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# Simple Agarose Gel Electrophoretic Method for the Identification and Characterization of Plasmid Deoxyribonucleic Acid

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Argarose gel electrophoresis may be employed effectively for the detection and preliminary characterization of plasmid deoxyribonucleic acid (DNA) present in clinical isolates and laboratory strains of gram-negative microorganisms. The method is sensitive and does not require radioisotopes or ultracentrifugation. The estimation of plasmid mass from the extent of DNA migration in gels compares favorably with results obtained by electron microscopy of plasmid DNA purified by equilibrium density centrifugation. The method has proved to be a useful tool for survey work and the epidemiological investigation of plasmid dissemination, as well as an important adjunct to the genetic analysis of plasmids.

Currently, the standard procedure for isolating and characterizing plasmid deoxyribonucleic acid (DNA) depends upon lysing host bacterial cells and subsequently treating the lysate so that the smaller circular plasmid DNA molecules are separated from the relatively huge mass of chromosomal DNA (6, 8, 13). An integral part of this procedure requires either the sedimentation of the plasmid DNA through an alkaline or neutral sucrose gradient or equilibrium density centrifugation with an intercalating dye such as ethidium bromide. Cesium chloride-ethidium bromide equilibrium density centrifugation requires an extensive period of centrifugation at high speed. The plasmid DNA may often be visualized directly in such density gradients by use of long-wave ultraviolet light (5), but if one wishes to determine the number and size of plasmid species it is necessary to either examine the plasmid DNA under an electron microscope or sediment labeled DNA through a sucrose gradient. Whole lysates containing labeled DNA may be sedimented directly in alkaline sucrose gradients to determine the number and size of resident plasmid species within a bacterial strain (8), but a large number of fractions must be counted in a liquid scintillation spectrometer. Moreover, it is often necessary to include a differentially labeled standard plasmid DNA of known molecular weight in sucrose gradients to calculate the size of any unknown plasmid species. Thus, current procedures for the isolation and superficial characterization of plasmid DNA in even a single strain can be relatively lengthy and costly, and the methods available are not well suited for survey work.

Our laboratory has been particularly interested in examining the plasmids present in clinical isolates of gram-negative bacteria (5, 11, 18). It has been also necessary for us to determine the number and molecular weight of covalently closed circular (CCC) plasmid DNA species in laboratory strains after their conjugation with a clinical isolate. Consequently, we have been searching for an inexpensive screening method for the detection of plasmids and the determination of their size that could be applied to a wide variety of bacterial species. Recently, agarose gel electrophoresis has been widely employed in the analysis of restriction endonuclease-generated fragments of plasmid and viral DNA (2, 14). The use of this methodology for the characterization of CCC DNA has received little attention. Aaij and Borst (1) reported that the migration rates of purified bacteriophage and mitrochondrial CCC DNAs ranging from $3.4 \times 10^6$ to $10 \times 10^6$ daltons were related inversely to the logarithm of their mass in 0.6% agarose gels. The migration properties of higher-molecular-weight CCC DNA has been overlooked, however.

In the present paper we report the use of agarose gel electrophoresis for the detection and preliminary characterization of CCC plasmid DNA present in clinical isolates and laboratory strains of gram-negative microorganisms. We have found the method to be suitable for the detection and estimation of plasmid DNA molecular weight ranging from $0.6 \times 10^6$

to 95 × $10^6$ in partially purified whole-cell lysates.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** The $F^-$ derivative of *Escherichia coli* K-12 strain J5-3, called SF400 [*pro-22 met-63* (λ)], was employed as a standard host for plasmid species. *E. coli* K-12 strain 711 [$F^-(\gamma)$ *lac-28 his-51 trp-30 proC23* Phe, $Nal^r$] was employed as recipient of plasmid species in genetic crosses. The molecular properties of the plasmids R1*drd*19 (62 × $10^6$ daltons), RP4 (34 × $10^6$ daltons), Sa (25 × $10^6$ daltons), RSF1030 (5.5 × $10^6$ daltons), and RSF1010 Ap113 (8.7 × $10^6$ daltons) were described previously (4, 7, 12). The 1.8 × $10^6$-dalton ColE1 derivative pMB8 was isolated by M. Betlach and H. W. Boyer. Strain B41, toxigenic for calves, was described previously (17, 18). Clinical isolates of toxigenic *E. coli* from cases of traveller's diarrhea were provided by E. Gangarosa, Center for Disease Control, Atlanta, Ga. *Haemophilus influenzae* strains containing plasmid-linked ampicillin resistance were described by Elwell et al. (5) The *Neisseria gonorrhoeae* isolates employed were also characterized previously (16).

