JOURNAL OF BACTERIOLOGY, Jan. 1977, p. 358-366
Copyright © 1977 American Society for Microbiology
Vol. 129, No. 1
Printed in U.S.A.

New Mini-ColE1 as a Molecular Cloning Vehicle

HANNA AVNI,* PATRICIA E. BERG, AND ALVIN MARKOVITZ

Department of Microbiology, The University of Chicago, Chicago, Illinois 60637

Received for publication 22 June 1976

A new mini-ColE1 plasmid, designated pAC105, was isolated. It has a molecular weight of 1.6×10^6 and carries information for its self-replication as well as information for conferring colicin E1 immunity upon its host. Furthermore, pAC105 undergoes replication in the presence of chloramphenicol even when a foreign deoxyribonucleic acid (pSC101) is inserted into its single *Eco*RI restriction site. Studies in minicell-producing strains demonstrate that pAC105 codes for only two or three polypeptides of low molecular weight. The advantages of using it as a molecular cloning vehicle are discussed.

The colicinogenic factor E1 (ColE1) is an extrachromosomal plasmid with a molecular weight of 4.4×10^6 which codes for the production of the antibiotic colicin E1 and confers colicin E1 immunity upon its host (colicinogenic bacteria) (13).

ColE1 deoxyribonucleic acid (DNA) has a unique site that is recognized by the restriction endonuclease *Eco*RI (21). This property permits one to ligate an *Eco*RI-cleaved ColE1 DNA with other *Eco*RI-cleaved plasmids; thus, ColE1 DNA can be used as a molecular cloning vehicle (15, 27, 28).

The insertion of a DNA segment in the *Eco*RI site gives rise to a new plasmid that can still confer colicin E1 immunity upon its host, but colicin E1 is no longer produced (15). During the course of ligating a segment of plasmid pMC52 (described below; 1) with the ColE1 DNA, a new mini-ColE1 plasmid was isolated. This plasmid, designated pAC105, has a molecular weight of 1.6×10^6, carries the information for colicin E1 immunity, is cleaved by the *Eco*RI enzyme, is amplified in the presence of chloramphenicol, and codes for two or three low-molecular-weight proteins.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains MC100 and MC171 have been described (4, 16). Strain χ984 is a minicell-producing strain obtained from R. Curtiss III (10). Strain C600, containing plasmid pSC101 (C600/pSC101), was obtained from S. Cohen. Strain JC411/ColE1 was obtained from P. Modrich. Plasmid pMC52 has been described (1). All strains are derivatives of *Escherichia coli* K-12.

Media, agarose gel electrophoresis, and electron microscopy. Media, agarose gel electrophoresis, and electron microscopy have been described (1).

Isolation of DNA. Covalently closed circular DNA was isolated by cesium chloride-ethidium bromide centrifugation (23) of cleared lysates prepared by the lysozyme-ethylenediaminetetraacetic acid-Triton X-100 procedure (19).

Enzymes. *Eco*RI was a gift from P. Modrich. The enzyme reaction has been described (22). T4 ligase was a gift from C. Richardson. The T4 ligase procedure from which pAC121 was isolated followed that of Sgaramella et al. (24), as indicated below. A mixture of pSC101 and pAC105 DNA (1:1.2, by weight) was cleaved by *Eco*RI (22). A 0.5-μg amount of the cleaved DNAs in the enzyme reaction mixture (minus adenosine 5'-triphosphate) was kept at 65°C for 3 min and then slowly (6 h) cooled to 4°C. Two units of the T4 ligase was added, and the mixture was kept for 30 min at 4°C. The reaction was then initiated by the addition of adenosine 5'-triphosphate. After 2 h at 4°C, 2 more units of T4 ligase was added and the reaction was continued at 4°C overnight.

Colicin E1 and E2 extractions. Colicin E1 and E2 extractions followed the procedure of Foulds and Barrett (9).

Transformation. Transformation was performed as described previously (1), using 1 μg of DNA per 4 × 10^9 $CaCl_2$-treated cells in a final volume of 0.3 ml. Tetracycline was omitted when selection was for colicin E1 immunity alone, and such transformants were selected on YET agar plates prespread with 50 units of colicin E1.

Purification of minicells and analysis of their proteins. Purification of minicells and labeling the proteins being synthesized followed the method described by Levy (20), except that the growth medium was M9 supplemented with 0.5% (wt/vol) Casamino Acids, adenine (50 μg/ml), and vitamin B6 (10 μg/ml). The polyacrylamide gel electrophoresis has been described (26). *N,N'*-diallytartardiamide was substituted for *N,N'*-methylene-bisacrylamide, and the main gel was 20% acrylamide with an *N,N'*-diallytartardiamide-acrylamide ratio of 1:350. Labeled proteins from minicells were detected by the fluorographic technique of Bonner and Laskey (3). The standards used and their molecular weights were: proinsulin, 9,000; lysozyme, 14,500; ovalbumin, 45,000; bovine serum albumin, 67,000.

Effect of chloramphenicol on thymidine incorpo-

ration. *E. coli* MC100 carrying various plasmids were grown in YET broth to exponential phase. At a cell density of 2×10^8 cells/ml, chloramphenicol (stock solution, 34 mg/ml in ethanol) was added to a final concentration of 170 μg/ml. After 3.5 h, the cultures were divided into two portions of equal volume. One portion of each culture received rifampin (final concentration, 5 μg/ml). At the times indicated, 1-ml samples (in duplication) were withdrawn into a tube containing 1 μCi of [^{3}H]thymidine. The tubes were shaken at 37°C for 5 min. Then 5% trichloroacetic acid was added, and the samples were filtered and counted.

