JOURNAL OF BACTERIOLOGY, Apr. 1979, p. 270-272
0021-9193/79/04-0270/03$02.00/0

Vol. 138, No. 1

Increased Amplification of pBR322 Plasmid Deoxyribonucleic Acid in *Escherichia coli* K-12 Strains RR1 and χ1776 Grown in the Presence of High Concentrations of Nucleoside

MICHAEL V. NORGARD, KIRSTEN EMIGHOLZ, AND JOHN J. MONAHAN*

Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey 07110

Received for publication 27 October 1978

When pBR322 plasmid-harboring *Escherichia coli* strains RR1 or χ1776 were grown in the presence of 1 mg of uridine or cytidine per ml and later treated with chloramphenicol, as much as three times more plasmid deoxyribonucleic acid was recovered than would normally be obtained by routine plasmid amplification procedures.

Recently there has been a tremendous surge in the advancement of plasmid DNA technology (1, 10). Various areas of plasmid DNA research routinely involve the preparation of a substantial quantity of plasmid DNA. Because plasmid DNA purification is somewhat laborious and time consuming, new methods are constantly being sought to increase yields of plasmid DNA from cultures of bacterial cells.

The National Institutes of Health EK2-certified plasmid vector pBR322 (3) is now widely used as a vehicle in DNA cloning experiments. Because previous studies showed that DNA from *Escherichia coli* and other gram-negative bacteria is more effectively labeled in vivo by radioactive nucleoside when labeling is performed in the presence of high concentrations of unlabeled nucleoside (4, 6, 13), we attempted to prepare a quantity of high-specific-activity pBR322 plasmid DNA by using these conditions. Our results surprisingly showed about a two- to threefold-higher recovery of purified plasmid DNA from *E. coli* cells grown in the presence of 1 mg of uridine or cytidine per ml than from untreated cells.

Bacteria tested included strains of *E. coli* RR1 (3) and χ1776 (2, 11), each harboring the plasmid pBR322 (3). *E. coli* RR1 cells were grown in M9-glucose medium (9) supplemented with 0.5% (wt/vol) Casamino Acids (Difco Laboratories, Detroit, Mich.) and 2 μg of thiamine HCl (Sigma Chemical Co., St. Louis, Mo.; T-4625) per ml. *E. coli* χ1776 was cultivated either in M9-glucose medium as above but containing additional supplements of 100 μg of DL-diaminopimelic acid (Sigma; D-1377) per ml, 20 μg of thymidine per ml, and 1 μg of biotin (Calbiochem, San Diego, Calif.) per ml [M9(χ1776)] or in "L broth" medium (15) designed for maintenance of this organism.

Overnight inocula of each organism were prepared and diluted 1:100 in 1 liter of each respective medium prewarmed to 37°C in a 2-liter Erlenmeyer flask, and cultures were incubated at 37°C while shaking at 125 rpm in a water bath shaker. When the optical density of the cultures reached 0.1 at 550 nm, uridine (Sigma, U-3750) or cytidine (Sigma, C-9505) was added to a final concentration of 1 mg/ml. Cultures were then returned to the incubator until an optical density at 550 nm of 0.3 was reached, at which time plasmid amplification was promoted by adding chloramphenicol at a final concentration of 12.5 μg/ml or 100 μg/ml for χ1776 or RR1 cells, respectively (7). Cultures were again returned to the water bath shaker, followed by final incubation periods of 5 h for χ1776 cells and 15 h for RR1 cells.

