parent's ability to utilize lactose.

We have found that *Klebsiella aerogenes* strain MK-1 carries resistance factors for at least chloramphenicol and streptomycin plus an unusual plasmid providing an increased level of β-galactosidase activity. This plasmid can be lost spontaneously or transferred to *Salmonella typhimurium* and *Escherichia coli* strains. Since a transducing phage exists (8) for this strain, it is a likely choice for further genetic studies and its transfer ability could be extremely valuable in developing a much needed conjugation system for these *Klebsiella* strains. Also, knowledge of the *lac* plasmid is essential for interpreting physiological experiments with β-galactosidase.

[1] Present address: Department of Microbiology, The Pennsylvania State University, University Park, Pa. 16802.

MATERIALS AND METHODS

Media. Minimal medium is that described previously (13) with carbon sources at 0.4%. Complex medium is LB (10). Streptomycin was added to LB at 500 μg/ml and chloramphenicol at 100 μg/ml for characterization of the drug-resistance plasmids. Streptomycin was added at 1 mg/ml to lactose minimal medium for selection of the *E. coli* recipient in transfer experiments with *Klebsiella* which is resistant to only about 100 μg of streptomycin/ml. Lactose-tetrazolium medium contained 25.5 g of antibiotic medium 2 (Difco), 50 mg of 2,,3,5-triphenyl-2H-tetrazolium chloride, and 10 g of lactose per liter.

Culture conditions. The cultures were grown at 37 C. Strains to be used as donors for transfer studies were grown as stationary LB cultures and then inoculated into LB at about 2×10^8 cells/ml and incubated about 1 hr before use. Recipient strains were grown in LB with shaking. Donor and recipient strains were cross-streaked on selective plates, and the phenotypes of colonies appearing at the junction were checked.

Acridine orange treatment was according to the procedures of Hirota (6). The sodium dodecyl sulfate (SDS) procedure was that described by Tomoeda et al. (15).

Strains. MK-1 is a derivative (10) of *K. aerogenes* strain W70 described by MacPhee, Sutherland, and Wilkinson (8) and is the host organism for the transducing phage PW52. Derivatives of MK-1 and other strains used are described in Table 1.

β-Galactosidase assays. β-Galactosidase assays were as described by Prival and Magasanik (10).

RESULTS

Several auxotrophic mutants isolated from

TABLE 1. *Characteristics of strains used*

Strain	Relevant phenotype[a]	Derivation
MK-1	Lac^H Sm^r Cm^r	*Klebsiella aerogenes* W70
MK-19	Lac^L Sm^r Cm^r Pur^-	Mutagenesis of MK-1
MK-189	Lac^L Sm^r Cm^r	Mutagenesis of MK-19
MK-247	Lac^L Sm^r Cm^r	Pur^+ transductant of MK-19
MK-195	Lac^H Sm^r Cm^r	Colony giving rise to Lac^L sector MK-196
MK-196	Lac^L Sm^r Cm^r	From Mk-195
MK-197	Lac^H Sm^r Cm^r	Colony giving rise to Lac^L sector MK-198
MK-198	Lac^L Sm^r Cm^r	From MK-195
MK-199	Lac^H Sm^r Cm^r Gal^- Bio^-	Chlorate resistant strain containing deletion of Gal-Hut-Bio region
MK-200	Lac^L Sm^r Cm^r Gal^- Bio^-	From MK-199
S-134	Lac^- Str^r	From *Escherichia coli* K12
4980	Lac^- Str^r	From *E. coli* K12
3.000	Lac^+	*E. coli* K12 Hfr Hayes
1033	Lac^+	*K. aerogenes*
JL-602	Met^- Lac^-	From *Salmonella typhimurium* LT-2
AC-100	Lac^+ Str^r Cm^r	Conjugation between MK-199 and 4980
AC-101	Lac^+ Str^r	Conjugation between MK-199 and 4980

[a] The abbreviations Sm and Cm refer to the streptomycin and chloramphenicol properties associated with the plasmid, whereas Str^r indicates the phenotype determined by chromosomal genes. The MK designation refers to *Klebsiella aerogenes* strains. All other strains are *E. coli* (excepting 1033 and JL-602 which are *Klebsiella* and *Salmonella*, respectively).