RESULTS

Isolation of pAC105. Plasmid pMC52 can be cleaved into a 6.1×10^6-dalton (29) fragment (pSC101) and a 2×10^6-dalton fragment (*capR*^{s}) by *Eco*RI (1). When mucoid bacteria carrying a *capR* mutation (strain MC171) are transformed with this plasmid, one can isolate clones that are resistant to tetracycline and are nonmucoid. Both ColE1 and pMC52 plasmids were cleaved by the *Eco*RI enzyme, and an attempt was made to ligate the *capR*^{s} and the ColE1 DNAs with T4 ligase. Since *capR*^{s} DNA does not contain an easily selectable marker, the primary selection was for colicin immunity. The transformants of strain MC171 were further screened for sensitivity to colicin E1 to distinguish between immune and tolerant bacteria (15). The transformants were also tested for sensitivity to tetracycline and for loss of colicin production, the latter as an indication that a DNA fragment was inserted into the *Eco*RI site (15). Six colicin E1-immune clones that did not produce colicin E1 and were tetracycline sensitive were isolated. They were still mucoid. Nonetheless, their DNAs were isolated, since it has been speculated (1) that *capR*^{s} DNA might cause cells to be nonmucoid only in conjunction with pSC101. Of course, a deletion of DNA specifying colicin E1 production would also yield the same phenotype. Two of the extracts showed, in CsCl-ethidium bromide equilibrium gradients, a characteristic covalently closed circular lower band in addition to the chromosomal upper band. This lower band DNA was examined by agarose gel electrophoresis and electron microscopy (Fig. 1).

The new plasmid, designated pAC105, has a contour length of 0.73 μm, which corresponds to a molecular weight of 1.6×10^6 using pSC101 DNA as a standard. Similar results were obtained from agarose gel electrophoresis of *Eco*RI-cleaved pAC105 with λ DNA fragments as standards (Fig. 2). The data, obtained from other agarose gels, also established the existence of an *Eco*RI site on pAC105, since the uncut supercoiled plasmid DNA migrates faster than the *Eco*RI cut plasmid (data not shown; 17, 27). Like the ColE1 plasmid, pAC105 transformed cells to colicin immunity; 1 μg of the DNA transformed 1.4×10^6 cells under the conditions described in Materials and Methods.

pAC105 as a cloning vehicle. The finding that pAC105 DNA contains an *Eco*RI restriction site suggested the possibility of using it as a cloning vehicle. pAC105 and pSC101 DNA were mixed together, celaved with *Eco*RI enzyme, and ligated by T4 DNA ligase, as described in Materials and Methods. This ligated DNA was used to transform strain MC100. Clones were selected for their ability to grow in the presence of both tetracycline (25 μg/ml) and colicin (50 units/plate). Twenty-eight transformants were obtained and all of them were sensitive to colicin E1. Two clones were selected for further analysis. DNA was purified from pure clones of transformed cells by CsCl-ethidium bromide centrifugation. The covalently closed circular DNA was used again to transform strain MC100, and the number of transformants on the single and double selection plates were compared. The number of cells transformed by the new plasmids pAC111 and pAC112 was somewhat higher when single selection was used as compared to double selection (Table 1). However, with pAC111 single selection was never as much as twofold higher than double selection, and with pAC112 single selection was never as much as threefold higher. In contrast, when a mixture of the two separate plasmids pAC105 (colicin E1 immunity) and pSC101 (tetracycline resistance) was used, single selection was 41 to 120 times greater than double selection (Table 1). These results indicated that a joint plasmid was present in preparations pAC111 and pAC112. The possibility that separate single-character plasmids were also present was ruled out by a subsequent transformation, using serial dilutions of pAC112 DNA. A clone transformed by the highest dilution of DNA that showed resistance to tetracycline and immunity to colicin E1 was purified. The supercoiled plasmid DNA was isolated from the transformant and characterized. The new plasmid, called pAC121, contained a homogeneous population of DNA circles which had a contour length of 3.6 μm, corresponding to a molecular weight of 7.7×10^6 (Fig. 3a). It was cleaved by *Eco*RI enzyme into two fragments corresponding in lengths to pSC101 and pAC105 (Fig. 2c and 3b) and transformed cells efficiently to both tetracycline resistance and immunity to colicin E1; 1 μg transformed 2.7×10^5 cells under the conditions described in Materials and Methods.