**Methods of DNA isolation. (i) Preparation of crude lysates for agarose gel electrophoresis.** Plasmid-containing strains were grown overnight in 30 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and harvested by centrifugation. The cells were suspended in 1.5 ml of 25% sucrose in 10 mM tris(hydroxymethyl)aminomethane (Tris)-1 mM ethylenediaminetetraacetate (EDTA), pH 8.0. Cleared lysates were then prepared by the sodium dodecyl sulfate (SDS)-salt precipitation method described by Guerry et al. (10). The volume of the cleared lysate was doubled by the addition of distilled water, 20 μg of ribonuclease per ml (bovine pancreas, type IA, Sigma Chemical Co., St. Louis, Mo.; 1 mg/ml in 50 mM sodium acetate [pH 5.0] heated for 10 min at 90°C) was added, and the lysate was incubated at 37° for 1 h. After ribonucleic acid digestion, 1 volume of Tris (50 mM)-saturated phenol was added. The tube was inverted gently several times and centrifuged at 12,100 × *g* for 30 min at 20°C in a refrigerated centrifuge to obtain a clear aqueous phase. Lysates from some clinical isolates required repeated rounds of phenol extraction and centrifugation to obtain a clear aqueous phase. When repeated phenol extractions were necessary, the loss of plasmid DNA was minimized by collecting the aqueous-phenol interface after each extraction. The combined interface material was pooled and extracted once more with phenol and this aqueous phase was added to the total aqueous volume.

The clear aqueous phase was brought to 0.3 M sodium acetate (final concentration), and twice the volume of cold (−20°C) 95% ethanol was added to precipitate the DNA. The tube was kept at −20°C for 2 to 3 h (or overnight if more convenient). The precipitated DNA was recovered by centrifugation at 12,000 × *g* at −10°C for 20 min. The ethanol was thoroughly drained from the tube, and the DNA was suspended in 100 μl of TES buffer (50 mM NaCl, 5 mM EDTA, 30 mM Tris, pH 8.0). The DNA sample was analyzed immediately by agarose gel electrophoresis or stored at −20°C until ready for use.

**(ii) Preparation of large quantities of purified plasmid DNA.** The polyethylene glycol (PEG) precipitation method (15) was used to obtain large quantities of plasmid DNA contained in *E. coli* K-12 or clinical isolates. The plasmid DNA was purified by cesium chloride-ethidium bromide equilibrium density centrifugation, and the purified plasmid DNA was collected by fractionation (4, 7). The ethidium bromide was removed by dialysis against TES. The purified DNA was stored at −20°C. Although plasmid DNA standards can be prepared individually and mixed, we found it convenient to simply co-cultivate different *E. coli* K-12 sublines containing standard plasmid species, starting with an equal inoculum of each strain ($10^6$ cells per ml) into 500 ml of brain heart infusion broth.

It should also be noted that the PEG method can also be employed as an alternative to the SDS-salt procedure described above. Rather than using density gradient centrifugation, one simply resuspends the PEG precipitate as obtained in this procedure in a small volume of 7 M CsCl. The PEG is insoluble at this concentration, so that removal of the aqueous phase, followed by dialysis against TES, yields a partially purified plasmid preparation that may be analyzed directly by gel electrophoresis.

**Agarose gel electrophoresis of DNA.** Ethanol-precipitated DNA (2 to 25 μl) from cleared lysates was subjected to electrophoresis in 0.7% agarose (Seakem, Marine Colloids, Inc.) dissolved in Tris-borate buffer (89 mM Tris base, 2.5 mM disodium EDTA, and 8.9 mM boric acid). A dye solution consisting of bromophenol blue (0.07%), SDS (7%), and glycerol (33%) in water was added at 5 μl per sample to DNA samples prior to electrophoresis. Electrophoresis was carried out in a vertical lucite slab gel apparatus (9). The dimensions of the gel were 9.6 by 14.2 by 0.6 cm. Sample wells were made by use of a Lucite comb with 14 teeth, each 0.508 cm wide and spaced by 0.478 cm. The power source was a Heathkit regulated high-voltage power supply, model 1P-17, and electrophoresis was carried out at 60 mA, 120 V, for 2 h or until the dye neared the bottom of the gel. The gel was then placed in a solution of ethidium bromide in water (0.4 μg/ml) and stained for 15 min.

**Electron microscopy of DNA.** The contour length of plasmid DNA was determined as described previously (7, 12). Molecular weights were calculated from the contour lengths by using the conversion factor of 1 μm = 2.07 × $10^6$.

## RESULTS AND DISCUSSION

**Relationship of molecular weight to migration in agarose gels.** Purified CCC plasmid DNAs from the previously characterized R plasmids R1*drd*19, RP4, Sa, RSF1010 (Ap113), and RSF1030, as well as monomeric and dimeric CCC molecules of the ColE1 derivative pMB8, were subjected to agarose gel electrophoresis. The migrations of these CCC plasmid