MK-1 without any apparent selection for altered growth on lactose were found to have one-tenth the β-galactosidase activity of the parent strain. The frequency with which this effect was observed in different strains suggested that it was not due to double mutations arising during mutagenesis or selection but to some unusual characteristic inherent in the strain. One of these mutants, MK-19, requires adenine for growth (Pur^-) and was used in reconstruction experiments to find a growth condition where it is possible to distinguish the strains with high β-galactosidase activity (Lac^H) from those with lowered activity (Lac^L). When a mixed population of MK-1 and MK-19 was grown on lactose-tetrazolium plates supplemented with 50 μg of adenine/ml to ensure full growth of MK-19, two colony types appeared. The colonies were scored, and it was found that the slightly larger white-edged colonies corresponded to the Lac^H phenotype of MK-1, whereas the smaller, brighter colonies which required adenine (MK-19) were Lac^L.

Using these characteristics, it was possible to examine a culture of MK-1 for spontaneous Lac^L colonies and also Lac^H colonies for sectors resembling the Lac^L type. From 1 to 5% of the MK-1 colonies had Lac^L segregrants (isolated colonies of the Lac^L type were also observed as about 0.1 to 1% of the colonies); several of these segregants were purified and their β-galactosidase levels were measured. Table 2 shows that both the uninduced and induced levels are about 10 times greater in the Lac^H strains than in the Lac^L derivatives or in the other *K. aerogenes* strain 1033. (Prior to the reallocation of nonmotile *Aerobacter* strains to the genus *Klebsiella*, this was referred to as *Aerobacter aerogenes* 1033.)

These Lac^L strains still contained significant levels of β-galactosidase activity and could grow on lactose minimal agar. However, growth experiments demonstrated that the change from Lac^H to Lac^L does affect the organism's ability to grow on lactose. Figure 1 shows the growth of MK-195 and MK-196 in glucose, galactose, and lactose media. The Lac^L strain exhibits a longer lag and about twice the generation time as the Lac^H strain in lactose medium. Growth in glucose and galactose media was normal. Thus, the loss of the high β-galactosidase activity is reflected by a decreased growth rate in lactose medium; however, the regulation of this remaining β-galactosidase by adenosine 3′,5′-monophosphate (cyclic AMP) is the same as that for the Lac^H strains. Table 3 shows that, when glucose is the carbon source, the β-galactosidase activity

TABLE 2. *β-Galactosidase levels in various Klebsiella strains*

Strain	IPTG[a] addition	β-Galactosidase activity[b] (units/OD_{420})
MK-1	−	40
MK-1	+	1,416
MK-195	−	26
MK-195	+	1,950
MK-196	−	2
MK-196	+	197
MK-197	+	1,890
MK-198	+	173
MK-199	+	1,835
MK-200	+	233
1033	+	237

[a] Isopropylthiogalactoside added at 5×10^{-3} M to induce β-galactosidase synthesis.

[b] One unit of enzyme activity is defined as producing 1 nmole of o-nitrophenol per min under the assay conditions used (10). The absorbancies of the cell suspensions used in the assays were determined at 420 nm. Cells were grown in citrate (0.4%) minimal medium plus appropriate nutritional supplements where required. Cultures were harvested at 100 Klett units.