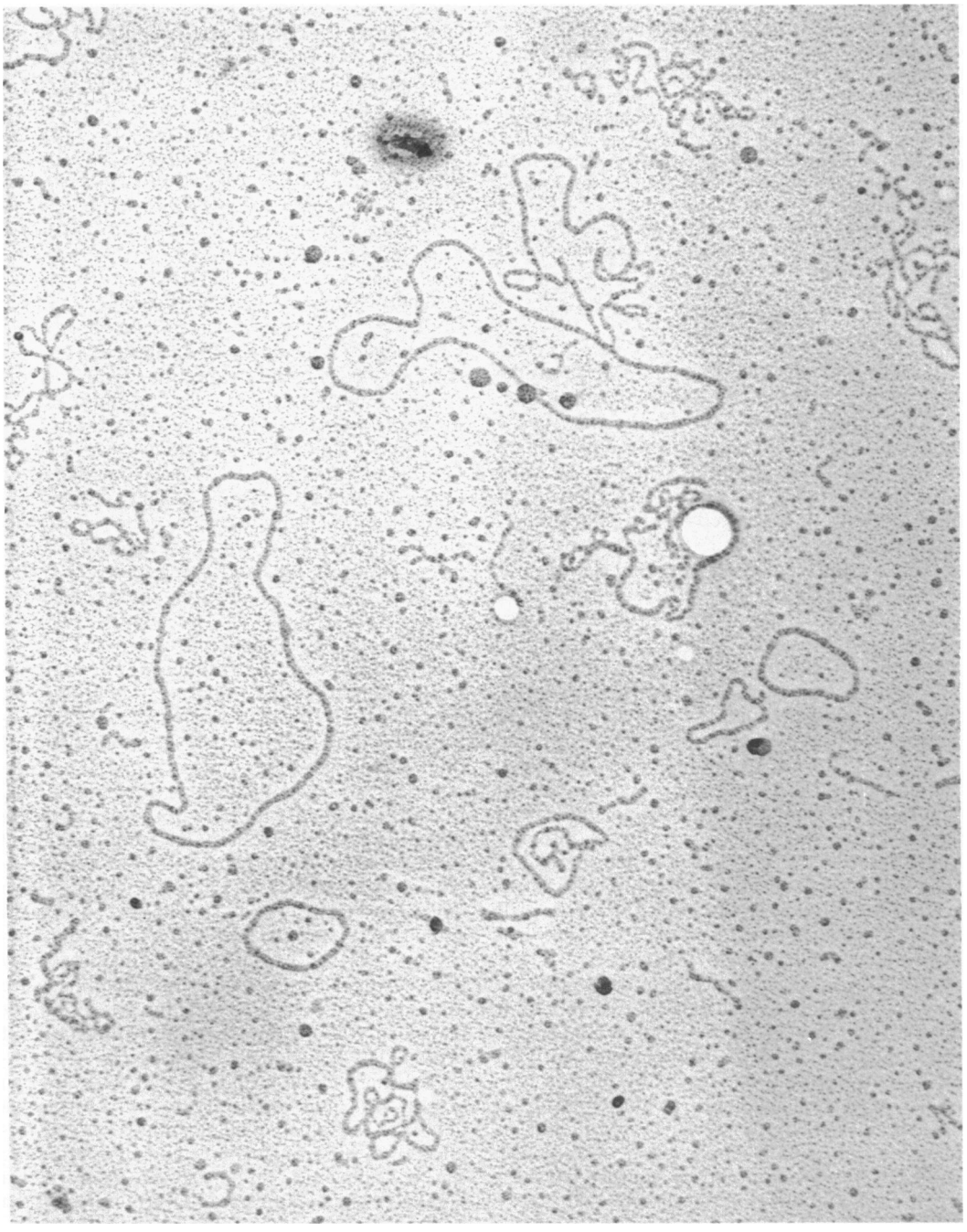

FIG. 1. *Electron micrograph of pAC105 and pSC101. The contour length of pAC105 is 0.73 ± 0.03 μm (32 molecules measured), whereas that of pSC101 is 2.78 ± 0.08 μm (11 molecules measured).*

Replication in the presence of chloramphenicol. ColE1 plasmid DNA continues to replicate in the presence of chloramphenicol after protein synthesis and chromosomal DNA synthesis have ceased (6). This DNA replication is sensitive to the ribonucleic acid polymerase inhibitor rifampin (2). *E. coli* cells of strain MC100 alone, or carrying plasmid pAC105,

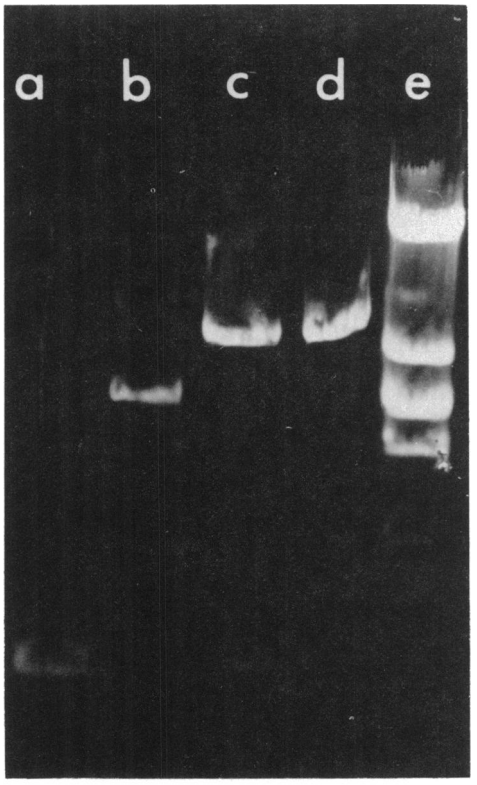

FIG. 2. *Agarose gel electrophoresis of EcoRI digests of plasmid DNA. Samples were treated with EcoRI endonuclease. (a) pAC105; (b) ColE1; (c) pAC121 (see text); (d) pSC101; (e) λ DNA.*

ColE1, pAC121, or pSC101, were grown to exponential phase and then treated with chloramphenicol (170 μg/ml). Three and one-half hours after chloramphenicol addition, each culture was divided into two portions, and rifampin (5 μg/ml) was added to only one portion. At the indicated times (Fig. 4), samples were withdrawn and pulse-labeled with [^{3}H]thymidine. The results shown in Fig. 4 indicate that chloramphenicol almost completely stops [^{3}H]thymidine incorporation in strain MC100 alone or in MC100/pSC101, and rifampin has no further effect. In contrast, [^{3}H]thymidine incorporation of strains containing ColE1, as well as plasmids pAC105 and pAC121, increases in the presence of chloramphenicol and this incorporation is inhibited by rifampin. These data indicate that pAC105 not only carries the gene(s) that determines colicin immunity, but, like its parental plasmid ColE1, follows the same relaxed replication pattern. The amplification of the supercoiled DNA in the presence of chloramphenicol is evident from the increase in the relative amount of the lower band in CsCl-ethidium bromide gradients of crude lysates of strain MC100/pAC105 (Fig. 5). The relaxed replication does not appear to be affected by the insertion of a DNA fragment at the *Eco*RI site, since the strain carrying the combined plasmid pAC121 continues to incorporate thymidine in the presence of chloramphenicol (Fig. 4).