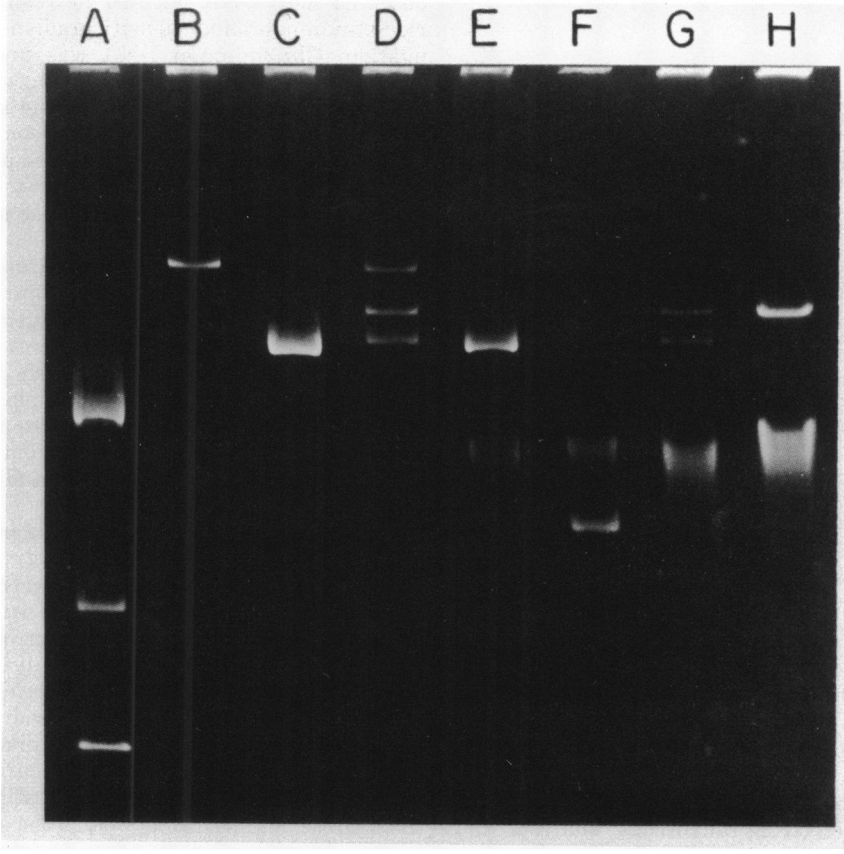


FIG. 1. *Agarose gel electrophoresis of plasmid DNA of known molecular weight. The plasmids were isolated from E. coli K-12 sublines, each subline containing one plasmid. The plasmid DNA was purified by cesium chloride-ethidium bromide centrifugation and fractionation, or was ethanol precipitated from a cleared lysate prepared by SDS lysis and salt precipitation. Migration was from top (cathode) to bottom (anode). (A) DNA (25 μl) purified by cesium chloride-ethidium bromide density centrifugation and fractionation; top band, RSF1010 Ap113 (molecular weight, 8.7 × 10⁶); middle band, dimer of pMB8 (molecular weight, 3.74 × 10⁶); bottom band, pMB8 (molecular weight, 1.8 × 10⁶); from E. coli K-12 sublines grown together in same culture flask. (B) Rldrd19, 25 μl of purified DNA. (C) Sa (molecular weight, 23 × 10⁶), 25 μl of purified DNA. (D) Upper band, R1; middle band, RP4 (molecular weight; 34 × 10⁶), and lower band, Sa; 10 μl of purified DNA of each plasmid species. (E) Ethanol-precipitated DNA (25 μl) of Sa; diffuse band is chromosomal fragments in this and following wells. (F) Ethanol-precipitated DNA (15 μl) of RSF1030 (molecular weight, 5.5 × 10⁶). (G) Ethanol-precipitated DNA (15 μl) of RP4 (upper band) and Sa (lower band) grown together in same culture flask. (H) Ethanol-precipitated DNA (30 μl) of RP4.*

DNA species were related inversely to their molecular weights (Fig. 1A through F); i.e., the larger the molecular weight, the slower the rate of migration. In each instance, only a single well-defined band of DNA was observed for each plasmid species. That the DNA was retained in the CCC state was confirmed by cutting out the DNA band from the gel, eluting the DNA from the agarose, and examining the DNA in an electron microscope. Neither open circular (OC) DNA, which migrates considerably slower than CCC DNA (1, 9), nor linear DNA, which generally migrates faster than CCC DNA (1, 9), was observed as long as the DNA was freshly prepared or had been stored at −20°C prior to use. Repeated freezing and thawing of the DNA samples or mild deoxyribonuclease treatment could convert the DNA to both the OC and linear form, however. Presumably, the presence of a "relaxation complex" (3) could result in detectable amounts of OC DNA molecules, although this has not been a problem in our experience thus far.

A plot of the logarithm of relative migration of the standard CCC plasmid DNA preparations through the gel versus the logarithm of