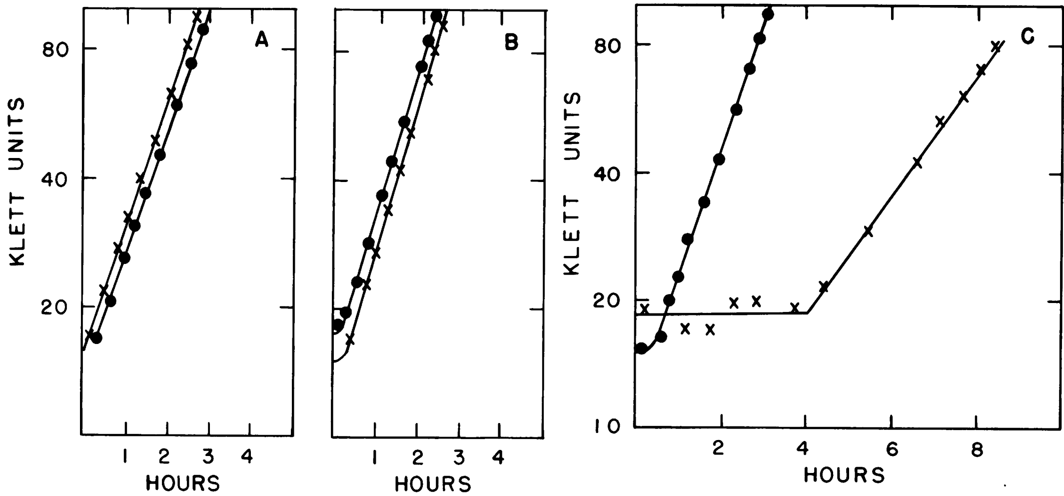

FIG. 1. *Cells were pregrown in glucose minimal medium, harvested by centrifugation, washed twice in sterile 0.85% sodium chloride, resuspended to their original volume, and used to inoculate the media containing different carbon sources as indicated. Symbols:* ●, *MK-195;* ×, *MK-196; A, galactose; B, glucose; and C, lactose.*

TABLE 3. *Cyclic AMP effect on β-galactosidase activities of Lac^H and Lac^L cultures*[a]

Strain	Cyclic AMP	β-Galactosidase	Ratio +/− cyclic AMP
MK-1	−	167	
MK-1	+	1,202	7.2
MK-189	−	13.8	
MK-189	+	94.3	6.9
MK-247	−	14.5	
MK-247	+	107.7	7.4

[a] Cultures were grown in glucose minimal medium plus 5×10^{-3} M IPTG. Cyclic AMP was added at 10^{-2} M.

is increased sevenfold in the presence of cyclic AMP for both Lac^H and Lac^L cultures.

The Lac^L strains were examined for their ability to recover the Lac^H phenotype as scored by their colony morphology on the tetrazolium plates. Of 5,000 colonies scored, none appeared as Lac^H. Thus, the high frequency of segregation from Lac^H to Lac^L and the stability of the resulting Lac^L strains suggest that a plasmid is responsible for the Lac^H phenotype. Since reports of plasmids conferring drug resistance have become common among several bacteria including *Klebsiella* (5, 7), MK-195 (Lac^H) and MK-196 (Lac^L) were examined for possible drug resistance as an indication of the presence of these plasmids.

Both MK-195 and MK-196 appear as sensitive to kanamycin, sodium cephalothin, tetracycline, mandelamine, and erythromycin as *K. aerogenes* 1033, *E. coli* K12, and *S. typhimurium* LT-2, but they are resistant to at least 100 μg of chloramphenicol or streptomycin per ml. No difference in sensitivity was found between MK-195 and MK-196. Several other Lac^L strains were examined; all were resistant to streptomycin and chloramphenicol (although one Lac^L was resistant to only 300 μg of chloramphenicol/ml and not to the usual 500 μg/ml concentration) showing that the Lac^H characteristic could be altered without necessarily changing the resistance to streptomycin or chloramphenicol.