TABLE 1. *Transformation of E. coli MC100 by ligated and separated plasmids*[a]

Plasmid DNA species	Transformation frequency for selective marker		
	Tetracycline	Colicin E1	Tetracycline and colicin E1
pAC111	4.5×10^5	2.9×10^5	2.5×10^5
pAC112	1.3×10^5	2.3×10^5	0.85×10^5
pAC105 and pSC101	7.5×10^5	22×10^5	0.18×10^5

[a] Transformation of *E. coli* strain MC100 by plasmid DNA was carried out as described in Materials and Methods. After DNA uptake, the DNA-cell mixtures were diluted 10-fold in L broth containing 10% glycerol and 2 μg of tetracycline per ml and were grown aerobically for 90 min at 37°C before plating on YET agar plates. Transformation frequency is expressed as transformants per microgram of DNA of each plasmid species. The ratio of pAC105 and pSC101 was the same ratio as their molecular weights, i.e., 0.2 μg of pAC105 and 0.8 μg of pSC101. Concentrations of the drugs were: 25 μg of tetracycline per ml; 50 units of colicin E1 per plate.

Proteins coded by pAC105. *E. coli* χ984 is a minicell-producing strain (10). When strain χ984 carries a plasmid, the plasmid DNA segregates into the minicells and is the only template available for ribonucleic acid and protein synthesis (10). Therefore, proteins coded by plasmid DNA can be selectively labeled in minicells. *E. coli* χ984 was transformed with ColE1 and pAC105 DNAs. Purified minicells were allowed to incorporate [^{35}S]methionine for 3 h at 37°C. At the end of the labeling period the cells were washed and disrupted with sodium dodecyl sulfate at 100°C for 5 min, and the proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (26). The gel was impregnated with 2,5-diphenyloxazole (PPO), and the labeled proteins were detected on an X-ray-sensitive film by the fluorographic technique of Bonner and Laskey (3). Figure 6 shows such a fluorogram. It is clear that ColE1 codes for many proteins spread over a wide range of molecular weights. In contrast, pAC105 codes for only two or three proteins with molecular weights of approximately 9,000, 14,000, and 15,000, as observed by the heavily exposed areas in Fig. 6b and c. Both the 14,500 lysozyme standard and the 9,000-dalton proinsulin standard (a gift from D. Steiner) diffused to the same extent as the unknown proteins coded by pAC105 plasmid in this gel system.