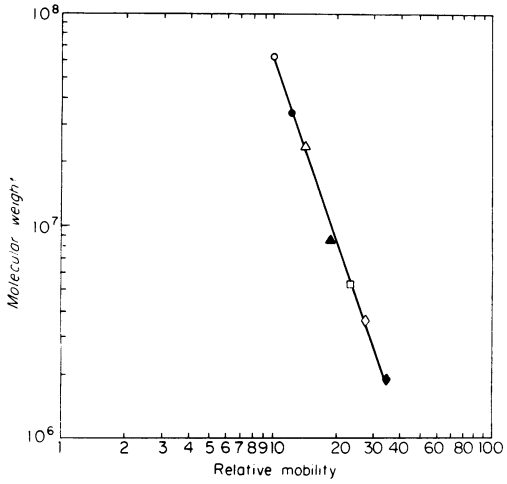


FIG. 2. *Molecular weight versus relative mobility of plasmid DNA from E. coli K-12 sublines carrying plasmids of known molecular weight. The mobility of plasmid R1 (molecular weight, 62 × 10⁶) was set arbitrarily at 10. Symbols: ○, R1 (molecular weight, 62 × 10⁶); ●, RP4 (molecular weight, 34 × 10⁶); △, Sa (molecular weight, 23 × 10⁶); ▲, RSF1010 Ap113 (molecular weight, 8.7 × 10⁶); □, RSF1030 (molecular weight, 5.5 × 10⁶); ◇, pMB8 dimer (molecular weight, 3.74 × 10⁶); ◆, pMB8 (molecular weight, 1.87 × 10⁶).*

plasmid molecular weight (previously determined by electron microscope analysis and physical measurements; 7) is shown in Fig. 2. A linear curve was observed consistently. One of the difficulties inherent in the estimation of plasmid molecular weight from its relative migration is shown in Fig. 1, wells C and D. Well D contains a mixture of R1*drd*19, RP4, and Sa plasmid DNA and represents the optimal band configuration that we attempt to achieve for accurate measurement. Well C contains an excess of Sa CCC DNA, and it may be observed that this higher concentration of Sa DNA bands at a position noticeably lower than that of the Sa DNA (the lower band) in well D. Taking the relative migration of the Sa DNA in well D as a standard, therefore, the estimation of the molecular weight of the plasmid DNA in well C from Fig. 1 would be somewhat lower. In our experience, however, the degree of error from differences in the amount of DNA was no greater than 10% of the true plasmid molecular weight (assuming contour length measurements as the standard for true molecular weight).

Plasmid DNA isolated by ethanol precipitation of DNA from an SDS-salt-cleared lysate (Fig. 1E through H) compared favorably to that observed with DNA purified by cesium chloride-ethidium bromide density gradient centrifugation. Chromosomal DNA was present in these preparations but generally appeared as a diffuse band well below CCC plasmid DNA, 10 × 10⁶ daltons in mass. The chromosomal fragments also did not interfere with the detection of CCC plasmid DNA of the 5.5 × 10⁶ molecular weight plasmid RSF1030 (Fig. 1F) or plasmids of lower molecular weight. Even in the presence of very large amounts of contaminating chromosomal fragments it was unusual for plasmid DNA to be obscured. Nevertheless, it should be noted that this is a potential drawback of the method examining such partially purified cell lysates, which contain plasmids of from 7 × 10⁶ to 15 × 10⁶ daltons in mass. A comparison of the plasmid DNA from cleared lysates of *E. coli* K-12 (Fig. 1E, G, and H) with the standard purified CCC DNAs (Fig. 1D) clearly demonstrates the utility and practicality of the method.

**Application of the agarose gel electrophoresis to clinical isolates and its use in genetic analysis.** The agarose gel electrophoresis method has been applied to the analysis of the plasmid complement of clinical isolates. Three toxigenic *E. coli* isolates from individuals participating in a study of traveller's diarrhea in Mexico were received from E. Gangarosa, and their plasmid complement was investigated by the agarose gel method as well as the standard cesium chloride-ethidium bromide density gradient centrifugation method. One strain, 514, was characterized as producing a heat-stable enterotoxin (ST) and was resistant to tetracycline (Tc) and sulfonamide (Su). Strain 471 also produced ST, whereas strain 322 produced both ST and a heat-labile (LT) enterotoxin. Strains 471 and 322 were susceptible to clinically relevant antibiotics. Figure 3 shows the migration of DNA extracted from these clinical isolates. Figure 3A shows the DNA from an *E. coli* K-12 strain, showing only chromosomal fragments; Fig. 3B shows the migration of standard CCC plasmid species. The latter sample was run routinely in every gel as a basis for the calculation for the molecular weight of unknown plasmid species. DNA from an *E. coli* K-12 $F^-$ strain or an *E. coli* K-12 R1*drd*19 strain was prepared each time to monitor the effectiveness of the lysis procedure and ethanol precipitation. In addition, Fig. 3 shows that each of the clinical isolates contained at least two plasmid species and that the plasmid DNA migrated to the same position in the gel whether the plasmid DNA was purified by cesium chloride-ethidium bromide centrifugation or was