It was then of interest to determine whether any of these characteristics could be transferred to other organisms. In conjugation experiments with *E. coli* 4980 and S-134 (containing a deletion in the lactose operon) and *S. typhimurium* JL-602, only the Lac^H culture, MK-199, transferred the ability to grow on lactose (Table 4). No growth resulted with the supernatant fluid of the MK-199 culture, indicating that phage or free deoxyribonucleic acid (DNA) was not responsibe for the transfer. The lactose-positive recipients retained the nutritional requirements associated with the original *E. coli* or *Salmonella* strains and were T4 and P22 sensitive, respectively; both were resistant to the *Klebsiella* PW52 phage. Some of the lactose-positive recipients were scored for their ability to grow on chloramphenicol, and about 50% were found to be chloramphenicol resistant. (The *E. coli* strains were already resistant to 1 mg of streptomycin/ml and this

TABLE 4. *Transfer of ability to grow on lactose from Klebsiella to other strains*

Donor	Recipient	Growth[a]
MK-199	4980	+
Supernatant fluid from MK-199 culture	4980	−
MK-200	4980	−
Supernatant fluid from MK-200 culture	4980	−
MK-199	S-134	+
MK-200	S-134	−
MK-199	JL-602	+
MK-200	JL-602	−
MK-199	None	−
MK-200	None	−

[a] Symbols: +, confluent growth at juncture of the cross-streak of the donor and recipient; −, no growth on the cross-streaks.

was used in addition to growth without biotin as a selection against the *Klebsiella* donor which is sensitive to the higher levels of streptomycin.)

Cultures of the Lac^+ Cm^R *E. coli* strain, AC-100, were compared with MK-199 for spontaneous and acridine orange-mediated curing of these markers. MK-199 segregates spontaneously to Lac^L at about 1 to 5%; this frequency does not increase with acridine orange or SDS treatment. No strains sensitive to streptomycin or chloramphenicol, or both, were found to arise from MK-199 under any of these conditions although preliminarly experiments with ethidium bromide treatments (2) indicate that this treatment may facilitate curing at a low frequency. The *E. coli* strain AC-100 produces about 1% Lac^- and about 13% chloramphenicol-sensitive colonies spontaneously. After treatment with 100 μg of acridine orange per ml, this was increased to 10% Lac^- and 19% chloramphenicol-sensitive colonies. Thus, it can be shown that both the Lac^H and chloramphenicol characters can be transferred from the original strains and that their segregation from the recipients occurs at high frequencies. Loss of the Lac^H character appears to be independent of the chloramphenicol and streptomycin factors, but it is not known whether it contains the genetic capability to promote its own transfer or whether it is dependent on a transfer mechanism provided by the other factors.

The level of β-galactosidase in the *E. coli* recipient was compared with that normally associated with a Lac^+ *E. coli* and with the Lac^H and Lac^L *Klebsiella* strains. Figure 2 shows the differential rate of β-galactosidase synthesis for *Klebsiella* strains MK-199, MK-200, and 1033, and *E. coli* strains AC-101 and 3.000 all growing in glycerol medium. Although a quantitative comparison can not be attempted because of differences in stains and possible number of plasmid copies, it is clear that the two *lac* plasmid-carrying strains and the other *E. coli* strain produce the enzyme at similar high differential rates, whereas the Lac^L derivative and the other *Klebsiella* 1033 strain produce it at low rates. The lac^- strain 4980 from which AC-101 is derived has no detectable β-galactosidase activity.

Several other observations are consistent with the presence of these plasmids in MK-1. (i) No lactose-negative strains have been isolated from MK-1 even when the mutagenesis produced several auxotrophs (M. Prival, *personal communication*); however, when another