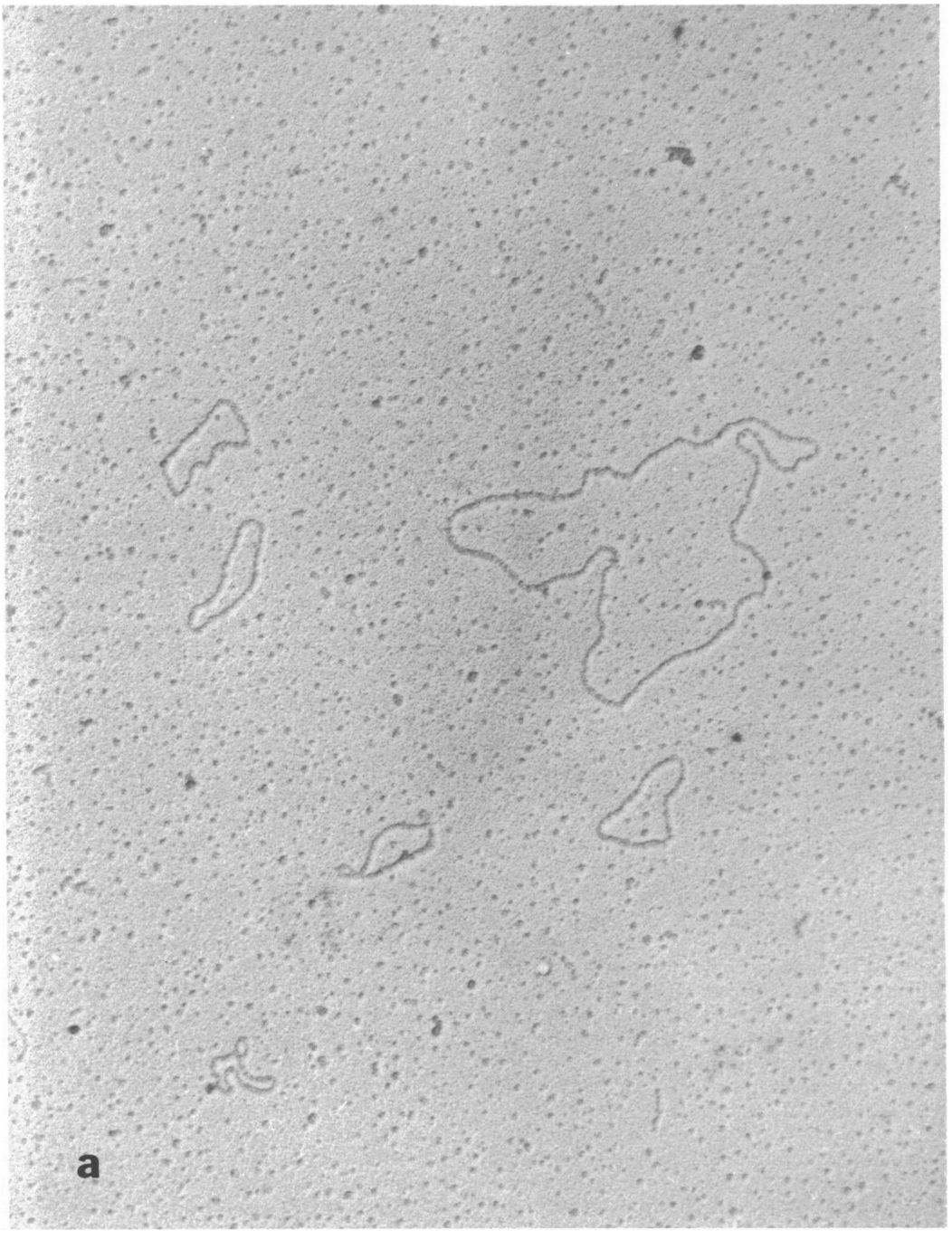

FIG. 3. *Electron micrograph of pAC121 and pAC105. (a) The average contour length of pAC121 is 3.6 ± 0.4 μm (36 molecules measured); that of pAC105 is 0.73 ± 0.03 μm. (b) EcoRI-treated pAC121; lengths, 0.75 ± 0.13 and 2.84 ± 0.17 μm (50 molecules measured).*

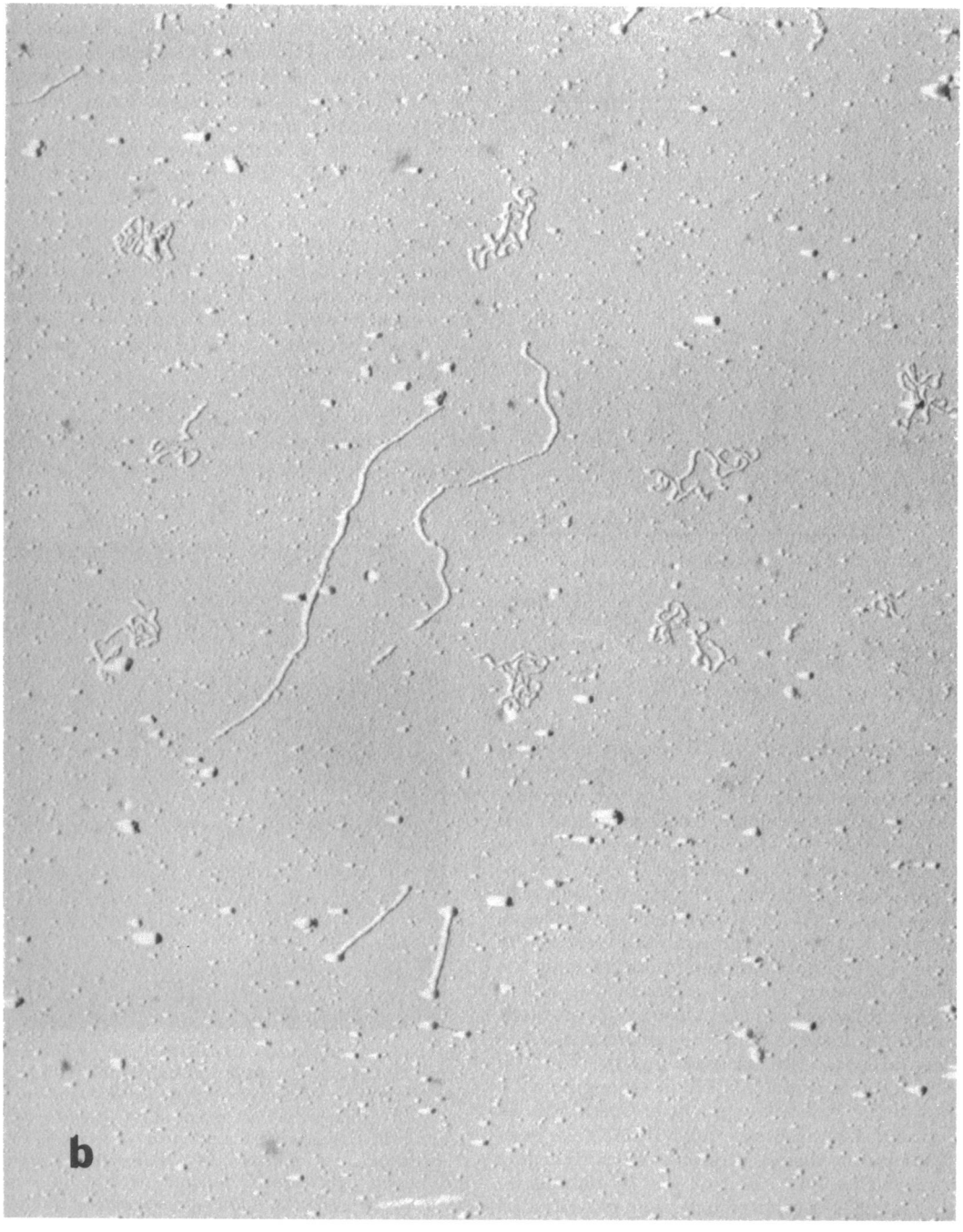

FIG. 3b

DISCUSSION

We have isolated a new mini-ColE1 plasmid, designated pAC105. It contains the genes responsible for its replication and for colicin E1 immunity, like its parental plasmid ColE1. Furthermore, it contains one *Eco*RI restriction site and has been used to clone the plasmid pSC101. It replicates in the presence of chloramphenicol even when a foreign DNA is inserted in its *Eco*RI site and codes for only two or three low-molecular-weight proteins.