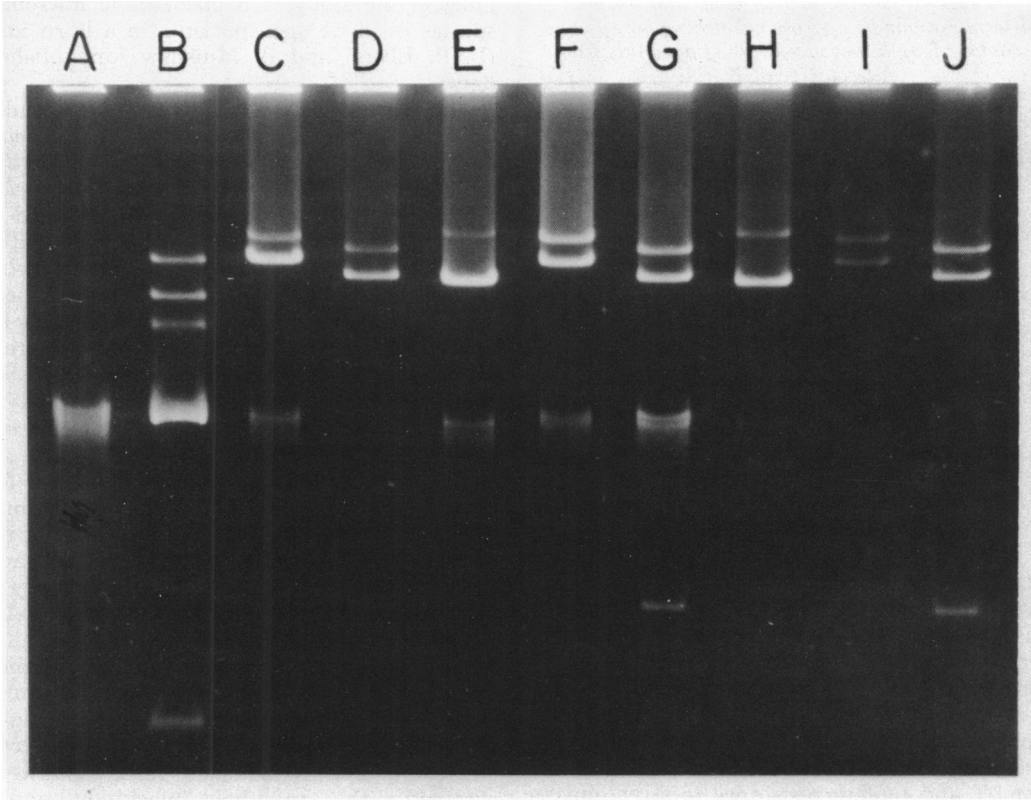


FIG. 3. *Agarose gel electrophoresis of plasmid DNA from enterotoxigenic E. coli. (A) Chromosomal fragments from E. coli K-12 F⁻; 15 μl of ethanol-precipitated DNA from cleared lysate. (B) Purified standard DNA (13 μl): upper band, R1drd19; second band, RP4; third band, Sa; fourth band, RSF1010 Ap113; fifth band, dimer of pMB8; sixth band, pMB8. (C) E. coli 514 ST⁺ Tcʳ Suʳ; 15 μl of ethanol-precipitated DNA from cleared lysate. (D) E. coli 471, 20 μl of DNA purified by cesium chloride-ethidium bromide density centrifugation and fractionation. (E) E. coli 322 ST⁺ LT⁺; 30 μl of ethanol-precipitated DNA from cleared lysate. (F) E. coli 514, 30 μl of ethanol-precipitated DNA from cleared lysate. (G) E. coli 471; 30 μl of ethanol-precipitated DNA from cleared lysate. (H) E. coli 322; 30 μl of DNA purified by cesium chloride-ethidium bromide density centrifugation and fractionation. (I) E. coli 514; 15 μl of DNA purified by cesium chloride-ethidium bromide density centrifugation and fractionation. (J) E. coli 471; 30 μl of DNA purified by cesium chloride-ethidium bromide density centrifugation and fractionation.*

simply ethanol precipitated from a cleared lysate (cf. Fig. 3C, F, and I; D, G, and J; 3E and H).

The purified plasmid DNA samples from strains 514, 471, and 322 were examined in an electron microscope. Isolated OC molecules were photographed and their contour lengths were measured (Table 1). The molecular masses of the plasmids of these strains were also estimated from their migration in gels relative to the standard plasmid DNA. The largest molecular species in the clinical isolates possessed a mass greater than the largest standard plasmid species (R1*drd*19), 62 × $10^6$ daltons. However, when the standard curve was projected as a straight line beyond the molecular mass of R1*drd*19, the relative migration of the larger plasmid species of strains 514, 471, and 322 fell precisely on the projected line (Fig. 4). Table 1 permits a comparison of the molecular mass obtained by electron microscopy and that estimated from migration in gels. There was a close agreement between the two methods.