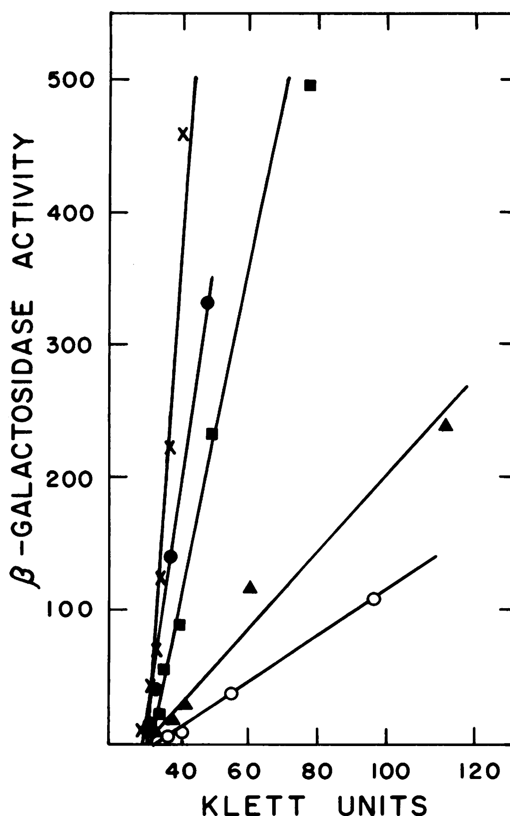

FIG. 2. *Differential rates of β-galactosidase synthesis by various strains. Symbols: ●, AC-101; ×, 3.000; ▲, 1033; ■, MK-109; and ○, MK-200. Cultures were grown in media containing 0.4% glycerol as the carbon source and 5 × 10^{-3} M IPTG to induce β-galactosidase synthesis.*

strain was used which was later shown to be Lac^L, lactose-negative mutants were isolated without difficulty (J. Brenchley, *unpublished results*). (ii) F-primes from *E. coli* and *Salmonella* could be introduced into the *Klebsiella* strains, but the reverse transfer occurred rarely. This is also consistent with the strain harboring other factors which repress synthesis of pili under most conditions (16). (iii) Although attempts to isolate a Cm^s Sm^s strain by treatment with acridine orange were unsuccessful, one such strain was isolated from a strain carrying an F factor (F′ *gal*) from *E. coli*. This Cm^s Sm^s Lac^L strain could now transfer the F episome to *Salmonella* and *E. coli* at a greater frequency than the Cm^r Sm^r Lac^L strains. This would be expected if the plasmids and F are unstable together, increasing the probability that one will be lost.

DISCUSSION

Many bacterial strains carry drug-resistance factors, and the observation of the presence of these factors in *K. aerogenes* MK-1 is not surprising. However, the presence of a *lac* plasmid, either as a separate entity or in conjunction with the drug-resistance factor(s), raises several interesting questions regarding its function, control, and derivation. The F_K-*lac* plasmid found by Reeve and Braithwaite (12) was also associated with antibiotic resistance factors (tetracycline and ampicillin instead of chloramphenicol and streptomycin). Their transfer studies suggested that unlike MK-1, their strains were completely unable to promote conjugation unless transfer genes were provided by another plasmid. Despite this transfer deficiency, the F_K-*lac* produced an active fi^+ repressor; our observation that strains resistant to chloramphenicol and streptomycin fail to transfer an F′ *gal* efficiently, whereas a drug-sensitive strain does transfer, suggests these resistance determinants also make an active fi^+ repressor.

Further, our results presented in Fig. 1 suggest that the possession of the *lac* plasmid by MK-1 provides the already lactose-positive organism with enhanced ability for growth on lactose, but not for growth on glucose or galactose. Cells that have lost the plasmid produce one-tenth the β-galactosidase activity and grow more slowly on lactose medium than the plasmid-carrying cells from which they are derived.

The differential rate of β-galactosidase production under plasmid control is approximately that found in cells of *E. coli* with a chromosomal *lac* operon. Although other explanations are possible, this observation suggests that the promoter and the structural gene for β-galactosidase in the plasmid are similar to the corresponding entities in the *lac* system of *E. coli*. The response of the plasmid β-galactosidase to control by inducer, glucose, and cyclic AMP also resembles that of the *E. coli* enzyme. The same responses to these regulatory agents are also observed in the chromosomally determined β-galactosidase of *K. aerogenes*, which is produced at a much lower differential rate. This lower rate may reflect a less-efficient promoter or a less-active enzyme. Additional work, including studies of the synthesis of permease and transacetylase in *Klebsiella* and a biochemical characterization of the β-galactosidase should help distinguish between these possibilities.