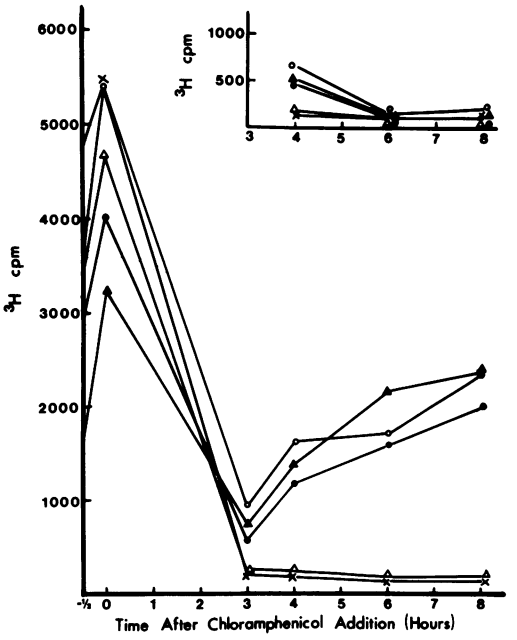

FIG. 4. *Effect of chloramphenicol on thymidine incorporation. Symbols:* ●, *MC100/pAC105;* ○, *MC100/ColE1;* ▲, *MC100/pAC121;* △, *MC100/pSC101;* ×, *MC100. In the inset are samples from the portions that were treated with rifampin.*

The presence of mini-ColE1 plasmids ranging in size from 1.8×10^6 to 2.2×10^6 daltons has been reported before (14, 15, 25). However, one of them, pVH51, probably contains genes of $\phi 80$ bacteriophage (14, 15) and the other contains a substitution of an unknown origin (25). Since all of these plasmids, including pAC105, can self-replicate and are all derived from the same plasmid, ColE1, they probably all contain the same basic genome fragment, containing the genes necessary for replication and for colicin E1 immunity, presumably the 0 to 27% segment from the *Eco*RI site (25). The importance of this segment has been discussed before (25). The majority of plasmid pVH51 is indeed homologous to the 0 to 49% segment of ColE1 (18). In addition, several minicircular DNAs have been isolated from various strains of *E. coli* varying in sizes from 1.5×10^6 to 2.3×10^6 daltons (8, 12). These DNAs shared base sequences, but no biological activity was reported. One of them, mini-DNA from *E. coli* strain 15, hybridized to an appreciable extent with ColE1 DNA (12). This may suggest a common origin for all of these small plasmids. The 1.5×10^6 daltons of DNA from *E. coli* strain 15 conferred no colicin immunity by transformation (data not shown). Therefore, our new mini-colE1 pAC105 is the smallest DNA isolated that carries a selectable character, colicin E1 immunity. The selectability of plasmid pAC105 allows its transformation into any host, and its replication can be studied in *E. coli* strains with known mutations in DNA replication and repair.

Observation by electron microscopy has revealed that ColE1 DNA contains a small percentage (5%) of minicircular DNA molecules with a contour length very close to that of pAC105 (11). We suspect that this DNA is, indeed, pAC105 that might have resulted from a deletion in the ColE1 plasmid. Heteroduplex studies with pAC105 and these other plasmid DNAs are necessary before further discussion of this topic is warranted.

Here we would like to compare the advantages of using pAC105 with those of other plasmids commonly used as cloning vehicles. When foreign DNA is inserted in the *Eco*RI site of

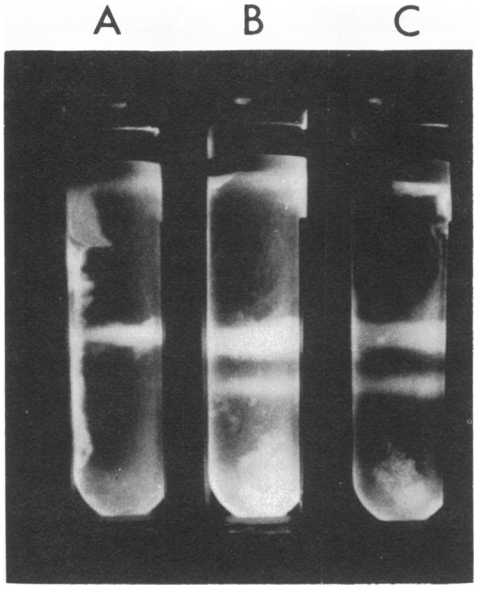

FIG. 5. *Amplification of DNA in the presence of chloramphenicol. A culture of MC100/pAC105 was grown in M9 medium (4) supplemented with 0.5% (wt/vol) Casamino Acids, adenine (50 μg/ml), and vitamin B1 (10 μg/ml). At a bacteria concentration of* 2×10^8*/ml, chloramphenicol (final concentration, 170 μg/ml) was added. Samples of 100 ml were removed at 0, 4, and 8 h after chloramphenicol addition. Lysates were prepared as described in Materials and Methods, except that the entire lysates were centrifuged to equilibrium in CsCl density gradients containing ethidium bromide. The gradients were photographed with an ultraviolet lamp. (A), (B), and (C) represent cells treated with chloramphenicol for 0, 4, and 8 h, respectively. Upper band represents chromosomal DNA; lower band represents covalently closed circular DNA.*