The observation that enterotoxigenic clinical isolates possessed several distinct plasmid species was by no means surprising. Earlier studies from this and other laboratories established that this is a common occurrence (6, 18). In the further analysis of such strains, it is generally necessary to mate the clinical isolate with a

TABLE 1. *Comparison of molecular weight determinations by agarose gel electrophoresis and contour length measurements of plasmids from enterotoxigenic E. coli*

| Strain | Contour length | | Gel electrophoresis | |
|---|---|---|---|---|
| | No. of molecules measured | Mol wt[a] ($\times 10^6$) | No. of determinations | Estimated mol wt ($\times 10^6$) from relative migration |
| 514 | 27 | 91.7 ± 2.5 | 3 | 90 |
| | 77 | 61.4 ± 2.1 | 3 | 61.5–63 |
| 322 | 14 | 93.2 ± 3.0 | 3 | 94–95 |
| | 35 | 43.9 ± 1.9 | 3 | 44–47 |
| 471 | 13 | 67.5 ± 2.3 | 3 | 70–72 |
| | 15 | 44.6 ± 2.1 | 3 | 46–47 |
| | 17 | 3.2 ± 0.2 | 3 | 3.4–3.6 |

[a] Molecular weights were calculated from contour lengths by using the conversion factor 1 $\mu$m = 2.07 $\times 10^6$.

standard laboratory recipient and to screen transconjugants for the inheritance of toxigenesis, (Ent$^+$) and other possible plasmid-mediated properties such as antibiotic resistance or K antigen synthesis. We have found the agarose gel method to be a useful adjunct for such screening. For example, toxigenic strain B41, isolated from scouring calves (17, 18), encodes for ST and confers resistance to streptomycin (Sm) and Tc. When strain B41 was analyzed by agarose gel electrophoresis it was observed to contain two plasmid species estimated to be 65 $\times 10^6$ and 46 $\times 10^6$ daltons (Fig. 5B). Genetic transfer experiments between strain B41 and *E. coli* K-12 strain 711 resulted in the isolation of Ent ST$^+$ Tc$^s$ Sm$^s$ transconjugants as well as Ent ST$^-$ Tc$^r$ Sm$^r$ transconjugants. The analysis of the DNA from these transconjugants by agarose gel electrophoresis revealed that the Ent ST$^+$ Tc$^s$ S$^s$ transconjugants harbored only the 65 $\times 10^6$-dalton plasmid (Fig. 5A), whereas the Ent ST$^-$ Tc$^r$ Sm$^r$ transconjugants possessed only the 45 $\times 10^6$-dalton plasmid species (Fig. 5C). Ent ST$^r$ Tc$^r$ Sm$^r$ transconjugants possessed both plasmids (Fig. 5B). Hence, the application of the agarose gel method permitted not only the identification of the plasmid species within strain B41, but also served as an invaluable aid in quickly determining the association between the plasmid species and a specific phenotypic trait. In a similar vein, the 61 $\times 10^6$-dalton plasmid of the human toxigenic isolate 514 (Fig. 3C, F, and I) has been associated with transmissible Tc Su resistance, whereas the larger plasmid species, 90 $\times 10^6$ daltons, appears to be solely associated with the Ent phenotype. We applied this methodology as an adjunct to the study of R plasmids of microbial species resident upon patients in a burn unit (L. P. Elwell and B. Minshew, unpublished data).

The agarose gel method has been successfully applied to clinical isolates of *E. coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Shigella dysenteriae*, *Enterobacter cloacae*, *Salmonella* sp., and other enterobacteria. As illustrated in Fig. 6 and Table 2, we also found the method to be directly applicable to strains of *H. influenzae* bearing R plasmids, as well as to clinical isolates of *N. gonorrhoeae*. The method may be applicable to many bacterial species of interest and, indeed, in several isolated instances we used the method to detect plasmid DNA in strains of *Bacteroides fragilis* and *Streptococcus faecalis*.

The agarose gel electrophoresis method for detecting CCC plasmid DNA and the estimation of plasmid mass is sensitive, does not require radioisotopes or ultracentrifugation, and compares favorably with electron microscopy of purified plasmid DNA. The method is relatively simple in that it employs readily available reagents and does not require, by current standards, sophisticated equipment. Although we employ a rather elaborate ultraviolet light transilluminator and camera system to record our results, in point of fact our initial data were obtained with a hand-held "blacklight" and a millimeter ruler. Undoubtedly, the basic meth-

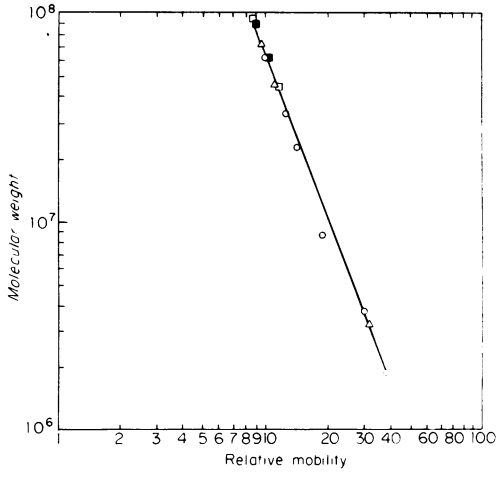


FIG. 4. *Molecular weight versus relative mobility of standard plasmid DNA from E. coli K-12 sublines and from toxigenic E. coli clinical isolates. The mobility of R1drd19 (molecular weight, 62 $\times 10^6$) was set arbitrarily at 10. Symbols: ○, standard plasmid DNA as described in the legend of Fig. 2; □, plasmids from E. coli 322; ■, plasmids from E. coli 514; △, plasmids from E. coli 471.*