Cells from each 500 ml of culture were then collected by centrifugation (4°C) at 16,300 × *g* for 10 min in 250-ml plastic bottles and washed once by centrifugation (4°C) at 1,500 × *g* for 10 min in 50 ml of cold 50 mM Tris-hydrochloride-5 mM EDTA, pH 7.6. Plasmid DNA was isolated from the cleared lysate of a lysozyme-Brij 58-sodium deoxycholate solution as described previously (8) and purified by CsCl buoyant density gradient centrifugation (16) in the presence of 100 μg of propidium iodide (PI) (Calbiochem) per ml. Purified covalently closed circular (CCC) plasmid DNA (lower CsCl gradient band) was collected, extracted 10 times with an equal volume of water-CsCl-saturated *n*-butanol to remove PI, diluted to twice the original volume with water, and precipitated with two volumes of cold ethanol. Open circular and linear plasmid forms found in the upper CsCl band were collected and treated similarly. Plasmid DNA was then pelleted by centrifugation (4°C) at 13,200 × *g* for 20 min, air dried, dissolved in 10 ml of

water, and again precipitated with ethanol and collected as above. The purified plasmid DNA was dissolved in 10 mM Tris–1 mM EDTA, pH 7.6, and the amount recovered was determined from its UV absorbance at 260 nm.

Figure 1 illustrates the effect of 1 mg of cytidine or uridine per ml (in M9-glucose medium) on the amplification of pBR322 plasmid DNA in *E. coli* RR1. In the absence of high concentrations of nucleoside (Fig. 1A), cleared lysates produced a distinct plasmid band on CsCl-PI gradients corresponding to CCC plasmid DNA. However, DNA from cleared lysates of RR1 cells grown and amplified in the presence of 1 mg of cytidine (Fig. 1B) or uridine (Fig. 1C) per ml yielded much heavier CCC plasmid bands on CsCl-PI gradients. Moreover, the discrete upper bands found in CsCl-PI gradients from cleared lysates of cytidine- or uridine-treated RR1 cells were found to be comprised chiefly of open circular and linear plasmid DNA, as demonstrated by 1% agarose gel electrophoresis (14) (data not shown). When the CCC (lower) bands were harvested from the CsCl-PI gradients and further purified, more than three times more plasmid DNA was recovered from uridine-treated cells than from untreated cells, whereas cytidine treatment produced a slightly lesser effect (Table 1). These increased plasmid yields were not the result of improved cell lysis of cells grown in the presence of high nucleoside concentrations. When cleared lysates of cells grown with or without nucleoside (and harvested before amplification by chloramphenicol) were assayed (5) for total DNA, identical amounts of DNA were found in these lysates.

The National Institutes of Health EK2-certified host *E. coli* χ1776 was also tested in a similar manner, but with two different types of media. When pBR322-harboring χ1776 cells were grown in L broth medium with or without uridine, no difference in plasmid DNA bands from cleared lysates were observed in CsCl-PI gradients. This was made more evident when the plasmid DNA was collected from the gradients and purified (Table 1). Only 160 μg from untreated χ1776 cells and 140 μg of plasmid DNA from uridine-treated cells could be recovered from bands originating from a lysate of χ1776 cells derived from 1 liter of cell culture. Indeed, it has been our experience that pBR322 plasmid amplification in χ1776 cells grown in L broth medium proceeds poorly under any conditions or treatments. On the other hand, we were able to achieve much better (ca. threefold) pBR322 plasmid amplification when χ1776 cells were grown in M9(χ1776) medium (Table 1). Furthermore, uridine-treated χ1776 cells produced almost twice as much plasmid DNA as untreated control cells in M9(χ1776) medium.

In all cases, CCC plasmid DNA preparations purified from CsCl-PI gradients appeared identical irrespective of growth medium employed, amplification conditions, or nucleoside treatments (Fig. 2). Analysis on 1% agarose gels showed that in each plasmid DNA preparation from χ1776 cells tested, plasmid DNA solutions contained a majority of CCC molecules with open circular forms also present as a result of "nicking," which took place during purification. Figure 2E also shows an *E. coli* χ1776 bulk chromosomal DNA control which shows the presence of both chromosomal and plasmid DNA. An identical electrophoretic pattern to Fig. 2 was observed when plasmid and chromosomal DNA preparations from pBR322-harbor-