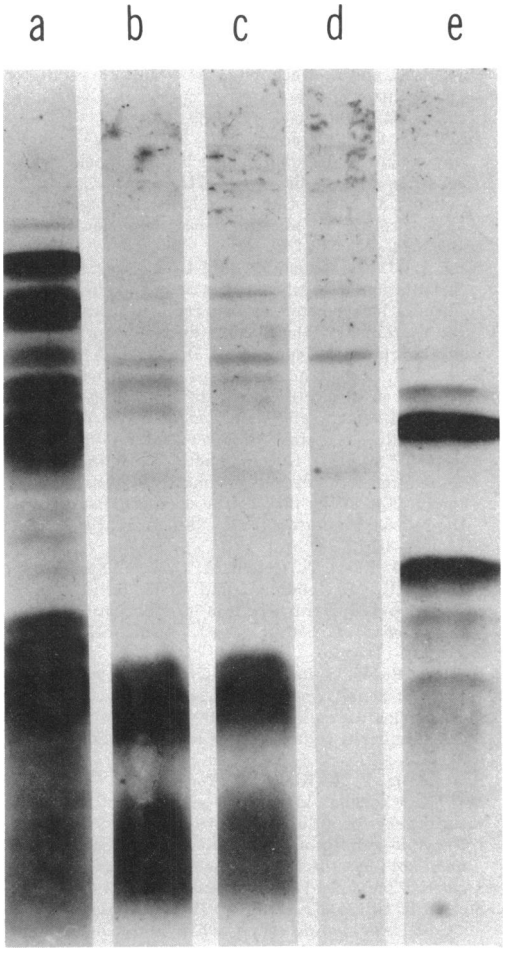

FIG. 6. *Fluorograph of proteins synthesized in minicells carrying plasmids. Purified minicells were concentrated into 1 ml of methionine-free medium (20) and were shaken for 3 h at 37°C with 100 μCi of [^{35}S]methionine (New England Nuclear Corp., 400 Ci/mmol) and 1 μg of nonradioactive methionine. A 50-μl amount of each sample, containing 5,000 cpm, was loaded on a sodium dodecyl sulfate-acrylamide gel. The dried gel was exposed for 5 days. (a) χ984/ ColE1; (b) and (c) χ984/pAC105; (d) χ984; (e) χ984/ pSC101.*

ColE1 DNA the ability to produce colicin E1 is lost (15). However, insertion of DNA in the *Eco*RI site of pAC105 does not interfere with expression of any particular known function, and this is a disadvantage of pAC105 as a cloning vehicle, just as it is with pSC101. pAC105 transforms cells to colicin E1 immunity as does ColE1. This selection occurs against a low background of spontaneous bacterial mutations to colicin resistance and tolerance, and in this respect the tetracycline resistance conferred by pSC101 is superior since spontaneous bacterial resistance to 25 μg of tetracycline per ml has not been observed in our laboratory. Nevertheless, we think that the small size of pAC105 is its main advantage for the following reasons. (i) The use of a cloning vehicle is aimed eventually at cloning a DNA fragment without a detectable marker. An important and helpful technique to isolate such a combined plasmid was developed by Cohen et al. (7) and Chang et al. (5). The heterogeneous DNA population extracted from a large culture of transformed cells can be fractionated on sucrose gradients according to the sizes of the various DNAs species. Since pAC105 is so small, any fragment of DNA inserted in its *Eco*RI site will increase its size significantly, thus allowing the separation of the joint plasmid from the cloning vehicle. (ii) pAC105 codes for only two or three low-molecular-weight proteins, as has been observed from a fluorogram of proteins synthesized in minicells. Once a DNA fragment has been cloned, one would like to investigate the proteins (if there are any) coded by the cloned fragment. With such a background of proteins as the one specified by pAC105, almost any new proteins larger than 15,000 daltons will be easily detected. (iii) As mentioned before, any DNA fragment inserted in the *Eco*RI site of pAC105 will be amplified in the presence of chloramphenicol. Using a rapid qualitative method (Fig. 4), we found that both pAC105 and pAC121 are amplified to approximately the same extent as is ColE1 DNA.

It is worth mentioning that we have isolated a joint plasmid containing the mini-ColE1 DNA of pAC105 and the DNA of the tetracycline resistance plasmid pSC101. This new plasmid, pAC121, might be a cloning vehicle of some advantage itself if a restriction endonuclease were obtained that cuts it in one site and if DNA inserted in such a site caused loss of either the colicin E1 immunity function or tetracycline resistance.

ACKNOWLEDGMENTS

We thank Stephanie Konodi and Carol Sanes for excellent technical assistance and R. C. Gayda for assistance with polyacrylamide gel electrophoresis.

This research was supported by an international fellowship from the American Association of University Women (H.A.), by the Logan Foundation (H.A.), by American Cancer Society grant VC116, and by Public Health Service grant A1-06966 from the National Institute of Allergy and Infectious Diseases.

ADDENDUM IN PROOF

The restriction endonuclease *Sal*I (New England BioLabs) cuts pAC121 only in the tetracycline gene, leaving the immunity to colicin E1 intact. Therefore, pAC121 is a good potential cloning vehicle.

The polypeptides coded by plasmid pAC105 in minicells were obtained as sharper bands on a 24% polyacrylamide gel. The molecular weights of these polypeptides were 15,000, 14,000, 10,000, and 9,000.