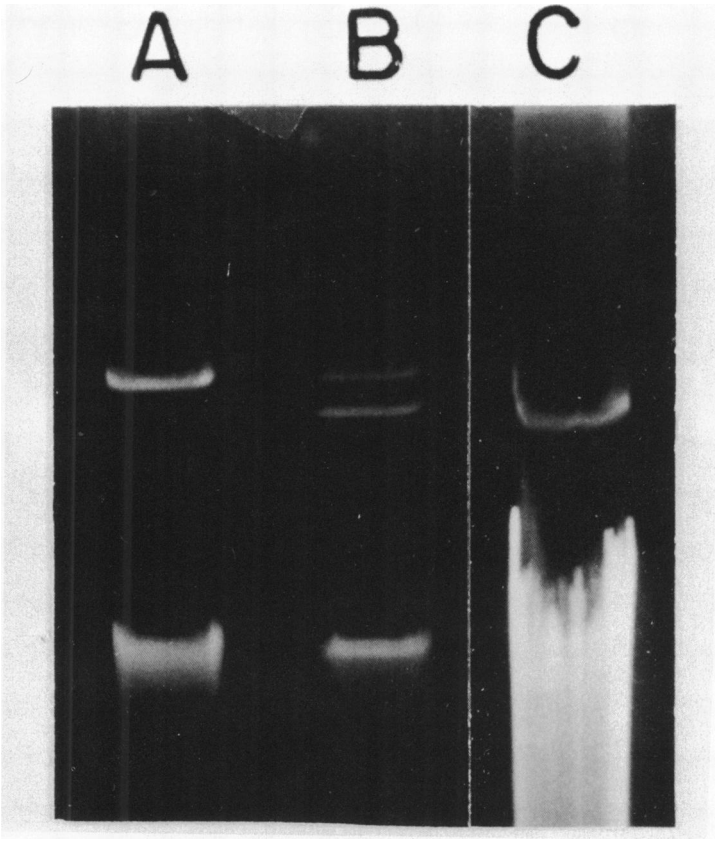


FIG. 5. *Electrophoresis of plasmid DNA from E. coli K-12 transconjugants derived from a mating with toxigenic E. coli B41. (A) Ethanol-precipitated DNA (25 μl) from cleared lysate of $Tc^s$ $Sm^s$ $ST^+$ transconjugant. (B) Ethanol-precipitated DNA (25 μl) from cleared lysate of $Tc^r$ $Cm^r$ $ST^+$ transconjugant. (C) Ethanol-precipitated DNA (25 μl) from cleared lysate of $Tc^r$ $Sm^r$ transconjugant.*

odology can be substantially simplified and refined. For example, ribonuclease digestion can be eliminated with no serious interference in DNA detection, and the time required for ethanol precipitation can be shortened substantially where maximal DNA recovery is not a critical factor. The method is also of considerable utility since from 8 to 10 cultures can be easily analyzed simultaneously by a single investigator within 3 days.

Despite the relative simplicity of the method, there are several important limitations. The effect of DNA concentration on gel migration has already been noted, as has the potential interference with the detection of some classes of CCC plasmid species by contaminating chromosomal fragments. Moreover, the amount of CCC plasmid DNA, as well as the concentration of contaminating chromosomal fragments, may vary from sample to sample, particularly with clinical isolates. For these reasons, we often initially run several different volumes (commonly 5, 10, and 25 μl) of material obtained from the ethanol precipitation of cleared lysates. Although the concentration of DNA in these samples is not known, as little as 50 ng of DNA in the band of least visibility need be present for the band to be seen (1, 9). As noted earlier, we have not experienced any difficulty thus far with OC DNA molecules as long as the DNA has been freshly prepared. We presume that, in part, this is a reflection that OC molecules are normally present in relatively low concentrations in our preparations and migrate considerably more slowly than the corresponding CCC form (1, 9). Nevertheless, we emphasize that the gel method alone does not permit discrimination between OC and CCC DNA. For example, in a previously uncharacterized isolate, one might not be able to discern between