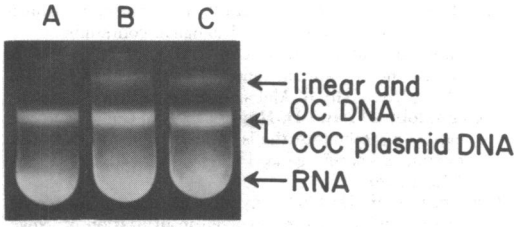

FIG. 1. *CsCl-PI buoyant density gradient centrifugation of DNA from cleared lysates of E. coli RR1 containing pBR322 plasmid DNA. The 30-ml polycarbonate Oak Ridge tubes containing DNA, CsCl (at a final refractive index of $n_D^{25°} = 1.3870$, and 100 μg of PI per ml were centrifuged in a Beckman 70 Ti rotor at 35,000 rpm (10°C) for 48 h. E. coli RR1 cells were previously grown in M9-glucose medium either without additional supplement (A) or in the presence of 1 mg of cytidine (B) or uridine (C) per ml, and then subjected to plasmid amplification by chloramphenicol.*

TABLE 1. *Recovery of pBR322 plasmid DNA from Escherichia coli strains RR1 and χ1776 grown in the presence of 1 mg of nucleoside per ml*

Strain	Medium	Treatment[a]	CCC pBR322 plasmid DNA recovered (mg/liter of cell culture)[b]
RR1	M9-glucose	None	0.85
RR1	M9-glucose	+Cytidine	2.33
RR1	M9-glucose	+Uridine	2.60
χ1776	L	None	0.16
χ1776	L	+Uridine	0.14
χ1776	M9(χ1776)	None	0.46
χ1776	M9(χ1776)	+Uridine	0.74

[a] Performed before plasmid amplification by chloramphenicol.

[b] Cultures were grown to an optical density at 550 nm of 0.3 before the addition of chloramphenicol.

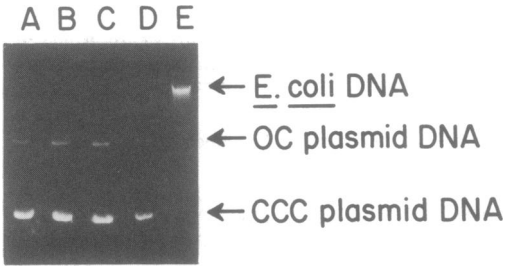

FIG. 2. *Agarose gel electrophoresis of DNA isolated from E. coli χ1776 harboring pBR322 plasmid DNA. Agarose (1%) gel electrophoresis was carried out as described previously (14). Slots: A, CCC plasmid DNA preparation from cells grown in L broth with no additional supplements and then treated with chloramphenicol; B, CCC plasmid DNA preparation from cells grown in L broth containing 1 mg of uridine per ml; C, CCC plasmid DNA preparation from cells grown in M9(χ1776) medium with no additional supplement; D, CCC plasmid DNA preparation from cells grown in M9(χ1776) medium containing 1 mg of uridine per ml; E, χ1776 bulk chromosomal DNA isolated from cells grown in M9(χ1776) medium containing 1 mg of uridine per ml. OC, Open circular.*

ing RR1 cells grown with or without cytidine or uridine were subjected to 1% agarose gel electrophoresis (data not shown).

The mechanism of nucleoside stimulation of pBR322 plasmid amplification reported here remains obscure. However, in *E. coli* (4, 13) and *Haemophilus influenzae* (6), previous reports have implicated the presence of a nonspecific nucleoside and/or nucleotide degrading enzyme(s) which is not a component of uptake mechanisms. Thus, it appears likely that increased levels of endogenous nucleoside accumulating from high exogenous supply (i.e., the medium), may effectively compete and block these degradative enzyme systems. This should allow greater endogenous sources of all four nucleotides to be used for plasmid DNA synthesis during amplification by chloramphenicol.