LITERATURE CITED

1. **Berg, P. E., R. Gayda, H. Avni, B. Zehnbauer, and A. Markovitz.** 1976. Cloning of *Escherichia coli* DNA that controls cell division and capsular polysaccharide synthesis. Proc. Natl. Acad. Sci. U.S.A. 73:697-701.
2. **Blair, D. G., D. J. Sherratt, D. B. Clewell, and D. R. Helinski.** 1972. Isolation of supercoiled colicinogenic factor E1 DNA sensitive to ribonuclease and alkali. Proc. Natl. Acad. Sci. U.S.A. 69:2518-2522.
3. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method of tritium-labelled proteins and nucleic acids in poly acrylamide gels. Eur. J. Biochem. 46:83-88.
4. **Bush, J. W., and A. Markovitz.** 1973. The genetic basis for mucoidy and radiation sensitivity in *capR* (*lon*) mutants of *E. coli* K-12. Genetics 74:215-225.
5. **Chang, A. C. Y., R. A. Lansman, D. A. Clayton, and S. N. Cohen.** 1975. Studies of mouse mitochondrial DNA in *Escherichia coli*: structure and function of the eucaryotic-procaryotic chimeric plasmids. Cell 6:231-244.
6. **Clewell, D. B.** 1972. Nature of ColE1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. 110:667-676.
7. **Cohen, S. N., and A. C. Y. Chang.** 1974. A method for selective cloning of eukaryotic DNA fragments in *Escherichia coli* by repeated transformation. Mol. Gen. Genet. 134:133-141.
8. **Cozzarelli, N. R., R. B. Kelly, and A. Kornberg.** 1968. A minute circular DNA from *Escherichia coli* 15. Proc. Natl. Acad. Sci. U.S.A. 60:992-999.
9. **Foulds, J., and C. Barrett.** 1973. Characterization of *Escherichia coli* mutants tolerant to bacteriocin JF46: two new classes of tolerant mutants. J. Bacteriol. 116:885-892.
10. **Frazer, A. C., and R. Curtiss III.** 1975. Production, properties and utility of bacterial minicells, p. 1-84. *In* W. Arber et al. (ed.), Current topics in microbiology and immunology, vol. 69. Springer-Verlag, New York.
11. **Goebel, W., and J. Kreft.** 1974. Complex ColE1 DNA in *Escherichia coli* and *Proteus mirabilis*. Mol. Gen. Genet. 129:149-166.
12. **Goebel, W., and H. Schrempf.** 1972. Isolation of minicircular deoxyribonucleic acids from wild strains of *Escherichia coli* and their relationship to other bacterial plasmids. J. Bacteriol. 111:696-704.
13. **Hardy, K. C.** 1975. Colicingeny and related phenomena. Bacteriol. Rev. 39:464-515.
14. **Hershfield, V., H. W. Boyer, L. Chow, and D. R. Helinski.** 1976. Characterization of a mini-ColE1 plasmid. J. Bacteriol. 126:447-453.
15. **Hershfield, V., H. W. Boyer, C. Yanofsky, M. A. Lovett, and D. R. Helinski.** 1974. Plasmid ColE1 as a molecular vehicle for cloning and amplification of DNA. Proc. Natl. Acad. Sci. U.S.A. 71:3455-3459.
16. **Hua, S. S., and A. Markovitz.** 1972. Multiple regulator gene control of the galactose operon in *Escherichia coli* K-12. J. Bacteriol. 110:1089-1099.
17. **Inselburg, J., and V. Johns.** 1975. Mapping of colicin E1 and colicin E3 plasmid deoxyribonucleic acid *Eco*RI-sensitive sites. J. Bacteriol. 121:381-389.
18. **Inselburg, J., and A. Oka.** 1975. Discontinuous replication of colicin E1 plasmid deoxyribonucleic acid. J. Bacteriol. 123:739-742.
19. **Katz, L., D. T. Kingsbury, and D. R. Helinski.** 1973. Stimulation by cyclic adenosine monophosphate of plasmid deoxyribonucleic acid replication and catabolite repression of the plasmid deoxyribonucleic acid-protein relaxation complex. J. Bacteriol. 114:577-591.
20. **Levy, S. B.** 1974. R factor proteins synthesized in *Escherichia coli* minicells: incorporation studies with different R factors and detection of deoxyribonucleic acid-binding proteins. J. Bacteriol. 120:1451-1463.
21. **Lovett, M. A., D. G. Guiney, and D. R. Helinski.** 1974. Relaxation complexes of plasmids ColE1 and ColE2: unique site of the nick in the open circular DNA of the relaxed complexes. Proc. Natl. Acad. Sci. U.S.A. 71:3854-3857.
22. **Morrow, J. F., S. N. Cohen, A. C. Y. Chang, H. W. Boyer, H. M. Goodman, and R. B. Helling.** 1974. Replication and transcription of eukaryotic DNA in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 71:1743-1747.
23. **Radloff, R., W. Bauer, and J. Vinograd.** 1967. A dye-buoyant density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in Hela cells. Proc. Natl. Acad. Sci. U.S.A. 57:1514-1521.
24. **Sgaramella, V., K. Kleppe, T. Terao, N. K. Gupta, and H. G. Khorana.** 1972. Total synthesis of the structural gene for an alanine transfer RNA from yeast. Enzymic joining of the chemically synthesized segments to form the DNA duplex corresponding to nucleotide sequence 17 to 50. J. Mol. Biol. 72:445-456.
25. **So, M., R. Gill, and S. Falkow.** 1975. The generation of a ColE1-Apr cloning vehicle which allows detection of inserted DNA. Mol. Gen. Genet. 142:239-249.
26. **Spear, P. G., and B. Roizman.** 1972. Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirus. J. Virol. 9:143-159.
27. **Tanaka, T., and B. Weisblum.** 1975. Construction of a colicin E1-R factor composite plasmid in vitro: means for amplification of deoxyribonucleic acid. J. Bacteriol. 121:354-362.
28. **Timmis, K., F. Cabello, and S. N. Cohen.** 1974. Utilization of two distinct modes of replication by a hybrid plasmid constructed *in vitro* from separate replicons. Proc. Natl. Acad. Sci. U.S.A. 71:4556-4560.
29. **Wensink, P. C., D. J. Finnegan, J. E. Donelson, and D. S. Hogness.** 1974. A system for mapping DNA sequences in the chromosomes of Drosophila melanogaster. Cell 3:315-326.