A question remains concerning the abundance of extrachromosomal-like factors carrying genes usually associated with chromosomal functions. Has the isolation of these strains containing the *lac* plasmid been selected for during growth and transfer in the laboratory, or is the presence of plasmids carrying "chromosomal genes" a common occurrence but the majority go undetected because their properties are not examined as routinely as β-galactosidase activities or growth on lactose? Future examinations of several different bacteria for their growth on various media and their ability to form segregants with altered growth properties might show that the occurrence of extrachromosomal genetic information in bacteria is not as rare as previously thought.

ACKNOWLEDGMENTS

We thank Michael Prival and Bonnie Tyler for helpful discussions.

This work was supported by Public Health Service research grants GM-07446 from the National Institute of General Medical Sciences and AM-13894 from the National Institute of Arthritis and Metabolic Diseases and grant GB-5322 from the National Science Foundation.

LITERATURE CITED

1. Anderson, E. S. 1968. The ecology of transferable drug resistance in the enterobacteria. Ann. Rev. Microbiol. 22:131–180.
2. Bouanchaud, D. H., M. R. Scavizzi, and Y. A. Chabbert. 1969. Elimination by ethidium bromide of antibiotic resistance in enterobacteria and staphylococci. J. Gen. Microbiol. 54:417–425.
3. Falkow, S., and L. S. Baron. 1962. Episomic element in a strain of *Salmonella typhosa*. J. Bacteriol. 84:581–589.
4. Falkow, S., J. A. Wohlhieter, R. V. Citarella, and L. S. Baron. 1964. Transfer of episomic elements to *Proteus* II. Nature of *lac*$^+$ *Proteus* strains isolated from clinical specimens. J. Bacteriol. 88:1598–1601.
5. Hinshaw, V., J. Punch, M. Y. Allison, and H. P. Dalton.

1969. Frequency of R factor-mediated multiple drug resistance in *Klebsiella* and *Aerobacter*. Appl. Microbiol. **17**:214:218.

6. Hirota, Y. 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **46**:57-64.
7. Iyer, R. V., and V. N. Iyer. 1969. Genetic and molecular properties of an infectious antibiotic resistance (R) factor isolated from *Klebsiella*. J. Bacteriol. **100**:605-616.
8. MacPhee, D. G., I. W. Sutherland, and J. F. Wilkinson. 1969. Transduction in *Klebsiella*. Nature (London) **221**:475-476.
9. Novick, R. P. 1969. Extrachromosomal inheritance in bacteria. Bacteriol. Rev. **33**:210-235.
10. Prival, M. J., and B. Magasanik. 1971. Resistance to catabolite repression of histidase and proline oxidase during nitrogen-limited growth of *Klebsiella aerogenes*. J. Biol. Chem. **246**:6288-6296.
11. Reeve, E. C. R. 1970. Transfer characteristics of two resistance determinants in a wild strain of *Klebsiella aerogenes* (V9A). Genet. Res. (Cambridge) **16**:235-240.
12. Reeve, E. C. R., and J. A. Braithwaite. 1970. F_K-*lac*, an episome with unusual properties found in a wild strain of a *Klebsiella* species. Nature (London) **228**: 162-164.
13. Smith, G. R., Y. S. Halpern, and B. Magasanik. 1971. Genetic and metabolic control of enzymes responsible for histidine degradation in *Salmonella typhimurium*. J. Biol. Chem. **246**:3320-3329.
14. Sutter, V. L., and F. Y. Foecking. 1962. Biochemical characteristics of lactose-fermenting *Proteus rettgeri* from clinical specimens. J. Bacteriol. **83**:933-935.
15. Tomoeda, M., M. Inuzuka, N. Kubo, and S. Nakamura. 1968. Effective elimination of drug resistance and sex factors in *Escherichia coli* by sodium dodecyl sulfate. J. Bacteriol. **95**:1078-1089.
16. Watanabe, T., and T. Fukasawa. 1962. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. IV. Interactions between resistance transfer factor and F-factor in *Escherichia coli* K-12. J. Bacteriol. **83**:727-735.