An interesting application of the nucleoside stimulation of pBR322 plasmid amplification may be found in the screening of bacterial colonies containing potentially cloned DNA. For example, in colony hybridization assays of Grunstein and Hogness (12), it would be a simple matter to incorporate nucleoside into the agar medium during growth of the potential clones. Filter disks containing the colonies could then be either transferred to similar medium containing chloramphenicol or simply sprayed with an appropriate solution of chloramphenicol from an atomizer, followed by incubation to promote plasmid amplification. A modification such as this may increase the overall sensitivity of the colony hybridization technique. In addition, the stimulatory effect of high nucleoside concentration on pBR322 plasmid amplification reported here may be applicable to other *E. coli* host-plasmid systems which are prone to plasmid amplification in the presence of chloramphenicol.

We thank Roy Curtiss III and his co-workers D. Pereira, J. E. Clark-Curtiss, S. Hull, R. Goldschmidt, J. C. Hsu, L. Maturin, R. Moody, and M. Inoue for developing and supplying χ1776, and H. W. Boyer for providing pBR322 plasmid.

LITERATURE CITED

1. **Abelson, J.** 1977. Recombinant DNA: examples of present-day research. Science **196**:159–160.
2. **Armstrong, K. A., V. Hershfield, and D. R. Helinski.** 1977. Gene cloning containment properties of plasmid Col E1 and its derivatives. Science **196**:172–174.
3. **Bolivar, R., R. L. Rodriguez, P. J. Green, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multi-purpose cloning system. Gene **2**:95–113.
4. **Budman, D. R., and A. B. Pardee.** 1967. Thymidine and thymine incorporation into deoxyribonucleic acid: inhibition and repression by uridine of thymidine phosphorylase of *Escherichia coli*. J. Bacteriol. **94**:1546–1550.
5. **Burton, K.** 1956. A study of the conditions and mechanism of diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62**: 315–323.
6. **Carmody, J. M., and R. M. Herriott.** 1970. Thymine and thymidine uptake by *Haemophilus influenzae* and the labeling of deoxyribonucleic acid. J. Bacteriol. **101**: 525–530.
7. **Clewell, D. B.** 1972. Nature of Col E_1 plasmid replication in *Escherichia coli* in the presence of chloramphenicol. J. Bacteriol. **110**:667–676.
8. **Clewell, D. B., and D. R. Helinski.** 1970. Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry **9**:4428–4440.
9. **Clowes, R. C., and W. Hayes (ed.).** 1968. Experiments in microbial genetics, p. 187. Blackwell Scientific Publications, Oxford.
10. **Cohen, S. N.** 1975. The manipulation of genes. Sci. Am. **233**:24–33.
11. **Curtiss, R., III, A. Pereira, J. Hsu, C. Hull, E. Clark, J. Maturin, R. Goldschmidt, R. Moody, M. Inoue, and L. Alexander.** 1976. Biological containment: the subordination of *Escherichia coli* K-12, p. 96–106. *In* Micro 76—the annual report. Department of Microbiology, University of Alabama, Birmingham.
12. **Grunstein, M., and D. Hogness.** 1975. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. U.S.A. **72**:3961–3965.
13. **Kammen, H. O.** 1967. Thymine metabolism in *Escherichia coli*. I. Factors involved in utilization of exogenous thymine. Biochim. Biophys. Acta **134**:301–311.
14. **McReynolds, L. A., J. J. Monahan, D. W. Bendure, S. L. C. Wood, G. V. Paddock, W. Salser, J. Dorson, R. E. Moses, and B. W. O'Malley.** 1977. The ovalbumin gene; insertion of ovalbumin gene sequences in chimeric bacterial plasmids. J. Biol. Chem. **252**:1840–1843.
15. **Norgard, M. V., K. Keem, and J. J. Monahan.** 1978. Factors affecting the transformation of *Escherichia coli* strain χ1776 by pBR322 plasmid DNA. Gene **3**:279–292.
16. **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.