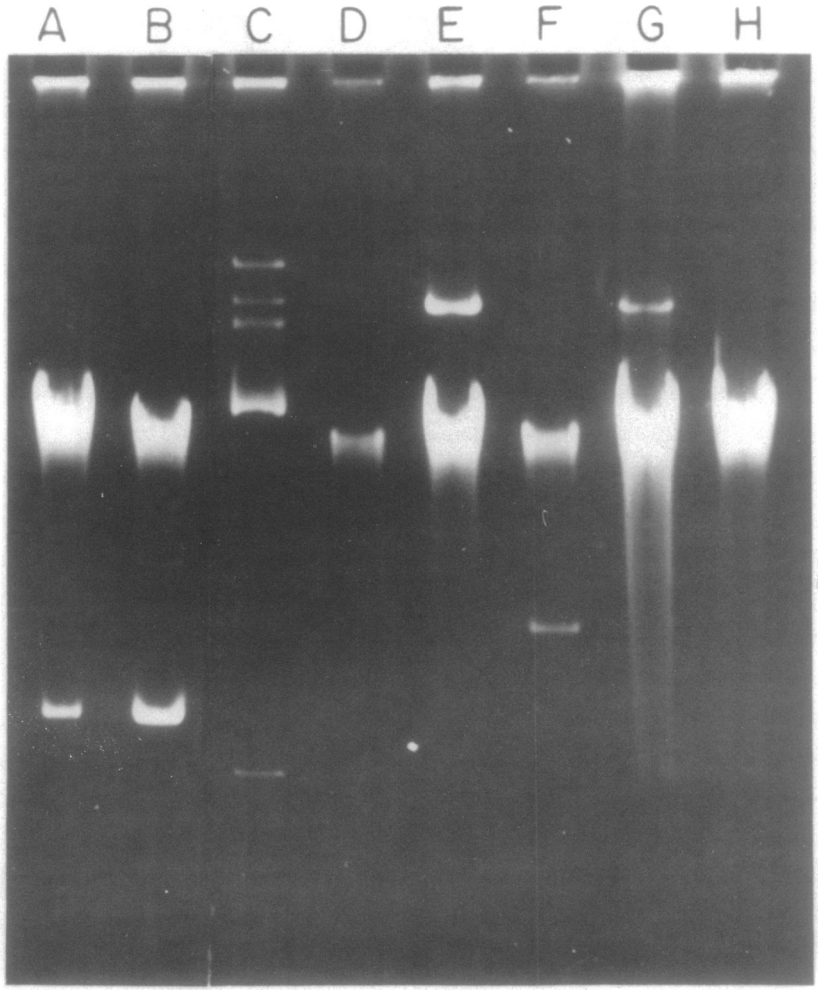


FIG. 6. *Agarose gel electrophoresis of ethanol-precipitated plasmid DNA from cleared lysates of N. gonorrhoeae and H. influenzae. (A) N. gonorrhoeae (GSF1911): molecular weight, 2.65 × 10⁶; cryptic plasmid. (B) N. gonorrhoeae (GFF1990): molecular weight, 2.5 × 10⁶; cryptic plasmid. (C) Standard plasmid DNA as described in the legend to Fig. 2. (D) Chromosomal DNA from E. coli K-12, lacking any plasmid DNA. (E) H. influenzae (UB2811); molecular weight, 31 × 10⁶; plasmid specifying Tetʳ. (F) H. influenzae (RSF0885): molecular weight, 3.6 × 10⁶; plasmid specifying Ampʳ. (G) H. influenzae (RSF007): molecular weight, 30 × 10⁶; plasmid specifying Ampʳ. (H) H. influenzae 4B, chromosomal DNA only.*

TABLE 2. *Comparison of molecular weight determinations by agarose gel electrophoresis and contour length measurements of plasmids from N. gonorrhoeae and H. influenzae*

| Strain and plasmid | Contour length | | Gel electrophoresis | |
|---|---|---|---|---|
| | No. of molecules measured | Mol wt[a] (×10⁶) | No. of determinations | Estimated mol wt (×10⁶) from relative migration |
| *N. gonorrhoeae* (GSF1911) | 109 | 2.39 ± 0.04[a] | 2 | 2.4–2.65 |
| *N. gonorrhoeae* (GSF1990) | 387 | 2.36 ± 0.12[a] | 2 | 2.3–2.5 |
| *H. influenzae* (UB2811)[b] | 3 | 31.1 | 1 | 30.0 |
| *H. influenzae* (RSF0885) | 56 | 3.19 ± 0.12[c] | 1 | 3.6 |
| *H. influenzae* (RSF007) | 56 | 30.2 ± 0.59[c] | 1 | 30.0 |

[a] Data taken from reference 16.
[b] Clinical isolate received from J. Saunders and M. H. Richmond.
[c] Data taken from reference 5.

two high-molecular-weight species of CCC DNA or one species that is easily nicked. If there is doubt on this point, it is possible to subject the lysate to freezing and thawing, mild deoxyribonuclease treatment (3), or X irradiation (8, 12) to deliberately convert the CCC DNA to the OC form. It should also be emphasized that, although we have easily detected plasmids ranging from $0.6 \times 10^6$ to $95 \times 10^6$ in molecular weight, the SDS-salt precipitation method is considerably less effective for higher-molecular-weight plasmids (10). Also, plasmids $120 \times 10^6$ daltons in mass or higher do not penetrate the gel under the stated experimental conditions. The latter drawback can be overcome by employing horizontal electrophoresis with gels containing a concentration of 0.35% agarose (D. Merlo and J. Meyers, unpublished data). Our choice of the SDS-salt precipitation method was dictated by our familiarity with the technique, as well as its utility in successfully lysing a wide variety of microbial species (10). However, the PEG precipitation method (15), as well as the Brij 58- or Triton X-100-cleared-lysate method (3), could be readily substituted.

The agarose gel electrophoresis method is not presented here as a means for precise plasmid characterization. It will, however, often rapidly and simply provide all of the information that is required by an investigator with regard to the plasmid complement of a bacterial strain. We found the method to be an important adjunct to genetic analysis, and it is invaluable for survey work as well as the epidemiological investigation of plasmid dissemination.

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